# INNOVATIVE PATTERNS AND TECHNOLOGIES OF CARDIAC REHABILITATION IN PATIENTS WITH CORONARY ARTERY DISEASE

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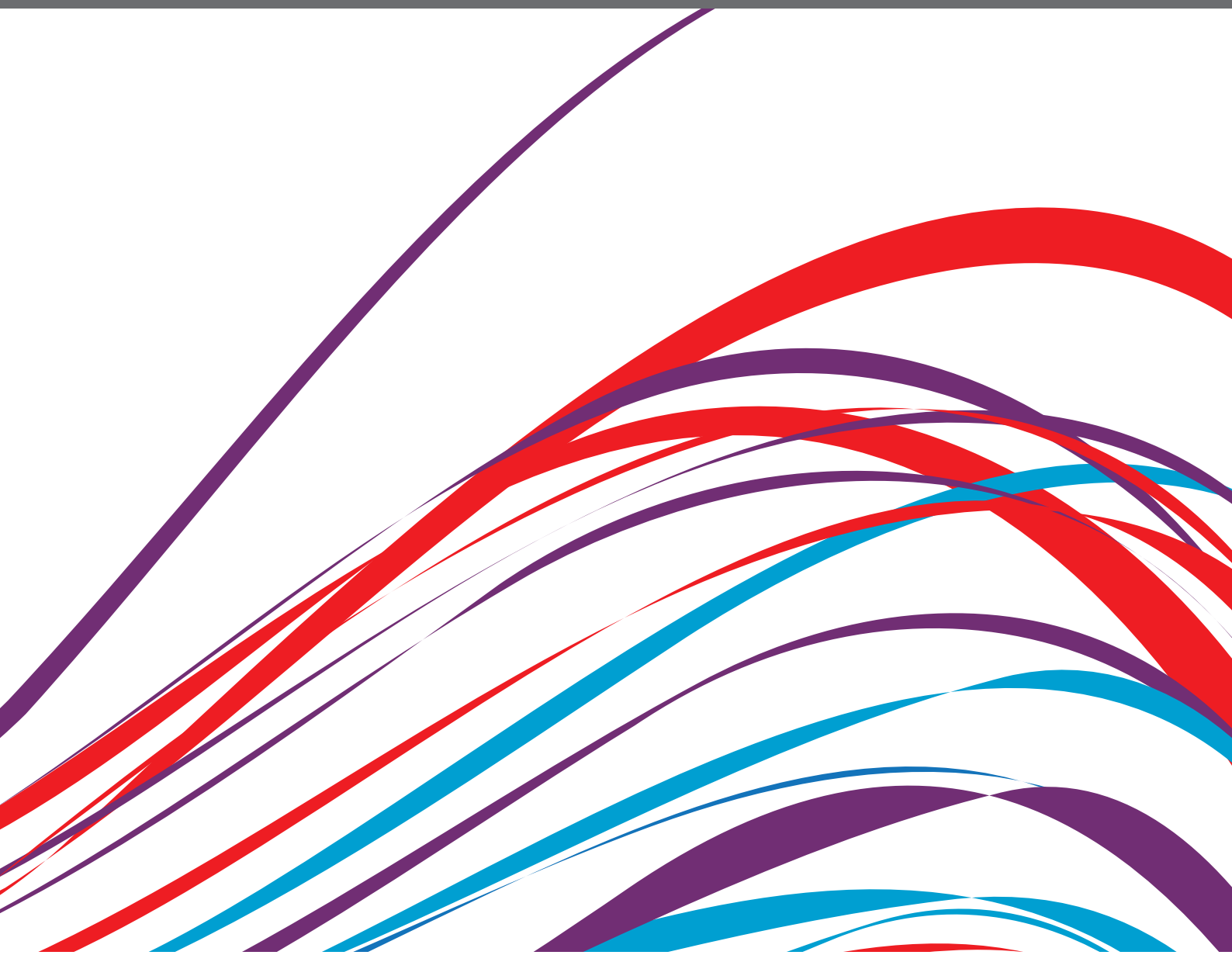

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# INNOVATIVE PATTERNS AND TECHNOLOGIES OF CARDIAC REHABILITATION IN PATIENTS WITH CORONARY ARTERY DISEASE

Topic Editors:
**Yan Zhang,** Peking University, China
**Matthew N. Bartels,** Albert Einstein College of Medicine, United States
**Han Xiao,** Peking University Third Hospital, China

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# Influence of Waist-to-Hip Ratio on the Prognosis of Heart Failure Patients With Revascularized Coronary Heart Disease

*Yingyue Zhang[1,2†], Yan Zhang[3†], Yajun Shi[1], Wei Dong[1], Yang Mu[1], Jing Wang[1], Yifan Gao[1,2], Rong Hu[1,2], Yong Xu[1,2*], Yundai Chen[1,2*] and Jing Ma[1*]*

[1] Department of Cardiology, Chinese PLA General Hospital, Beijing, China, [2] Medical School of Chinese PLA, Beijing, China, [3] The First Affiliated Hospital of Dalian Medical University, Liaoning, China

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***Edited by:***
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*Peking University, China*

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*Xijing 986 Hospital, Fourth Military Medical University, China*

****Correspondence:***
*Yong Xu*
*13701141929@163.com*
*Yundai Chen*
*cyundai@vip.163.com*
*Jing Ma*
*crystalma@126.com*

*† These authors have contributed equally to this work and share first authorship*

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**Background:** Heart failure (HF) is considered one of the most common complications of coronary heart disease (CHD), with a higher incidence of readmission and mortality. Thus, exploring the risk factors related to the prognosis is necessary. Moreover, the effect of the waist-to-hip ratio (WHR) on HF patients with revascularized CHD is still unclear. Thus, we aimed to assess the influence of WHR on the prognosis of HF patients with revascularized CHD.

**Methods:** We collected data of HF patients with revascularized CHD who were referred to the Cardiac Rehabilitation Clinic of PLA Hospital from June 30, 2015, to June 30, 2019. Cox proportional hazard regression analysis was used to determine the relationship between WHR and prognosis of HF patients with revascularized CHD. Patients were divided into higher and lower WHR groups based on the cutoff WHR value calculated by the X-tile software. Cox regression analysis was used to analysis the two groups. We drew the receiver operating characteristic curve (ROC) of WHR and analyzed the differences between the two groups. Endpoints were defined as major adverse cardiac events (MACE) (including all-cause mortality, non-fatal myocardial infarction, unscheduled revascularization, and stroke).

**Results:** During the median follow-up of 39 months and maximum follow-up of 54 months, 109 patients were enrolled, of which 91.7% were males, and the mean age was 56.0 ± 10.4 years. WHR was associated with the incidence of MACE in the Cox regression analysis ($p = 0.001$); an increase in WHR of 0.01 unit had a hazard ratio (HR) of 1.134 (95%CI: 1.057–1.216). The WHR cutoff value was 0.93. Patients in the higher WHR group had a significantly higher risk of MACE than those in the lower WHR group (HR = 7.037, 95%CI: 1.758–28.168). The ROC area under the curve was 0.733 at 4 years. Patients in the higher WHR group had a higher body mass index (BMI; 26.7 ± 3.5 vs. 25.4 ± 2.4, $P = 0.033$) than patients in the lower WHR group.

**Conclusions:** WHR is an independent risk factor of the long-term prognosis of Chinese HF patients with revascularized CHD. Patients with WHR ≥ 0.93 require intensified treatment. Higher WHR is related to higher BMI and ΔVO2/ΔWR.

**Keywords: heart failure, coronary heart disease, waist-to-hip ratio, abdominal obesity, forecasting**

## INTRODUCTION

The incidence rate of heart failure (HF) ranges from 1 to 2% of the population in a developed country. However, its incidence exceeds 10% in people aged over 70 (1, 2). Recently, the distribution of the etiology of HF in developed and developing countries gradually became similar: coronary heart disease (CHD) becomes the leading cause of HF (3, 4). One Chinese survey of 42 regions and over 10 thousand hospitalized HF patients showed that CHD accounted for 56% of the cause of HF (5). The progress in the treatment of CHD, such as revascularization techniques and optimal medical therapy, has reduced the mortality rate, consequently it also increased the number of HF patients with CHD (6, 7). This subgroup of patients usually brings a significant burden to social and medical insurance because of the high incidence of rehospitalization and mortality; however, strong evidence from diagnosis to treatment is still lacking (6, 8, 9). Therefore, finding the prognostic factors of HF patients with revascularized CHD is an urgent issue.

Studies reported a strong association between abdominal obesity and cardiac metabolic characteristics (10, 11). Moreover, abdominal obesity is established as one of the risk factors of CHD (12, 13). However, the effect of abdominal obesity on the prognosis of HF is still controversial. Some researchers pointed out that abdominal obesity was a risk factor of HF and is related to the increase of all-cause mortality (14, 15). Other researchers proposed that abdominal obesity was a "protective" factor of HF and related to the improvement of HF prognosis (16, 17). Surprisingly, little attention has been devoted to the influence of abdominal obesity on the prognosis of HF patients with CHD.

Abdominal obesity is measured by different methods such as computerized tomography (CT), magnetic resonance imaging (MRI), anthropometry measurements, and other bioelectrical impedance analysis. Anthropometry measurements includes waist circumference (WC), waist-to-height ratio (WHtR), and waist-to-hip ratio (WHR) (18). CT, MRI, and bioelectrical impedance analysis are the methods using direct and precise measurements of abdominal fat, but they are not widely used in the clinical work due to the high cost or concerns about radiation. Moreover, the measurement of obesity based on CT doesn't seem much better than WC measurement in patients with subclinical coronary heart disease (19). In contrast, anthropometry measurements, like WC and WHR, which have been proven to be related to visceral fat, are easy to perform at a low cost. Thus, anthropometry measurements are widely used to measure body fat distribution and widely applied in the clinic (20, 21). Although there is no significant difference between WHR, WC, and WHtR in terms of their influence on clinical outcomes (22), WHR is considered more accurate to define abdominal obesity than WC for patients with large body size. Individuals with large body size without abdominal obesity may be misdiagnosed as having abdominal obesity because of the high WC (23). WHR is demonstrated to be related to the risk of CHD (24), therefore, we used WHR as a measurement of abdominal obesity in this study.

Although cardiopulmonary function is closely related to the prognosis of HF, it is not well-utilized in the clinical practice due to measuring difficulty and lack of standard (25). As an essential measurement of cardiopulmonary function, cardiopulmonary exercise test (CPET) and its indices are recognized as a certain influencing factor of the prognosis of HF (26–28). With the above background, this study aimed to assess the influence of WHR on the prognosis of HF patients with revascularized CHD.

## MATERIALS AND METHODS

### Study Population

Consecutive patients who were referred to the Cardiac Rehabilitation Clinic of PLA Hospital from June 30, 2015, to June 30, 2019, were invited in our study. The Ethics Committee of PLA General Hospital approved the study and all participants provided written informed consent (registration number: ChiCTR2000035048). This study is a prospective study.

The inclusion criteria were as follows: (1) age between 18 and 80 years, (2) diagnosis of CHD [in accordance with the 2012 ACCF/AHA focused update of the guideline for the management of patients with unstable angina/Non-ST-elevation myocardial infarction (29)] and underwent revascularization, (3) diagnosis of HF [in accordance with 2013 ACCF/AHA guideline for the management of heart failure (30)], (4) available WHR data and CPET results, and (5) left ventricular ejection fraction (LVEF) lower than 50%. The exclusion criteria were as follows: (1) HF due to non-ischemic cardiomyopathy (such as dilated cardiomyopathy and hypertrophic cardiomyopathy), (2) severe angina, (3) uncontrolled arrhythmia, and (4) untreatable carcinoma. Baseline information including demographic characteristics (such as age, sex, etc.), clinical features (such as diagnosis, history of the disease, etc.), complications (such as hypertension, diabetes, etc.), medicine, CPET results, cardiac ultrasound results, and laboratory results were collected 3 months before and after CPET from the database of the Cardiac Rehabilitation Clinic of PLA hospital.

### Waist and Hip Circumference Measurement

Trained nurses used uniform standards during measurement. The waist and hip circumference were measured when patients were standing, wearing light clothing. At the end of expiration and the beginning of inspiration, WC was measured at the midpoint between the lowest point of the rib and the upper edge of the iliac crest. Hip circumference was measured at the most prominent part of the buttocks. The measurement of WC and hip circumference had an accuracy of 0.1 cm. The average WC and hip circumference were calculated from three measurements. WC divided by hip circumference was defined as WHR.

### CPETs

Every patient enrolled in our study performed the cardiopulmonary exercise test using the stationary cycle ergometer and gas analysis apparatus (CS-200, Schiller, Obfelden, Switzerland). The breath-by-breath method was used to analyze gas exchanges. Mixed gases ($4\%CO_2/16\%O_2/N_2$) were used for calibration before each test, and the test was performed using the ramp protocol. The exercise duration

was 8–12 min. The ramp protocol was carried out as follows: the patient rested for 1 min with a load of 0 W, performed warm-up exercises for 2 min with a load of 0 W, and continued the exercise with an initial load of 5 W. The load was further increased in the ramp-incremental exercise (25 W/min in men, 20 W/min in women). The speed ranged from 55 to 65 rpm until the maximal load. For the recovery protocol, the patient performed exercise with load of 0 W for at least 2 min. When ST depression was $\geq$3 mm, the systolic blood pressure or average blood pressure decreased by $\geq$10 mmHg, angina or severe arrhythmia occurred, or the patient requested to stop the exercise, the exercise load was removed and the test was stopped.

## Outcomes

The primary outcome in this study was the occurrence of major adverse cardiac events (MACE), including all-cause mortality, non-fatal myocardial infarction, unscheduled revascularization, and stroke. All-cause mortality was defined as death from any cause. Non-fatal myocardial infarction was defined according to ESC guidelines (31). Unscheduled revascularization was defined as balloon dilatation, percutaneous coronary intervention, or coronary artery bypass grafting surgery unexpectedly. Stroke was defined as ischemic or hemorrhagic nervous system disease which is not secondary to brain tumors, brain trauma, or other reasons. Clinical end-point events were determined by the steering committee.

## Follow-Up

Clinical outcomes were collected from clinic visits, 6-month telephone interviews, or medical history from our hospital's database. We contacted the patients or their families by phone call prior to recording their outcomes.

## Statistical Analysis

Normal data are presented as mean ± standard deviation, and non-normal data are presented as median (25th percentile, 75th percentile). Cox proportional hazards models were used to determine whether WHR was independently associated with MACE. Patients were divided into two groups according to the WHR cutoff value calculated by the X-tile software. Kaplan–Meier analysis and log-rank test were used to assess significant differences in survival time and survival differences between the two groups. Hazard ratio (HR) and confidence interval (CI) between the higher and lower WHR groups were calculated by Cox proportional risk regression. Further adjustments included possible predictors of abdominal

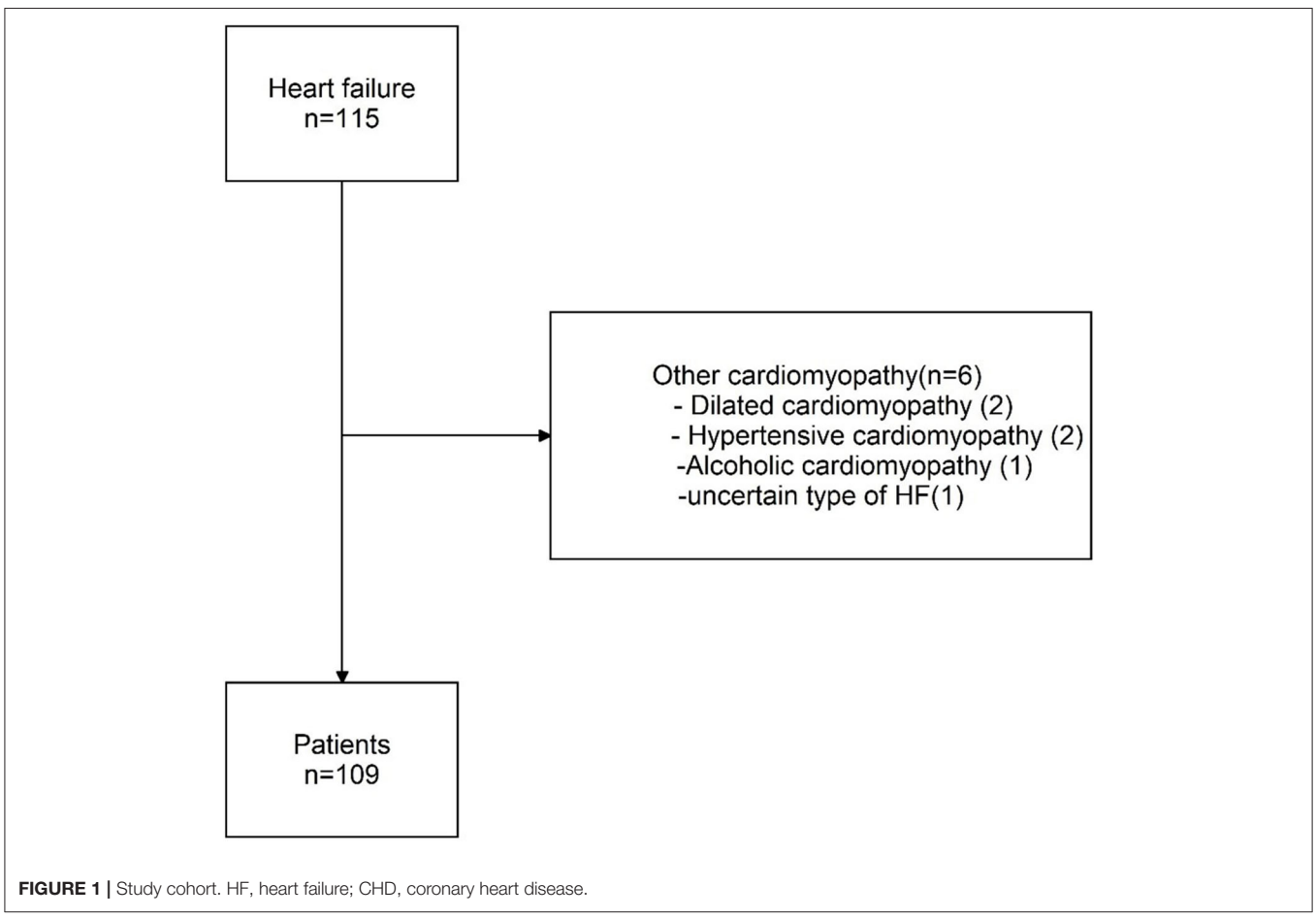


**FIGURE 1 |** Study cohort. HF, heart failure; CHD, coronary heart disease.

**TABLE 1 |** Baseline characteristics of study participants.

| Characteristic | Mean ± std/median (25–75 p)/No of cases (%) |
|---|---|
| **Demographic characteristics** | |
| Sex, male | 100 (91.7) |
| Age, years | 56.0 ± 10.4 |
| WHR | 0.93 (0.90–0.96) |
| BMI, kg/m$^2$ | 26.1 ± 3.1 |
| **BMI grade** | |
| <18.5 kg/m$^2$ | 0 (0) |
| 18.5–24.9 kg/m$^2$ | 37 (33.94) |
| 25.0–29.9 kg/m$^2$ | 60 (55.05) |
| ≥30.0 kg/m$^2$ | 12 (11.01) |
| **NYHA class** | |
| I | 55 (50.5) |
| II | 39 (35.8) |
| III | 8 (7.3) |
| IV | 7 (6.4) |
| **LVEF (%)** | |
| HFrEF | 17 (15.6) |
| HFmrEF | 92 (84.4) |
| SBP | 125.7 ± 14.6 |
| DBP | 80.2 ± 10.4 |
| LVEF, % | 44.5 (41–46) |
| Smoking history | 81 (79.4) |
| **Medical history** | |
| MI | 99 (90.8) |
| Hypertension | 59 (54.1) |
| Diabetes | 32 (29.4) |
| Hyperlipidemia ($n$ = 107) | 56 (52.3) |
| **Pharmacotherapy ($n$ = 101)** | |
| ACEI | 31 (30.7) |
| ARB | 11 (10.9) |
| Beta-blocker | 81 (80.2) |
| Statins | 96 (95.1) |
| Diuretic | 17 (16.8) |
| Antiplatelet agents | 98 (97.0) |
| Digoxin | 5 (5.0) |

*WHR, waist-to- hip ratio; BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection function; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-ranged ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; MI, myocardiopathy infraction; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker.*

obesity, HF, or coronary heart disease. We computed receiver operating characteristic (ROC) curves to evaluate the value of WHR in predicting MACE incidence within 1, 2, 3, and 4 years. The characteristics of the two groups were also compared. Chi-square test, Fisher's exact test, or Mann–Whitney $U$-test was used to compare categorical variables, while Student's $t$-test or Mann–Whitney $U$-test was used to compare continuous variables, as appropriate. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 19 and R Language Version 3.6.3.

**TABLE 2 |** Outcomes.

| Outcomes | No of cases (%) |
|---|---|
| MACE | 16 (14.7) |
| Death | 3 (2.8) |
| Cardiac death | 2 (1.8) |
| Non-cardiac death | 1 (0.9) |
| Unscheduled revascularization | 13 (11.9) |
| Non-fatal myocardial infarction | 1 (0.9) |
| Stroke | 0 (0) |

*MACE, major adverse cardiac events.*

**TABLE 3 |** Outcomes in groups stratified by WHR.

| | WHR < 0.93 ($n$ = 54) | WHR ≥ 0.93 ($n$ = 55) | *P* |
|---|---|---|---|
| MACE | 3 (5.6) | 13 (23.6) | 0.008 |
| Death | 1 (1.9) | 2 (3.6) | 1 |
| Cardiac death | 0 (0) | 2 (3.6) | 0.495 |
| Non cardiac death | 1 (1.9) | 0 (0) | 0.495 |
| Unscheduled revascularization | 2 (3.7) | 11 (20.0) | 0.015 |
| Non-fatal myocardial infarction | 0 (0) | 1 (1.8) | 1 |

*Data are expressed as No of cases (%). MACE, major adverse cardiac events.*

# RESULTS

## General Characteristics of the Study Participants

Of the 115 patients, 109 were enrolled in our study, and the reasons of exclusion are shown in **Figure 1**. The majority [101 (91.7%)] of the patients were men, with a mean age of 56 ±10 years. The patients tended to be obese with higher WHR [0.93 (0.90–0.96)] and higher BMI (26.1 ± 3.1) kg/m$_2$. Patients were more often at New York Heart Association I [55 (50.5%)] and were more often classified as having HF with mid-range ejection fraction [92 (84.4%)]. Cardiac ultrasonography showed a median LVEF of 44.5% (5). Most of the patients [81 (79.4%)] had smoking history. The majority of the patients [99 (90.8%)] were diagnosed of old myocardial infarction. Common complications were diabetes [32 (29.4%)], hypertension [59 (54.1%)], and hyperlipidemia [56 (52.3%)]. Nearly all patients took antiplatelet drugs [98 (97.0%)] and beta-blockers [81 (80.2%)] (**Table 1**).

## Outcomes

The median survival time was 39 months (interquartile range, 14), and the maximum survival time was 54 months. During the follow-up, 3 (2.8%) patients died due to cardiac-related death ($n$ = 2) or gastrointestinal hemorrhage ($n$ = 1); 1 (0.9%) patient had a non-fatal myocardial infarction, and 13 (11.9%) patients had unscheduled revascularization (**Table 2**).

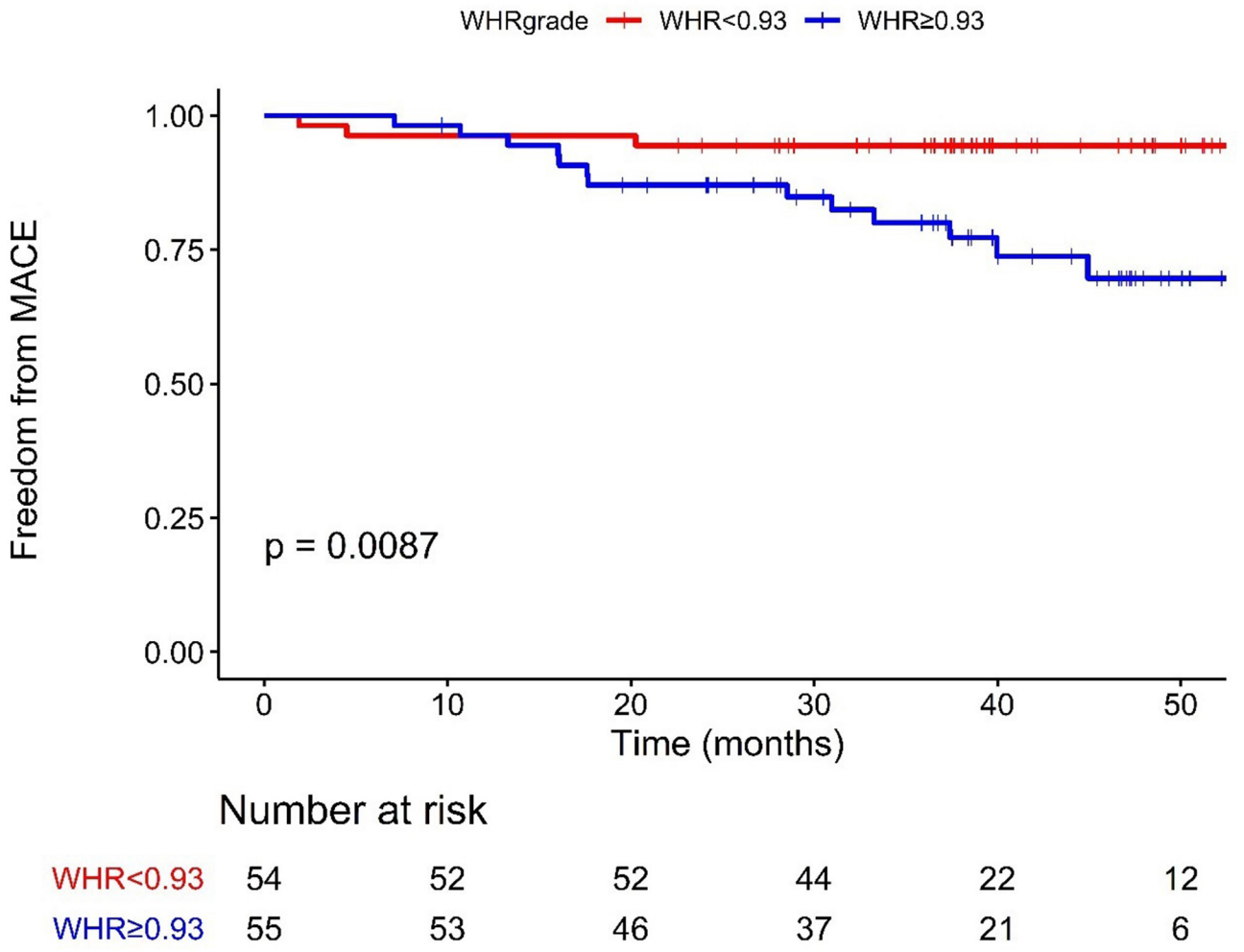


**FIGURE 2 |** Kaplan–Meier curves of MACE stratified according to the cut-off value. WHR < 0.93 (54 cases), ≥0.93 (55 cases); MACE, major adverse cardiac events; WHR, waist-to-hip ratio.

## Association of WHR With Unfavorable Outcomes of HF Patients With Revascularized CHD

Cox analysis results demonstrated WHR might be an independent predictor of the incidence of MACE ($P < 0.001$); i.e., an increase of 0.01 unit in WHR correspond to a HR of 1.134 (95%CI: 1.057–1.216). We divided the patients into two groups according to WHR cutoff value (0.93) calculated by X-tile. The incidence of MACE differed between the higher and lower WHR groups: there was a significant difference in the incidence of MACE (23.6 vs. 5.6%, $P = 0.008$) and in the incidence of unscheduled revascularization (20.0 vs. 3.7%, $P = 0.015$). No statistically significant difference was noted in the all-cause mortality or incidence of non-fatal myocardial infraction between the two groups ($P > 0.05$; **Table 3**).

**Figure 2** presents the Kaplan–Meier survival curves between the higher and lower WHR groups. Patients in the lower WHR group presented much higher event-free survival possibility than patients in the higher WHR group [$P = 0.0087$]. In the univariate analysis, patients in the higher WHR group were more likely

**TABLE 4 |** Hazard ratio and *P*-value of higher WHR group vs. lower WHR group and MACE.

| | HR (95%CI) | *P* |
|---|---|---|
| Crude | 4.611 (1.313–16.193) | 0.017 |
| Model 1 | 4.921 (1.391–17.406) | 0.013 |
| Model 2 | 5.487 (1.507–19.973) | 0.01 |
| Model 3 | 7.037 (1.758–28.168) | 0.006 |

*Model 1 was adjusted for age and sex.*
*Model 2 further adjusted for age, sex, BMI, diabetes, hypertension, and HR rest.*
*Model 3 further adjusted for age, sex, BMI, diabetes, hypertension, HR rest, VO2_kg_AT, VO2_max, VE/VCO2_slope.*
*WHR, waist-to- hip ratio; BMI, body mass index; LVEF, left ventricular ejection function; HR, heart rate; SBP, systolic blood pressure; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker.*

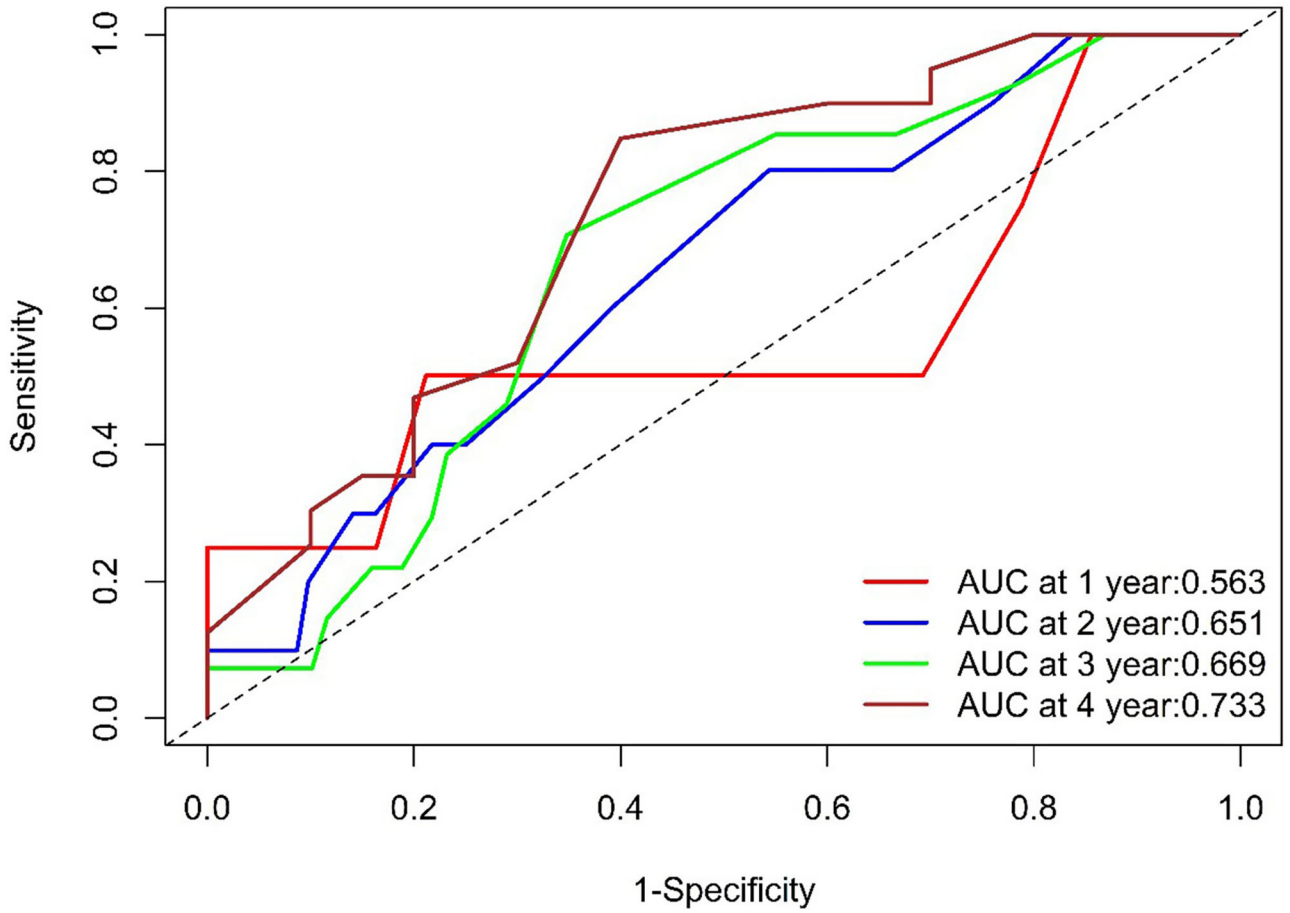


**FIGURE 3 |** ROC of WHR. The WHR AUC was 0.563 at 1 year (104 survivors and 4 events), 0.651at 2 years (92 survivors and10 events), 0.669 at 3 years (69 survivors and 13 events), and 0.733at 4 years (20 survivors and 16 events). ROC, Receiver Operating Characteristic; WHR, waist-to- hip ratio; AUC, area under the curve.

to experience MACE (HR = 4.611, 95%CI: 1.313–16.193). After adjustment for age, sex, BMI, diabetes, hypertension, HR rest as well as CPET parameters, including VO2_kg_AT, VO2_max, VE/VCO2_slope, patients in the higher WHR group were still more likely to experience MACE (HR = 7.037, 95%CI: 1.758–28.168; **Table 4**).

The ROC curves for WHR and MACE are displayed in **Figure 3**. The ROC area under curve (AUC) was 0.563 at 1 year, 0.651 at 2 years, 0.669 at 3 years, and 0.733 at 4 years.

The descriptions of patients' characteristics are shown in **Table 5**, dichotomized by the WHR cutoff value. Patients with higher WHR had a higher level of BMI (26.7 ± 3.5 vs. 25.4 ± 2.4, $P = 0.033$) and ΔVO2/ΔWR [10.2 (8.7–13.5) vs. 12.0 (9.9–14.0), $P = 0.025$] than those with lower WHR. No significant difference was found between the two groups in terms of age, sex, cardiac function classification, blood pressure, smoking history, medical history, medication, cardiac ultrasound results and other CPET parameters ($P < 0.05$).

## DISCUSSION

Abdominal obesity may be related to the prognosis of CHD, while the value is not sure in HF patients, especially in HF patients with revascularized CHD. In this study, by collecting the data of 109 revascularized CHD patients with HF with a maximum follow-up time of 54 months, we for the first time found WHR was associated with the incidence of MACE in Chinese HF patients with revascularized CHD ($P < 0.001$). Every 0.01 increase in WHR had a corresponding ∼13.4% higher risk to develop MACE. Patients in the higher WHR group had a higher risk of MACE than patients in the lower WHR group. The HR increased to 7.037 after adjustment for multivariables (**Table 4**). The ROC AUC was 0.733 at 4 years. Thus, our main finding was that WHR might be an independent risk factor of the long-term prognosis. Additionally, BMI level and ΔVO2/ΔWR in the higher WHR group demonstrated higher than that in the lower WHR group.

**TABLE 5 |** Comparison of clinical characteristic between groups stratified by WHR.

| Characteristic | WHR < 0.93 | WHR ≥ 0.93 | *P* |
|---|---|---|---|
| Male, *n* (%) | 50 (92.6) | 50 (90.9) | 1 |
| Age, years | 57.5 ± 10.0 | 54.7 ± 10.6 | 0.16 |
| BMI, kg/m$^2$ | 25.4 ± 2.4 | 26.7 ± 3.5 | 0.033 |
| **NYHA class** | | | 0.821 |
| I | 29 (53.7) | 26 (47.3) | |
| II | 16 (29.6) | 23 (41.8) | |
| III | 4 (7.4) | 4 (7.3) | |
| IV | 5 (9.3) | 2 (3.6) | |
| **LVEF (%)** | | | 0.201 |
| HFrEF | 6 (11.1) | 11 (20) | |
| HFmrEF | 48 (88.9) | 44 (80) | |
| SBP, mmHg | 126.1 ± 14.8 | 125.2 ± 14.5 | 0.739 |
| DBP, mmHg | 79.5 ± 9.7 | 80.9 ± 11.2 | 0.503 |
| LVEF, % | 44 (41–46) | 45 (40–46) | 0.732 |
| Smoking history | 41 (78.9) | 40 (80) | 0.885 |
| **Medical history, *n* (%)** | | | |
| Hypertension | 31 (57.4) | 28 (50.9) | 0.496 |
| Diabetes | 17 (31.5) | 15 (27.3) | 0.630 |
| Hyperlipidemia | 28 (52.8) | 28 (51.9) | 0.995 |
| **Pharmacotherapy, *n* (%)** | | | |
| ACEI | 14 (26.9) | 17 (34.7) | 0.397 |
| ARB | 6 (11.5) | 5 (10.2) | 0.507 |
| Statins | 48 (92.3) | 48 (98.0) | 0.363 |
| Beta-blocker | 44 (84.6) | 37 (75.5) | 0.251 |
| Diuretic | 8 (15.4) | 9 (18.4) | 0.689 |
| Antiplatelet | 50 (96.2) | 48 (98.0) | 1 |
| **CPET** | | | |
| Peak VO2, ml/ (kg·min) | 16.3 ± 0.6 | 17.3 ± 0.6 | 0.246 |
| VO2 at AT, ml/ (kg·min) | 12.3 (10.0–14.2) | 12.3 (10.0–14.1) | 0.058 |
| O2 pulse, ml/bpm | 9.8 ± 0.4 | 11.0 (8.9–12.2) | 0.110 |
| VE/$VCO_2$ slope | 26.5 (23.1–29.6) | 28.2 ± 0.6 | 0.063 |
| ΔVO2/ΔWR, ml/ (min·W) | 10.2 (8.7–13.5) | 12.0 (9.9–14.0) | 0.025 |

*Data are expressed as mean ± std, median (25–75 p), No of cases (%).*
*WHR, waist-to- hip ratio; BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection function; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-ranged ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker.*

Abdominal obesity is associated with the high mortality in CHD (32) and is a risk factor of the prognosis of CHD. Although the prognosis of CHD patients improved by the development of optimal medical therapy and revascularization technology, the incidence of HF due to myocardial infarction was still high (33), and the risk of death for HF patients due to myocardial infarction increased to 3–4-folds, compared with myocardial infarction patients without HF (34). Patients with HF due to CHD have poor prognosis. Thus, there is a need to find predictors to improve the prognosis of these patients.

The all-cause mortality of our cohort was 2.8% (**Table 2**). In this study, the median LVEF was 44.5 (35–40) %, which may be one possible reason of the low mortality. Furthermore, although 90% of the patients in this study had HF due to myocardial infarction, all patients were successfully revascularized. In addition, 97.0% of the patients received antiplatelet agents and 80.2% took beta-blockers. Most patients have received optimal medical therapy which might attribute to the low mortality.

Further Cox regression analysis showed WHR independently related to the incidence of MACE for HF patients with revascularized CHD (P<0.001); an increase of WHR by 0.01 unit correspond to ∼13.4% higher risk. To better guide clinical practice, we divided the patients into two groups stratified by WHR cutoff value of 0.93. Patients in the higher WHR group had a significantly higher risk of MACE than patients in the lower WHR group. After multivariable adjustment, the higher WHR remained significantly associated with a higher incidence of MACE. Kaplan–Meier and log-rank test analysis showed similar tendency.

Our findings were consistent, to a certain extent, with the findings of some studies reporting that WHR was associated with rehospitalization due to HF (41). WHR might affect the prognosis of HF patients with revascularized CHD in the following mechanism: WHR increased in patients with abdominal obesity, which is an external manifestation of visceral fat accumulation. To our knowledge, the accumulation of visceral adipose tissue regulates sympathetic hyperactivity through the direct influence of the autonomic nervous system. Meanwhile, the increase in visceral fat will change the secretion mode of adipocytokines (including leptin, adiponectin, etc.), which plays a vital role in insulin resistance, dyslipidemia, prethrombotic state, and chronic inflammatory state. These abnormal clinical conditions will eventually promote the development of coronary atherosclerosis and adverse events of HF (15, 42–46). Besides, abdominal obesity is associated with LV longitudinal strain and increased epicardial adiposity, which also increases the incidence of adverse cardiovascular effects (35, 36). Higher WHR may influence the prognosis of HF patients with revascularized CHD directly or indirectly through the above mechanisms.

We observed that the AUC was 0.563, 0.651, 0.669, and 0.733 (>0.7) at 1, 2, 3, and 4 years, respectively (**Figure 3**), which meant that WHR had more prognostic value in the long-term in HF patients with revascularized CHD. However, further studies with large sample are required to validate the above finding.

In this study, we also compared the differences in age, sex, cardiac function, and complications between the higher and the lower WHR groups. The difference in BMI was statistically significant. Streng et al. (23) found that the increase in WHR was associated with an increase in weight, which led to an increase in BMI. Ortega et al. (37) thought that BMI in abdominal obesity patients was higher than patients without abdominal obesity. The relationship between BMI and abdominal obesity still existed in HF patients with revascularized CHD, which may be related to common risk factors, including unhealthy diet patterns, low physical activities, and low cardiorespiratory fitness. ΔVO2/ΔWR, as a sensitive indicator of abnormal muscle oxygen transport or utilization during exercise, was higher in the higher WHR group, which may be attributed to the increased functional impairment and higher cardiopulmonary stress (38,

39). In addition, no significant differences were observed in other indicators including age, sex, cardiac function classification, medical history, and medication.

WHR was focused in Chinese revascularized HF patients for the first time. The World Health Organization recommended WHR $\geq$ 0.9 for men and WHR $\geq$ 0.85 for women as the standard diagnostic of abdominal obesity. In our study, we determined 0.93 as the cutoff value by calculation using X-tile software, which is extensively used (40). Consequently, we found a significant difference in the prognosis between patients with higher and lower WHR. Thus, patients with a WHR $\geq$ 0.93 in Chinese HF patients with revascularized CHD population, deserve more attention and intensified treatment. The meaning of abdominal obesity deserves further research in revascularized HF patients.

This study has some limitations. First, this study had a small sample size and involved a single center, although the follow-up duration was long. Thus, a multicenter study with large size is under consideration. Second, sex and ethnicity might influence fat distribution (47). The WHR cutoff value (0.93) in our study did not distinguish between male and female patients, and it was only aimed at the Chinese population. Further study with larger size and more stratification might resulted in more accurate WHR cutoff value. Incomplete revascularization may affect MACE, while the information of incomplete revascularization was not collected.

## CONCLUSION

In conclusion, this study shows that WHR demonstrated to be an independent risk factor of the long-term prognosis of Chinese HF patients with revascularized CHD. Patients with higher WHR are more vulnerable to develop MACE in the long term. Thus, Patients with WHR $\geq$ 0.93 need more concern and require intensified treatment. Higher WHR is related to higher BMI and higher $\Delta$VO2/$\Delta$WR, which may be associated with the common risk factors of abdominal obesity and obesity.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of PLA General Hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JM designed and helped in the data analysis and manuscript writing. YX and YC contributed to the conception of the study. YiZ and YaZ performed the data analyses and wrote the manuscript. YS performed the CPET and collected the data. WD, YM, JW, YG, and RH referred the participants and helped perform the analysis with constructive discussions. All authors contributed to the article and approved the submitted version.

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# Chinese Home-Based Cardiac Rehabilitation Model Delivered by Smartphone Interaction Improves Clinical Outcomes in Patients With Coronary Heart Disease

*Jing Ma[1†], Cheng Ge[1†], Yajun Shi[1], Yong Xu[1], Chenghui Zhao[1], Ling Gao[1], Dongling Wen[1], Tengjing Li[1], Jinli Wang[1], Sherry Yan[2], Sidney C. Smith Jr.[3] and Yundai Chen[1*]*

[1] Department of Cardiology, First Medical Center of Chinese People's Liberation Army (PLA) General Hospital, Beijing, China, [2] Center of Health System Research, Sutter Health, Walnut Creek, CA, United States, [3] Heart and Vascular Center, University of North Carolina, Chapel Hill, NC, United States

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**Edited by:**
Yan Zhang,
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**Reviewed by:**
Danyan Xu,
Central South University, China
Junnan Wang,
Jilin University, China

***Correspondence:**
Yundai Chen
cyundai@vip.163.com

[†] These authors share first authorship

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**Purpose:** We evaluated the long-term effect of a smartphone-facilitated home-based cardiac rehabilitation (HBCR) model in revascularized patients with coronary heart disease (CHD) on major adverse cardiac events (MACE), and secondary outcomes, including safety, quality of life, and physical capacity.

**Methods:** It was a prospective observational cohort study including a total of 335 CHD patients after successful percutaneous coronary intervention (PCI) referred to the CR clinic in China between July 23, 2015 and March 1, 2018. Patients were assigned to two groups: HBCR tailored by monitoring and telecommunication *via* smartphone app (WeChat) (HBCR group, $n = 170$) or usual care (control group, $n = 165$), with follow-up for up to 42 months. Propensity score matching was conducted to match patients in the HBCR group with those in the control group. The patients in the HBCR group received educational materials weekly and individualized exercise prescription monthly, and the control group only received 20-min education at baseline in the CR clinic. The primary outcome was MACE, analyzed by Cox regression models. The changes in the secondary outcomes were analyzed by paired *t*-test among the matched cohort.

**Results:** One hundred thirty-five HBCR patients were matched with the same number of control patients. Compared to the control group, the HBCR group had a much lower incidence of MACE (1.5 vs. 8.9%, $p = 0.002$), with adjusted HR = 0.21, 95% CI 0.07–0.85, and also had reduced unscheduled readmission (9.7 vs. 23.0%, $p = 0.002$), improved exercise capacity [maximal METs (6.2 vs. 5.1, $p = 0.002$)], higher Seattle Angina Questionnaire score, and better control of risk factors.

**Conclusions:** The Chinese HBCR model using smartphone interaction is a safe and effective approach to decrease cardiovascular risks of patients with CHD and improve patients' wellness.

**Clinical Trial Registration:** http://www.chictr.org.cn, identifier: ChiCTR1800015042.

**Keywords: coronary heart disease (CHD), exercise training, smartphone, home-based cardiac rehabilitation, major adverse cardiac events**

## INTRODUCTION

Coronary heart disease (CHD) remains the leading cause of death worldwide after decades of major advances in treatment (1, 2). In the USA, over 370,000 people die annually from CHD (cdc.gov), whereas in economically developing countries, China, for instance, the incidence of CHD exceeds 11 million with a death rate of about 110 per 100,000.

Cardiac rehabilitation (CR), as a continuum of care for patients with CHD after initial treatment, has been approved to significantly promote wellness, improve exercise capacity, and preserve cardiac function (3, 4). In 2018, multiple medical professional agencies established referral to CR as a performance measure (5, 6). However, the referral rate and completion rate for CR are still suboptimal, with 53–74% of patients referred to CR (5, 6) and participation rates of 19–34% (7, 8). The participation rate is much lower in developing countries. In China, the referral rate is <1%, although a large number of patients are eligible (8). The major barriers to referral or participation to CR include lack of center-based cardiac rehabilitation (CBCR) facilities and lack of patient awareness. According to a survey, In China, there are only ∼500 CR centers nationwide and mostly located in major cities; the majority of eligible patients are from rural areas and they do not have an access to CR. To increase participation and promote health and wellness, a more accessible and flexible model of CR is needed in China.

Home-based cardiac rehabilitation (HBCR) programs were thus introduced to increase access and patient acceptance and are reported to be equally effective as conventional CR in improving physical capacity and cholesterol control (9–12). HBCR, conducted at non-clinical settings, including home and other community-based facilities, is more accessible to patients and costing less (12). One study reported a more than 50% participant rate for HBCR in the United Kingdom after a cardiac event (13). However, compared to conventional CR, HBCR raised concerns about safety due to inadequate monitoring and instant communication with the care team. HBCR facilitated by telecommunication and monitoring offers a new opportunity.

HBCR with mobile communication has been used during the past decade, and studies, mostly conducted with text messaging, have shown promising results on improving CR enrollment, physical activity, and physical exercise capacity (14–17). The smartphone provides a flexible platform to deliver patient education, monitor physical activity, exchange patient data, and provide real-time communication and clinical support, and is a better form of telemedicine that can be delivered to patients compared to telephone call-/text message-based care (18, 19).

Most HBCR studies have revealed short-term benefits for CHD patients (i.e., 6 months or less) (14–16, 18, 20, 21), except one study with 24 months of follow-up, a reported exercise capacity, and clinical biometric outcomes (22). Major adverse cardiac events (MACE), however, are rarely reported in studies due to limited follow-up time. To this end, we evaluate the impact on MACE with up to 42 months follow-up, along with other clinical outcomes, including cardiovascular disease risk factors, exercise capacity, quality of life, and psychological outcome in a Chinese population. Similar to Dorje's study, we used WeChat, a social app widely used as the main tool to deliver HBCR with tele-education, telecommunication, telemonitoring, and data transferring functions (19).

## METHODS

### Study Population

The study population included patients > 18 years of age who were referred to the cardiac rehabilitation clinic at First Medical Center of Chinese PLA General Hospital after successful PCI between July 23, 2015 and March 1, 2018. Successful PCI was defined as residual stenosis of the target lesion <30% without procedural complications and TIMI flow grade 3. Inclusion criteria also required smartphone ownership with an active WeChat account; 350 patients agreed to participate in the study.

After obtaining informed consent, all participants were rigorously screened for comorbidities, and 15 patients were excluded because they had any of the following conditions: unstable angina, myocardial infarction (MI) within 2 weeks, new ST-segment deviation, severe arrhythmias, decompensated heart failure, uncontrolled hypertension, severe pulmonary hypertension, obstructive hypertrophic cardiomyopathy, severe valvular heart disease, dementia, and inability to exercise as a result of orthopedic or neurological limitations. Among 335 remaining patients, 170 chose to participate in the smartphone HBCR program, while 165 patients declined and automatically became controls.

The study was approved by the ethics committee of Chinese PLA General Hospital and registered on http://www.chictr.org.cn (ChiCTR1800015042).

### Study Design and Procedures

This was a prospective observational cohort study. Participants were followed up to 42 months, and the last follow-up date was December 31, 2018. We stopped following up patients when the last follow-up date was reached, or patients had unscheduled rehospitalization due to worsening angina, or patients expired or developed a primary outcome. The primary outcome is the incidence of composite MACE, including cardiovascular death, non-fatal acute myocardial infarction, unscheduled coronary revascularization, and non-fatal stroke.

### Baseline Assessment for All Participants

Patients' demographics, socioeconomic status, disease history, current medications, and laboratory tests were collected at baseline, and physical examination was also conducted. Cardiopulmonary exercise testing (CPET) was performed to evaluate exercise intolerance and cardiopulmonary function (**Supplementary Material**).

A 12-lead electrocardiogram was monitored throughout CPET; the rated perceived exertion (RPE) on the original Borg scale was recorded at the end of each stage; oxygen uptake ($VO_2$) and carbon dioxide output ($VCO_2$) were measured every 10 s. Peak $VO_2$ was defined as the average oxygen consumption during the last 15 s of cycle ergometry. $VE/VCO_2$ slope was measured by plotting minute ventilation volume (VE) against $VCO_2$ obtained every 10 s of exercise.

All cardiopulmonary exercise tests were reviewed by special medical staff blinded to the study protocol.

Patients completed baseline questionnaires to assess psychological stress, angina symptoms, and quality of life. Psychological stress was assessed by the Generalized Anxiety Disorder 7 (GAD-7) (23) and Patient Health Questionnaire 9 (PHQ-9) (24). The Seattle Angina Questionnaire (SAQ) was used to measure the effect of angina on physical limitation, anginal stability, anginal frequency, treatment satisfaction, and disease perception (25). The World Health Organization Quality of Life (WHOQOL) was used to assess the life quality of the participants.

All participants received 20 min of health education by CR doctors and nurses, including counseling on lifestyle modification, smoking cessation, and medication adherence.

All procedures complied with the *Helsinki Declaration* standards.

## CR Intervention Delivered by Smartphone

The CR intervention plan was based on standardized HBCR and secondary prevention guidelines (5, 26), including exercise prescription adjusted monthly and health education material.

### Smart Phone Interaction System

The Smartphone Interaction System, a built-in WeChat plug-in app was developed by Halents Life-Info Technologies. It contains several modules, including the electronic medical management (EMM) software, an education module displaying educational materials; an exercise data collection module, which collects data from wearable devices; and a reminder module, to remind patients of upcoming clinic visits. The EMM software contains a database storing patient demographics, clinical measurement, and cardiopulmonary exercise testing results. An exercise prescription is also in the software, which sends prescriptions automatically to the intervention group monthly. After participant enrollment in the CR management system, remote data transmission makes the patient information accessible to both HBCR participants and staff.

Health educational materials were delivered to the intervention group weekly. The educational materials, including education about hypertension, diabetes, cardiovascular health, healthy nutritional advice, medications, psychological well-being, and smoking cessation, are in text-based education articles and video format. Educational topics also cover how to exercise and the appropriate exercise (e.g., exercise type, duration, intensity) for patients with CHD. The health educational materials were created based on evidence-based recommendations and were approved by a physician advisory board.

The heart rate, recorded by wearable devices such as monitoring watch or single chest straps, was monitored remotely by CR staff weekly. If the patient's heart rate exceeds the individualized target heart rate, the system will automatically alarm the participant *via* heart rate watch or smartphone and advise the patient to slow down. Besides uploading the heart rate records, a structured lab value collection table was sent to patients in this system; the patient can add body mass index (BMI) measure and lab values if new labs are available. Patients can also take a picture of lab reports and upload them through the system.

### Exercise Prescription

An exercise prescription was determined using the target $HR/HR_{AT}$ principle from the ninth edition of ACSM's guidelines for exercise (27). The target heart rate is defined as the sum of the resting heart rate or 50–80% of the reserve heart rate, sometimes combined with $HR_{AT}$, depending on which value is lower than others. Exercise starts with 10 min of warm-up, followed by 30-min aerobic exercise (fast walking or cycling or slow jogging) or alternative exercise types to meet target heart rate, 10–15 minutes resistance, stretching,and balance training, and ended by 5-min cool down. Aerobic or stretching exercise is recommended five to six times weekly, while resistance and balance training are two to three times weekly. Patients were instructed to maintain all types of exercise intensity between "relatively easy" and "slightly tiring" which is equivalent to Borg Index 11–13 (28).

## MACE and Home Exercise Follow-Up

Clinic staff called or used WeChat to communicate with participants (HBCR and control groups) to collect MACE every 3 months. If an event occurred, the recall date and time were recorded. Patient medical records were also reviewed every 3 months by well-trained clinical staff to identify MACE events. Besides, several other questions were asked to patients, including whether exercise-related adverse events occurred, family support, length of exercise per day, and the number of days exercising per week. The length of exercise and number of days with exercise were scored according to the adherence scale developed by our group (29). Adherence to exercise is calculated based on a scoring algorithm: if a patient reported exercising 7 days a week was scored 5, 5–6 days scored 4, 3–4 days scored 3, 1–2 days scored 2, and not exercising scored 1. Patients exercising daily for more than 1 h scored 5, 30 min to 1 h scored 4, 10–30 min scored 3, and $<$10 min scored 2. For each individual, the total score is the product of the frequency score and duration of exercise score. A score above 12 (e.g., exercise 5–6 days a week and 10–30 min each time, or 3–4 days a week and 30 min−1 h each time) is considered good adherence to exercise.

An independent committee blinded to treatment assignment adjudicated clinical outcomes.

## Follow-Up Procedures

All participants (HBCR and control) were instructed to return to the clinic every 6 months for a follow-up visit. At the follow-up visit, blood pressure (BP), BMI, and waist-to-hip ratio were measured, and a series of tests were conducted, including lab tests [lipid panel, uric acid (UA), homocysteine (Hcy)], ECG, CPET, and echocardiogram. Patients were asked to answer GAD-7 and PHQ-9 questionnaires at follow-up visits. WHOQOL was administrated at the baseline and at the last follow-up. No incentives were given to participants who made follow-up visits.

## Control Group

Participants in the control group received standard care. Except for the same initial 20 min of education given to the intervention

**TABLE 1 |** Patients' baseline characteristics, by case and intervention groups.

| | Total ($n = 335$) | Control ($n = 165$) | HBCR ($n = 170$) | $p$-value |
|---|---|---|---|---|
| **Age (years)** | 56.3 ± 9.5 | 56.5 ± 8.9 | 56.2 ± 10.1 | 0.755 |
| **Sex, No.** | | | | |
| Male participant (%) | 295 (88.1) | 143 (86.7) | 152 (89.4) | 0.502 |
| Female participant (%) | 40 (11.9) | 22 (13.3) | 18 (10.6) | |
| Manual workers (%) | 91 (27.2) | 46 (27.9%) | 45 (26.5%) | 0.807 |
| BMI [kg/m] | 26.2 ± 3.0 | 26.2 ± 2.9 | 26.2 ± 3.1 | 0.896 |
| WHR | 0.93 ± 0.05 | 0.93 ± 0.05 | 0.93 ± 0.05 | 0.216 |
| Exercise history (%) | 184 (54.9) | 82 (49.7%) | 102 (60.0%) | 0.063 |
| Smoking history (%) | 255 (76.1) | 124 (75.2%) | 131 (77.1%) | 0.702 |
| Systolic blood pressure (mmHg) | 129.0 ± 14.1 | 128.7 ± 14.4 | 129.3 ± 13.9 | 0.699 |
| Systolic blood pressure target-reached (%) | 190 (56.7) | 97 (58.8) | 93 (54.7) | 0.508 |
| Diastolic blood pressure (mmHg) | 81.0 ± 10.2 | 81.2 ± 10.3 | 80.9 ± 10.2 | 0.786 |
| Diastolic blood pressure target-reached (%) | 158 (47.2) | 74 (44.8) | 84 (49.4) | 0.444 |
| **Labs** | | | | |
| LDL (mmol/L) | 1.97 ± 0.71 | 1.99 ± 0.75 | 1.95 ± 0.66 | 0.720 |
| LDL target-reached (%) | 85 (46.4) | 40 (46.0) | 45 (46.9) | 1.000 |
| Total cholesterol (mmol/L) | 3.45 ± 0.88 | 3.48 ± 0.92 | 3.43 ± 0.85 | 0.670 |
| Total triglyceride (mmol/L) | 1.46 ± 0.90 | 1.51 ± 1.00 | 1.43 ± 0.81 | 0.581 |
| Uric acid (μmol/L) | 351.1 ± 80.0 | 347.8 ± 84.7 | 353.9 ± 76.1 | 0.625 |
| Homocysteine (μmol/L) | 15.4 ± 5.7 | 16.1 ± 6.5 | 14.9 ± 5.0 | 0.355 |
| **Comorbidities** | | | | |
| Myocardial infarction history (%) | 128 (38.2) | 60 (36.4%) | 68 (40.0%) | 0.499 |
| Hypertension (%) | 184 (54.9) | 87 (52.8%) | 97 (57.1%) | 0.444 |
| Hyperlipidemia (%) | 218 (65.1) | 113 (68.5%) | 105 (61.8%) | 0.209 |
| Diabetes mellitus (%) | 70 (20.9) | 28 (17.0%) | 42 (24.7%) | 0.106 |
| **Echocardiography** | | | | |
| LVEF (%) | 57.6 ± 8.9 | 59.0 ± 7.2 | 58.0 ± 7.1 | 0.182 |
| Regional wall motion abnormality or ventricular aneurysm (%) | 41 (12.2) | 19 (11.5) | 22 (12.9) | 0.691 |
| LVIDd (mm) | 46.5 ± 4.7 | 46.4 ± 4.6 | 46.5 ± 4.8 | 0.861 |
| IVS (mm) | 10.8 ± 1.2 | 10.8 ± 1.1 | 10.8 ± 1.3 | 0.974 |
| **Percutaneous coronary intervention** | | | | |
| Number of stents/patients | 2.3 ± 1.4 | 2.2 ± 1.2 | 2.5 ± 1.6 | 0.071 |
| Untreated stenosis (%) | 187 (55.8) | 87 (52.7%) | 100 (58.8%) | 0.273 |
| **Medication** | | | | |
| Anti-platelet (%) | 327 (97.6) | 159 (96.4) | 168 (98.8) | 0.169 |
| Statins (%) | 271 (80.9) | 133 (80.6) | 138 (81.2) | 0.502 |
| β-Blocker (%) | 198 (59.1) | 95 (57.6) | 104 (60.6) | 0.581 |
| ACEI/ARB (%) | 74 (22.1) | 34 (20.6) | 40 (23.5) | 0.513 |
| Nitrates (%) | 125 (37.3) | 61 (37.0) | 64 (37.6) | 0.911 |
| Diltiazem (%) | 62 (18.5) | 29 (17.6) | 33 (19.4) | 0.676 |
| Trimetazidine (%) | 119 (35.5) | 57 (34.5) | 62 (36.5) | 0.733 |
| **CPET** | | | | |
| METS | 5.43 ± 1.32 | 5.60 ± 1.31 | 5.27 ± 1.32 | 0.023* |
| Peak oxygen pulse (ml $O_2$/beat) | 11.70 ± 4.09 | 12.04 ± 3.64 | 11.38 ± 4.47 | 0.142 |
| $VO_2$ AT ($ml.kg^{-1}.min^{-1}$) | 14.60 ± 4.43 | 15.08 ± 4.09 | 14.13 ± 4.71 | 0.050 |
| $VE/VCO_2$ | 25.50 ± 4.55 | 25.07 ± 4.81 | 25.92 ± 4.26 | 0.086 |
| $\Delta VO_2/\Delta WR$ ($ml.min^{-1}.W^{-1}$) | 11.72 ± 2.93 | 12.05 ± 3.13 | 11.39 ± 2.69 | 0.039* |
| **Psychological stress** | | | | |
| GAD-7 | 3.24 ± 4.14 | 3.62 ± 4.60 | 2.87 ± 3.63 | 0.107 |
| PHQ-9 | 4.46 ± 3.70 | 4.78 ± 4.18 | 4.16 ± 3.16 | 0.137 |

*(Continued)*

**TABLE 1** | Continued

| | Total ($n = 335$) | Control ($n = 165$) | HBCR ($n = 170$) | $p$-value |
|---|---|---|---|---|
| **Symptoms** | | | | |
| SAQ-PL | 67.01 ± 14.77 | 68.15 ± 13.95 | 66.08 ± 15.41 | 0.279 |
| SAQ-AS | 64.95 ± 29.40 | 64.53 ± 28.39 | 65.31 ± 30.31 | 0.837 |
| SAQ-AF | 88.81 ± 17.67 | 88.84 ± 18.77 | 88.78 ± 16.82 | 0.977 |
| SAQ-TS | 79.34 ± 14.10 | 78.44 ± 14.92 | 80.06 ± 13.41 | 0.377 |
| SAQ-DP | 60.51 ± 20.87 | 61.85 ± 22.25 | 59.43 ± 19.71 | 0.371 |

*BMI indicates body mass index; WHR, waist hip rate; LDL, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole; IVS, interventricular septum; ACEI, angiotensin converting enzyme inhibitor; ARB, adrenergic receptor blockers. Untreated Stenosis was defined as stenosis >50% left untreated after successful coronary stenting. METS indicates metabolism equivalents; $VO_2$, oxygen consumption; AT, Anaerobic Threshold; $VCO_2$, carbon dioxide production; $VE/VCO_2$, minute ventilation/carbon dioxide production relationship; $\Delta VO_2/\Delta WR$, $VO_2$/work rate relationship; GAD-7, Generalized Anxiety Disorder-7; PHQ-9, Generalized Anxiety Disorder-7; SAQ, Seattle Angina Questionnaire; PL, physical limitation; AS, anginal stability; AF, anginal frequency; TS, treatment satisfaction; DP, disease perception. Continuous parameters are shown as mean ± standard deviation (SD). Categorical variables are presented as numbers and proportions. For continuous variables, comparisons between groups were made using two-sample t-test. Categorical variables were compared using the chi-square test. *p value <0.05 was considered to be of statistical significance.*

group at baseline, control participants did not receive any additional CR-related intervention.

## Statistical Analysis

Descriptive statistics were conducted for baseline measures, where continuous measures were summarized by the mean and standard deviation (SD), and categorical variables were summarized by proportion. To assess whether the HBCR group was similar to the control group at baseline measures, we conducted the Student $t$-test if the variables were normally distributed, or Wilcoxon rank-sum test otherwise for continuous variables, and the chi-square test for categorical variables.

We conducted propensity score matching (30), where propensity score was derived from a logistic regression model with HBCR/control status as the outcome, and with baseline characteristics (age, gender, BMI, systolic BP, physical capacity, comorbidities, and length of time between baseline and last follow-up time) to balance HBCR and control and to reduce the bias due to the natural limitation of the cohort study. Nearest neighbor matching within a 0.1 caliper distance was used to find matched cases and controls.

For the primary outcome (i.e., MACE event), a Kaplan–Meier survival plot was created and stratified by intervention group and control group, and the log-rank test was used to compare the survival probability. Multivariable Cox regression was used with (i.e., model 1 to model 3 in **Table 3**) and without (i.e. crude model in **Table 3**) adjustment for baseline characteristics. In the final model (model 3), baseline demographic, medication, and baseline exercise capacity were controlled. Hazard ratio and 95% confidence interval for intervention status were estimated.

Secondary outcomes included changes of CPET, symptoms, risk factors, exercise capacity, quality of life, and psychological outcomes. Changes in secondary outcomes were calculated by subcontracting baseline measures from follow-up measures. Paired $t$-test was used to compare changes between matched HBCR and control.

The absence of a measure for each secondary outcome during follow-up was assessed, if the overall absence rate was above 50% (in both HBCR and control groups), the outcome was dropped from the final analysis due to a high missing rate. For remaining secondary outcomes, we applied multiple imputations to impute missing values for follow-up measures; 100 imputed values for each missing measure were created based on Markov Chain Monte Carlo methods, and standard error was estimated based on Rubin's Rules wherein standard error was calculated by combining within-imputation variance and between-imputation variance (31).

In addition, as the comparison purpose, we conducted analyses for unmatched raw data as well as comparison. Similar multivariable Cox regression was applied to the primary outcome. For the secondary outcomes, due to potentially unbalanced baseline characteristics between HBCR and control groups, we applied the generalized linear model (GLM) to test the difference of changes between the HBCR group and control group after controlling baseline demographic and clinical variables. In order to adjust for different follow-up time, we also included time between the follow-up date and baseline date as a covariate in the model. The results were reported in **Supplementary Material**.

Statistical analysis was performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

# RESULTS

At baseline, the average age was 56.3 (±9.5) years old, and the majority of participants were male (88%) and smokers (76%). Regarding comorbidities, 38% had an MI history, 55% had hypertension, 65% had hyperlipidemia, and 21% had diabetes. About half of the participants reached the targeted blood pressure (130/80 mmHg). Almost all patients received anti-platelet medications (98%), 81% statins, 59% β-blockers, and 22% ACEI/ARB (**Table 1**). The majority of participants in both the HBCR and control group did not report strong anxiety or depression. The average GAD-7 score was 3.24 (±4.14), and the PHQ-9 score was 4.46 (±3.7).

The baseline characteristics of the HBCR group were similar to those of the control group, including demographics (age,

**TABLE 2 |** Summary statistics of the Home-Based Cardiac Rehabilitation program on primary and second outcomes at end of follow-up period for matched case and control.

| | Control($n = 135$) | HBCR ($n = 135$) | $p^{\dagger}$ |
|---|---|---|---|
| **Primary outcomes** | | | |
| **MACE** | 12 (8.9) | 2 (1.5) | 0.002* |
| Incidence of myocardial infarction | 0 (0.0) | 0 (0.0) | – |
| Unscheduled revascularization | 12 (8.9) | 2 (1.5) | 0.002* |
| Stroke | 0 (0.0) | 0 (0.0) | – |
| Cardiac death | 1 (0.6) | 0 (0.0) | 0.493 |
| **Second outcomes** | | | |
| **Unscheduled hospitalization worsened due to worsening angina** | 31 (23.0) | 13 (9.7) | 0.002* |
| **CPET** | | | |
| METS | 5.1 ± 1.4 | 6.2 ± 1.3 | 0.001* |
| Peak oxygen pulse (ml $O_2$/beat) | 12.4 ± 3.7 | 12.6 ± 3.4 | 0.27 |
| $VO_2$ AT ($ml.kg^{-1}.min^{-1}$) | 13.7 ± 4.1 | 16.2 ± 4.3 | <0.001* |
| $VE/VCO_2$ | 25.4 ± 3.7 | 24.70 ± 3.8 | 0.34 |
| $\Delta VO_2/\Delta WR$ ($ml.min^{-1}.W^{-1}$) | 11.1 ± 2.9 | 11.9 ± 2.5 | 0.41 |
| **Risk factors control** | | | |
| SBP (mmHg) | 130.1 ± 13.9 | 122.2 ± 13.7 | <0.001* |
| Target-reached ratio of SBP | 75 (55.5) | 115 (85.2) | <0.001* |
| DBP (mmHg) | 81.1 ± 10.4 | 77.5 ± 11.6 | 0.09 |
| Target-reached ratio of DBP | 76 (56.3) | 99 (73.3) | 0.03 |
| LDL (mmol/L) | 2.2 ± 0.8 | 1.5 ± 0.6 | <0.001* |
| Target-reached ratio of LDL | 55 (40.7) | 118 (87.4) | <0.001* |
| **Other labs** | | | |
| TC (mmol/L) | 3.7 ± 1.2 | 3.1 ± 0.9 | 0.02* |
| TG (mmol/L) | 1.6 ± 1.0 | 1.4 ± 0.9 | 0.12 |
| UA (mmol/L) | 340.0 ± 86.2 | 347.3 ± 77.9 | 0.71 |

*$p^{\dagger}$ is the p-value for raw data (without imputation).*

*METS indicates metabolism equivalents; $VO_2$ AT, oxygen consumption at Anaerobic Threshold; VE, pulmonary ventilation; $VCO_2$, carbon dioxide production; $VE/VCO_2$, minute ventilation/carbon dioxide production relationship; $\Delta VO_2/\Delta WR$, $VO_2$/work rate relationship; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, total triglyceride; UA, uric acid.*

*Data are shown as mean ± standard deviation (SD).*

*Time effects of exercise (±) on the second endpoints were analyzed using paired t-test. *p-value < 0.05 was considered to be of statistical significance.*

gender), health behavior and lifestyle (smoking history, exercise history), vitals (BMI, BP), comorbidities (MI, hypertension, hyperlipidemia, diabetes), baseline lab measures (LDL, total cholesterol, uric acid, and homocysteine), echocardiographic results (LVEF, regional wall motion abnormality, LVID, and IVS), medication, PCI procedures, exercise capacity (heart rate reserve, peak oxygen pulse, $VO_2$ AT, $VE/VCO_2$), psychological stress, and symptoms (**Table 1**). Only two differences were observed for METS (5.3 ± 1.3 for HBCR group vs. 5.6 ± 1.3 for the control group, $p = 0.02$), and $\Delta VO_2/\Delta WR$ (12.1 ± 3.1 for control group vs. 11.4 ± 2.7 for the HBCR group, $p = 0.04$).

After matching, 135 patients remained in each group. The difference of baseline characteristics between HBCR and control groups were diminished, except for two lab measures ($VO_2$ AT and $\Delta VO_2/\Delta WR$) where measures for the control group were slightly higher than those in the HBCR group ($p = 0.05$ and 0.039, respectively, **Supplementary Table 1**).

There were no missing data for primary outcomes (**Supplementary Table 2**) and very little missing for MET, HR, and CPET measures at the final follow-up (0.9–1.2%). GAD-7 and PHQ-9 were missing for ∼8% of participants. SAQ, LDL, TC, and TG have a moderate missing rate (35–49.9%), while UA and Hcy were not included in the final analysis due to the high missing rate (>50%). MET, HR, and CPET measures at 6, 12, 18, 24, or 30 months were also missing for more than half of participants; thus, these intermittent measures were not imputed and were not used in the analysis.

Among the matched cohort, a total of 14 (10.4%) MACE occurred during follow-up, including one cardiovascular death and 14 unscheduled revascularizations (**Table 2**). The HBCR group had a significantly lower incidence for MACE compared to the control group (1.5 vs. 8.9%, $p = 0.002$) (**Figure 1**); the hazard ratio of the incidence of MACE in HBCR reached 0.21 after adjusting for baseline characteristics (HR = 0.21, 95% CI 0.07–0.85) (**Table 3**, model 3).

The participants in the HBCR group demonstrated much lower incidence of unscheduled hospitalizations due to worsened angina than those in the control group (23.0 vs. 9.7%, $p = 0.002$).

**TABLE 3 |** Multivariate Cox regression analysis of the correlation between Home-Based Cardiac Rehabilitation program and incidence of clinical events, with and without adjusting for confounding variables.

| | HR (95% CI) | *p*-value |
|---|---|---|
| Crude | 0.21 (0.07, 0.85) | 0.008 |
| Model 1 | 0.21 (0.070, 0.85) | 0.008 |
| Model 2 | 0.21 (0.07, 0.85) | 0.008 |
| Model 3* | 0.21 (0.07, 0.85)* | 0.008* |

**The fully adjusted result, which is used in interpreting the association between Home-Based Exercise and Incidence of Clinical events.*
*Model 1: after adjustment for age and gender.*
*Model 2: after further adjustment for history of previous myocardial infarction, history of diabetes mellitus, number of stents implanted, whether has residual stenosis or not.*
*Model 3: after further adjustment for BMI, WHR, history of Hypertension, history of smoking, history of exercise, maximal METs, oxygen uptake at AT, VE/$VCO_2$, $\Delta VO_2/\Delta WR$, use of at least one antiplatelet drug, use of statins, use of trimetazidine, use ofβ-blockade, Whether received emergency PCI or not, systolic blood pressure value and diastolic blood pressure value at the baseline level.*
*BMI indicates body mass index; WHR, waist hip rate; METS, metabolism equivalents; AT, anaerobic threshold; VE/$VCO_2$, minute ventilation/carbon dioxide production relationship; $\Delta VO_2/\Delta WR$, $VO_2$/work rate relationship; PCI, percutaneous coronary intervention.*

The exercise capacities in the HBCR group were also better at the end of follow-up compared to controls: maximal METs were higher in the HBCR group (6.2 vs. 5.1, $p = 0.001$) than that for the control group, and oxygen uptake at anaerobic threshold ($VO_2$ AT) was also improved (16.2 vs. 13.7, $p < 0.001$) in the HBCR group. Regarding the risk factor control, at the final follow-up, the HBCR group had lower SBP (122 vs. 130, $p < 0.001$) and a higher proportion of patients with controlled systolic blood pressure (85.2 vs. 55.5%, $p < 0.001$) as well as diastolic blood pressure (73.3 vs. 56.3%, $p = 0.03$). Similarly, the HBCR group also had lower LDL-C (1.5 vs. 2.2, $p < 0.001$) and a higher proportion of patients with controlled LDL-C (87.4 vs. 40.7%, $p < 0.001$) (**Table 2**).

**Table 4** shows the changes in secondary outcomes using imputed data. Among CPET measures, the HBCR group (mean = 0.9, 95% CI: 0.9–1.5) showed significant increase in METs compared to the control group (mean = 0, 95% CI: −0.3 to 0.1). The HBCR groups also had more significant increase in $VO_2$ at AT (mean = 1.9, 95% CI 0.9–2.2), $\Delta VO_2/\Delta WR$ (mean = 0.6, 95% CI −0.1 to 0.9) and decrease in VE/$VCO_2$ (mean −1.2, 95% CI −1.6 to 0.6) compared to controls. Systolic BP was reduced for the HBCR group (mean = −5.2, 95% CI = −7.7 to 1.1), but not for the control group (mean = 0.5, 94% CI −2.0 to 3.0). The differences of those changes between the HBCR and control group were significant ($p = 0.003$).

We assessed the psychological status and cardiac symptoms at the final follow-up (**Table 4**). Among matched patients, the changes of GAD-7 and PHD-9 did not show significant difference between the HBCR and control groups ($p > 0.20$), while three dimensions of SAQ showed significant difference between the HBCR and the control groups, which were SAQ physical limitation, angina frequency, and disease perception. The SAQ physical limitation measure increased 6.9 (95% CI: 3.8–10.2) for the HBCR group, which was significantly higher than that for the control group (mean = −0.7, 95% CI: −4.1 to 2.2) ($p = 0.001$). A similar tendency was found in the increase of SAQ angina frequency and disease perception (2.9 vs. 0.8, $p = 0.04$).

## DISCUSSION

This HBCR model which delivers via smartphone using the WeChat built-in application in China found a significant reduction of MACE in the HBCR group compared to the control group (1.5 vs. 8.9%), confirming a clear benefit for HBCR during the 24–48-month follow-up period. To our knowledge, this is the first published study to assess the effect of smartphone-based HBCR programs on MACE for patients with CHD with a long follow-up time. No physical exercise-related adverse events were observed during the follow-up period for both groups.

MACE is rarely reported as an outcome in CR studies. Soga et al. reported the impact of exercise training on MACE events after coronary stenting for CHD patients within 30 days as a safety concern of early exercise after the coronary procedure (32). In our study, we extend the safety concern of HBCR to a long-term effectiveness measure, and MACE was evaluated after more than 2 years of follow-up. Smartphone-facilitated HBCR can effectively reduce MACE and is suggested to be suitable for long-term use to prevent secondary severe outcomes in order to promote general wellness to post-cardiac patients.

Consistent with another HBCR study, our study indicated improved exercise capacity for patients, particularly for METs, and oxygen consumption at Anaerobic Threshold ($VO_2$ AT) (**Table 2**). Moreover, our study showed that the improvement persists longer than 2 years (3, 9, 10). Previous studies have established the connection between exercise capacity and health outcome: each increment of 1 metabolic equivalent (MET) (3.5 ml $O_2$ $kg^{-1}.min^{-1}$) in peak $VO_2$ corresponds to 13% reductions in all-cause mortality and 15% in cardiovascular mortality (32). In our study, although the baseline levels of peak METs, oxygen uptake, and $\Delta VO_2/\Delta WR$ in the control group were slightly higher than those in the HBCR group, after 2 years of intervention, peak METS were significantly improved in the HBCR group. Moreover, $VO_2$ at the anaerobic threshold ($VO_{2AT}$) was observed to be higher in the HBCR group than the control group, which indicates the further benefit of HBCR on cardiorespiratory fitness. The improvements of exercise capacity in the HBCR group were also more significant than those in the control group.

Our study also found better adherence to exercise in the HBCR group (85%) compared to the control (31%) during the entire follow-up period, suggesting that smartphone-based HBCR can be used as a long-term secondary prevention tool. Though it is not clear which components of this smartphone-based HBCR directly lead to improved exercise adherence, the real-time monitoring, communication about exercise-related concerns, individualized exercise prescription, and repeated education may work together to promote patient engagement. Regardless of the mechanism, long-term adherence to exercise, resulting in improved exercise capacity, was associated with a reduction of MACE.

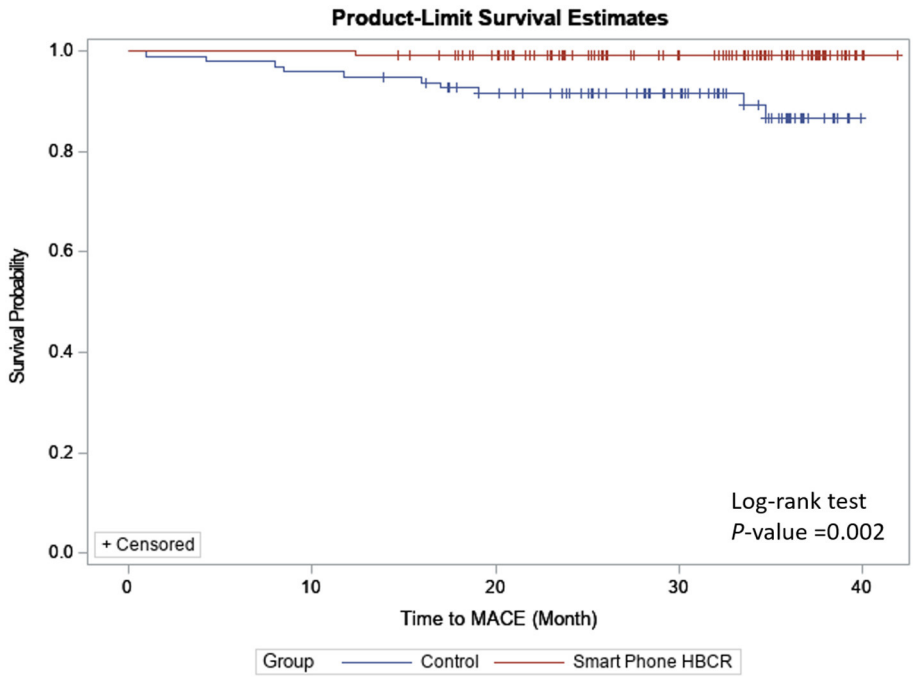


**FIGURE 1 |** Kaplan–Meier plot for MACE, stratified by intervention status (for the matched cohort).

Improved LDL-C and systolic BP control observed at the final follow-up visit were consistent with findings from another smartphone-based HBCR study (19). In that study, systolic BP in HBCR was 8 mmHg lower compared to the control group at 6-month follow-up, which is slightly higher than the 5 mmHg reduction in our study after 2 years of follow-up. LDL-C was reduced 0.5 mmol/L more for HBCR in our study, compared to 0.22 mmol/L in Dorje's study at 12-month follow-up (19). It has been suggested that the 10 mmHg reduction of systolic BP is associated with 20% reduction of major cardiovascular events (e.g., stroke, MI, HF) (33, 34). Although our results did not reach the same degree of blood pressure reduction, the proportion of patients who achieved the target BP control was significantly improved (55% at baseline vs. 85% at the final follow-up visit) for the HBCR group, compared to worsened BP control for the control group (59% at baseline vs. 56% at the final follow-up visit) (**Tables 1**, **2**). Similarly, we also observed significantly improved LDL-C control proportion (47 vs. 87%) for HBCR, compared to 46 vs. 41% for the control group (**Tables 1**, **2**). BP and LDL-C control are closely related to lifestyle modification (e.g., exercise, diet), medication adherence, the educational material, exercise prescription, and monitoring provided to the HBCR group, which are likely to be associated with improved BP and LDL-C (27). Moreover, LDL-C control is directly associated with the reduced risks of cardiovascular disease and has been strongly recommended by clinical guidelines since 2000. Educational materials in the present study, particularly lifestyle modification and diet education (35, 36), are thought to contribute to the improvement in BP and LDL-C.

Smartphone-based HBCR makes it possible for real-time communication and data exchange between medical staff and patients (*via* WeChat) and promotes personalized exercise prescription and exercise safety. Besides this individualized care, which is usually an advantage of center-based CR, smartphone-based HBCR is conducted in the home environment and is more acceptable and accessible for patients. Thus, smartphone-based intervention represents a new form of telemedicine wherein care can be effectively delivered through telecommunication. This form of telemedicine is particularly useful when patients are not able to access clinic facilities (e.g., current lockdown due to COVID-19). It is also a better option for countries such as China where the smartphone is commonly available but health care facilities are limited, particularly if intervention focuses on improving knowledge and awareness of the disease through educational materials. Moreover, a social media communication tool such as WeChat has widespread use and extended functions that can be connected to other applications (e.g., wearable devices) thereby making the social media tool (e.g., WeChat) an ideal and flexible platform to deliver care (19). It can be easy to adapt as one form of telemedicine. The experience from our study should be easily generalized to other disease areas.

There are several limitations of the study. First, participants were not randomized and participant assignment was based on their willingness to participate in HBCR which may result in a self-selection bias. We conducted propensity score matching (30) to reduce the potential bias; the baseline characteristics were balanced among HBCR and control groups among the matched cohort. The impact of HBCR in reducing MACE

**TABLE 4 |** Effects of the Home-Based Cardiac rehabilitation program on psychological state, cardiac symptoms and biochemical metric changes for the matched cohort.

| | Control Change† ($n = 135$) Mean (95%CI) | HBCR Change† ($n = 135$) Mean (95%CI) | Difference (HBCR-Control) Mean (95% CI) | $p^{††}$ |
|---|---|---|---|---|
| **Psychological stress** | | | | |
| GAD-7 | −0.2 (−0.9, 0.4) | −0.5 (−1.0, 0.5) | −0.3 (−1.4, 7) | 0.20 |
| PHQ-9 | 0.2 (−0.4, 0.8) | −0.3 (−0.9, 0.5) | −0.5 (−1.3, 0.6) | 0.22 |
| **Symptoms** | | | | |
| SAQ-PL | −0.7 (−4.1, 2.2) | 6.9 (3.8, 10.2) | 7.6 (3.2, 12.1) | 0.001 |
| SAQ-AS | −1.9 (−10.1, 5.3) | 5.9 (−1.4, 12.8) | 7.7 (−1.9, 13.8) | 0.09 |
| SAQ-AF | 2.0 (−0.4, 6.0) | 6.9 (4.2, 10.7) | 4.9 (1.0, 7.4) | 0.04 |
| SAQ-TS | 0.8 (−2.1, 4.0) | 2.9 (−0.4, 5.9) | 2.1 (−1.7, 5.1) | 0.21 |
| SAQ-DP | 4.3 (1.9, 10.2) | 8.9 (4.1, 13.4) | 4.6 (0.9, 6.1) | 0.04 |
| **Biometric metric** | | | | |
| SBP | 0.5 (−2.0, 3.0) | −5.2 (−7.7, −1.1) | −5.7 (−7.8, −2.2) | 0.003 |
| DBP | −0.7 (−2.2, 0.7) | −3.2 (−4.4, −0.9) | −2.5 (−3.5, 0.2) | 0.09 |
| LDL-C | 0.2 (−1.0, 1.3) | −0.3 (−1.3, 0.7) | −0.5 (−1.7, −0.1) | 0.03 |
| **CPET** | | | | |
| Maximal MET | 0.0 (−0.3, 0.1) | 0.9 (0.7, 1.2) | 0.9 (0.9, 1.5) | < 0.001 |
| Peak oxygen pulse (ml $O_2$/beat) | 0.0 (−0.3, 0.6) | 1.8 (0.7, 2.0) | 1.8 (0.4, 1.7) | 0.05 |
| $VO_2$ AT ($ml.kg^{-1}.min^{-1}$) | −0.7 (−1.4, −0.2) | 1.9 (0.9, 2.2) | 2.6 (1.6, 3.3) | < 0.001 |
| $VE/VCO_2$ | 0.4 (−0.6, 1.2) | −1.2 (−1.6, −0.6) | −1.6 (−2.1, −0.7) | 0.001 |
| $\Delta VO_2/\Delta WR$ ($ml.min^{-1}.W^{-1}$) | −0.6 (−1.3, −0.1) | 0.6 (−0.1, 0.9) | 1.2 (0.8, 1.5) | 0.004 |

† *Changes were summarized based on imputed data.* $p^{††}$ *is the p-value after imputation to test the changes between the HBCR and control groups.*
*GAD-7 indicates Generalized Anxiety Disorder-7; PHQ-9, Generalized Anxiety Disorder-7; SAQ, Seattle Angina Questionnaire; PL, physical limitation; AS, anginal stability; AF, anginal frequency; TS, treatment satisfaction; DP, disease perception; SBP, systolic blood pressure; DBP, diastolic blood pressure; METS, metabolism equivalents;* $VO_2$*, oxygen consumption; AT, anaerobic threshold;* $VCO_2$*, carbon dioxide production;* $VE/VCO_2$*, minute ventilation/carbon dioxide production relationship;* $\Delta VO_2/\Delta WR$*,* $VO_2$*/work rate relationship.*
**p-value < 0.05 was considered to be of statistical significance.*

was even more appealing compared to the unmatched cohort (**Supplementary Tables**).

Secondly, the sample size was relatively small and all patients were recruited from a single center. Therefore, a large-scale multi-center is needed to further verify the results. A multi-center smartphone-based HBCR randomized trial, led by our clinic center, has been initiated and is now in the patient recruitment stage. Nevertheless, randomized control trials with a greater number of patients are needed to confirm the findings of this study.

Thirdly, because all follow-up visits are voluntary, more than half of patients missed intermittent follow-up measures because of secondary outcomes. Patients have to pay a ∼500 Chinese Yuan (∼US $70) out-of-pocket expense at each follow-up visit, which is a financial burden for most patients. Ideally, health insurance would pay for tests as a continuum of care for CHD patients. Because it generally takes a long time to advocate health policy change, this may limit the practical use of HCBR.

## CONCLUSION

To our knowledge, this study provides the longest follow-up evaluation of smartphone-based HBCR for successfully revascularized CHD patients. After more than 2 years of follow-up, smartphone-based HBCR facilitated by a social network application (WeChat) was effective in decreasing the incidence of major adverse cardiovascular events, improving exercise capacity, and risk factor control.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors.

## ETHICS STATEMENT

The study involved human subjects and was approved by the Ethics Committee of the Chinese PLA general hospital. The participants provided written consent to the study.

## AUTHOR CONTRIBUTIONS

The study was initiated by JM and YC. JM, CG, and SY performed the statistical analysis and drafted the manuscript. YS, YX, CZ, LG, DW, TL, and JW were helpful for data collection. YC and SS contributed substantially to its revision. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.731557/full#supplementary-material

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# Low-to-Moderate-Intensity Resistance Exercise Effectively Improves Arterial Stiffness in Adults: Evidence From Systematic Review, Meta-Analysis, and Meta-Regression Analysis

*Yong Zhang*[1], *Ya-Jun Zhang*[1]*, *Weibing Ye*[2] *and Mallikarjuna Korivi*[2]*

[1] Department of Rehabilitation Medicine, College of Medicine, Shaoxing University, Shaoxing, China, [2] Exercise and Metabolism Research Center, College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua, China

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**Edited by:**
Yan Zhang,
Peking University, China

**Reviewed by:**
Yuanzheng Ye,
Xinjiang Medical University, China
Hung-Ju Lin,
National Taiwan University Hospital, Taiwan

***Correspondence:**
Ya-Jun Zhang
zhangyajunqq12@163.com
Mallikarjuna Korivi
mallik.k5@gmail.com;
mallik@zjnu.edu.cn
orcid.org/0000-0002-4038-1368

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**Background/Purpose:** Resistance exercise (RE) is known to improve cardiovascular health, but the role of RE variables on arterial stiffness is inconclusive. In this systematic review and meta-analysis, we investigated the influence of RE and its intensities on arterial stiffness measured as pulse wave velocity (PWV) in young and middle-aged adults.

**Methods:** Web of Science, PubMed/MEDLINE, Scopus, EMBASE, Cochrane Library, ScienceDirect, CINAHL, Wiley Online Library, and Google Scholar were searched for relevant studies. RE trials that reported PWV data, and compared with respective controls were included. The Cochrane Collaboration tool was used to assess the risk of bias.

**Results:** Data were synthesized from a total of 20 studies, involving 981 participants from control ($n$ = 462) and exercise ($n$ = 519) trials. The test for overall effect (pooled outcome) showed RE intervention had no effect on arterial stiffness (SMD = −0.09; 95% CI: −0.32, 0.13; $P$ = 0.42), but risk of heterogeneity ($I^2$) was 64%. Meta-regression results revealed a significant correlation ($P$ = 0.042) between RE intensity and PWV changes. Consequently, the trials were subgrouped into high-intensity and low-to-moderate-intensity to identify the effective RE intensity. Subgroup analysis showed that low-to-moderate-intensity significantly decreased PWV (SMD = −0.34; 95% CI: −0.51, −0.17; $P$ < 0.0001), while high-intensity had no effect (SMD = 0.24; 95% CI: −0.18, 0.67; $P$ = 0.26). When trials separated into young and middle-aged, low-to-moderate-intensity notably decreased PWV in young (SMD = −0.41; 95% CI: −0.77, −0.04; $P$ = 0.03) and middle-aged adults (SMD = −0.32; 95% CI: −0.51, −0.14; $P$ = 0.0007), whereas high-intensity had no effect in both age groups.

**Conclusions:** Our findings demonstrated that RE intensity is the key variable in improving arterial stiffness. Low-to-moderate-intensity can prescribe as an effective non-pharmacological strategy to treat cardiovascular complications in young and middle-aged adults.

**Keywords: pulse wave velocities, arterial stiffness, resistance training, cardiovascular, meta-analyses**

## INTRODUCTION

Arterial stiffness, measured from pulse wave velocity (PWV) is an independent risk factor for the development of cardiovascular disease (CVD). Increased arterial stiffness is closely associated with increased risk of morbidity and mortality in older populations and also in patients with chronic diseases (hypertension, type 2 diabetes, kidney disease, and stroke) (1–4). Arterial stiffening is represented by a gradual fragmentation and loss of elastin fibers, and accumulation of stiffer collagen fibers in the arterial wall (5). Several confounding factors, including aging, life style, diet, and concurrent disease are said to be involved in arterial stiffening and hypertension (6). Among various non-invasive and simplified protocols to measure the elastic properties of arteries, PWV is a widely recognized gold standard measure of arterial stiffness. Carotid-femoral PWV (cfPWV) and brachial-ankle PWV (baPWV) are the novel and most frequently used indices to determine arterial stiffness (7). The cfPWV is used to assess the central arterial stiffness, and baPWV is used to assess the whole-body arterial stiffness. Increased cfPWV and baPWV are the valid predictors of future incidence of CVD and mortality (1, 7). Given that, 1.0 m/s increase in cfPWV or baPWV can increase the risk of total cardiovascular events (12–14%), mortality (13–15%), and all-cause mortality (13–15%) (8). Therefore, reversing arterial stiffness (decreasing PWV) is a major achievement to prevent the development of hypertension and other clinical complications.

For decades, physical exercise, either resistance or aerobic, has been prescribed as a non-pharmacological intervention to promote overall health and to treat cardiovascular complications (9). Studies on exercise interventions are emerging due to the widespread benefits of exercise on human health (improving antioxidant status, lowering blood pressure, decreasing CVD risk factors, improving arterial stiffness, etc.). It has been stated that more time spent in physical activity is associated with lower arterial stiffness, whereas more time spent in sedentary behavior is associated with higher arterial stiffness (10). About the type, aerobic exercise has been confirmed to improve arterial stiffness in young, middle-aged, and older adults (11–13), as well as in patients with hypertension, metabolic syndrome, and diabetes (14–16).

However, the influence of RE on arterial stiffness or changes in PWV is still controversial. Some studies reported that RE training can improve the arterial stiffness in young healthy subjects (17, 18) and older hypertensive females (19). While others reported RE had no effect on arterial stiffness in young subjects (20) and individuals with metabolic syndrome (21). Contrary, RE training was reported to increase the arterial stiffness in healthy young subjects (22, 23), which decreases the vascular compliance. Besides, meta-analyses of research trials reported inconclusive results of RE intervention on changes in arterial stiffness. For instance, a meta-analysis reported increased arterial stiffness with high-intensity RE in young subjects, but moderate-intensity RE did not show such an association in middle-aged adults (24). Two recent meta-analyses based on the available evidence concluded that RE alone does not improve or impair the arterial stiffness in patients at risk for CVD (25) and in healthy individuals (26). It is worth noting that the included studies in these meta-analyses used different protocols to assess the arterial stiffness. Importantly, these meta-analyses reported moderate to high heterogeneity, and did not address the source of heterogeneity on exercise-induced changes in PWV.

High heterogeneity signifies the involvement of variables related to exercise protocol and/or patients' characteristics. In 2020, Ceciliato et al., who reported high heterogeneity without altering the PWVs, recommended further studies to identify the responsible variable of RE intervention (26). Typically, characteristics of RE (frequency, intensity, number of sets/repetitions, and duration) or participants (age, sex, and health status) are involved in the changes of arterial stiffness following intervention. Professional organizations like the American Heart Association (AHA) and American College of Sports Medicine (ACSM) also recommended practicing of RE training for further improvement of overall health and to overcome the practical limitations of aerobic exercise (27, 28). Therefore, we designed this study to systematically review and statistically analyze the impact of RE on arterial stiffness. We further aimed to identify the responsible RE variable that is involved in altering the PWV. Besides, the significance of RE "intensity" on improved arterial stiffness in young and middle-aged adults was emphasized based on the evidence from meta-regression and subgroup analyses.

## METHODS

### Data Sources and Search Strategy

We used major electronic databases, including Web of Science, PubMed/MEDLINE, Google Scholar, EMBASE, Cochrane Library, ScienceDirect, CINAHL, Scopus, and Wiley Online Library for article search. The articles search was conducted until April 2021 using the main keywords: "resistance" or "strength" and "arterial stiffness." In addition, "exercise" or "training" or "physical activity" should be in the title and abstract. The keywords "exercise," "training," or "physical activity" were independently used with "resistance" or "strength" and "arterial stiffness" and all searches were performed separately. In the search process, a filtering function of the databases was applied to filter the preliminary search results using "article," "randomized controlled trial," and "journals" options wherever applicable.

### Study Inclusion and Exclusion Criteria

The two authors (Y.Z. and Y.J.Z.) conducted the article search and selection independently. The author W.B.Y. provided additional review and insight. M.K. discussed and confirmed the disagreements on inclusion or exclusion of trials into the study. Initially, the titles and abstracts of searched articles were screened for relevance, and then the full text of the specified articles was obtained and carefully reviewed for the inclusion criteria. The following criteria were used to include the trials in this systematic review and meta-analysis: (1) studies were randomized controlled trials (RCTs) published in English; (2) resistance training is the only intervention in the trials, and is not combined with other interventions; (3) the control trial did not participate in any exercise, and maintained daily behavior or

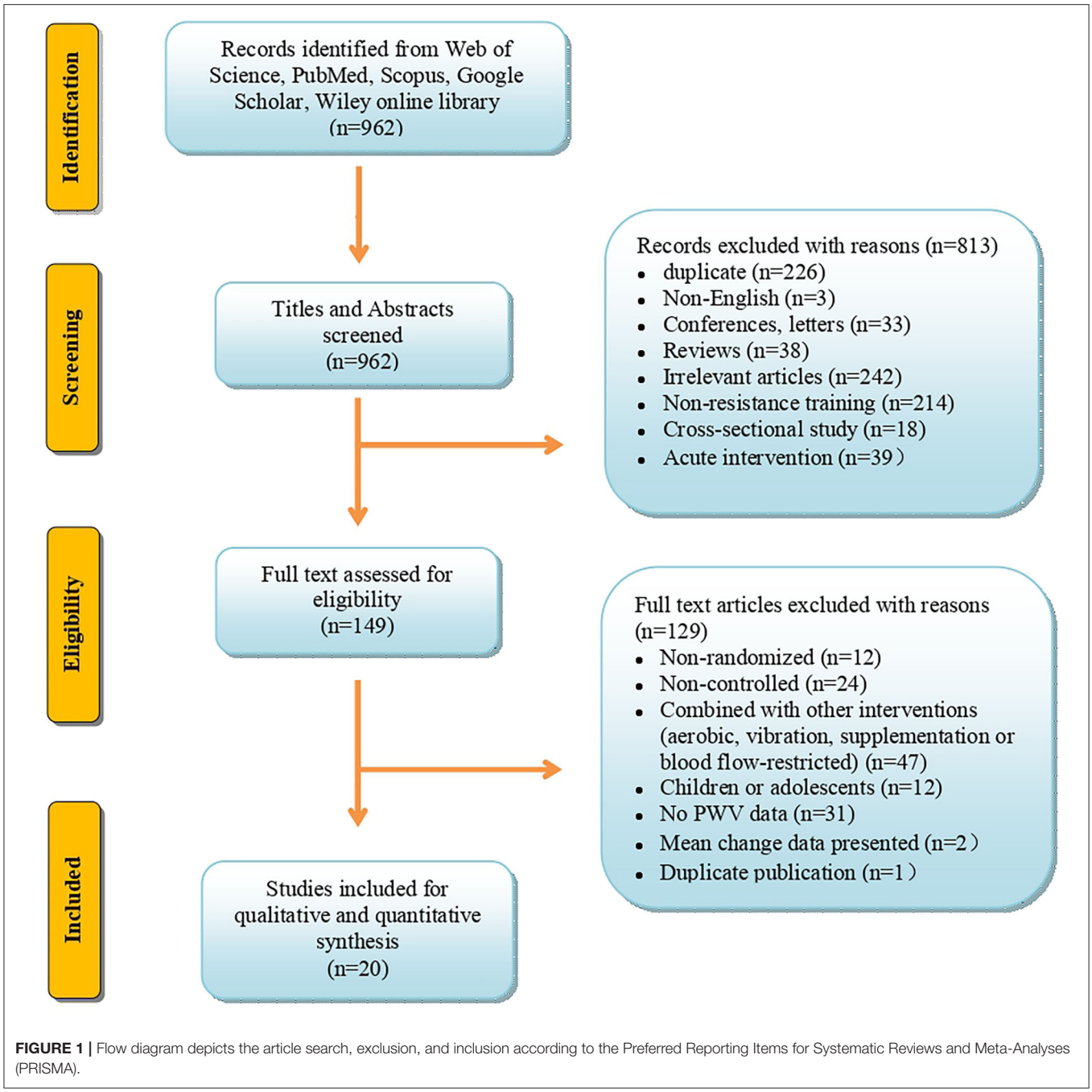


**FIGURE 1 |** Flow diagram depicts the article search, exclusion, and inclusion according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

was sedentary; (4) participants were adults aged ≥18 years; (5) the duration of resistance exercise was 4 weeks or more; and (6) the outcome assessment was "arterial stiffness" measured as carotid-femoral PWV (cfPWV) or brachial-ankle PWV (baPWV), which is typically used to assess the central and whole-body arterial stiffness, respectively. We excluded studies according to these criteria: (1) non-randomized controlled trials or without control group; (2) combined with other interventions (aerobic exercise, vibration, supplementation, blood flow-restriction); (3) if participants were children or adolescents; (4) acute intervention study; (5) study did not report cfPWV or baPWV data; and (6) articles with repeated results, non-English, poor quality, or insufficient information about RE.

The specific details of the selection process, inclusion, and exclusion of articles for this study are presented in **Figure 1**. The article search and selection were performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines as shown in **Figure 1**.

## Data Extraction From the Included Trials

The data from 20 eligible articles (30 trials), including basic information (authors, publishing year, and country in which the study was conducted), characteristics of participants (sex and age), resistance exercise protocols (intensity, repetitions, sets, frequency, and duration), and clinical outcomes (cfPWV and baPWV) were extracted and presented in **Table 1**. The data extraction was done by three independent review authors (Y.Z., Y.J.Z., and M.K.), and PWV was presented as mean and standard deviation (SD). If mean and SD were not available in the trials, we contacted the corresponding author for further information. If authors did not respond, standard errors were converted to SD, quartile data were converted to mean and SD (41), and data represented in tables were extracted to the nearest number by WebPlotDigitizer.

## Risk of Bias Assessment

The risk of bias for the included articles was determined according to the Cochrane Collaboration tool (42). Two of the three review authors (Y.Z., Y.J.Z., or W.B.Y.) independently assessed the risk of bias, and possible discrepancies were resolved by discussing with the other review author (M.K.). The source of bias, such as selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other bias were detected. The detailed judgment of the risk of bias of included trials is summarized in the Results section.

## Subgroup Division and Observed Outcomes

The intensity of RE performed by individuals in each trial was converted and presented as 1-RM percentages (43, 44). Based on the intensity, included trials were categorized into two subgroups, including low-to-moderate-intensity and high-intensity trials. The intensity between 30 and 70% 1-RM is considered as low-to-moderate-intensity RE, and intensity between 70 and 100% 1-RM is considered as high-intensity RE. This subgroup category was followed according to the ACSM Guidelines for Exercise Testing and Prescription (28). When two or more different intensities were used in training, the average intensity was used to classify into low-to-moderate-intensity or high-intensity RE.

As a gold standard approach for assessing the arterial stiffness, the outcome values of cfPWV (used to assess central arterial stiffness) and baPWV (used to assess whole-body arterial stiffness) were included in the meta-analysis.

## Statistical Analyses

The data analysis was performed using statistical software of the Cochrane Collaboration Review Manager (RevMan, version 5.3, Copenhagen, Denmark). The main statistical procedures were heterogeneity analysis, computation, and verification of combined effect size. The fixed effect model was used for meta-analysis, if no significant difference was found in heterogeneity analysis ($p > 0.05$). The random effect model was used, if heterogeneity was found significant ($p < 0.05$). We used STATA version 12 (StataCorp, College Station, TX) for the analyses of sensitivity, publication bias, and meta-regression. The changes in pulse wave velocity after exercise were found to be correlated with the intensity variable (Coef. = 0.382, T = 2.13, $p$ = 0.042), and not with other variables (intervention duration, frequency, sets, and repetitions). Hence, trials were categorized into two subgroups, high-intensity (70–100%, 1-RM) and low-to-moderate-intensity (30–70%, 1-RM) to identify the effective intensity of RE (28). The differences between the subgroups were also analyzed, and indicated as a significant difference.

Upon the heterogeneity significance (pooled outcome), we performed another subgroup analysis to examine the association between age and RE intensities on the outcomes (cfPWV and baPWV). According to the participants' age, trials were subgrouped into young (<40 years) and middle-aged (≥40 years) (24), and the influence of RE intensities on outcome changes was analyzed in both age groups. Taking into account the differences of outcomes in the studies and the evaluation of the effect size, the standardized mean difference (SMD) was used to determine the magnitude of the RE effect, where the value <0.2 was defined as trivial, 0.2–0.3 as small, 0.4–0.8 as moderate, and >0.8 as large (45). The SMD was expressed as 95% confidence interval (CI). The statistical heterogeneity across different trials in the meta-analysis was assessed by the $I^2$ statistic, where <25% indicates a low risk of heterogeneity, 25–75% indicates a moderate risk of heterogeneity, and >75% indicates a considerable risk of heterogeneity (46).

# RESULTS

## Search Results and Article Selection

We identified a total of 962 articles from the electronic databases, Web of Science, PubMed/MEDLINE, Scopus, ScienceDirect, EMBASE, Cochrane Library, CINAHL, Google Scholar, and Wiley Online Library. After screening the titles and abstracts, 813 articles were excluded, and the remaining 149 were selected for the full-text assessment. Of these, 129 articles were further excluded with reasons explained in **Figure 1**. Finally, 20 articles met the inclusion criteria, and were included in the systematic review and meta-analysis. The informative flow-chart of article search and selection according to PRISMA guidelines was summarized in **Figure 1**.

## Description of the Included Articles

The included trials in this systematic review and meta-analysis ($n = 20$) were intercontinental, published between 2005 and 2020. A majority of the articles ($n = 9$) were conducted in Japan (17, 19, 23, 35–38, 40), followed by the U.S. ($n = 7$) (20–22, 29, 31, 33, 39) and each one from Canada (18), Brazil (30), England (32), and New Zealand (34). Of these, five studies recruited only male participants, seven studies recruited only females, and eight studies recruited a combination of both males and females. The number of participants in the RE group ranged from 10 to 55, and the number of participants in the control group ranged from 9 to 53. The total sample size was 519 (187 males and 332 females) in the RE trial, and 462 (156 males and 306 females) in control trial. The age of participants ranged from 18 to 88 years old. The

**TABLE 1 |** Characteristics of the included studies.

| Study | Country | Age (Y) | Participants (M/F) | | Description of RE | Intensity (%1RM) | Repetitions | Sets | Frequency (t/wk) | Duration (wk) | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RE/Control | RE | Control | | | | | | | |
| Au et al. (18) | Canada | 23 ± 2/ 23 ± 2 | 16 (16/0) | 14 (14/0) | Leg press, seated row, bench press, cable hamstring curl, front planks, shoulder press, bicep curls, triceps extension, wide grip pull downs, and knee extension | 75–90% | 8–12 | 3 | 4 | 12 | cfPWV |
| Au et al. (18) | Canada | 23 ± 3/ 23 ± 2 | 16 (16/0) | 14 (14/0) | Leg press, seated row, bench press, cable hamstring curl, front planks, shoulder press, bicep curls, triceps extension, wide grip pull downs, and knee extension | 30–50% | 20–25 | 3 | 4 | 12 | cfPWV |
| Beck et al. (29) | U.S. | 21.1 ± 2.3/ 21.6 ± 3.1 | 15 (11/4) | 15 (10/5) | Leg extension, leg curl, leg press, lat pull down, chest press, overhead press, and biceps curl | 60% | 8–12 | 2 | 3 | 8 | cfPWV |
| Cahu Rodrigues et al. (30) | Brazil | 61 ± 8.25/ 59 ± 8 | 17 (6/11) | 16 (5/11) | Four sets of 2-min isometric contractions using a performed handgrip dynamometer | 30% | nr | 4 | 3 | 12 | cfPWV |
| Casey et al. (20) | U.S. | 21 ± 2.45/ 22 ± 2.97 | 24 (11/13) | 18 (8/10) | Leg extension, leg curl, leg press, lat pulldown, chest press, overhead press, and bicep curl | 70% | 8–12 | 2 | 3 | 12 | cfPWV |
| Cortez-Cooper et al. (22) | U.S. | 29 ± 1/ 27 ± 2 | 23 (0/23) | 10 (0/10) | Bench press, overhead press, weight-assisted parallel bar dip, dumbbell crossover pull, dumbbell rowing motion, latissimus dorsi pulldown, dumbbell curl, squat/leg press, high pull, deadlift, medicine ball drills, and abdominal exercises | 75–85% | 5–10 | 3–4 | 4 | 11 | cfPWV |
| Croymans et al. (31) | U.S. | 21.5 ± 2.34/ 21.85 ± 1.79 | 28 (28/0) | 8 (8/0) | Dumbbell (DB) squat, cable row, DB front lunge, DB row, barbell dead lift, DB triceps extension, DB bicep curl, DB step-up, barbell chest press, machine squat, DB overhead press, DB incline chest press, DB side raise, DB reverse fly, and abdominal crunches | 70–85% | 8–12 | 2–3 | 3 | 12 | cfPWV |
| Devallance et al. (21) | U.S. | 49 ± 12/ 44 ± 10.4 | 16 (4/12) | 12 (3/9) | Leg press, chest press, lat pull down, leg curl, shoulder press, and leg extension | 70–85% | 8–12 | 3 | 3 | 8 | cfPWV |
| Devallance et al. (21) | U.S. | 51 ± 11/ 51 ± 16 | 13 (4/9) | 16 (4/12) | Leg press, chest press, lat pull down, leg curl, shoulder press, and leg extension | 70–85% | 8–12 | 3 | 3 | 8 | cfPWV |

*(Continued)*

**TABLE 1** | Continued

| Study | Country | Age (Y) | Participants (M/F) | | Description of RE | Intensity (%1RM) | Repetitions | Sets | Frequency (t/wk) | Duration (wk) | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RE/Control | RE | Control | | | | | | | |
| Greenwood et al. (32) | England | 54.6 ± 10.6/ 49.5 ± 10.6 | 13 (7/6) | 20 (10/10) | Bench press, latissimus pulldown, bicep curl, triceps pull down, leg press, knee extension, hamstring curl, and calf raises | 80% | 8–10 | 3 | 3 | 12 | cfPWV |
| Jaime et al. (33) | U.S. | 64 ± 3.46/ 67 ± 2.83 | 12 (0/12) | 8 (0/8) | Leg press, leg extension, leg flexion, and calf raise | 40% | 15 | 2 | 3 | 12 | baPWV cfPWV |
| Jones et al. (34) | New Zealand | 55.8 ± 7.2/ 55.9 ± 7.1 | 26 (0/26) | 25 (0/25) | Leg press, leg extension, lying hamstring curl, machine bench press, lat pulldown, cable row, dumbbell shoulder press, dumbbell bicep curl, triceps pushdown, V-sit, abdominal crunches, and reverse abdominal crunches | 60% | 10–12 | 2–4 | 2 | 12 | cfPWV |
| Miura et al. (35) | Japan | 69.0 ± 6.5/ 68.9 ± 7.5 | 29 (0/29) | 23 (0/23) | Chest fly, biceps curl, push-up, bent-over row, upright row, overhead press, squat, front lunge, side lunge, straight-leg extension, heal raise, and outer thigh lift | 50–60% | 15–20 | 3–5 | 1 | 12 | baPWV |
| Miura et al. (35) | Japan | 69.5 ± 7.0/ 68.9 ± 7.5 | 25 (0/25) | 23 (0/23) | Chest fly, biceps curl, push-up, bent-over row, upright row, overhead press, squat, front lunge, side lunge, straight-leg extension, heal raise, and outer thigh lift | 50–60% | 15–20 | 3–5 | 2 | 12 | baPWV |
| Miura et al. (19) | Japan | 72.9 ± 5.7/ 69.7 ± 6.7 | 45 (0/45) | 47 (0/47) | Chest fly, biceps curl, push-up, bent-over row, upright row, overhead press, squat, front lunge, side lunge, straight-leg extension, heal raise, and outer thigh lift | 50–60% | 15–20 | 3–5 | 2 | 12 | baPWV |
| Miura et al. (19) | Japan | 72.0 ± 7.1/ 71.8 ± 5.6 | 55 (0/55) | 53 (0/53) | Chest fly, biceps curl, push-up, bent-over row, upright row, overhead press, squat, front lunge, side lunge, straight-leg extension, heal raise, and outer thigh lift | 50–60% | 15–20 | 3–5 | 2 | 12 | baPWV |
| Okamoto et al. (36) | Japan | 18.9 ± 0.3/ 19.9 ± 1.2 | 10 (0/10) | 9 (0/9) | Arm curl (2s eccentric phase) | 80% | 10 | 5 | 3 | 8 | baPWV |
| Okamoto et al. (36) | Japan | 19.1 ± 0.3/ 19.9 ± 1.2 | 10 (0/10) | 9 (0/9) | Arm curl (2s concentric phase) | 80% | 10 | 5 | 3 | 8 | baPWV |
| Okamoto et al. (37) | Japan | 19.4 ± 0.2/ 19.4 ± 0.2 | 10 (10/0) | 9 (9/0) | Chest press, arm curl, lateral pull down, seated row, shoulder press, leg extension, leg curl, leg press, and sit-up (3-s lowering phase and 3-s lifting phase) | 40% | 10 | 5 | 2 | 8 | baPWV |

*(Continued)*

**TABLE 1** | Continued

| Study | Country | Age (Y) | Participants (M/F) | | Description of RE | Intensity (%1RM) | Repetitions | Sets | Frequency (t/wk) | Duration (wk) | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RE/Control | RE | Control | | | | | | | |
| Okamoto et al. (23) | Japan | 20.2 ± 0.4/ 20.1 ± 0.3 | 10 (7/3) | 10 (6/4) | Chest presses, arm curls, seated rows, shoulder presses, and lat pull downs | 80% | 8–10 | 5 | 2 | 10 | baPWV |
| Okamoto et al. (23) | Japan | 20.0 ± 0.5/ 20.1 ± 0.3 | 10 (7/3) | 10 (6/4) | Leg presses, squats, seated calf raises, leg extensions, and leg curls | 80% | 8–10 | 5 | 2 | 10 | baPWV |
| Okamoto et al. (23) | Japan | 19.6 ± 1.26/ 19.7 ± 0.95 | 10 (10/0) | 10 (10/0) | Chest presses, arm curls, seated rowing, leg curls, leg presses, and sit-ups (1-s lifting phase and 3-s lowering phase) | 80% | 8–10 | 5 | 2 | 10 | baPWV |
| Okamoto et al. (23) | Japan | 19.2 ± 0.95/ 19.7 ± 0.95 | 10 (10/0) | 10 (10/0) | Chest presses, arm curls, seated rowing, leg curls, leg presses, and sit-ups (3-s lifting phase and 1-s lowering phase) | 80% | 8–10 | 5 | 2 | 10 | baPWV |
| Okamoto et al. (17) | Japan | 18.5 ± 0.5/ 18.6 ± 0.5 | 13 (10/3) | 13 (9/4) | Chest press, arm curl, seated row, lateral pull down, leg press, leg extension, leg curls, and sit-ups | 50% | 10 | 5 | 2 | 10 | baPWV |
| Okamoto et al. (38) | Japan | 19.3 ± 0.7/ 19.1 ± 0.6 | 10 (5/5) | 10 (5/5) | Chest presses, arm curls, seated rowing, leg curls, and leg presses | 50/80% | 10 | 2/3 | 2 | 10 | cfPWV |
| Okamoto et al. (38) | Japan | 19.1 ± 0.7/ 19.1 ± 0.6 | 10 (5/5) | 10 (5/5) | Chest presses, arm curls, seated rowing, leg curls, and leg presses | 80/50% | 10 | 3/2 | 2 | 10 | cfPWV |
| Werner et al. (39) | U.S. | 22.9 ± 2.9/ 21.2 ± 2.8 | 10 (10/0) | 10 (10/0) | Back squats, flat bench press, seated rows, shoulder press, bicep curls, triceps extension, standing calf raises, seated leg curls, and seated leg extension | 80–90% | 3–8 | 2–3 | 3–5 | 12 | cfPWV |
| Werner et al. (39) | U.S. | 20.9 ± 3.2/ 21.2 ± 2.8 | 10 (10/0) | 10 (10/0) | Back squats, flat bench press, seated rows, shoulder press, bicep curls, triceps extension, standing calf raises, seated leg curls, and seated leg extension | 50–70% | 10–15 | 3–4 | 3–5 | 12 | cfPWV |
| Yoshizawa et al. (40) | Japan | 47 ± 6.63/ 49 ± 10.39 | 11 (0/11) | 12 (0/12) | Leg curl, leg press, hip adduction, hip flexion, vertical press, and sit-ups | 60% | 10 | 3 | 2 | 12 | cfPWV |

*Y, years; M/F, male/female; RE, resistance exercise; 1RM, one-repetition maximum; t/wk, times/week; cfPWV, carotid-femoral pulse wave velocity; baPWV, brachial-ankle PWV; nr, not reported.*

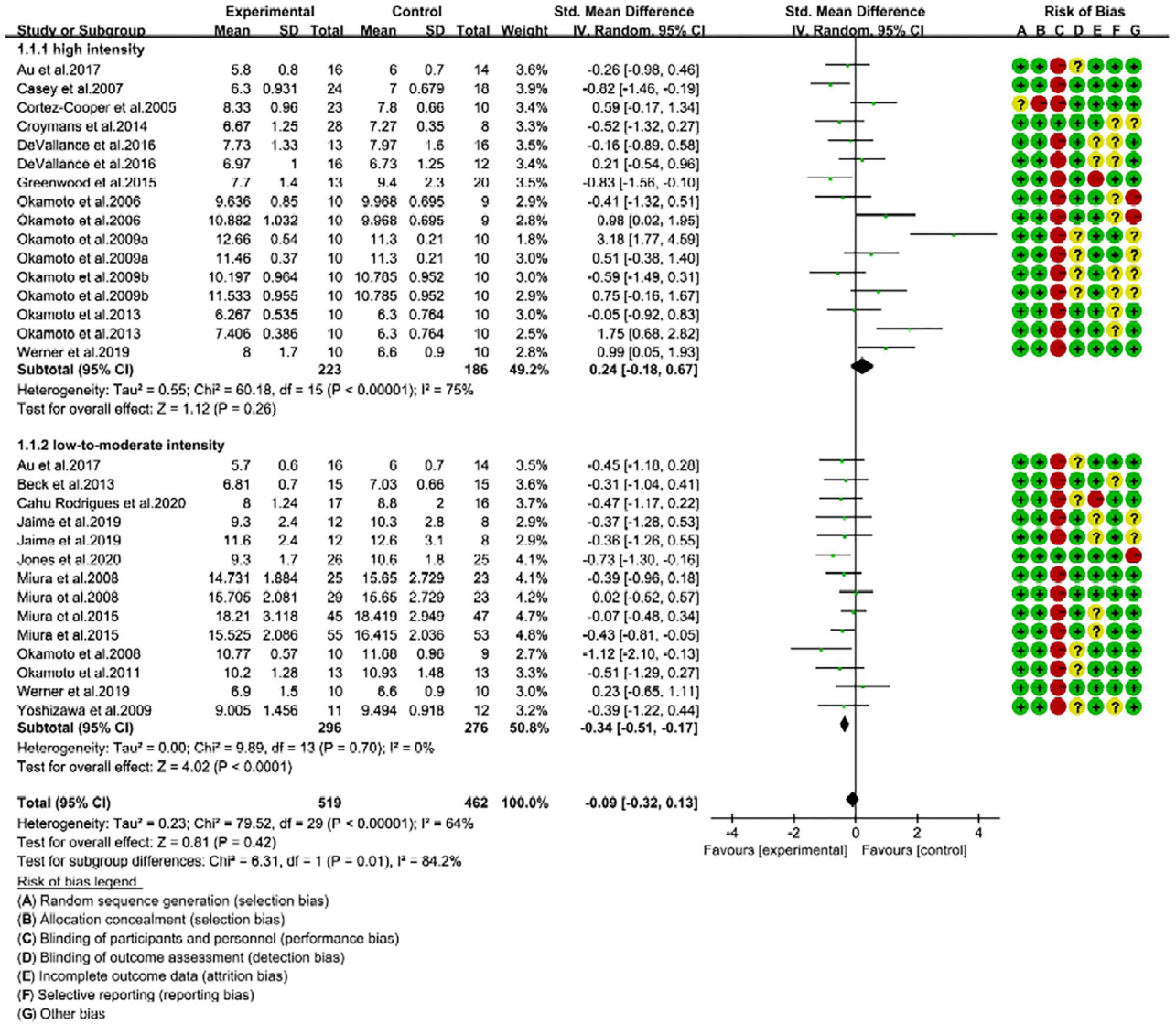


**FIGURE 2 |** Forest plot of pulse wave velocity (PWV) changes with different intensities of RE in adults (m/s). SD, standard deviation; IV, inverse variation; CI, confidence interval; df, degrees of freedom. Risk of bias: green (+) indicates low risk of bias; red (–) high risk of bias; and yellow (?) unclear risk of bias.

duration of RE intervention was between 8 and 12 weeks with a frequency of 1–5 times per week. The intensity of RE (%1-RM) ranged from 30 to 90% 1-RM, and sets of repetitions ranged from 3 to 25. The characteristics of participants and RE intervention along with publication details were summarized in **Table 1**.

## Summary of the Risk of Bias

The Cochrane Collaboration method was employed to assess the risk of bias for the included trials, and the detailed statement was presented in **Figure 2**. For the selection bias, all the trials except one (22) were randomly assigned, and five trials reported the methods used for randomization (30–34). For the performance bias, except two trials (31, 34), all trials were judged to have high risk of bias for blinding participants to an exercise intervention. In those two studies (31, 34) all participants and authors (except research coordinator) were blinded to the recruitment, randomization, and experiment execution. Typically it is not possible to blind the participants in an exercise intervention, and reporting such a high risk of bias does not mean it influences or compromises the quality of the study (47, 48). Instead, other variables, including the level of study attrition, poor intervention adherence, and selective reporting bias are the most common issues around the high risk of bias that would impact quality of the study (49). In our assessment, two studies reported to have attrition bias because of the high attrition rate (30, 32). In addition, two trials were identified with other risks of bias (34, 36), one conducted a circuit resistance training mixed with a small amount of aerobic exercise components (34), and the other

**TABLE 2 |** Meta-regression analysis for the changes in pulse wave velocity (PWV, m/s) and resistance exercise (RE) variables.

| RE variables | Coefficient | Standard error | *T* value | *P*-value | [95% Conf. interval] | |
|---|---|---|---|---|---|---|
| Intensity | 0.382 | 0.179 | 2.13 | **0.042*** | 0.015 | 0.749 |
| Frequency | 0.007 | 0.175 | 0.04 | 0.969 | −0.352 | 0.366 |
| Duration | −0.095 | 0.087 | −1.10 | 0.282 | −0.273 | 0.083 |
| Sets | 0.126 | 0.134 | 0.94 | 0.354 | −0.148 | 0.401 |
| Repetitions | −0.055 | 0.040 | −1.38 | 0.179 | −0.136 | 0.027 |

**Represents a significant correlation between PWV change and RE variables.*

one conducted local training limited to only arm curls on the left side (36).

The sensitivity analyses results showed that no trial had a significant impact on the total effect size. However, the funnel plot and Egger linear regression test (Egger's test) ($t = 2.52$; $p = 0.018$; 95% CI: 0.46, 4.46) showed a publication bias (**Supplementary Figure 1**). It can be seen from the funnel plot that one trail had relatively large bias (23), but when it was not considered, the publication bias was eliminated ($t = 1.75$; $p = 0.092$; 95% CI: −0.29, 3.57). This trial reported significantly increased baPWV (SMD = 3.18, 95% CI: 1.77, 4.59) with upper limb resistance training.

## Influence of Resistance Exercise Intervention on Arterial Stiffness

Arterial stiffness is determined by monitoring the changes of cfPWV and baPWV in adults (7). In our meta-analysis ($n = 30$), 17 trials reported cfPWV data and 13 reported baPWV data. We combined the effect size of two outcomes (cfPWV and baPWV) under the random effects model, and used SMD to determine the effect size of RE intervention on arterial stiffness. The pooled results showed that RE intervention had no effect on PWV in adults (SMD = −0.09; 95% CI: −0.32, 0.13). The overall effect of RE on arterial stiffness was not statistically significant ($P = 0.42$), but heterogeneity was moderate ($I^2 = 64\%$; **Supplementary Figure 2**). These results direct us to find out the source of heterogeneity.

## RE Intensity Is Associated With Reduction of PWV

We performed meta-regression analysis to determine the influence of RE variables, such as frequency, intensity, duration, sets, and repetitions on PWV changes. The results revealed that only the "intensity" variable is significantly correlated with the changes of PWV after RE intervention (Coef. = 0.382, $t = 2.13$, $P = 0.042$), while frequency (Coef. = 0.007, $t = 0.04$, $P = 0.969$), duration (Coef. = −0.095, $t = -1.10$, $P = 0.282$), sets (Coef. = 0.126, $t = 0.94$, $P = 0.354$), and repetition (Coef. = −0.055, $t = -1.38$, $P = 0.179$) were not correlated with the changes of PWV (**Table 2**).

Based on intensity, we then categorized the trials into two subgroups, namely high-intensity (70–100% 1-RM, 16 trials) and low-to-moderate-intensity (30–70% 1-RM, 14 trials). Consequently, we determined the effectiveness of two different RE intensities on reduction of arterial stiffness in adults.

## Low-to-Moderate-Intensity Effectively Reduces PWV Than High-Intensity RE

We performed subgroup analysis to identify the effective RE intensity that could improve arterial stiffness in adults. The subgroup analysis results showed that the changes in PWV were not significant after high-intensity RE (SMD = 0.24; 95% CI: −0.18, 0.67; $P = 0.26$), which indicates high-intensity RE was unable to improve the arterial stiffness (**Figure 2**). Interestingly, we noticed that low-to-moderate-intensity RE significantly improved arterial stiffness in adults. This was evidenced by a significant reduction of PWV in RE trials (SMD = −0.34, 95% CI: −0.51, −0.17; $P < 0.0001$) with no risk of heterogeneity ($I^2 = 0\%$). From the subgroup analysis, we further noticed that there is a significant difference ($P = 0.01$) in the effect size of RE on arterial stiffness (SMD changes) between high- and low-to-moderate intensity trials ($Chi^2 = 6.31$, $I^2 = 84.2\%$) (**Figure 2**).

## RE Improves Arterial Stiffness in Middle-Aged Adults

We next hypothesized that the beneficial effects of RE on arterial stiffness could be influenced by the "age" of the individuals. To explore this phenomenon, we categorized the trials into young (<40 years, 18 trials) and middle-age or old groups (≥40 years, 12 trials) (24). Regardless of intensity, the overall RE intervention had no effect on PWV changes in young participants (SMD = 0.14; 95% CI: −0.25, 0.53; $P = 0.49$, **Figure 3**). Nevertheless, as reported in **Figure 4**, middle-aged adults showed positive response to RE intervention, and the decreased PWV was statistically significant (SMD = −0.31; 95% CI: −0.49, −0.14; $P = 0.0003$) with no risk of heterogeneity ($I^2 = 0\%$). These findings indicate that RE improved arterial stiffness in middle-aged adults, but not in young individuals.

## Association Between RE Intensities and Age on Arterial Stiffness

We performed another subgroup analysis to reveal the association between RE intensities and age on improvement of arterial stiffness. For the young individuals, high-intensity RE (13 trials) had no effect on PWV changes (SMD = 0.38; 95% CI: −0.13, 0.89; $P = 0.14$), and trials indicated considerable

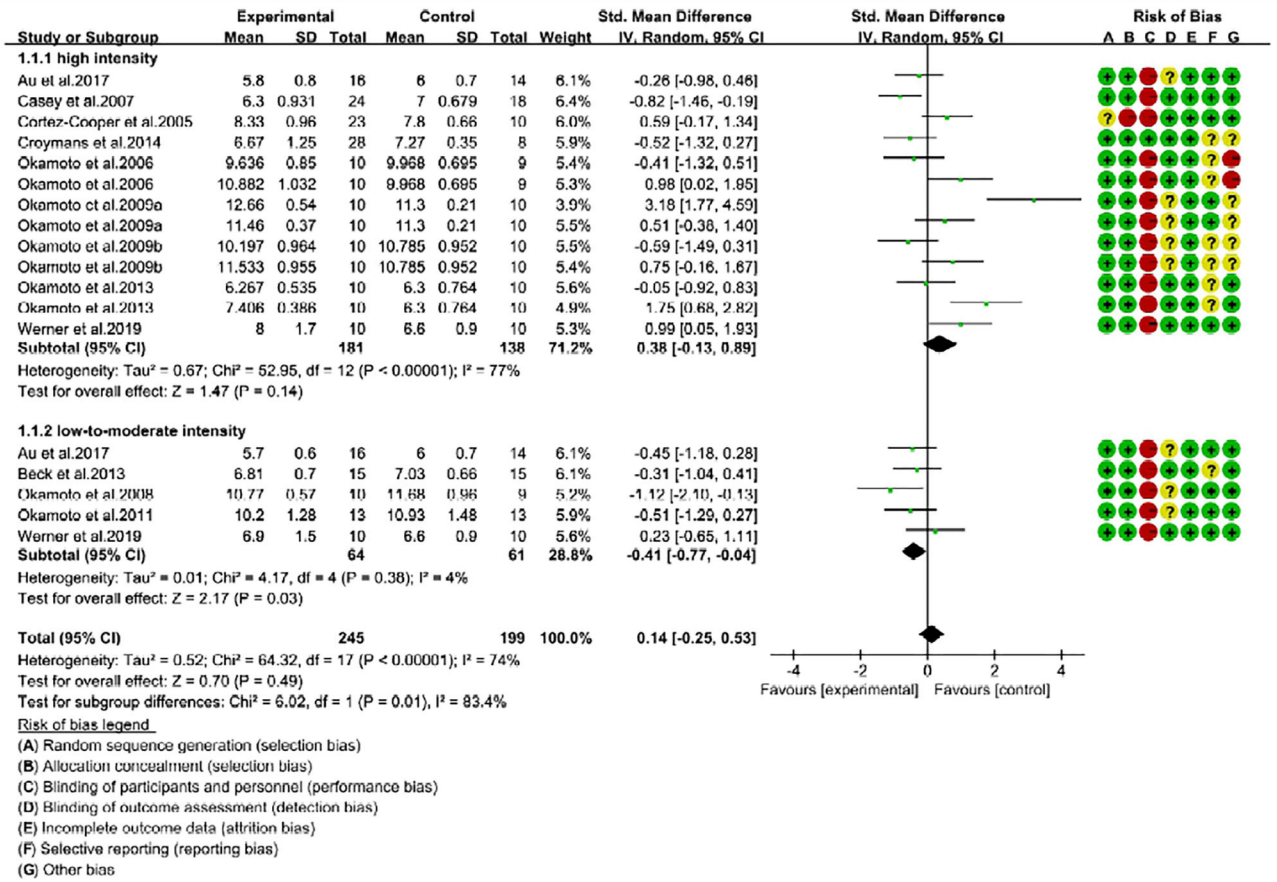


**FIGURE 3 |** Forest plot of pulse wave velocity (PWV) changes with different intensities of RE in young individuals (m/s). SD, standard deviation; IV, inverse variation; CI, confidence interval; df, degrees of freedom. Risk of bias: green (+) indicates low risk of bias; red (–) high risk of bias; and yellow (?) unclear risk of bias.

risk of heterogeneity ($I^2 = 77\%$). In contrast, low-to-moderate-intensity (5 trials) resulted in a significant ($P = 0.03$) reduction of PWV in young adults (SMD = −0.41; 95% CI: −0.77, −0.04) with low risk of heterogeneity ($I^2 = 4\%$). Furthermore, the test for subgroup difference between low-to-moderate-intensity and high-intensity was significantly different ($Chi^2 = 6.02$; $P = 0.01$; $I^2 = 83.4\%$). These results witnessed that only low-to-moderate-intensity improved the arterial stiffness in young individuals (**Figure 3**).

Next, we found that middle-aged adults were not positively responded to high-intensity RE, as reported insignificant changes of PWV after intervention (SMD = −0.26; 95% CI: −0.86, 0.33; $P = 0.39$) (**Figure 4**). Noteworthy, middle-aged adults following low-to-moderate-intensity were represented with a significant improvement in arterial stiffness. The PWV mean change in low-to-moderate-intensity trials was extremely significant ($P = 0.0007$) with SMD of −0.32, 95% CI is −0.51 to −0.14, and low risk of heterogeneity ($I^2 = 0\%$). Subgroup analysis showed no significant difference ($P = 0.85$) between high- and low-to-moderate intensity trials (**Figure 4**). Taken together, our analysis revealed that RE with low-to-moderate-intensity promoted arterial stiffness in young and middle-aged adults, while high-intensity is ineffective in both age groups.

## DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis to demonstrate the influential role of RE intensities on arterial stiffness in young and middle-aged adults. Arterial stiffness is a growing global-health burden associated with increased risk of cardiovascular events, hypertension, dementia, and mortality. However, reversing arterial stiffness or decreasing PWV (m/s) could prevent the incidence of such diseases (4, 6, 8). Here we determined the changes of PWV (a gold standard measure of arterial stiffness) in adults, who participated in RE intervention at least for 8 weeks. Meta-analysis results showed RE (irrespective of intensity) had no effect on arterial stiffness, but risk of heterogeneity was moderate. Meta-regression analysis revealed that RE intensity is correlated with decreased PWV, which indicates "intensity" is the key variable in improving arterial stiffness. Subgroup analysis showed

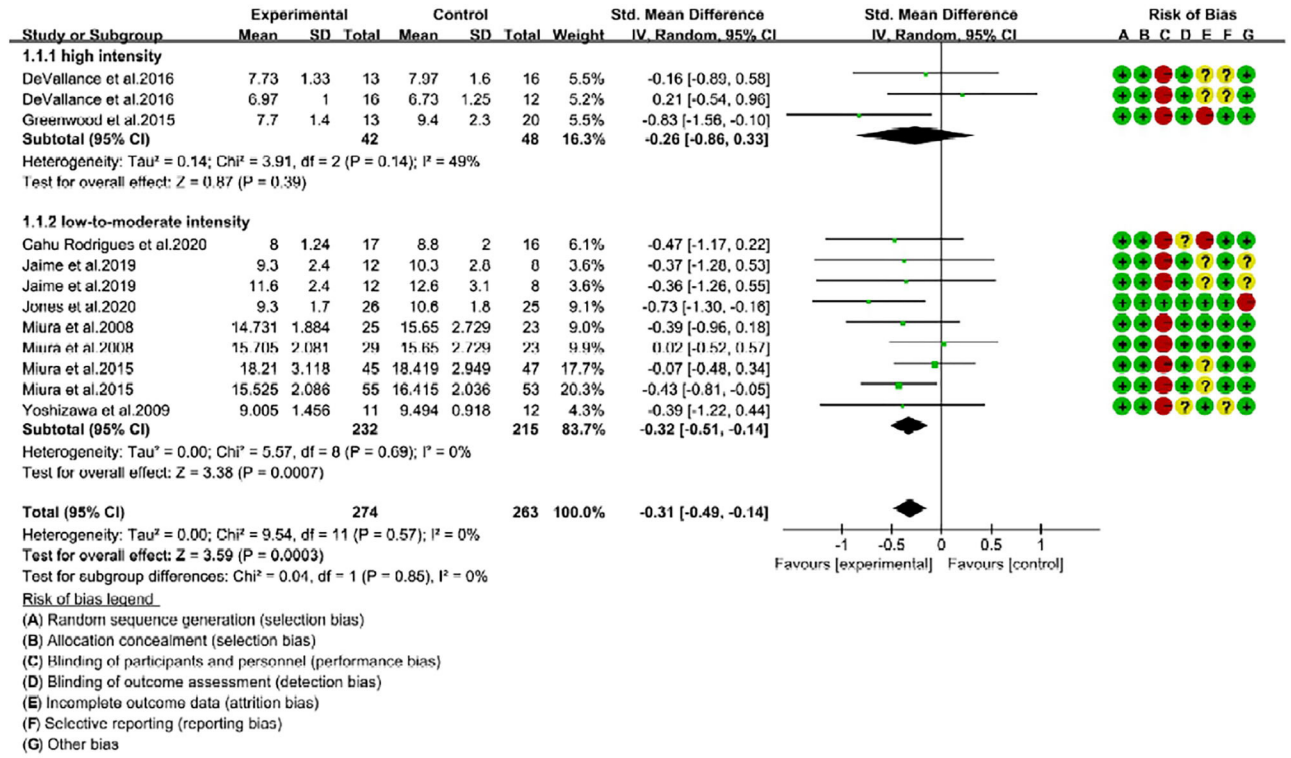


**FIGURE 4 |** Forest plot of pulse wave velocity (PWV) changes with different intensities of RE in middle-aged adults (m/s). SD, standard deviation; IV, inverse variation; CI, confidence interval; df, degrees of freedom. Risk of bias: green (+) indicates low risk of bias; red (–) high risk of bias; and yellow (?) unclear risk of bias.

decreased PWV is effective (−0.34) with low-to-moderate-intensity. Conversely, high-intensity RE is ineffective to decrease the PWV. About the age factor, we further reported low-to-moderate-intensity decreased PWV in young and middle-aged adults, while high-intensity is unable to improve the arterial stiffness in either age group. These findings provided evidence that RE interventions with low-to-moderate-intensity can reverse the arterial stiffness, and thereby prevent the occurrence and/or progression of cardiovascular events. Our meta-regression and subgroup analyses are the newly added evidence to the existing review that summarizes RE intensity effect on arterial stiffness without statistical analysis (50).

It is well-documented that arterial stiffness determined by increased PWV is associated with age and systolic blood pressure, and is an independent risk factor for CVD and mortality (7, 8, 51). The included trials in our study addressed the effect of resistance training on arterial stiffness of young and middle-aged adults by reporting the changes in cfPWV and/or baPWV. The test for overall effect (pooled outcome) showed RE intervention for a period of 8–12 weeks did not influence the arterial stiffness in adults. Similar to our findings, two recent meta-analyses concluded that resistance training had no effect on arterial stiffness in persons with high risk of CVD (25) and also in healthy individuals (26). However, the source of heterogeneity ($I^2$) that is 40% in Evans' study (25) (5 trials) and 86% in the study by Ceciliato et al. (26) (15 trials) was not addressed clearly. The moderate heterogeneity ($I^2 = 64\%$) reported in our analysis suggests the possible involvement of exercise variables on PWV changes. Through meta-regression analysis, we found exercise intensity is associated with reduction of PWV rather than exercise frequency, duration, sets, and repetitions. To be particular, low-to-moderate-intensity had a greater beneficial effect in improving the arterial stiffness, while high-intensity was ineffective to do so.

Our findings are quite interesting and are different from the previous reviews and meta-analyses, which investigated RE intensities effect on arterial stiffness. For instance, a meta-analysis by Miyachi demonstrated that high-intensity RE was associated with increased arterial stiffness, but moderate-intensity RE did not show such association in adults (24). In contrast, another meta-analysis showed RE had no effect (positive or negative) on PWV, and there was no association between RE intensity and arterial stiffness (52). A recent systematic review emphasized that 4-week RE intervention with a frequency of 2 times per day per week may decrease arterial stiffness. Nevertheless, the influence of intensity on arterial stiffness was not addressed in this review (53). To address the RE intensity effect, a review by Figueroa et al. stated that low- and high-intensity resistance training may not influence arterial stiffness. Irrespective of intensity, the overall RE may decrease central and peripheral blood pressure in middle-aged and older adults with elevated blood pressure at baseline (50). However, these two reviews (50, 53) did not explore the

correlations between PWV and exercise characteristics, and did not provide any statistical or meta-analysis evidence to convince their conclusions. In our study, through meta-regression and subgroup analyses, we provided evidence that low-to-moderate-intensity RE effectively improved arterial stiffness in young and middle-aged adults.

Regardless of intensity, we found RE intervention (the overall effect) significantly decreased PWV in middle-aged adults, but not in young individuals. However, when trials were subgrouped based on the intensity, only low-to-moderate-intensity improved arterial stiffness in young and middle-aged adults, while high-intensity had no effect in both age groups. These findings opened for debate why high-intensity RE is not beneficial to improve arterial stiffness in individuals separated into similar age groups. Relatively with a lesser number of trials (4), Miyachi (2013) reported significantly increased arterial stiffness in young subjects after high-intensity RE, while moderate-intensity (3 trials) had no effect on middle-aged adults. This tendency might be due to the lower baseline values in young adults, and higher arterial stiffness in middle-aged adults (24). It is further suggested that low-intensity may decrease the systemic arterial stiffness (baPWV) in young healthy adults or not influence the arterial stiffness in middle-age and older adults (50). The latest systematic review stated that acute low-intensity RE with blood flow restriction intervention (2 articles) had positive or negative effects on arterial stiffness in healthy young adults, while chronic intervention (3 articles) had neutral effects on healthy young and older adults (54). In middle-aged women, moderate-intensity RE (60%, 1-RM) for 12 weeks did not produce any unfavorable effects on vasculature, as revealed by unchanged cfPWV and femoral-ankle PWV; however, muscle strength was increased (40). A study conducted on young healthy men reported increased arterial stiffness (cfPWV) and aortic augmentation index (Aix) following acute RE program (60%, 1-RM), and advised future studies to examine the long-term effect of RE on arterial stiffness (55). A few years later, another study on young and older women represented with unchanged cfPWV or femoral-tibialis posterior arterial stiffness after 8 weeks of high-intensity resistance training (3-time/week, ∼80% 1-RM) (56). These equivocal conclusions from reviews and research trials may be due to the variances in article inclusion criteria, RE protocols (frequency, intensity, sets, repetitions, duration), and/or subjects' characteristics (age, sex, bodyweight, health status).

## Mechanism and Factors Involved in Regulation of Arterial Stiffness

The detailed mechanism for the diverse effect of RE on arterial stiffness has yet to be fully elucidated. We postulated that internal factors, including muscle tone, sympathetic nerve activity, blood pressure, blood circulation, and endothelial function could influence the arterial stiffness following exercise intervention. Okamoto and team demonstrated that upper- but not lower-limb high-intensity RE increased arterial stiffness (baPWV) in young adults. This was accompanied by an increased plasma norepinephrine concentration, which reflects sympathetic nervous system activity. The activation of the sympathetic system may acutely affect the arterial distensibility through complex interactions between large arterial smooth muscle tone and distending blood pressure (23). The central arterial function is influenced by endothelial function. The key vasoactive agents, nitric oxide (NO) and endothelin-1 (ET-1) produced by endothelial cells can alter the smooth muscle tone, and thereby regulate large artery stiffness (57). On the other hand, decreased conduit artery endothelial function is associated with increased peripheral artery PWV and central pulse pressure (58).

Decreased central blood pressure and peripheral PWV (not cfPWV) in pre-hypertensive patients after whole-body resistance training (60%, 1RM, 8 weeks) are associated with improved endothelial function and vasoactive substances (29). It is further disclosed that RE reduced blood pressure and improved brachial artery FMD (flow-mediated dilation) in young pre-hypertensive patients with concurrently increased NO bioavailability and decreased circulating ET-1 (29). Higher ET-1 production is associated with increased arterial stiffness in young strength-trained men (weight lifters), while plasma NO concentrations remain unchanged (59). Improved arterial stiffness in obese adolescent girls after RE plus aerobic exercise intervention was represented by an increased plasma NO level and unchanged ET-1 (60). Besides, aerobic exercise combined with low-intensity RE reported to increase basal NO production, and decrease arterial stiffness without changing the bodyweight in healthy older adults (61). Improved endothelial NO-mediated vasodilatory function may result in decreased PWV (50). Literature revealed that high-intensity RE may not produce favorable effects on endothelial function in healthy men (23, 62). High-intensity RE may increase the blood pressure acutely and sympathetic activity chronically, which contribute to an increase in arterial stiffness. Besides, moderate- or high-intensity RE is favorable on brachial artery or forearm endothelial function in overweight postmenopausal women (63, 64) and middle-aged adults (65) with elevated blood pressure. Interestingly, low-intensity RE with slow lifting and lowering, and short inter-set rest periods showed positive effects on endothelial function in healthy adults (17, 37). Taken together, high-intensity RE can increase the sympathetic nerve activity, muscle tone, blood pressure, and circulation resistance. Such elevations eventually lead to deleterious adaptation of vascular smooth muscle, and thereby increase arterial stiffness. On the other hand, low-intensity RE may not increase sympathetic nerve activity or muscle tone. The proper muscle contraction with low-intensity also promotes blood circulation and thereby improves vascular endothelial function and arterial stiffness.

# LIMITATIONS

In our analyses, we mixed the trials that investigated the effect of RE in healthy individuals as well as patients. Although our results showed that low-to-moderate intensity RE is beneficial in improving the arterial stiffness in young and middle-aged adults, the beneficial effects of RE intensity in specific population,

like hypertensive or diabetic patients, remains uncertain. The small sample size in the included trials (not many) might be a limitation for those studies. Very small sample size can undermine the internal and external validity of the study, and it is hard to determine whether the changes in outcome measures are true or statistically different. Further analyses with large-scale sample size are required to confirm negative or neutral effects of high-intensity RE on the arterial stiffness of an aged population with or without the existence of chronic diseases.

## CONCLUSIONS

The evidence from our systematic review, meta-analysis, and meta-regression analysis confirmed that RE intensity is the key variable to promote arterial stiffness. Precisely, low-to-moderate-intensity RE is effective in improving arterial stiffness in young and middle-aged adults. In contrast, high-intensity RE is ineffective in decreasing the PWV. Therefore, practicing high-intensity RE should be cautious in particular age groups/patients due to the unfavorable effects on arterial stiffness. Practicing of low-to-moderate-intensity RE is beneficial to promote arterial stiffness that may aid to reduce the risk for cardiovascular diseases.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

## AUTHOR CONTRIBUTIONS

YZ, Y-JZ, and MK designed the study, performed article search and screening, reviewed the full-text articles, and extracted the data. YZ and Y-JZ performed statistical analyses and drafted the manuscript. WY provided additional suggestions and assisted in interpretation of data. YZ and MK revised and finalized the manuscript. All authors have read and approved the submission.

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# Impacts of Cigarette Smoking Status on Metabolomic and Gut Microbiota Profile in Male Patients With Coronary Artery Disease: A Multi-Omics Study

*Xiaomin Hu[1,2*†], Yue Fan[1†], Hanyu Li[1], Ruilin Zhou[1], Xinyue Zhao[1], Yueshen Sun[1] and Shuyang Zhang[1*]*

[1] Department of Cardiology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, China, [2] Department of Medical Research Center, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, China

**Background:** Cigarette smoking has been considered a modifiable risk factor for coronary artery disease (CAD). Changes in gut microbiota and microbe-derived metabolites have been shown to influence atherosclerotic pathogenesis. However, the effect of cigarette smoking on the gut microbiome and serum metabolites in CAD remains unclear.

**Method:** We profiled the gut microbiota and serum metabolites of 113 male participants with diagnosed CAD including 46 current smokers, 34 former smokers, and 33 never smokers by 16S ribosomal RNA (rRNA) gene sequencing and untargeted metabolomics study. A follow-up study was conducted. PICRUSt2 was used for metagenomic functional prediction of important bacterial taxa.

**Results:** In the analysis of the microbial composition, the current smokers were characterized with depleted *Bifidobacterium catenulatum, Akkermansia muciniphila*, and enriched *Enterococcus faecium*, *Haemophilus parainfluenzae* compared with the former and never smokers. In the untargeted serum metabolomic study, we observed and annotated 304 discriminant metabolites, uniquely including ceramides, acyl carnitines, and glycerophospholipids. Pathway analysis revealed a significantly changed sphingolipids metabolism related to cigarette smoking. However, the change of the majority of the discriminant metabolites is possibly reversible after smoking cessation. While performing PICRUSt2 metagenomic prediction, several key enzymes (wbpA, nadM) were identified to possibly explain the cross talk between gut microbiota and metabolomic changes associated with smoking. Moreover, the multi-omics analysis revealed that specific changes in bacterial taxa were associated with disease severity or outcomes by mediating metabolites such as glycerophospholipids.

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*Chinese Academy of Medical Sciences and Peking Union Medical College, China*
*Peidong Han,*
*Zhejiang University, China*

***Correspondence:**
*Xiaomin Hu*
*huxiaomin2015@163.com*
*Shuyang Zhang*
*shuyangzhang103@nrdrs.org*

*†These authors have contributed equally to this work*

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**Conclusions:** Our results indicated that both the gut microbiota composition and metabolomic profile of current smokers are different from that of never smokers. The present study may provide new insights into understanding the heterogenic influences of cigarette smoking on atherosclerotic pathogenesis by modulating gut microbiota as well as circulating metabolites.

**Keywords: smoking, coronary artery disease, gut microbiota, metabolomics, sphingolipids metabolism**

## INTRODUCTION

Cigarette smoking is a major modifiable cardiovascular risk factor (1). Epidemiological evidence has shown that current smokers with coronary artery disease (CAD) undergo percutaneous coronary intervention (PCI) at a younger age and have a significantly higher short-term and 3-year mortality compared with non-smokers (2, 3). However, smoking cessation in patients with CAD can substantially lower the risk of recurrent cardiovascular events and all-cause mortality (4).

In the last few decades, there has been a surge of interest in the pathophysiologic role of gut microbiota in atherosclerosis and cardiovascular disease. Possible mechanisms involve symbiont microbiota influencing the host immune system or generating microbial-derived products such as trimethylamine N-oxide (TMAO) and short-chain fatty acids (SCFAs) (5, 6). TMAO can accelerate the progression of atherosclerosis by enhancing the accumulation of cholesterol in macrophages and foam cells in artery walls as well as enhancing platelet hyperreactivity and thrombosis (7, 8). Moreover, elevated plasma levels of TMAO were associated with an increased risk of major adverse cardiovascular events, which is independent of traditional risk factors, even in the low-risk population (9). Based on bracing discoveries in microbiota-host interaction in atherosclerosis, novel therapeutic targets have been proposed for the treatment of cardiometabolic diseases, such as bacterial enzyme inhibitors and dietary substrate analogs (10, 11).

Cigarette smoking may influence host microbiota through various mechanisms including upregulating oxidative stress-associated enzymes in gut immune cells, altering the gut mucin layer, and increasing the intestinal pH (12–14). A prior study showed that gut microbiome compositions of smokers differed significantly from those of never smokers in the healthy population (15), but whether this change can be reversed by smoking cessation remains a matter of controversy (16, 17). Besides, the smoking-induced intestinal microbiota changes under the background of chronic diseases like Crohn's disease and ankylosing spondylitis have been investigated but whether this change contributes to disease progression is not determined (18, 19). Whereas, little is known about the effect of smoking on gut flora and its association with disease progression in patients with CAD. In this study, we sought to address the knowledge gap by evaluating the effects of smoking status on gut microbiota and serum metabolome to explain the role of smoking on CAD pathogenesis from a multi-omics view.

## MATERIALS AND METHODS

### Study Participants and Sample Collection

We consecutively recruited patients who were hospitalized for coronary angiography at Peking Union Medical College Hospital (PUMCH). Male patients with ≥50% stenosis in at least one main coronary artery were included in this study. All female patients were excluded since the percentage of current smokers was too low (4/50). Participants were excluded if they had infectious diseases, gastrointestinal diseases, malignant tumors, autoimmune disorders, renal dysfunction (severe renal disease or creatinine >3.0 mg/dl), a history of gastrointestinal surgery in the previous year, or antibiotics usage lasting for more than 3 days in the previous 3 months. A total of 113 male patients with CAD were enrolled and further split into the following three groups: (1) current smokers, (2) former smokers, and (3) never smokers. The coronary atherosclerotic burden of each patient was assessed using the Gensini score by two professional cardiologists as described in our previous publication (20).

After admission, in-hospital participants were given stool samplers and provided with detailed instructions on sample collection. Freshly collected stool samples were immediately transported to the laboratory and frozen at −80°C to prevent microbiota structure shift. Fasting peripheral venous blood was collected in the morning of the day after admission, and all clinical, as well as smoking information, were collected. The study was performed in accordance with the principles of the Declaration of Helsinki. All subjects provided written, informed consent for participation in this study.

### Smoking Information

Smoking information was obtained via questions while hospitalization at PUMCH. Participants who were active smokers at the point of admission were classified as current smokers. Participants who had a smoke history but quitted smoking prior to admission (smoking cessation > 2 months before the time of interview) were defined as former smokers. Smoking intensity (cigarettes per day, only for current smokers) and smoking burden (pack-years) were also collected.

### Sample Collection, 16S rRNA Processing, and Sequencing

Bacterial DNA was isolated from fecal samples by utilizing the bead-beating method and then proceeded to PCR amplification and sequencing of the V3–V4 region of the 16S rRNA gene under raw data quality control. A sequencing library of the V3–V4 regions of the 16S rRNA gene was established. The purified

products were mixed at an equal ratio for sequencing using an Illumina MiSeq system (Illumina Inc., USA). EasyAmplicon was utilized for the analysis of downstream amplicon information (21). Operational taxonomic units (OTUs) were delineated at a cutoff value of 97% by using USEARCH v.8.0 after dereplication performed by the *-derep_fullength* command of VSEARCH32 (v2.15) (22). Taxonomic classification of OTUs was achieved using the sintax algorithm of USEARCH based on the Ribosomal Database Project (RDP) training set v16.

## Analysis of the Taxonomic Composition and Prediction of Gut Microbiota Phenotype

The OTU feature table was created with *-usearch_global* command and taxonomic annotation was generated by USEARCH *-otutab* command based on the Greengenes database. Alpha diversity analysis was carried out using the vegan package (v2.5-6) in R v4.0.2 (23). Differences in Shannon's index and the ACE index between groups were evaluated using Tukey's honestly significant difference (HSD) test. The weighted UniFrac distance matrix was generated using *usearch -beta_div*. Beta diversity calculations were performed by principal coordinate analysis (PCoA) and the Adonis test was applied to test for significant differences between groups. The R package ggplot2 was used to visualize the results of the diversity analyses. The taxonomic composition of each group was visualized as a stacked bar plot at the phylum and genus level by the ggplot2 package. For OTU comparisons between groups, EdgeR was utilized to identify significantly differential features and the Benjamini-Hochberg method was applied to control the false discovery rate (FDR).

PICRUSt2 was used to predict the metagenomic functional compositions (24). STAMP software (v2.1.3) was utilized for statistical analyses to compare the microbiota structure at different levels (Welch's $t$-test) and predicted pathways (White's non-parametric test). Linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/galaxy) was used to compare the gut composition structure.

## Untargeted Metabolomics Study

Sample analysis was performed by a Waters ACQUITY ultra-high-performance liquid chromatography (HPLC) system (Milford, MA) coupled with a Waters Q-TOF Micromass system (Manchester, UK). Sample analysis was performed in both positive and negative ionization modes, while both polar ionic and lipid modes were used depending on the properties of metabolites. The detailed procedures for sample preparation, HPLC-mass spectrometry (MS) experiments, and peak-ion intensity matrix preparation were described in our previous publication (20). The matrix was further reduced by removing peaks with missing values in more than 80% of the samples and those with isotope ions from each group to obtain consistent variables. The coefficient of variation (CV) of metabolites in the quality control (QC) samples was set at a threshold of 30% for the assessment of repeatability in the metabolomics datasets. Partial least squares discriminant analysis (PLS-DA) was applied by SIMCA software (v14.1, Umetrics, Sweden) to calculate variable importance in the projection (VIP) values. Significant differential metabolites were selected on the basis of VIP value $> 1$ and $p < 0.05$. Annotation and classification of metabolites were achieved by online databases, as described in our previous publication (20). MetaboAnalyst (http://www.metaboanalyst.ca) (version 4.0) was used for the identification of metabolic pathways and analysis.

## Follow-Up Study

Post-discharge, a follow-up study was conducted by return visit at PUMCH or by telephone interviews with patients or close family members. The composite endpoint of this study consisted of all-cause mortality and/or stroke and/or reoccurrence of acute coronary syndrome (ACS) and/or readmission for cardiac causes. The identification of composite endpoint events was based on the electronic medical record system of PUMCH or telephone interviews in cases of events outside PUMCH. Binary logistic regression analysis was employed to explore the relationship between smoking status and the outcome of patients with CAD after adjusting for potential confounding factors using IBM SPSS (v26.0, SPSS Inc., Chicago, IL, USA). The results of binary logistic regression were visualized as forest plots using the R package ggplot2 (25).

## Statistical Analysis

A total of 113 study participants were categorized into three groups: current smokers ($N = 46$), former smokers ($N = 34$), and never smokers ($N = 33$). Spearman correlations between important bacterial taxa, serum metabolomic features, and clinical parameters were calculated in IBM SPSS v.26.0 software. Correlations between features were visualized using the pheatmap R package and corrplot R package. A Sankey plot was utilized to present the multi-omics correlation with the R package networkD3.

# RESULTS

## Characteristics of the Study Population

A total of 113 male participants who were diagnosed with CAD at admission were consecutively enrolled at PUMCH and were further divided into the following three groups based on their smoking status: current smokers ($N = 46$), former smokers ($N = 34$), and never smokers ($N = 33$). The characteristics and traditional cardiovascular risk factors for the participants are summarized in **Table 1**. In terms of the number of stenosed vessels, we observed that the current smokers and former smokers exhibited a higher proportion of two- or three-stenosed vessels than the never smokers. However, the difference in the Gensini score had no significant difference. In general, the difference in disease severity was inconspicuous in participants with different smoking statuses according to the biochemical data at the baseline.

## Relatively Worse Clinical Outcome of Current Smokers Compared With Former and Never Smokers

Among the enrolled 113 male patients, 106 patients were followed up by interview through phone or electronic medical record and seven patients were out of touch, or personal

**TABLE 1 |** Characteristics of the study cohort.

| | Current, $N = 46$ | Former, $N = 34$ | Never, $N = 33$ | $p$-values |
|---|---|---|---|---|
| Age* | 58.48 ± 9.96 | 61.44 ± 9.3 | 62.52 ± 11.82 | 0.198 |
| Systolic blood pressure (SBP) (mmHg)* | 131.32 ± 20.43 | 128.97 ± 13.43 | 129.54 ± 16.36 | 0.836 |
| BMI* | 26.41 ± 3.22 | 26.05 ± 3.01 | 26.00 ± 3.15 | 0.831 |
| **Type of CAD (%)** | | | | 0.604 |
| SCAD | 11 (23.9) | 11 (32.4) | 10 (30.3) | |
| UA | 24 (52.2) | 12 (35.3) | 16 (48.5) | |
| MI | 11 (23.9) | 11 (32.4) | 7 (21.2) | |
| Gensini# | 32 (14.5, 65.5) | 45.0 (23.5,65.8) | 32.5 (20.0, 46.0) | 0.455 |
| **No. of vessels (%)** | | | | 0.043 |
| 0 | 4 (8.7) | 0 (0) | 12 (6.1) | |
| 1 | 10 (21.7) | 5 (14.7) | 13 (39.4) | |
| 2 | 13 (28.3) | 6 (17.6) | 7 (21.2) | |
| 3 | 19 (41.3) | 23 (67.6) | 11 (33.3) | |
| **History (%)** | | | | |
| OMI | 4 (8.7) | 9 (26.5) | 4 (12.1) | 0.076 |
| DM | 13 (28.3) | 10 (29.4) | 12 (36.4) | 0.724 |
| FLD | 9 (19.6) | 4 (11.8) | 7 (21.2) | 0.546 |
| HTN | 31 (67.4) | 21 (61.8) | 18 (54.5) | 0.51 |
| **Medication (%)** | | | | |
| HTNdrug | 28 (60.9) | 21 (61.8) | 18 (54.5) | 0.802 |
| OAD | 10 (21.7) | 7 (20.6) | 8 (24.2) | 0.934 |
| Statin | 15 (32.6) | 11 (32.4) | 13 (39.4) | 0.782 |
| **Laboratory data** | | | | |
| TC (mmol/L)* | 3.97 ± 1.01 | 3.6 ± 0.63 | 3.99 ± 1.27 | 0.176 |
| TG (mmol/L)* | 1.92 ± 1.75 | 1.46 ± 0.61 | 1.4 ± 0.59 | 0.103 |
| HDL-C (mmol/L)* | 0.89 ± 0.19 | 0.93 ± 0.21 | 0.97 ± 0.18 | 0.235 |
| LDL-C (mmol/L)* | 2.28 ± 0.75 | 2.03 ± 0.58 | 2.37 ± 1.1 | 0.218 |
| hsCRP (mg/L)# | 2.02 (1.04, 3.56) | 1.78 (0.48, 4.48) | 1.86 (0.54, 7.35) | 0.712 |
| cTnI (ng/ml)# | 0.011 (0.000,0.037) | 0.012 (0.000,0.075) | 0.003 (0.000, 0.065) | 0.706 |

*Values are presented as *mean ± SD, #median (IQR).*
*One-way ANOVA and Kruskal-Wallis H-test were employed in cases of continuous data. Categorical variables were compared by the $\chi^2$ test or Fisher's exact test.*
*SCAD, stable coronary artery disease; UA, unstable angina; MI, myocardial infarction; No. of vessels, number of stenosed vessels;I OMI, old myocardial infarction; DM, diabetes; FLD, fatty liver disease; HTN, hypertension; OAD, oral antidiabetic drugs.*

rejection in rare cases. The median follow-up time was 3.95 [interquartile range (IQR): 3.69–4.27] years. In the current smokers (42/46 successfully followed up), composite endpoint events were observed in 14 subjects, including one cardiac death [myocardial infarction (MI)], 11 recrudescent ACS, and 13 readmissions for cardiac issues. In the former-smoker group (33/34 followed up), composite endpoint events were observed in nine patients, including one non-cardiac death, six recrudescent ACS, and six readmissions for cardiac causes. Lastly, in the never-smoking patients with CAD (31/33 successfully followed up), composite endpoint events were observed in three patients, including one cardiac death (MI) as well as one non-cardiac death, one recrudescent ACS, and one readmission for cardiac causes. Binary logistic analyses demonstrated that current active smoking was associated with an increased risk of ACS reoccurrence [odds ratio (OR) = 10.855, 95% CI: 1.236–95.360, $p = 0.031$] and an increased risk of readmission due to cardiac issues (OR = 7.181, 95% CI: 1.411–36.553, $p = 0.018$) after adjusting for confounding factors, including age, history of old myocardial infarction (OMI), diabetes, oral antidiabetic drugs (OAD), hypertension (HTN), drugs for HTN, and fatty liver disease (FLD) (**Supplementary Figures S1A,B**, **Supplementary Table S1**). However, in the binary logistic regression, the $p$-value for former vs. current smokers is not significant for both ACS reoccurrence ($p = 0.114$) and readmission for cardiac issues ($p = 0.074$), indicating statistically no difference between the outcome of former and never group in our study cohort.

## Gut Microbiome Composition in Male Patients With CAD Varies With Different Smoking Status

In the 16s gut microbiota investigation, a total of 2,830,519 high-quality 16S rRNA reads were obtained, with a median read count of 22,842 (range: 11,202–44,385) per sample. A total of 626 OTUs were obtained by clustering sequences within a percent sequence similarity threshold of 97%. In terms of

the diversity of gut microbiota, we observed no significant differences in either alpha or beta diversity (PCoA based on the weighted UniFrac distances) among the three groups (shown in **Supplementary Figures S2A,B**). At the phylum level, the relative proportions of six phylas were assessed and their contributions in each group are shown in **Figure 1A**. The percentage of Firmicutes increased and Proteobacteria decreased among current smokers compared with never smokers (relative abundance: Firmicutes 51.7 vs. 46.9%; Proteobacteria 5.9 vs. 7.8%), while the former group was at an intermediate level. It is consistent with the previous report on the increased general abundance of Firmicutes in smokers (16). LEfSe analysis was utilized to compare the bacterial composition between groups. Taxa with LDA scores >2 were displayed in **Figure 1B**. The bacterial communities were different between current smokers and never smokers, while the latter was characterized by a bloom of members of the *Desulfovibrionaceae*, and decreased *Veillonella* and *Lactobacillaceae*. *Desulfovibrionaceae* was the most discriminant feature for the current smokers (LDA score 3.06, $p = 0.039$), whereas the *Veillonella* genus was the most discriminative for the never smokers (LDA score 3.47, $p = 0.007$). It was reported that *Desulfovibrio* DNA progressively increased with the smoking burden (pack-years) (26). The overall decreased *Veillonella* abundance in smokers at the genus level is in line with an earlier study (15). Manhattan plots showed the contributions of differentially abundant OTUs at the class level (**Figure 1C**). The comparison of relative abundance at phylum and genus levels is also carried out between current smokers and never smokers, and these discriminant taxa were correlated with clinical indicators (**Supplementary Figure S3**C, Welch's *t*-test). At the genus level, we found decreased abundance in *Akkermansia* and increased *Roseburia* in current smokers.

To compare the detailed composition differences between groups, edgeR was utilized with a threshold of $p < 0.05$ and FDR < 0.2. A total of 26 OTUs exhibited significantly different abundances in the comparison between the current and never smokers (16 depleted and 10 enriched OTUs), as shown in the volcano plot (**Supplementary Figure S2D**). These discriminant OTUs with FDR < 0.05 were summarized in **Supplementary Table S2** and displayed in **Figure 1D**. Several smoking-associated OTUs decreased in current smokers belonged to *Bifidobacterium*, such as OTU 397 (*Bifidobacterium faecale*) and OTU 13 (*Bifidobacterium catenulatum*). We noticed that OTU 13 (*B. catenulatum*) is negatively correlated to blood glucose, implying its antidiabetic effect (**Supplementary Figure S3A**). OTU 29 (*Akkermansia muciniphila,* FDR < 0.001*)* was found to be significantly depleted in current smokers compared with former smokers and is negatively correlated to several inflammation indicators (IL-6&IL-18). OTU 37 (*Collinsella aerofaciens*) showed a negative correlation with low-density lipoprotein cholesterol (LDL-C). The smoking-positive OTUs include potential pathogens such as OTU19 (*Enterococcus faecium*), OTU 98 (*Haemophilus parainfluenzae*), and OTU 32 (*Klebsiella*). OTU 98 (*Desulfovibrio piger)* under the genus *Desulfovibrio* also had an elevated abundance among smokers. We also noticed that OTU 17 (uncultured clone 218002-1-48, belonging to the Lachnospiraceae family) is positively correlated to total cholesterol (TC), triglyceride (TG), LDL-C, and free fatty acid (FFA), indicating the potential detrimental role of OTU 17 in lipid metabolism.

## Untargeted LC-MS Analysis Reveals Smoking-Specific Metabolomic Signatures

We then explored the serum metabolome among different smoker groups by the untargeted LC-MS method. After QC and removal of the low-abundance peaks, metabolomic (polar ionic mode, positive, and negative) and lipidomic (lipid mode, positive and negative) profiling yielded 14,585 (PP, 7,246 annotated), 7,394 (NP, 3,304 annotated), 5,193 (PLP, 1,973 annotated), and 4,974 (LPN, 2,491 annotated) features, respectively. The PLS-DA analyses were carried out to discriminate the metabolomic profiles of current-smoking and never-smoking patients with CAD. The PLS-DA scatter plots under the four modes are shown in **Supplementary Figure S4**. A total of 304 metabolites (VIP value > 1 and Wilcoxon rank-sum $p$-value < 0.05) whose abundance significantly changed in current smokers compared to never smokers were selected, including 248 features annotated and classified based on the online databases (**Supplementary Table S3**). The VIP values of the top 20 discriminant metabolites are visualized in a bar plot in **Supplementary Figure S5**.

We subsequently assessed the correlation between the smoking-related serum metabolites and clinical indicators with special attention to the smoking intensity and burden. As shown in **Figures 2A**, 24 metabolites were significantly correlated with the indicators of CAD severity [evaluated by Gensini score, number of stenosed vessels, and cardiac troponin I (cTnI) levels] and the follow-up outcomes. And the fold of change and concentration of these differential metabolites are displayed in **Figures 2B,C**. Notably, PP553 (belonging to pyrrolidines) was identified to be positively related to disease severity as well as clinical outcomes. In contrast, we observed that three smoking-negative (decreased in current smokers) metabolic features (LPN4423, LPN4452, and LPN4453, all belonging to glycerophospholipids) and LPN4896 (TetraHCA, belonging to the class bile acid) were negatively correlated with disease severity and adverse outcomes of patients ($p < 0.05$, Spearman correlation). Compounds including PP12572, PP13266 (N-acetylarylamine), PP12921 (Estrone), and PP11995 (L-Histidine) were shown to be strongly related to smoking intensity and positively related to the adverse clinical outcomes. PP8242 (Riboflavin) is another smoking-related metabolite of interest in our study, which is negatively related to smoking burden and Gensini score. Earlier researches have demonstrated that smoking can induce downregulation in circulating vitamin B, including riboflavin and the deprivation of riboflavin may aggravate cardiovascular illnesses (27, 28).

**Figure 3A** presented the discriminant smoking-related metabolites in four major groups (including sphingolipids, fatty acyl carnitines, glycerophospholipids, and pyrimidine and derivatives) and their relationship with clinical parameters. Notably, the sphingolipid family is strongly correlated to

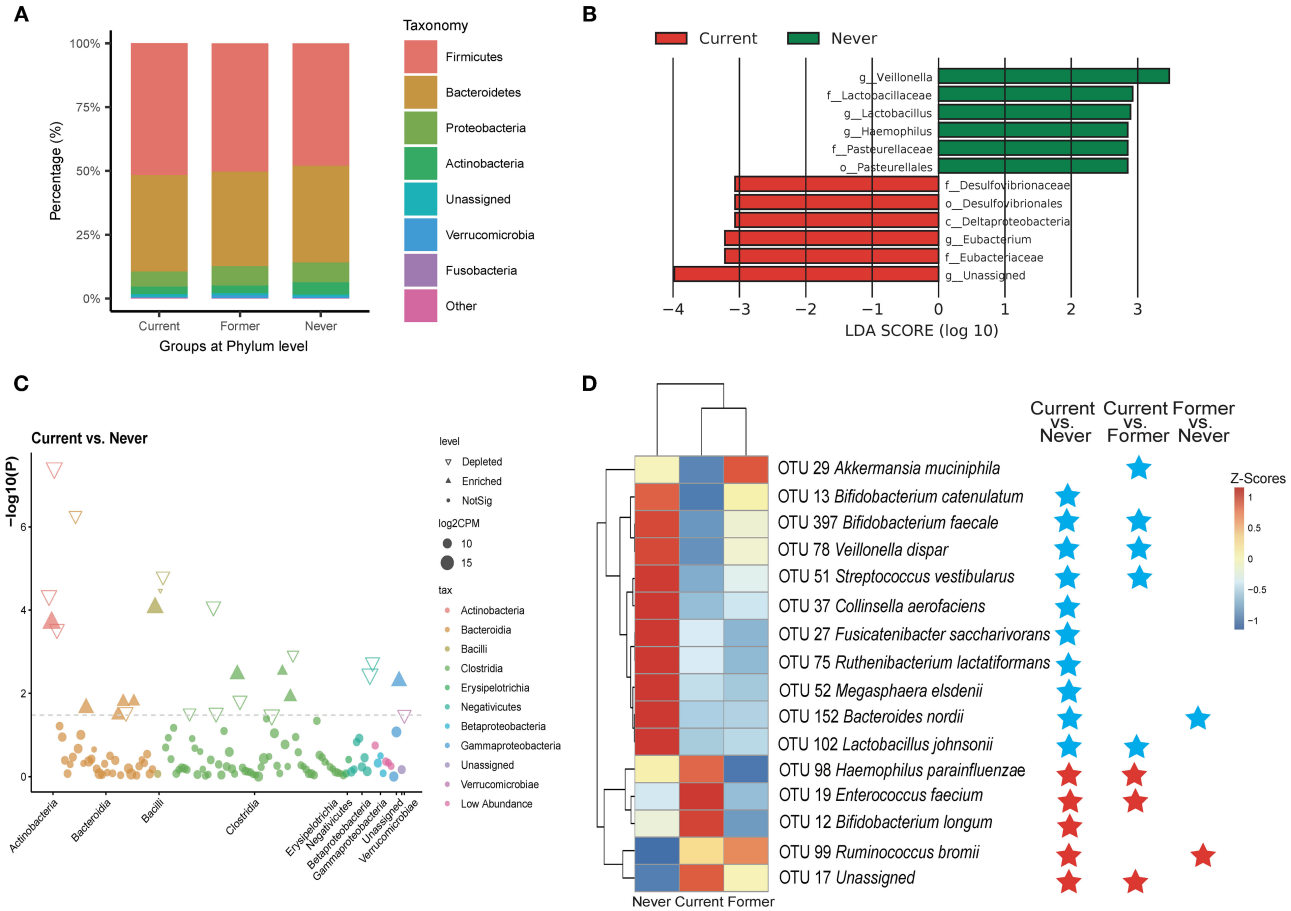


**FIGURE 1 |** Alterations in the composition of gut microbiota associated with smoking status. **(A)** Dominant phyla in each group. **(B)** Linear discriminant analysis (LDA) effect size (LEfSe) analyses of the differential taxa between current and never smokers. **(C)** A Manhattan plot showing the distribution of differential taxa. **(D)** A heatmap illustrating the relative abundance of smoking-associated operational taxonomic units (OTUs) across the three groups. A threshold of FDR < 0.05 calculated by the edgeR test was considered statistically significant. Blue stars indicate the depleted OTUs in comparison; red stars indicate the enriched OTUs.

the lipidomic profile among patients with CAD. PLP 28 [Ceramide(d18:1/22:0)], PLP 67 [Ceramide(d18:1/24:0)], PLP 379 (Glucosylceramide), and PLP 2,763 [GlcCer(d18:1/22:0)] are all correlated with smoking burden or intensity and LDL-C, TC, and apolipoprotein B (ApoB). The smoking-negative LPN4127 [SM(d18:1/14:0)] was positively corelated to cardioprotective HDL and ApoA1, implying the general lipidotoxic effect of shifts in sphingolipids induced by smoking. Among the six sphingolipids, PP11445 (Sphinganine) is positively correlated to the number of stenosed vessels but interestingly negatively correlated to the relative abundance of the Phylum Bacteroidetes (Rho $= -2.48$, $p = 0.008$, Spearman correlation), which will be explained in the discussion part. Furthermore, we found a declined L-serine in current smokers, which also plays a vital role in sphingolipids metabolism. The relative abundances of the identified metabolites involved in sphingolipids metabolism across different groups are shown in **Figure 3B**, while the mutual conversion between the sphingolipids is summarized in a simplified pathway map in **Figure 4A**. 9-Decenoylcarnitine, median-chain acyl carnitine, which is positively associated with smoking and tightly correlated with MB isoenzyme of creatine kinase (CKMB), cTnI, and hsCRP in our study, was reported that to be associated with incident atrial fibrillation (29).

We also noticed the depletion of several bile acids (PP7591, LPN4896, PP1057, LPN3069, and PP6142) as well as taurine (NP6888) in smokers. The previous study has shown that microbial enzymes include bile salt hydrolase (BSH) and bile acid-inducible (BAI) enzymes are essential for bile acid homeostasis in the host, which has a further influence on the host lipidomic profile (30). We correlated the genera capable of deconjugation (*Lactobacillus*, *Bacteroides*, and *Bifidobacterium*) and dehydroxylation (*Clostridium*, *Eubacterium*) with three groups of differential smoking-related metabolites (bile acids, sphingolipid, and glycerophospholipids) to investigate the possible interrelationship (31). The correlation map was visualized with corrplot R package in **Supplementary Figure S6**. Generally, the genera capable of bile acid transformation were negatively correlated to the abundance of the metabolites,

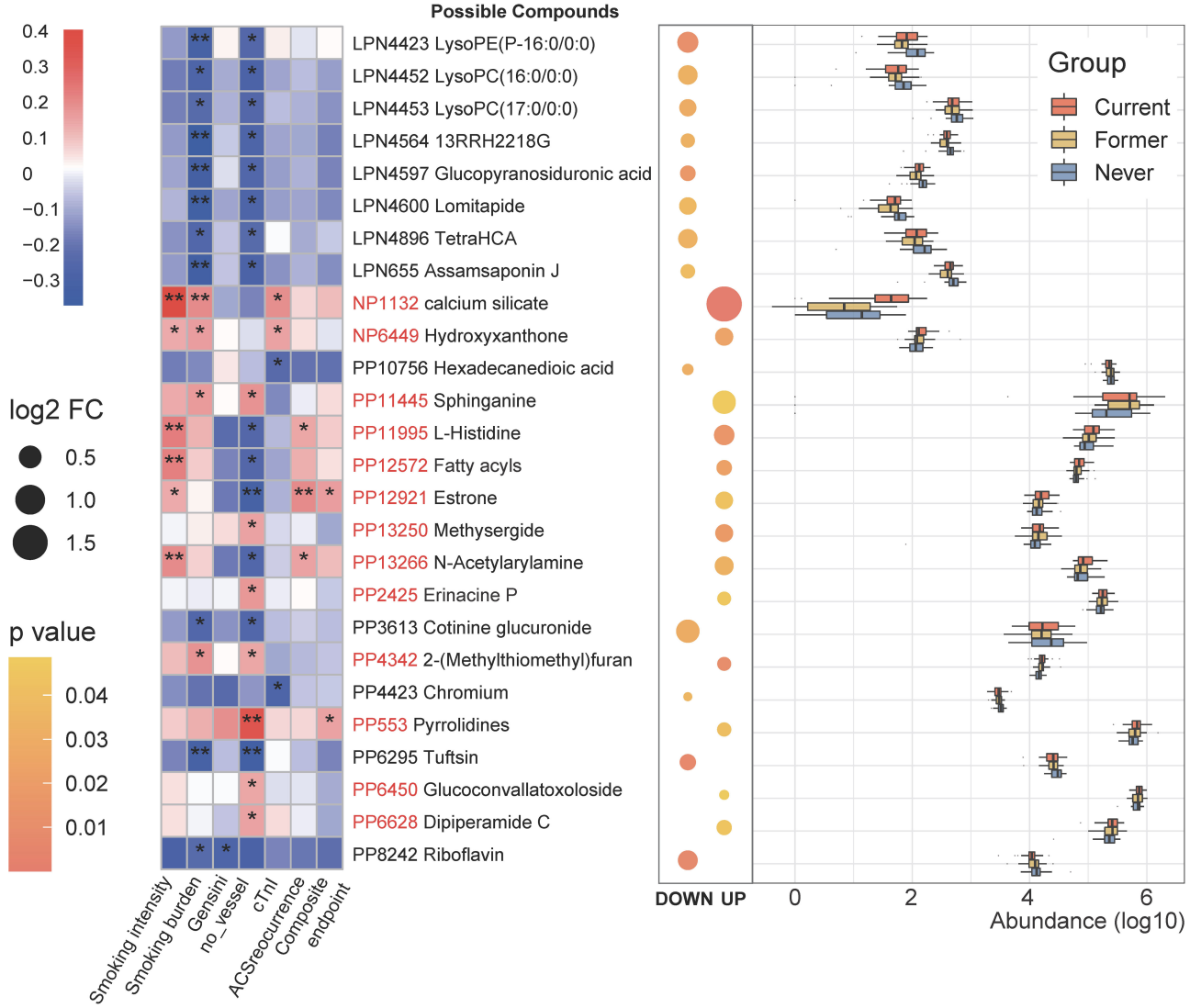


**FIGURE 2 |** Identification of the major smoking-associated serum metabolomic features correlated with clinical phenotypes. **(A)** Spearman correlations between smoking-associated metabolic features and clinical outcomes as well as indicators of disease severity in patients with CAD. *$p < 0.05$, **$p < 0.01$. **(B)** Fold of change and *p*-values of important metabolomic features. The left column shows the smoking-negative metabolomic features; the right column shows the smoking-positive metabolites. Bubble size indicates the relative fold change in comparison between current and never smokers. Log2 FC, Log2 (the fold of change), Current vs. Never smokers. **(C)** Changes in important smoking-associated metabolomic features among the three groups.

especially the glycerophospholipids. Among the genera, *Eubacterium* and *Bacteroides* showed a tighter correlation with glycerophospholipids.

## Further Comparison Between Former and Never Smokers Revealed the Reversibility of Metabolomic Changes

*T*-tests were also performed between the former smokers and never smokers to further explore the effect of smoking cessation behavior on the serum metabolomic profile (**Supplementary Table S3**). Seventy out of 304 metabolites remained differential metabolites ($p < 0.05$, Former vs. Never), while 234 showed no significant differences, revealing the partial reversibility in metabolomic change after smoking cessation. Most of the irreversible metabolites were correlated with smoking burden instead of smoking intensity. Some of these metabolites correlated tightly with the inflammation indicators.

The related metabolic pathway analysis was performed on MetaboAnalyst 4.0. Among the top 10 pathways, sphingolipid metabolism had the most significant *p*-value and a relatively big impact ratio. Other involved pathways include D-Glutamine and D-glutamate metabolism and Glycerophospholipid metabolism (**Figure 3C**, **Supplementary Table S4**). The critical irreversible

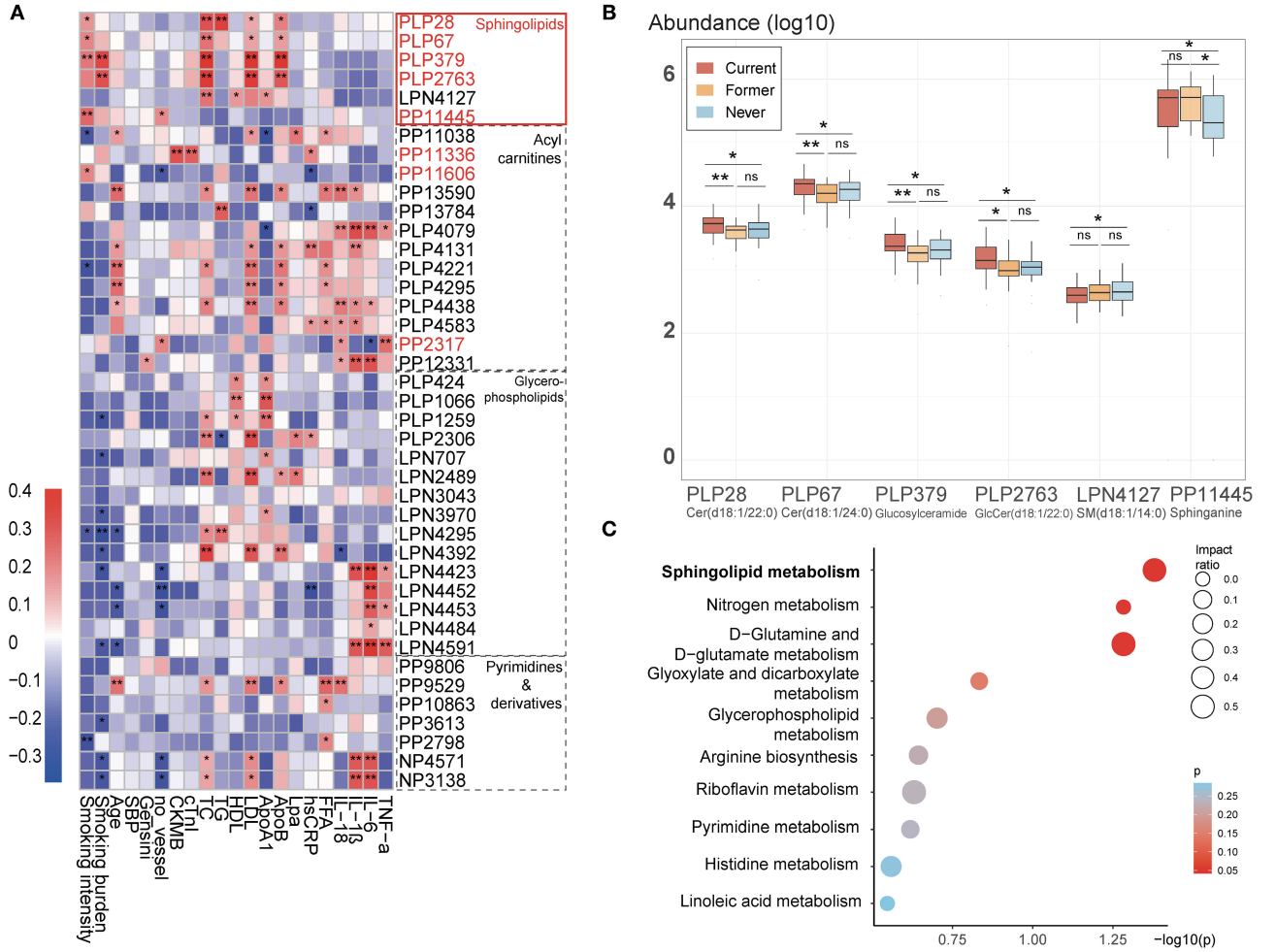


**FIGURE 3 |** Changes of four groups of important smoking-associated serum metabolomic features. **(A)** Spearman correlations between smoking-associated metabolomic features in four main groups and major risk factors for CAD. The IDs of metabolic features highlighted in red are smoking-positive. Lpa, lipoprotein (a); ApoB, apolipoprotein B; FFA, free fatty acid; ApoA1, apolipoprotein A1. There is a remarkable elevation in sphingolipids, which is correlated with CAD phenotypes. $^{*}p < 0.05$, $^{**}p < 0.01$. **(B)** Relative abundance of six metabolites belonging to the class of sphingolipids among three groups. ns: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, Mann-Whitney *U* test. **(C)** Pathway analysis of differential metabolites (Current *vs.* Never smokers).

pathways generated with 70 possibly irreversible metabolites mainly comprise glycerophospholipid, sphingolipid, and linoleic acid pathways.

## Prediction of Bacterial Metagenomic Functions Associated With Smoking Status

The functional potentials of the gut bacterial community were predicted using the PICRUSt2 tool based on the MetaCyc database (24), including pathway prediction and enzyme functional prediction. A total of 10 pathways were found to differ in the pairwise comparison among the three groups (**Supplementary Figures S7, S8**, **Supplementary Tables S5, S6**), and these smoking-related pathways were correlated with the discriminant OTUs. The smoking-positive pathways are mainly involved in sugar nucleotide biosynthesis (PWY-7332 and PWY-7090), whose downstream pathways include D-Glutamine and D-glutamate metabolism. PWY-7090 (UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis) contains several enzymes wbpA, wbpB, wbpD, and wbpI that are shown to be significantly elevated in current smokers (K13015, K13019, and K13018). We also found that PWY6895, which is a part of thamin biosynthesis, is significantly changed in smokers. When taken together, the changed riboflavin metabolism revealed by metabolomic analysis, we speculated that a change in vitamin B metabolism may be associated with active smoking. Besides, the phenotype analysis revealed that some key enzymes involved in nicotinate and nicotinamide metabolism (KEGG database: ko00760) were related to smoking status, including elevated nicotinamide-nucleotide adenylyltransferase (K00952), enamidase (K15358), and 2-hydroxymethylglutarate dehydrogenase (K19647). Some sphingolipids-related enzymes were also elevated in smokers, including homoserine phosphotransferase (K02203)

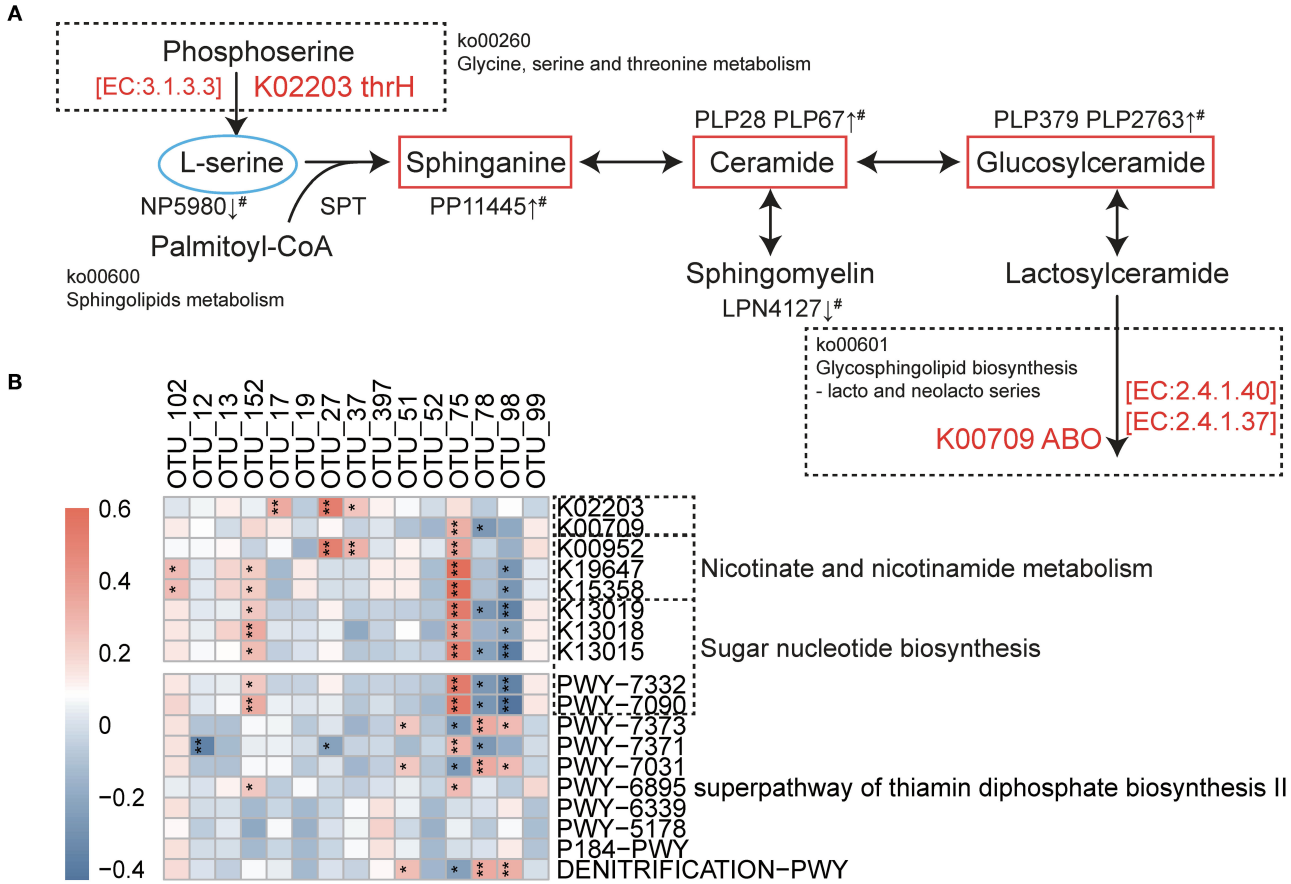


**FIGURE 4 |** Association between predicted metagenomic functional changes and differential OTUs as well as sphingolipids metabolism. **(A)** Relationship between sphingolipids pathway and two related differential enzymes (K02203 and K00709) predicted by PICRUSt2. The features are highlighted in red (smoking-positive) or blue (smoking-negative). #Observed metabolomic changes in smokers with CAD compared with never smokers in the present study. IDs and names of enzymes and pathways were based on the KEGG database. SPT, serine pamitoyl-transferase; K02203, homoserine phosphotransferase; K00709, histo-blood group ABO system transferase. **(B)** Spearman correlations between smoking-associated OTUs and metagenomic prediction including differential pathways as well as important enzymes. IDs of enzymes were according to the KEGG database; IDs of pathways were according to the MetaCyc database. *$p < 0.05$, **$p < 0.01$.

and histo-blood group ABO system transferase (K00709). The relationship between these two key enzymes and the sphingolipids metabolism is shown in **Figure 4A.** Correlations between the OTUs and predicted pathways as well as important enzymes are shown in **Figure 4B**. By offering insights into the possible function of microbial community and the relationship with metabolome, we believe that gut microbiota has contributed to the changed metabolomic profile in smokers.

## Multi-Omics Analysis Reveals the Relationship Between the Gut Microbiota and Serum Metabolites Associated With Smoking Status

We subsequently assessed the correlation between the gut microbiota and serum metabolites to further explore the interrelationship between gut microbiota, metabolomic features, and clinical phenotypes associated with smoking. As demonstrated in **Figure 5**, a total of nine smoking-associated OTUs that contributed were significantly ($p < 0.05$) correlated with 18 metabolomic features, which were further correlated with indicators of disease severity and/or the clinical outcome. At the OTU level, we observed that some of the smoking-positive OTUs were found to be positively correlated with poor CAD phenotypes possibly through the mediation of metabolites. Notably, OTU 99 and OTU 19 were positively correlated to disease severity by mediating PP13250 and PP4342, respectively. Moreover, we also discovered some negative relationships between some of the smoking-positive metabolites and smoking-negative OTUs (PP553 with OTU 29; NP1132 with OTU 29 and OTU 397), implying that accumulation of pernicious metabolites may be related to the depletion of potentially beneficial bacteria. Whereas, we were not able to find the correlation between some of the important metabolites with any of the discriminant OTUs, such as PP12921 that is tightly correlated to poor clinical prognosis. This implies that cigarette smoking may exert an influence on metabolomic and microbial features of individuals through more diversified and

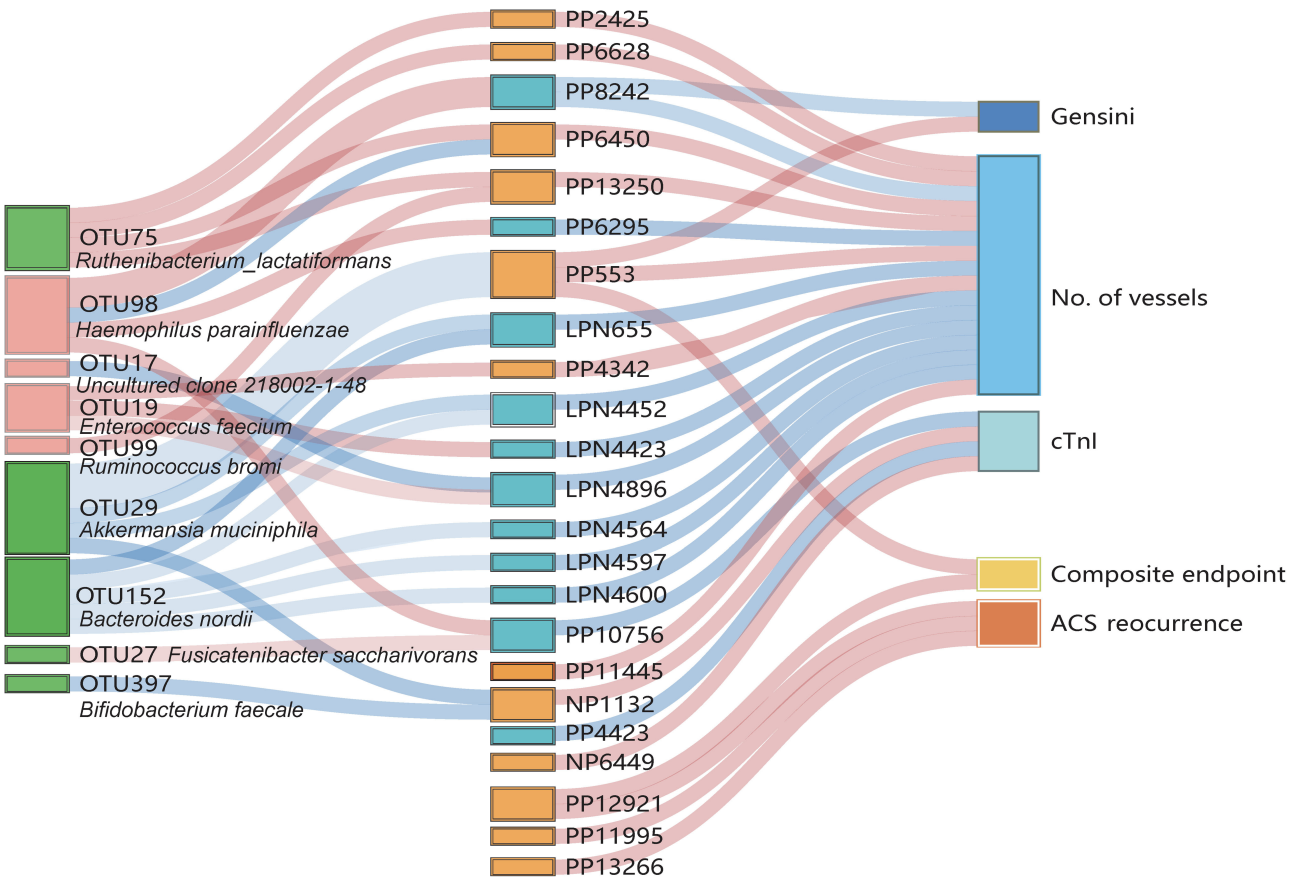


**FIGURE 5 |** Interrelationship among smoking-associated gut flora, serum metabolic features, and major CAD phenotypes. A Sankey plot was utilized to examine and visualize the correlation between important smoking-associated OTUs and the severity and outcomes of CAD in the mediation of key serum metabolic features. Connections in red indicate positive correlations while blue connections indicate negative correlations (Spearman correlation analysis, $p < 0.05$). In the left column, red boxes indicate smoking-positive OTUs, and green boxes indicate smoking-negative OTUs. In the metabolic features column, orange boxes indicate smoking-positive metabolic features and blue boxes indicate smoking-negative metabolic features. A composite endpoint was defined as all-cause mortality and/or reoccurrence of ACS and/or readmission for cardiac causes. No. of vessels: ANthe number of stenosed vessels; cTnI: cardiac troponin I.

complicated mechanisms. The results of the multi-omics analysis are summarized in **Supplementary Table S7**.

Overall, the smoking-associated microbial and metabolic features are shown above may provide further evidence of the microbial dysbiosis and changed metabolomic profile in CAD smokers, which has the potential to explain the cross talk of gut-heart axis in the pathogenesis of CAD.

## DISCUSSIONS

In response to environmental perturbations such as cigarette smoking, bacteria in the human gut may thrive or decline as a functional community. We demonstrated that smoking patients with CAD had significantly different gut microbiota composition and serum metabolomic profiles compared with never smokers. Besides, through multi-omics correlative study, our study found that these discriminant microbe features and metabolomic features were correlated and also correlated well with clinical indicators.

We evaluated the smoking-related microbial change sequentially from phylum to OTU level. From a relatively macro perspective, we discovered an increasing gradient of the ratio of Firmicutes phylum in current smokers compared with former and never smokers. As the most abundant phylum, it was reported that the ratio of Firmicutes is increased in active smokers but can shift back after smoking cessation (16). The ratio of Firmicutes to Bacteroidetes in patients with CAD is higher than in healthy controls (32) and in the present study, a negative correlation was found between *Bacteroidetes* and inflammation indicators. Furthermore, the LEfSe analysis was applied to identify differential bacteria composition between smoking and non-smoking patients with CAD. We found out some smoking-negative genera (*Lactobacillus* and *Veillonella)* as well as some smoking-positive taxa (*Desulfovibrionaceae* and *Eubacterium)*. A similar pattern of increased *Desulfovibrionaceae*

and decreased *Lactobacillus* spp. were previously observed when mice were fed a high-fat "Western" diet (33). *Desulfovibrionaceae* may have pro-inflammatory effects for their sulfate-reducing capacities producing toxic hydrogen sulfide ($H_2S$). Ijssennagger presented that $H_2S$ produced by gut bacteria may damage the intestinal mucus layer by reducing disulfide bonds, resulting in the invasion of toxins and stimulation of host inflammation (34). *Lactobacillus* and *Eubacteria* are both BSH-producing genera, which may act as cholesterol-lowering agents by deconjugating bile salts and decrease cholesterol reabsorption (35). Besides, specific *Eubacteria* spp. has bacterial 7alpha-dehydroxylases to convert primary BAs to secondary BAs [deoxycholic acid (DCA) and lithocholic acid (LCA)] (36). Secondary bile acids can be reabsorbed and impact host lipid and glucose metabolism through several nuclear receptors [liver X receptor (LXR), pregnane X receptor (PXR), and specific G-protein-coupled receptors (GPCRs) like takeda G-protein-coupled receptor 5 (TGR5)] (37–39). Quite surprisingly, the depletion of several bile acids and taurine were significant in the current smokers, implying a changed bile acids profile associated with cigarette smoking. The previous study has shown that smoking might break the homeostasis of bile acids metabolism (40). Based on the above analysis, we believe that further investigations are acquired to explore the causal link between smoking, gut microbiota, and bile acids metabolism.

At the OTU level, a previous study conducted in a healthy population suggested that the smoking-related microbiota composition profile features the increased *R. bromii* and depleted *A. muciniphila* and *B. nordii,* which is in line with our study in CAD smokers (15). We found via intergroup comparison that the OTUs enriched in current smokers comprise several opportunistic pathogens such as *H. parainfluenzae* and *Klebsiella sp.* The gram-negative bacteria infection can induce cytokine burst by releasing lipopolysaccharide (LPS) to affect the plaque stability and also the development of atherosclerosis (41). *Klebsiella* was reported to be associated with the hypertensive population and may play a part in hypertensive progression (42). The major discriminant feature of the current smokers may be the deprivation of some potentially beneficial taxa, such as *A. muciniphila* and *Bifidobacterium* spp. *A. muciniphila* is considered to have multiple probiotic roles in host metabolic modulation, immune regulation, and gut barrier protection (43). The protective role of *A. muciniphila* against atherosclerosis is also promising due to its lipid-lowering and anti-inflammation abilities (44, 45). *B. catenulatum* was reported to have an anti-inflammation role and to assist other probiotics to produce butyrate (46, 47). Other possibly beneficial bacteria depleted in the smoker population include *Fusicatenibacter saccharivorans and Collinsella aerofaciens. F. saccharivorans* was reported to have an anti-inflammation role to relieve ulcerative colitis (UC) in the murine model, while a novel subsp. of *C. aerofaciens* was isolated and proved to be capable of butyrate synthesis (48, 49). Some probiotic strains have been investigated to exhibit beneficial effects on CAD (50). For instance, a 12-week intake of *Lactobacillus rhamnosus* GG (LGG) exhibited beneficial effects in reducing mega inflammation and metabolic endotoxemia in participants with CAD (51). Moreover, co-supplementation of probiotics (LGG) and prebiotic inulin in subjects with CAD for 8 weeks had beneficial effects on depression, anxiety, as well as inflammatory biomarkers (52). The underlying mechanisms of probiotics on CAD are complicated and are yet to be elucidated.

The human gut microbial ecosystem is now considered an endocrine organ, which interacts intensively with the host through circulating metabolites. Metabolomics analysis also revealed the significant change in patients with CAD with different smoking statuses. As presented above, several sphingolipids were found to be elevated in current smokers, including Cer(d18:1/22:0) (PLP28), Cer(d18:1/24:0) (PLP67), 2 glucosylceramides (PLP379 & PLP2763), and sphinganine (PP11445). In pathway analysis conducted by MetaboAnalyst 4.0, sphingolipids metabolism in current smokers was significantly changed compared with the never smokers; and the significant difference between former and never smokers revealed the partial irreversibility of this change. Prior to this study, Tong et al. (53) has elucidated that cigarette smoking can interfere with insulin secretion through induction of ceramide accumulation and activation of oxidative stress. Also, the detrimental effect continued even during smoking cessation, which is consistent with the irreversibility presented in our study. Animal experiments also confirmed the changed ratio of Cer(d18:1/24:0) to Cer(d18:1/18:0) as markers of CS exposure in the lungs, plasma, and liver (54). As the metabolites of sphingolipids, ceramides are considered as lipotoxic inducers of disturbed glucose homeostasis and also an active player in the progression of atherosclerosis (55). Cer(d18:1/22:0) and Cer(d18:1/24:0) are both associated with stroke severity at admission and future risk (56). Studies in rodent models revealed that the inhibition of ceramide synthesis reduces ischemic cardiomyopathy-related heart failure post-MI or tissue hypoxia and preventing ventricular remodeling (57). According to Edsfeldt et al. (58), six sphingolipids (particularly GluCer) can boost plaque inflammation and promote vascular smooth muscle cell apoptosis.

In recent decades, the gut-heart axis has emerged as a novel concept and provided new insights into atherosclerotic pathogenesis. Previous studies have shown that an imbalance in the gut-heart axis due to the gut microbiota plays an important role in atherosclerosis progression. The gut microbiota promotes the development of atherosclerosis by producing intermediate metabolites, including TMAO, LPS, Phenylacetylglutamine (PAGln), and reducing SCFAs (59). This theory may also help to explain the discovered gut microbiota change and elevated ceramide level that may be related to different CAD prognoses. A prior study of our group has convincingly shown that intestinal farnesoid X receptor (FXR) may modulate atherosclerosis by elevating ceramide metabolism (60). FXR was identified as an orphan nuclear receptor that plays multiple roles in regulating bile acid homeostasis, lipid, and glucose metabolism (61). Noticing the possible influence on the bile acid profile of smoking as mentioned above, we speculated that the ceramide and bile acid dysregulation may be related to FXR. The activation of intestinal FXR can decrease bile acid absorption, while hepatic FXR has a role in attenuating cholesterol metabolism/bile acid synthesis by suppression of CYP7A1 and CYP8B1 expression,

both contributing to a decreased level of circulating bile acids (62). Intestinal FXR activation also induces genes involved in ceramide synthesis that potentiate metabolic disorders (63). Several therapeutic strategies have been designed to improve metabolic diseases by inhibiting FXR activity. For instance, metformin, tempol, or antibiotics can reduce the abundance of BSH-secreting gut microbiota, and thus increase levels of endogenous FXR antagonists [especially tauro-β-muricholic acid (T-β-MCA)] (64, 65). Also, direct oral administration of FXR antagonists including ursodeoxycholic acid and Gly-MCA can affect bile acid and lipid metabolism (66, 67). A previous publication has indicated a potential association between smoking and FXR in pulmonary inflammation (68). The interaction of cigarette smoking, gut microbiota composition shift, and ceramide and bile acids dysregulation needs to be further elucidated.

Despite *de novo* generation in mammalian tissue and dietary uptake, sphingolipids can also be produced by the *Bacteroidetes* spp., which is one of the dominant phyla of the gut microbiome (on an average constitute of 30–40%) (69). Bacteroidetes have the necessary enzyme serine palmitoyl-transferase (SPT), making them the only gut commensal group known to produce sphingolipids (70). Recent studies have shown that deficiency of Bacteroidetes-derived sphingolipids can affect host sphingolipid metabolism resulting in elevated ceramide levels and subsequent amplification of host inflammation (71, 72). In our study, several OTUs under the Bacteroidetes phylum were depleted (e.g., *Bacteroides nordii* and *Prevotella copri*) in smokers compared with never smokers. Although no significant change was detected in the abundance of Bacteroidetes, the negative correlation between Bacteroidetes and sphinganine (PP11445, positively correlated with the number of stenosed vessels) may suggest the possible interrelation. Cigarette smoking may play a role in promoting vulnerable plaque formation through interfering with gut microbiota sphingolipid production and regulating host sphingolipid levels. Possible therapeutic targets on *Bacteroidetes*-derived sphingolipids may be beneficial for both current and former smokers.

We also noticed a decreased level of L-glutamate and L-glutamine in current smokers, while the difference is no more significant between former and never smokers. Besides, pathway analysis revealed a changed D-glutamine and D-glutamate metabolism, which is in line with the microbial functional prediction conducted by PICRUSt2. In the nervous system, glutamate is an important excitatory transmitter and plays an important role in the addiction to nicotine and other drugs. Cigarette smoking was found to be associated with decreased regional brain glutamate as well as circulating glutamate (73, 74). However, third-hand smoking and alcohol consumption can also induce imbalanced Glu-Gln metabolism, making the issue more complicated (75). When ACS occurs, glutamate is important in energy metabolism and promoting survival of cardiac cells subjected to hypoxia/reoxygenation (76). Glutamine was reported to inhibit the progression of atherosclerosis and promote plaque stability by activating M2 macrophages (77). The downregulated Gln and Glu levels in current smokers may need more investigation to possibly improve the post-ACS prognosis in smokers.

Results from epidemiological studies have identified cigarette smoking as a major risk factor for poor CAD prognosis. By the same time, evidence has shown that gut microbiota might play an important role in CADs. However, to our knowledge, this is the first multi-omics study to investigate the role of smoking in atherosclerosis pathogenesis. Our results showed that certain alterations in the gut microbial community and serum metabolites are related to smoking status. Compared with simple mono-omic microbiome analysis, the addition of metabolome study can directly reflect the functional capacity of symbiont gut flora by circulating microbe-related metabolites. Based on these differential bacteria and metabolites, we are provided with novel biomarkers or therapeutic targets for CAD progression beyond the traditional concepts like smoking-induced endothelial dysfunction. Taken the heavy coronary disease burden and high global smoking prevalence into consideration, our study may open up the possibility of modulating gut microbiota to improve CAD prognosis in smokers and even high-risk former or never smokers. To further explore the interaction of smoking and smoking-related microbes, functional studies are urgently needed.

Our study has some limitations as well. First, due to the small proportion of current and former smokers in female patients with CAD, we excluded all the female participants to avoid possible confounding factors in our study. An intriguing epidemiological issue is the vulnerability of female smokers to develop CADs. The pooled adjusted female-to-male relative risk ratio of smoking compared with non-smoking for CAD was 1.25; however, the possible mechanism remains unclear (78). It was also reported that the influence of smoking status on the metabolomic profile may be gender-specific (79). To make sure how smoking status interacts with cardiovascular disease progression, the female population should be taken into consideration. Another limitation of our study is the unmeasurable influence of passive smoking. Passive smoking can also have a detrimental effect on cardiovascular health but it is hard to be measured or documented (80).

## CONCLUSIONS

Our study demonstrated that smoking can influence the physical condition of patients with CAD from a multi-omics perspective. Gut microbiota analysis revealed that smoking may influence the composition of host gut flora by increasing potentially pathogenic bacteria such as *Desulfovibrionaceae*, *H. parainfluenzae*, and *Klebsiella*, and reducing possibly beneficial bacteria such as *Bifidobacterium* spp. and *A. muciniphila*, thus increasing the metabolic risk of CAD smokers. The metabolomic study showed that smoking is associated with concentration variations in sphingolipids, glycerophospholipids, and amino acid metabolism. Moreover, the serum metabolite profile of smokers is partially reversible after stopping smoking, which indicates the benefits of smoking cessation to improve CAD prognosis. Our findings provide new insights into the heterogenic roles of cigarette smoking and the multi-omics interactions in CAD, and the identified microbiota or metabolites may serve as biomarkers of smoking cessation status or novel therapeutic targets. However, more functional and interventional

studies will be needed to elucidate the role of smoking in CAD pathogenesis and progression.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, SRP167862.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Review Board of the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

XH and SZ designed and supervised the study. XH and YF managed the clinical research. XH, YF, HL, RZ, XZ, and YS obtained the samples and clinical information. YF and XH performed the data analysis. YF, XH, and SZ wrote, reviewed, and revised the manuscript. All the authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.766739/full#supplementary-material

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# The Effects of Moderate Alcohol Consumption on Circulating Metabolites and Gut Microbiota in Patients With Coronary Artery Disease

*Xinyue Zhao*[1†], *Ruilin Zhou*[1†], *Hanyu Li*[1], *Yue Fan*[1], *Yueshen Sun*[1], *Xiaomin Hu*[1,2*] and *Shuyang Zhang*[1*]

[1] State Key Laboratory of Complex Severe and Rare Diseases, Department of Cardiology, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, China, [2] Department of Medical Research Center, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, China

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Yahan Liu,
Peking University Health Science Centre, China

***Correspondence:**
Xiaomin Hu
huxiaomin2015@163.com
Shuyang Zhang
shuyangzhang103@nrdrs.org

[†]These authors have contributed equally to this work

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**Background:** Epidemiological studies confirmed that moderate alcohol consumption was associated with a reduced risk of adverse cardiovascular events. It is increasingly recognized that the composition of gut microbiota and metabolites is involved in modulating the cardiovascular health of the host. However, the association of moderate alcohol consumption with serum metabolites and gut microbiome and its impact on coronary artery disease (CAD) is not fully investigated.

**Method:** Serum untargeted metabolomics analysis and fecal 16S rRNA sequencing were performed on 72 male patients with CAD having various alcohol consumption (36 non-drinkers, 18 moderate drinkers, and 18 heavy drinkers) and 17 matched healthy controls. MetaboAnalyst and PICRUSt2 were utilized to analyze the possible involved metabolic pathways. Multi-omics analysis was achieved by Spearman correlation to reveal the interactions of alcohol consumption with gut microbiome and serum metabolites in patients with CAD.

**Results:** We noted distinct differences between patients with CAD, with varying levels of alcohol consumption and healthy controls in aspects of serum metabolome and the gut microbiome. Moderate alcohol consumption significantly changed the lipidomic profiles, including reductions of sphingolipids and glycerophospholipids in moderate drinkers with CAD when compared with non and heavy drinkers with CAD. Moreover, we also found the reduction of microbial-derived metabolites in moderate drinkers with CAD, such as 2-phenylacetamide and mevalonic acid. To be noted, the gut microbiota of moderate drinkers with CAD tended to resemble that of healthy controls. Compared with non-drinkers, the relative abundance of genus *Paraprevotella*, *Lysinibacillus* was significantly elevated in moderate drinkers with CAD, while the genus *Bifidobacterium*, *Megasphaera*, and *Streptococcus* were significantly reduced in moderate drinkers with CAD. Multi-omics analysis revealed that specific metabolites and microbes associated with moderate alcohol consumption were correlated with the severity of CAD.

**Conclusions:** Our study revealed that the impact of moderate alcohol consumption on serum metabolites and gut microbiota in patients with CAD seemed to be separated from that of heavy and non-alcohol consumption. Moderate drinking tended to have more positive effects on metabolic profiles and commensal flora, which may explain its beneficial effects on cardiovascular health. Overall, our study provides a novel insight into the effects of moderate alcohol consumption in patients with CAD.

**Keywords: coronary artery disease, alcohol, serum metabolites, gut microbiome, multi-omics**

## INTRODUCTION

Coronary artery disease is one of the most common causes of death in the general population (1), and alcohol abuse is a widely recognized risk factor in adverse cardiovascular events. Alcohol affects the cardiovascular system of human beings in several ways. It was reported that alcohol abuse can be associated with various adverse cardiovascular events, such as stroke, acute heart failure, and sudden cardiac death (2). However, clinical studies demonstrated that moderate drinking presented an inverse correlation with the incidence of coronary artery disease, perhaps due to its effects on lipoprotein levels (3). Epidemiological studies illustrated that moderate alcohol intake (one to two cups a day, or 100 grams per week) was associated with a decreased incidence of CAD (4, 5). What is more, the association between the severity of cardiovascular diseases and different alcohol consumption presented a *U*-shaped curve. In other words, light to moderate alcohol intake had a reduced risk of cardiovascular events, while the relationship reversed in chronic alcohol overconsumption (4–8). In addition, recent studies have reported possible mechanisms for the protective effects of moderate alcohol consumption on patients with CAD. Some research reported that moderate alcohol consumption may elevate the level of high-density lipoprotein cholesterol (HDL-C), apolipoprotein A, and adiponectin levels (9, 10), which were considered as protective factors in cardiovascular disease. It was also reported that moderate alcohol consumption may affect the modulation of insulin resistance and chronic inflammation. By suppressing inflammation and promoting vasodilation, adverse cardiovascular events can be reduced (11). Furthermore, by moderate alcohol consumption, the hypercoagulable state of patients with CAD may be reduced by the anticoagulation effect of alcohol (12). Overall, it was believed that moderate alcohol consumption was associated with a reduced risk of cardiovascular events by various possible mechanisms.

Meanwhile, it is increasingly recognized that the composition of gut microbiota and serum metabolites is involved in modulating the cardiovascular health of the host. In recent years, more and more studies have confirmed that alterations in the gut microbiome were associated with the severity of CAD (13–15). Gut microbiota participates in modulating the cardiovascular health of the host through its metabolites, such as short-chain fatty acids (SCFA), trimethylamine N-oxide (TMAO), and phenylacetylglutamine (PAGln) (16–18). Alterations in serum metabolome, such as C34:2 hydroxy-phosphatidylcholine, N-acetylneuraminic acid, sphingomyelin (SM), were reported to be significantly associated with CAD and were considered as potential biomarkers (19–22). Moreover, the metabolic features of alcohol drinkers were investigated and showed profound modifications of lipidomic profiles and amino acids in humans (23–25). Alcohol use was associated with lower levels of phosphatidylcholine acyl-alkyls, hydroxy-sphingomyelin, glutamine, and citrate, and higher concentration of fatty acids, phosphatidylcholine diacyls, tyrosine, and alanine (23). Many metabolic profiles showed U-shape associations with alcohol consumption, such as total triglycerides and phenylalanine (24). Thus, there may exist the possibility that moderate alcohol consumption may have a protective effect on cardiovascular health by modulating the gut microbiome and serum metabolome.

To address the question above, we recruited a total of 89 patients with CAD enrolled at Peking Union Medical College Hospital (PUMCH) and divided them into four groups (detailed in the "Materials and Methods" section): healthy controls (HC) ($n = 17$), non-drinkers with CAD (ND-CAD) ($n = 36$), moderate drinkers with CAD (MD-CAD) ($n = 18$), and heavy drinkers with CAD (HD-CAD) ($n = 18$). Detailed clinical data of all 89 subjects were collected. Moreover, 16S rRNA sequencing and liquid chromatography-mass spectrometry (LC-MS) were applied. The aim of this study was to reveal the

**Abbreviations:** CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; SCFA, short-chain fatty acids; TMAO, trimethylamine N-oxide; PAGln, phenylacetylglutamine; SM, sphingomyelin; PUMCH, Peking Union Medical College Hospital; LC-MS, liquid chromatography-mass spectrometry; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in the projection; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; CPCoA, constrained principal coordinate analysis; PICRUSt2, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2; BMI, body mass index; SCAD, stable coronary artery disease; UA, unstable angina; MI: myocardial infarction; HTN, hypertension; T2DM, type 2 diabetes mellitus; HLP, hyperlipidemia; FLD, fatty liver disease; CK-MB, creatine kinase-MB; TC, total cholesterol; TG, triacylglycerol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; PI, phosphatidylinositol; PC, phosphatidylcholine; PS, phosphatidylserine; cTnI, cardiac troponin I; Hs-CRP, high-sensitivity C-reactive protein; S1P, sphingosine-1-phosphate; SBP, systolic blood pressure; UA, uric acid; Phe, phenylalanine; PAA, phenylacetic acid; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; 12-HPEPE, 12S-hydroperoxy-eicosapentaenoic acid; HTN drug, antihypertensive drugs; OAD, oral hypoglycemic drugs; AST, aspartate aminotransferase; CK, creatine kinase; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; FFA, free fatty acid; Lpa, lipoprotein (a); Cr, creatine; Glu, fasting blood glucose; BUN, blood urea nitrogen; no. vessel, number of stenosed vessels.

association between moderate drinking and gut microbiome, serum metabolome in patients with CAD, as well as key alterations related to clinical benefits.

# MATERIALS AND METHODS

## Study Population

The participants in the study were recruited consecutively at the department of cardiology in Peking Union Medical College Hospital from 2016 to 2018. The inclusion criteria were as follows: (1) male patients (to exclude the influence of hormone); (2) patients who exhibited $\geq$ 50% stenosis in at least one main coronary artery in coronary angiography; exclusion criteria included the complication of gastrointestinal diseases, malignant tumor, autoimmune disorders, infectious diseases, renal dysfunction (creatine $>$ 3 mg/dl), a history of gastrointestinal surgery within a year, and had antibiotics over 3 days in the last 3 months. In addition, the study focused on current drinkers and current non-drinkers, excluding the abstinent drinkers, who may interfere with the results. The subjects enrolled in the study had detailed questionnaires about their demographic features, living habits, and clinical information. Peripheral venous blood and stool samples were collected the next morning after admission. The preparation and the storage of blood and stool samples were described in our previous study (26). The freshly collected samples from each participant were immediately transported to our laboratory and stored in a $-80^{\circ}$C refrigerator.

The level of alcohol consumption was assessed based on the information collected in the questionnaire, including drinking history, frequency, amount, and the most often consumed type of alcoholic beverage. The alcohol intake (g/d) was obtained by calculating drinking frequency, amount, ethanol density (0.8 g/L), and the alcohol content of each beverage (%v/v): 50.0% for liquor, 12.9% for wine, and 5.3% for beer (27). Based on the cut-off value for different drinking categories in other research (28–30), 72 patients with CAD were divided into three groups: (1) non-drinkers with CAD (ND-CAD): patients with CAD who denied a drinking history; (2) moderate drinkers with CAD (MD-CAD): patients with CAD who had an ongoing regular light-to-moderate drinking habit (the average alcohol intake was 0–40 g pure alcohol per day); (3) heavy drinkers with CAD (HD-CAD): patients with CAD who had alcoholism habits or chronic alcohol over-intake (more than 40 g pure alcohol per day).

In addition, 17 healthy volunteers who met the following criteria were enrolled as the healthy control (HC): (1) male, (2) did not suffer from CAD, (3) and did not meet any of the exclusion criteria above.

The statistical analysis of the characteristics in the study population was described in detail in our previous work (31). The study complied with the principles of the Declaration of Helsinki. All the participants in the study provided written informed consent.

## Untargeted Metabolomics Analysis

Serum metabolome analysis was conducted on a Waters ACQUITY ultra-high-performance liquid chromatography system (Milford, MA), coupled with a Waters Q-TOF Micromass system (Manchester, UK) in positive and negative ionization modes. Polar ionic and lipid modes were performed based on the properties of metabolites. Sample preparation and LC-MS experiment procedure were detailed and described previously (26). The peak-ion intensity matrix was then filtered by removing peaks with a zero value in more than 80% of samples. The threshold of the coefficient variation value of quality control samples was set at 30%. Wilcoxon rank-sum test was adopted to identify the metabolites with significant differences between groups. Partial least squares discriminant analysis (PLS-DA) was conducted by SIMCA software (v14.1, Umetrics, Sweden). Variable importance in the projection (VIP)-value $>$ 1 and $P < 0.05$ was adopted for select important peaks. Online databases Human Metabolome Database (https://hmdb.ca), LipidMaps (https://www.lipidmaps.org), and PubChem (https://pubchem.ncbi.nlm.nih.gov/) were used for classifying peaks according to the molecular mass data (m/z). MetaboAnalyst (https://www.metaboanalyst.ca) was used to conduct pathway enrichment analysis. Pathways were regarded as potential targets with a threshold of impact-value $>$ 0.10 when utilizing MetaboAnalyst (32).

## 16S rRNA Gene V3–V4 Region Sequencing of Fecal Microbiota and Data Analysis

Microbial DNAs were extracted from the stool samples using the bead-beating method (33). Then, the amplification of the V3–V4 region of 16S rRNA genes was performed using PCR (34). The sequencing library was established as described previously (35), and purified products were sequenced with the Illumina Miseq system (Illumina Inc., USA). The downstream amplicon analysis was conducted by EasyAmplicon v1.0 (36). Dereplication was performed by the *-derep_fulength* command of VSEARCH (v2.15) (37). Operational taxonomic units (OTU) were clustered *via* the *-cluster_otus* command of USEARCH (v10.0) at the cutoff of 97% (38). The feature table was created with *vsearch—usearch_global*. Taxonomic annotation was generated according to Greengenes database using *usearch–otutab* (39).

The sequences of all samples were downsized to the sample with the least sequences to calculate the diversity indices. Alpha diversity was evaluated with Shannon's index and Chao1 index. Beta diversity was performed by principal coordinate analysis (PCoA) and constrained PCoA (CPCoA) with Bray-Curtis distances. The composition of each group was represented as a boxplot plot at the phylum level and as a Chord diagram at the genus level with R package ggplot2. As for the comparison of differences, edgeR was applied to identify the differences between groups and the Benjamini-Hochberg method to control the FDR (40). A threshold of $P < 0.05$ with FDR $< 0.2$ was considered statistically significant. Bugbase and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) were utilized to perform functional predication of the gut microbiota (41, 42). Furthermore, PICRUSt2 was utilized to predict the metagenomic pathways based on the MetaCyc database (43). Pathways that were significantly different between the moderate drinkers and non-drinkers were identified by

Welch's *t*-test, and Storey FDR was utilized for multiple pathways. STAMP software (v2.1.3) was applied for statistical analysis and visualization of the identified pathways.

## Identification of the Key Metabolites or OTUs Associated With Moderate Alcohol Consumption

For metabolomics analysis, Wilcoxon rank-sum test was conducted, and the threshold of VIP $> 1$ and $P < 0.05$ was used to identify differential metabolites. To be detailed, the metabolites that were significantly higher or lower in MD-CAD than those in both ND-CAD and HD-CAD groups were defined as key metabolites associated with moderate alcohol consumption. In terms of the microbiota, using edgeR with a threshold of $P < 0.05$ and FDR $< 0.2$, there were a total of 21 differential OTUs between MD-CAD and ND-CAD. Among the 21 OTUs, key OTUs were defined as those whose average relative abundances represented a U-shaped or convex-shaped relationship with different levels of alcohol consumption (i.e., the key OTUs present either the highest or the lowest average relative abundance in MD-CAD when comparing with ND-CAD and HD-CAD).

## Multi-Omics Correlation Study

Spearman correlation analysis was conducted between key bacterial taxa, metagenomic pathway, serum metabolites, and clinical parameters with SPSS (v. 24.0), and visualized as a heatmap using the R package pheatmap. A Sankey plot was drawn by R package networkD3 to illustrate the interrelationship among the differential OTUs, metabolic features, and clinical parameters.

## Statistical Analysis and Visualization

Continuous normally distributed data among three groups were analyzed by one-way ANOVA. Kruskal-Wallis H-test was employed to analyze continuous data with non-normal distribution among three groups, and Mann—Whitney U test was applied for that between two groups. Categorical variables were analyzed by $\chi 2$ test or Fisher's exact test. Data were analyzed using SPSS (v.24.0). Figures were made by utilizing R 4.0.4.

# RESULTS

## Clinical Characteristics of the Study Population

The clinical characteristics of the study cohort were summarized in **Table 1**. In general, among different alcohol consumption groups, there was no significant difference in age, body mass index (BMI), and systolic blood pressure. In terms of alcohol consumption, there were significant differences between the three disease groups. The amount of alcohol intake was significantly higher in HD-CAD when compared with MD-CAD ($p < 0.001$), and liquor was the most common alcohol intake type. Drinking history, drinking years, and drinking frequency showed no significant difference. The three disease groups showed no significant difference in hypertension (HTN), type 2 diabetes mellitus (T2DM), hyperlipidemia (HLP), and fatty liver disease (FLD). In terms of CAD severity, although there was no significant difference in Gensini score. Gensini score is an assessment of the CAD severity using coronary angiography and positively associated with disease severity (44). The average Gensini score of MD-CAD [27.50 (16.00, 42.75)] was the lowest when compared with ND-CAD [39.50 (19.25, 66.75)] and HD-CAD [34.75 (24.75, 56.00)]. Also, the average level of CK-MB [CK-MB is a cardiac biomarker that represents cardiovascular injury (45)] in MD-CAD was the lowest when compared with non-drinkers and heavy drinkers, so as the level of hs-CRP [The level of hs-CRP represents the inflammation status in the human body (46), and chronic inflammation plays a central role in the development of cardiovascular disease (47)]. The other laboratory tests, such as total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein cholesterol (LDL-C), HDL-C, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), showed no significant difference among the three disease groups. Thus, moderate alcohol consumption was associated with the improvement of CAD severity, that is, MD-CAD presented relatively better status than other patients with CAD.

## Serum Metabolome Presented Significant Alterations in Patients With CAD, With Different Alcohol Consumption

Since it was reported that alcohol may affect serum metabolic profiles, serum metabolome in ND-CAD, MD-CAD, and HD-CAD was explored through the untargeted LC-MS method. After QC and removal of peaks with low abundance, a total of 10,416 metabolites in polar ionic mode and 3,131 in lipid mode were obtained. PLS-DA modeling was utilized to reveal the alteration of serum metabolites. The score scatter plots of four modes were shown in **Figures 1A–D**, and the parameters of each mode were provided in **Supplementary Table 1**. It was obvious that the metabolic feature of three diseased groups (ND-CAD, MD-CAD, and HD-CAD) showed significant differences in score scatter plot, especially in polar ionic positive mode (**Figure 1B**) and lipid positive mode (**Figure 1D**).

## Serum Metabolome Alterations in MD-CAD Were Associated With Cardiovascular Health Status

A total of 203 serum metabolites were identified as key serum metabolites associated with moderate alcohol consumption with a threshold of VIP $> 1$ and Wilcoxon rank-sum $P < 0.05$ (**Supplementary Table 2**). All the 203 key metabolites in MD-CAD were significantly different from those in ND-CAD or HD-CAD, either the most abundant or the least in the three disease groups (**Supplementary Table 2**). In other words, the relative abundance of these 203 metabolites showed a "*U*" shaped or convex curve with alcohol consumption. Since MD-CAD tended to have better cardiovascular health according to the previous studies, we speculated that the most abundant metabolites (i.e., convex-shaped metabolites) in MD-CAD may have protective effects in cardiovascular health, while the most deprived metabolites (i.e., "U" shaped metabolites) may be harmful. Among these 203 metabolites, we observed

**TABLE 1 |** Characteristics of the study population.

| Variable | HC ($n$ = 17) | ND-CAD ($n$ = 36) | MD-CAD ($n$ = 18) | HD-CAD ($n$ = 18) | $P$-Value |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Age, years* | 57.7 ± 12.2 | 65.2 ± 11.1 | 60.2 ± 10.5 | 58.9 ± 8.3 | 0.058 |
| BMI, kg/m$^2$* | 24.85 ± 3.01 | 25.50 ± 2.86 | 25.72 ± 3.51 | 26.19 ± 1.96 | 0.582 |
| SBP, mmHg# | 120.00 (113.00, 130.00) | 130.00 (118.25, 137.00) | 133.50 (123.00, 139.25) | 127.00 (112.75, 134.50) | 0.061[a] |
| **Type of CAD** | | | | | |
| SCAD† | NA | 13 (36.1) | 5 (27.8) | 5 (27.8) | NA |
| UA† | NA | 19 (52.8) | 10 (55.6) | 7 (38.9) | NA |
| MI† | NA | 4 (11.1) | 3 (16.7) | 6 (33.3) | NA |
| Gensini# | NA | 39.50 (19.25, 66.75) | 27.50 (16.00, 42.75) | 34.75 (24.75, 56.00) | 0.613 |
| **Drinking information** | | | | | |
| Drink history† | 5 (29.4) | 0 (0) | 18 (100) | 18 (100) | <0.001[b,c] |
| Drink years# | NA | NA | 30.0 (18.8, 40.0) | 27.5 (20.0, 40.0) | 0.736 |
| Frequency, per week# | NA | – | 7 (1,7) | 7 (7,7) | 0.203 |
| Types† | NA | NA | Liquor: 16 (88.9)<br>Wine: 1 (5.6)<br>Beer: 1 (5.6) | Liquor: 18 (100)<br>Wine: 0 (0)<br>Beer: 0 (0) | NA |
| Alcohol intake, g/d# | NA | NA | 21.4 (5.4, 25.0) | 87.5 (60.3, 131.3) | <0.001[d] |
| **Medications** | | | | | |
| HTN, %† | 5 (29.4) | 20 (55.6) | 11 (61.1) | 15 (83.3) | 0.036[a] |
| T2DM, %† | 1 (5.9) | 13 (36.1) | 4 (22.2) | 7 (38.9) | 0.070[a] |
| HLP, %† | 5 (29.4) | 22 (61.1) | 9 (50.0) | 13 (72.2) | 0.065[a] |
| FLD, %† | 4 (23.5) | 5 (13.9) | 5 (27.8) | 3 (16.7) | 0.593 |
| Statin, %† | 1 (5.9) | 12 (33.3) | 5 (27.8) | 9 (50.0) | 0.031[a] |
| HTN-Drugs, %† | 5 (29.4) | 20 (55.6) | 10 (55.6) | 16 (88.9) | 0.010[a,c,d] |
| OAD, %† | 1 (5.9) | 6 (16.7) | 2 (11.1) | 7 (38.9) | 0.086 |
| **Laboratory test** | | | | | |
| TC, mmol/L* | 4.34 ± 0.68 | 3.93 ± 1.25 | 3.79 ± 1.02 | 3.78 ± 0.89 | 0.363 |
| TG, mmol/L# | 1.36 (0.73, 2.34) | 1.17 (0.98, 1.54) | 1.67 (1.06, 2.15) | 1.18 (0.94, 2.55) | 0.339[b] |
| LDL-C, mmol/L# | 2.45 (2.03, 3.00) | 2.12 (1.57, 2.81) | 2.06 (1.77, 2.40) | 1.98 (1.43, 2.47) | 0.083[a] |
| HDL-C, mmol/L* | 1.11 ± 0.28 | 0.96 ± 0.20 | 0.90 ± 0.20 | 0.95 ± 0.21 | 0.040[a] |
| ALT, U/mL# | 20.00 (13.50, 33.00) | 23.00 (18.25, 29.00) | 20.00 (14.00, 37.75) | 26.00 (18.75, 40.00) | 0.333 |
| CK, U/mL# | 112.00 (91.00, 128.50) | 105.50 (72.75, 142.00) | 91.00 (75.50, 129.00) | 81.50 (70.50, 103.50) | 0.192 |
| CK-MB, U/mL# | 070 (0.50, 0.95) | 0.80 (0.50, 1.75) | 0.60 (0.45, 1.08) | 0.70 (0.60, 1.00) | 0.530 |
| cTnI, ug/L# | 0.00 (0.00, 0.00) | 0.00 (0.00, 0.02) | 0.00 (0.00, 0.01) | 0.03 (0.01, 0.13) | <0.001[a,c,d] |
| Hs-CRP, mg/L# | 0.50 (0.35, 1.01) | 1.86 (0.90, 4.37) | 1.08 (0.63, 4.51) | 2.39 (1.80, 4.11) | 0.002[a] |

*Data are presented with *mean ± SD, #median (IQR), or †number (%).*

*Continuous normally distributed data among three groups were analyzed by one-way ANOVA. Kruskal-Wallis H-test was employed to analyze continuous data with non-normal distribution among three groups, and Mann—Whitney U-test was applied for that between two groups. Categorical variables were analyzed by $\chi^2$ test or Fisher's exact test. N/A, not available; SBP, systolic blood pressure; BMI, body mass index; SCAD, stable coronary artery disease; UA, unstable angina; MI, myocardial infarction; HTN, hypertension; T2DM, type 2 diabetes mellitus; HLP, hyperlipidemia; FLD, fatty liver disease; HTN drug, antihypertensive drugs; OAD, oral antidiabetic drugs; TC, total cholesterol; TG, triacylglycerol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase-MB; cTnI, cardiac troponin I; HsCRP, high-sensitivity C-reactive protein; [a]$P < 0.05$ for HC vs. all patients with CAD. [b]$P < 0.05$ for ND-CAD vs. MD-CAD. [c]$P < 0.05$ for ND-CAD vs. HD-CAD. [d]$P < 0.05$ for MD-CAD vs. HD-CAD.*

unignorable quantities of metabolites were lipids and lipids-like molecules, such as sphingolipids, glycerophospholipids, prenol lipids, and fatty acyls (**Figure 2A**), which were consistent with other existing reports (23–25). It was reported that a high plasma level of SM was an independent risk factor in cardiovascular diseases and predicted a poor prognosis of CAD (48, 49). Consistently, we observed sphingolipids, such as SM (d18:0/22:1) (LPN77) and SM (d18:1/20:0) (LPN288), were depleted in MD-CAD when compared with the other two diseased groups. Moreover, we observed all annotated glycerophospholipid showed significant depletion in MD-CAD when compared with other two CAD groups (ND-CAD and HD-CAD) (**Figure 2A**), including phosphatidylinositol [PI, such as PI (18:1/22:6) (LPN207) and PI (20:4/18:0) (LPN208)],

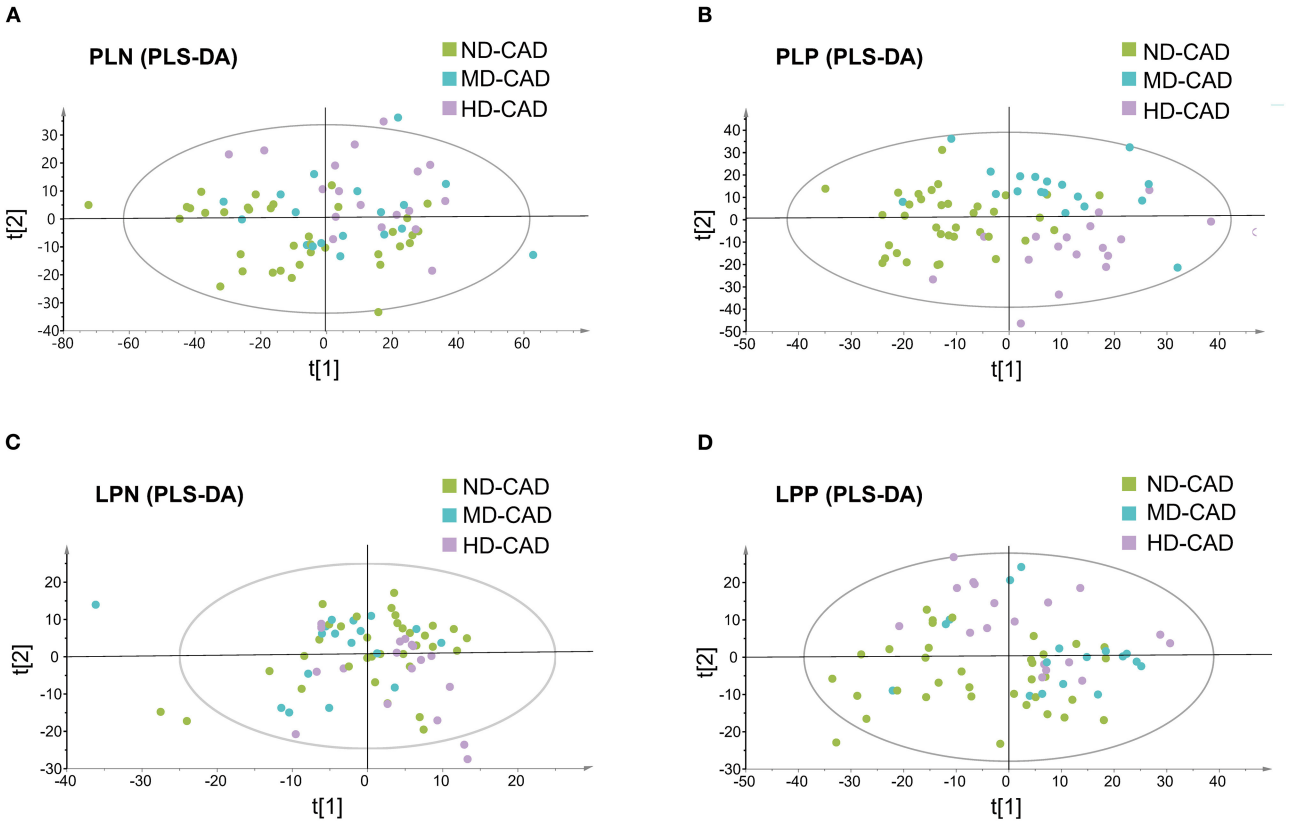


**FIGURE 1 |** Serum metabolites profiles of the patients with coronary artery disease with different alcohol consumption. **(A–D)** Separation of serum metabolites in polar ionic negative (PLN) mode, polar ionic positive (PLP) mode, lipid negative (LPN) mode, and lipid positive (LPP) mode, revealed by PLS-DA modeling.

phosphatidylcholine [PC, such as PC (18:3/20:5) (LPP1438)], phosphatidylserine [PS, such as PS (22:1/22:6) (LPP 1453), PS (22:4/22:5) (LPP1458), PS (22:0/22:1) (LPP863), PS (22:0/22:2) (LPP911), PS (22:0/22:1) (LPP863), PS (20:0/22:2) (LPP960), PS (20:0/22:1) (LPP932), and PS (22:0/22:2) (LPP960)]. Since glycerophospholipid was associated with macrophage-driven inflammation (50) and atherosclerosis (51), the depletion of glycerophospholipid in MD-CAD may be associated with cardiovascular health. What is more, PC can be degraded by the gut microbiome and thus converted to TMAO, a well-known risk factor in the major adverse cardiovascular events (17, 52, 53). Thus, a higher level of PC was often associated with higher cardiovascular risk (17, 52, 53). Consistently, PC was observed to be depleted in MD-CAD when compared with the other two diseased groups.

To further elucidate the cause of serum metabolome change, metabolic pathway enrichment was performed by MetaboAnalyst. The results showed that these differential metabolites mainly clustered in 11 pathways (**Figure 2B**, **Supplementary Table 3**). Among the 11 pathways, terpenoid backbone synthesis ($P = 0.28955$, impact-value = 0.0.11429), and lipoic acid metabolism ($P = 0.14053$, impact-value = 0.16667) were regarded as potential target pathways. Terpenoid had significant effects on the prevention and treatment of cardiovascular disease (54), while lipoic acid was reported to be beneficial in cardiovascular disease by reducing atherosclerosis (55, 56).

## Gut Microbiome Taxonomic Features of Moderate Drinkers With CAD Resembles That of Healthy Controls

Since some of the differential metabolites may be microbiome derived, such as PC, we further elucidate the taxonomic features of the gut microbiome by 16S rRNA sequencing with sufficient depths (**Supplementary Figure 1A**). A total of 626 OTUs were obtained. In terms of alpha-diversity, no significant difference was observed in HC, ND-CAD, MD-CAD, and HD-CAD (**Supplementary Figures 1B,C**). As for beta-diversity, although no significant difference was observed (**Supplementary Figure 1D**) among three diseased groups in PCoA analysis, the taxonomic features of MD-CAD were almost overlapping with HC while the other two disease groups separated clearly from HC in CPCoA analysis (**Figure 3A**). Firmicutes and Bacteroides were the two most dominant phyla in all groups (**Figure 3B**, **Supplementary Table 4**). We noted that MD-CAD and HC presented similar proportions of


**FIGURE 2 |** Identification of the key metabolites associated with alcohol consumption in patients with CAD. **(A)** Heatmap showing part of the key metabolites associated with moderate alcohol consumption across three groups with CAD. **(B)** Bubble map demonstrating the potential metabolic pathways associated with moderate alcohol consumption using MetaboAnalyst. The IDs and the annotated potential metabolites are highlighted in red (enriched in MD-CAD) or blue (depleted in MD-CAD).

Actinobacteria and Proteobacteria, while ND-CAD and HD-CAD presented a much higher proportion of the two phyla (**Figure 3B**). It could be inferred that the taxonomic features of the gut microbiome in MD-CAD were more similar with healthy people when compared with the other two disease groups. What is more, *Bacteroides*, *Prevotella*, and *Faecalibacterium* were the

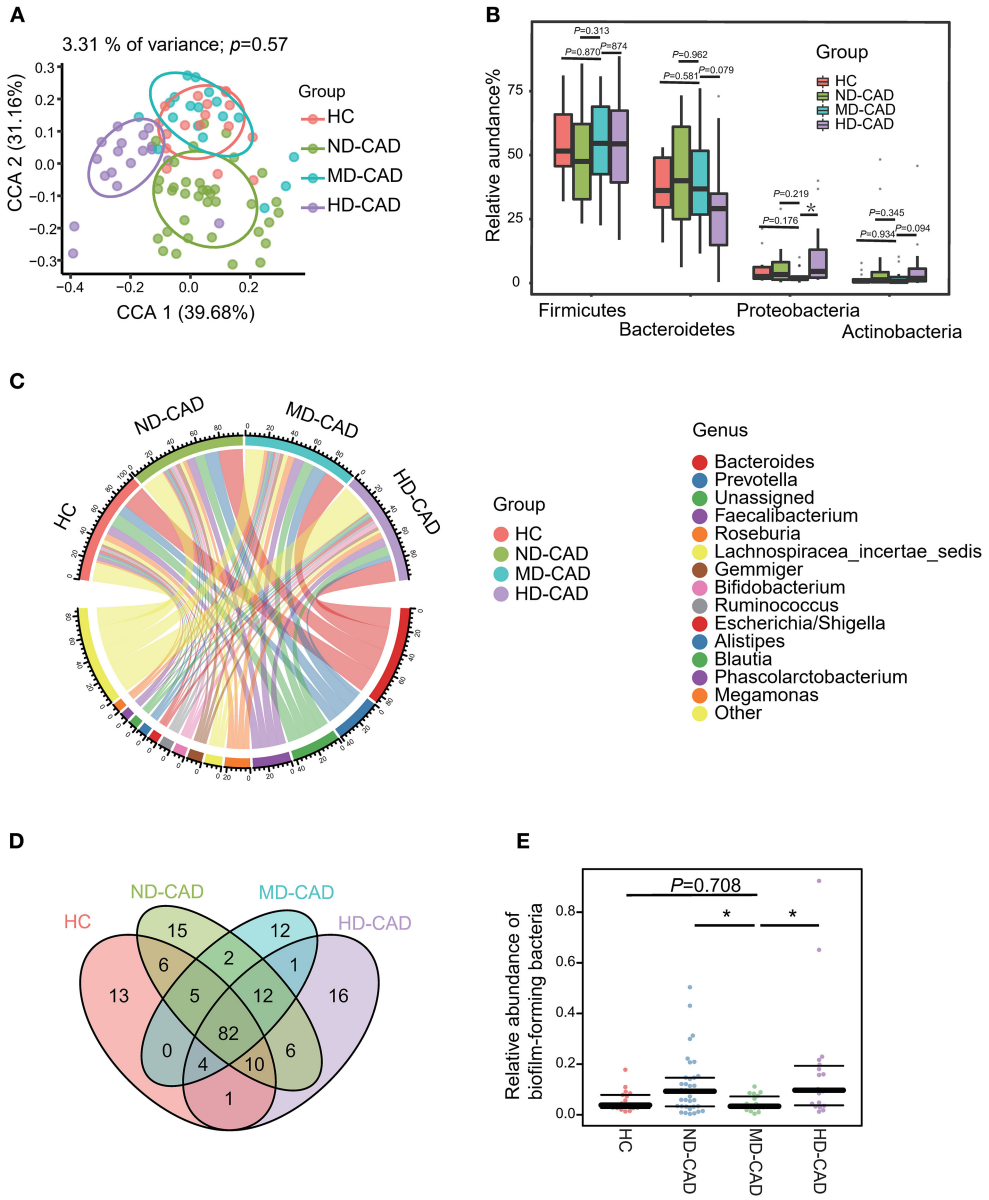


**FIGURE 3 |** Different alcohol consumption affects the taxonomic features of gut microbiota in patients with CAD. **(A)** Beta diversity analyzed by CPCoA plot based on Bray-Curtis distances ($P = 0.57$, Adonis Test). **(B)** Comparison of the relative abundance of gut microbiota at the phylum level (ns, not significant, $^*P < 0.05$, Mann—Whitney *U*-test. Phylum Proteobacteria: MD-CAD vs. HC: $P = 0.176$, MD-CAD vs. ND-CAD: $P = 0.219$, MD-CAD vs. HD-CAD: $P = 0.019$). **(C)** Chord plot showing the dominant genera and their contribution to each group. **(D)** Venn plot indicating the number of overlapped OTUs. **(E)** Relative abundance of biofilm-forming bacteria predicted by BugBase (ns, not significant, $^*P < 0.05$, Mann—Whitney *U*-test. MD-CAD vs. HC: $P = 0.708$, MD-CAD vs. ND-CAD: $P = 0.015$, MD-CAD vs. HD-CAD: $P = 0.007$).

top three abundant genera (**Figure 3C**) in all four groups. Venn plot was utilized to further illustrate the taxonomic features with a relative abundance >0.1% in HC, ND-CAD, MD-CAD, and HD-CAD (**Figure 3D**). A total of 82 OTUs coexisted in four groups, and 94 were specifically shared by three CAD groups. Twelve OTUs were unique in MD-CAD, while those in ND-CAD and HD-CAD were 15 and 16. We further predicted the phenotypes of the gut microbiome based on their 16S rRNA sequence utilizing BugBase. Notably, HC and MD-CAD had a similar abundance of "biofilm-forming" bacteria, while ND-CAD and HD-CAD had a significantly higher abundance of "biofilm-forming" bacteria (**Figure 3E**). Thus, it was clear that MD-CAD presented more similar taxonomic and functional features with HC than ND-CAD and HD-CAD. This taxonomic similarity may

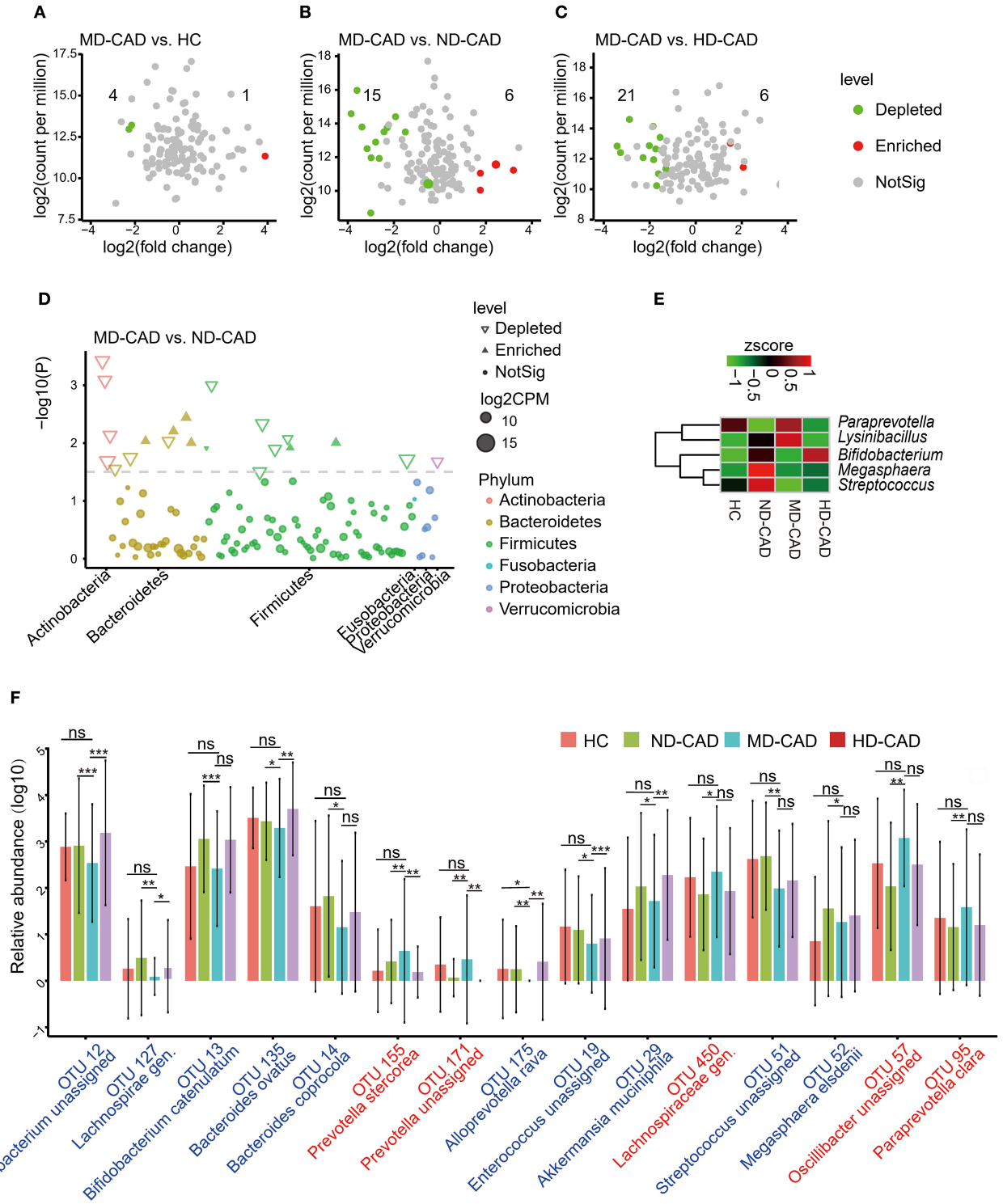


**FIGURE 4 |** Moderate alcohol consumption was associated with certain changes of intestinal flora in patients with CAD. **(A–C)** Volcano plots showing differential OTUs in patients with CAD with different levels of alcohol consumption. **(A)** MD-CAD vs. HC. **(B)** MD-CAD vs. ND-CAD. **(C)** MD-CAD vs. HD-CAD. The numbers labeled in the figures indicated significantly depleted OTUs (highlighted in green) or enriched OTUs (highlighted in red) in MD-CAD when compared with HC, ND-CAD, and HD-CAD, respectively. **(D)** Manhattan plot demonstrating the differentially abundant OTUs and their contributions to each phylum. **(E)** Heatmap showing the relative abundance of the genera that were significantly different between MD-CAD and ND-CAD. **(F)** An overview of the relative abundance of the differential OTUs associated with moderate alcohol consumption. The differential OTUs were filtered with $P < 0.05$ as well as FDR < 0.2. ns, not significant. *$P < 0.05$ and FDR < 0.2, **$P < 0.01$ and FDR < 0.2, ***$P < 0.001$ and FDR < 0.2, analyzed by edgeR.

partially explain the healthier cardiovascular status discovered in MD-CAD.

## Variations of Microbiota in MD-CAD Were Associated With Cardiovascular Health

To further elucidate the alterations of the gut microbiome in MD-CAD, edgeR was utilized with a threshold of $P < 0.05$ and FDR $< 0.02$. Only five OTUs showed significant differences between HC and MD-CAD (**Figure 4A**). However, there were 21 significant differentials OTUs between MD-CAD and ND-CAD (**Figure 4B**), while there were 27 significant differentials OTUs between MD-CAD and HD-CAD (**Figure 4C**). It was obvious that, regarding MD-CAD, more differential OTUs were found when comparing with ND-CAD and HD-CAD than comparing with HC. This further confirmed the similarity of gut microbiome taxonomic features between HC and MD-CAD. We suspected that it was moderate alcohol consumption that tended to alter the gut microbiome of patients with CAD to a more similar feature with HC. We further looked into the differential microbiome between ND-CAD and MD-CAD. At the phylum level, Actinobacteria, Firmicutes, and Bacteroidetes were found significantly different (**Figure 4D**). At the genus level, *Paraprevotella* and *Lysinibacillus* were significantly elevated in MD-CAD while *Bifidobacterium*, *Megasphaera*, and *Streptococcus* were significantly depleted in MD-CAD when compared with ND-CAD (**Figure 4E**). At the species level, we paid attention to 15 key OTUs associated with moderate alcohol consumption (key OTUs were defined in the "Materials and Methods" section) that showed significant differences between MD-CAD and ND-CAD (**Figure 4F**, **Supplementary Table 5**). *Bacteroides ovatus* (OTU 135, depleted in MD-CAD) was found to drive the production of IgA (57), an autoimmune disease inflammatory mediator (58), and was thus regarded as a driver of cardiovascular diseases. *Prevotella stercorea* (OTU 155, enriched in MD-CAD) was reported to be beneficial in overweight patients (59). *Bacteroides coprocola* (OTU 14, depleted in MD-CAD) was reported to be enriched in patients with hypertension (60). Our result indicated that potentially beneficial bacteria were possibly promoted, and potentially pathogenic bacteria were possibly suppressed by moderate alcohol consumption.

In addition, we predicted possible functional pathways by utilizing PICRUSt2. We found 12 significantly affected pathways in MD-CAD when compared with ND-CAD (**Supplementary Figure 2**, **Supplementary Table 6**). Moreover, we found that the microbes depleted in MD-CAD (OTU 19, OTU 13, OTU 12, OTU 51, and OTU 135) were all positively correlated with pathways that were depleted in MD-CAD, especially the methionine-related pathways [superpathway of L-methionine biosynthesis (transsulfuration), L-methionine biosynthesis I, and superpathway of S-adenosyl-L-methionine biosynthesis]. In addition, we found most of these pathways were closely associated with metabolites degradation or biosynthesis, such as glucose and glucose-1-phosphate degradation, lactose, and galactose degradation I. Thus, we inferred that there may be exhibited functional changes of the gut microbiome that led to the alterations of serum metabolome in MD-CAD.

## Multi-Omics Analysis Revealed the Association Between Alcohol Consumption, Gut Microbiome, and Serum Metabolome

We analyzed the association between key OTUs and serum metabolites by utilizing the Spearman correlation (**Figure 5A**). Several key OTUs were significantly associated with serum metabolites. We inferred that it may be the alterations in the gut microbiome that may affect the serum metabolites features. Furthermore, we correlated the key metabolites with clinical indexes and found several significant Spearman correlations (**Figure 5B**). We represented these interrelationships in a Sankey map, shown in **Figure 6**. We noted that *Bifidobacterium longum* (OTU 12, depleted in MD-CAD), *Lachnospiraceae gen*. (OTU 127, depleted in MD-CAD), and *Oscillibacter valericigenes* (OTU 57, enriched in MD-CAD) were the three main species that impacted the serum metabolome. In terms of clinical indexes, Gensini score was positively correlated with 9R-hydroxy-octadecadienoic acid (PLP716, elevated in MD-CAD) and syringic acid (PLP7081, elevated in MD-CAD), and negatively correlated with β-citronellol (PLP3157, depleted in MD-CAD) and trans,cis-3,6-nonadien-1-ol (PLP4864, depleted in MD-CAD). Moreover, cTnI negatively correlated with 12S-hydroperoxy-eicosapentaenoic acid (12-HPEPE, PLP2108, elevated in MD-CAD). Correlation analysis showed that the relative better cardiovascular health of MD-CAD may be associated with serum metabolome and gut microbiome alterations.

# DISCUSSION

Alcohol presented complex effects on the cardiovascular system that vary with dose (61). Observational and prospective studies consistently showed a lower risk of cardiovascular and all-cause mortality in people with low levels of alcohol consumption when compared with people with alcohol abuse and non-drinkers (62). In our study, we found patients with CAD with moderate alcohol consumption presented a relatively healthier cardiovascular status, that is, a lower level of Gensini score, CK-MB, and hs-CRP. Thus, we looked further into the serum metabolome profiles and gut microbiome taxonomic features to reveal the possible associations between moderate alcohol consumption and healthier cardiovascular status.

Serum metabolome presented significant alterations in MD-CAD when compared with ND-CAD and HD-CAD. The representative metabolites were sphingolipids and glycerophospholipids, which were both significantly depleted in MD-CAD. Sphingolipids accumulate in the formation of atherosclerotic plaque both in humans and primates (63, 64). Emerging studies uncovered that a high plasma level of sphingolipids was an independent risk factor in CAD (48, 49). Moreover, studies on animal models confirmed that sphingolipids contributed to the development of obesity and insulin resistance (65). The medication targeting sphingolipid metabolism may improve the condition and prognosis of metabolic disorders (66). Consistently, in our study, the

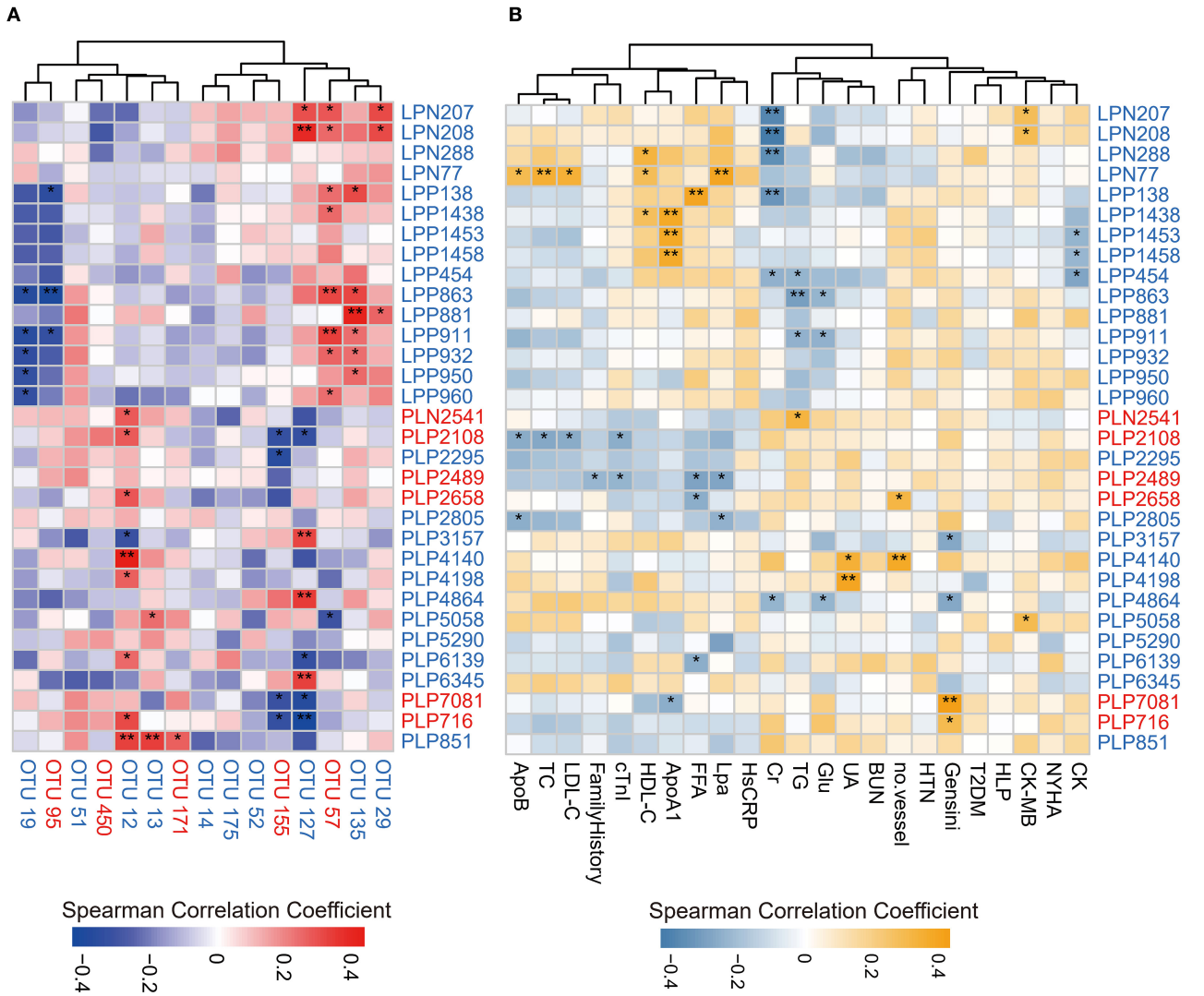


**FIGURE 5 |** Spearman correlations between differential serum metabolites and OTUs associated with moderate alcohol consumption or major clinical parameters for CAD. **(A)** Spearman correlations between differential serum metabolites and OTUs associated with moderate alcohol consumption. **(B)** Spearman correlations between differential serum metabolites and major clinical parameters associated with moderate alcohol consumption. The IDs of metabolites or OTUs are highlighted in red (enriched in MD-CAD) and blue (depleted in MD-CAD). $^{*}P < 0.05$, $^{**}P < 0.01$. ApoB, apolipoprotein B; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; family history: having a family history of CAD; cTnI, cardiac tropinin I; HDL-C, high-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; FFA, free fatty acid; Lpa, lipoprotein (a); HsCRP, high-sensitivity C-reactive protein; Cr, creatine; TG, triglyceride; Glu, fasting blood glucose; UA, uric acid; BUN, blood urea nitrogen; no. vessel, number of stenosed vessels; HTN, hypertension; T2DM, type 2 diabetes mellitus; HLP, hyperlipidemia; CK-MB, creatine kinase-MB; CK, creatine kinase; NYHA, grade of NYHA classification.

depletion of sphingolipids associated with moderate alcohol consumption tended to relieve cardiovascular stress. In addition, C19 sphingosine-1-phosphate (S1P, LPP138, depleted in MD-CAD) was reported to contribute to the pathogenesis of multiple cardiovascular disorders (67) by activating the proliferation and migration of vascular smooth muscle cells (68). Hence, we suspected that moderate alcohol consumption may suppress the level of sphingolipids and thus present a cardiovascular protective effect. As for glycerophospholipids, including PC, PI, and PS, accumulating evidence showed that glycerophospholipids promoted the progression of cardiovascular diseases (69, 70). Studies showed that PC was positively associated with the prevalence of CAD (71), while moderate alcohol consumption was found to reduce the level of PC (25). Consistently, in our study, we found a decrease of PC (LPP1438, HMDB0008182) in MD-CAD when compared with HD-CAD and ND-CAD, suggesting that moderate alcohol consumption may reduce PC and lead to better cardiovascular health. In addition, it was reported that, in patients with hypertension, PI was found positively related to systolic blood pressure (SBP) (72). What is more, researchers found that alcohol-treated rats exhibited a decrease in PI and PS than

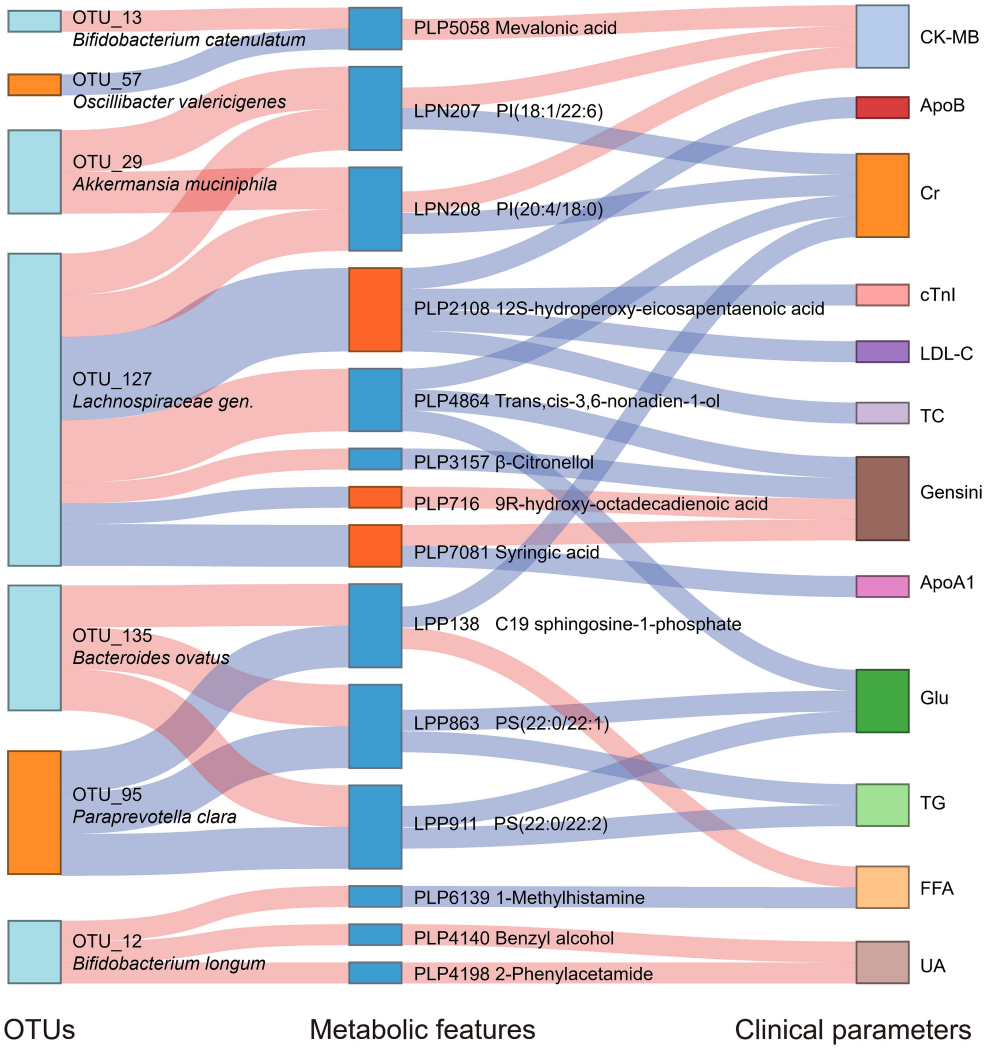


**FIGURE 6 |** Interrelationship between intestinal flora, serum metabolic features, and major clinical parameters associated with moderate alcohol consumption with CAD. A Sankey plot was utilized to examine the relationship among the differential OTUs and serum metabolites associated with moderate alcohol consumption, and major clinical parameters of CAD. Red connections indicate significant positive correlations, and blue connections indicate significant negative correlations (Spearman correlation analysis, $P < 0.05$). In the left column, light blue boxes indicate OTUs that are significantly depleted in MD-CAD when compared with ND-CAD and HD-CAD, and light orange boxes indicate OTUs that are significantly elevated in MD-CAD when compared with ND-CAD and HD-CAD. In the middle column, dark blue boxes indicate metabolic features that are significantly decreased in MD-CAD when compared with ND-CAD and HD-CAD, and dark orange boxes indicate metabolic features that are significantly increased in MD-CAD when compared with ND-CAD and HD-CAD. The right column exhibits some major clinical parameters of CAD. CK-MB, creatine kinase-MB; cTnI, cardiac tropinin I; Cr, creatine; ApoA1, apolipoprotein A1; Glu, fasting blood glucose; TG, triglyceride; FFA, free fatty acid; UA, uric acid; ApoB, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

control (25). Consistently, in our study, PI (LPN 207, LPN208, both reduced in MD-CAD) was positively correlated with CK-MB, a biomarker for myocardial injury. Overall, the reduction of glycerophospholipids associated with moderate alcohol consumption tended to be beneficial for cardiovascular health.

In addition to sphingolipids and glycerophospholipids, several other metabolites also caught our attention. Firstly, it was worth mentioning that 2-phenylacetamide (PLP4198, reduced in MD-CAD) positively correlated with *Bifidobacterium longum* (OTU 12, depleted in MD-CAD) ($P < 0.029$, Rho = 0.302) and uric acid (UA) ($P = 0.003$, Rho = 0.409). A high concentration of UA was associated with cardiovascular diseases (73). It was reported that 2-phenylacetamide (PLP4198) was the intermediate product in the bacteria fermentation of aromatic amino acid phenylalanine (Phe) into phenylacetic acid (PAA) (74). The downstream product of PAA in the host is PAGln, which acts via adrenergic receptors and increases the risk of thrombosis and CAD (18). Thus, we may infer that the reduction of 2-phenylacetamide (PLP4198) in MD-CAD may be associated with relevant bacteria, and thus improve the health of the cardiovascular system.

Secondly, mevalonic acid (PLP5058, depleted in MD-CAD) was the key intermediate product in terpenoid backbone synthesis catalyzed by key enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase. Wang et al. observed a considerable elevation of mevalonic acid in hyperlipidemic rats and a decrease after lipid-lowering therapy with a folk medicine in China (75). As for the key enzyme in terpenoid backbone synthesis, genomic and phylogenetic analysis revealed that multiple bacteria had the similar HMG-CoA reductase as in human beings (76). According to the results of our study, we speculated that moderate drinking might inhibit cholesterol synthesis by restraining the proliferation of bacteria with the ability to synthesize mevalonic acid. Thirdly, 1-methylhistamine (PLP6139, depleted in MD-CAD) was a biomarker of allergic response, which was the main catabolite of histamine. Depleted 1-methylhistamine concentrations were observed in moderate drinkers when compared with heavy drinkers (77), and increased 1-methylhistamine was found in alcohol-preferring rats (78). Besides, 1-methylhistamine was a vital cardiac substrate that activated monoamine oxidase and induced reactive oxygen species production in the heart during oxidative stress (79). Our result was consistent with these findings and indicated that the reduction of 1-methylhistamine (PLP6139, depleted in MD-CAD) in moderate drinkers may play a role in the amelioration of allergic response and oxidative stress, owing to moderate alcohol consumption. In addition, 12-HPEPE (PLP2108, increased in MD-CAD) drew our special attention. An early study *in vitro* confirmed the potent platelet aggregation inhibitor 12-HPEPE could inhibit platelet aggregation and serotonin release (80). The rise of 12-HPEPE observed in MD-CAD may suggest a positive effect on the progression of atherosclerotic plaque.

Since several metabolites were microbiome derived, we paid attention to gut microbiome alterations in MD-CAD. The taxonomic features of MD-CAD resembled that of HC, and several gut microbes in MD-CAD altered significantly when compared with HD-CAD and ND-CAD. *Oscillibacter valericigenes* (OTU 57, elevated in MD-CAD) caught our special attention. It was reported that *Oscillibacter valericigenes* was depleted in people with Crohn's disease (81), and the decrease of the *Oscillibacter* genus may promote inflammation (82). Furthermore, genus *Oscillibacter* was reported to be able to produce butyric acid (83) and valeric acid (84). Butyric acid and valeric acid were both well-known beneficial substances for gut health. Thus, *Oscillibacter valericigenes* was considered a beneficial species. Our results indicated that *Oscillibacter valericigenes* (OTU 57, elevated in MD-CAD) may be promoted by alcohol and thus be beneficial to cardiovascular health. The relative abundance of *Enterococcus villorum* (OTU 19) was reduced in MD-CAD when compared with HD-CAD and ND-CAD. It was reported that *Enterococcus faecalis* was identified as a cause of hepatocyte death and liver injury (85). Since *Enterococcus villorum* (OTU 19) belonged to the same genus as *Enterococcus faecalis,* we suspected that the depletion may be beneficial to the liver. Our result indicated that moderate alcohol consumption may possibly promote potentially beneficial bacteria and may inhibit potentially pathogenic bacteria.

In addition, the multi-omics correlation revealed that moderate alcohol consumption was beneficial for cardiovascular health possibly by affecting the gut microbiome and serum metabolome. In our study, by moderate alcohol consumption, potential beneficial gut microbes (such as *Oscillibacter valericigenes* OTU 57 and *Paraprevotella clara* OTU 95) presented the highest abundance in MD-CAD among three disease groups, while potential pathogenic gut microbes (such as *Bacteroides ovatus* OTU 135 and *Bacteroides coprocola* OTU 14) showed the lowest abundance in MD-CAD when compared with ND-CAD and HD-CAD. Furthermore, the elevation of *Oscillibacter valericigenes* OTU 57 and *Paraprevotella clara* OTU 95 were both negatively associated with sphingolipids and glycerophospholipids (such as LPP138, LPP863, and LPP911), which were known to promote the progression of cardiovascular diseases (69, 70) by activating the proliferation and migration of vascular smooth muscle cells (68). Moreover, the depletion of potential sphingolipids and glycerophospholipids was positively associated with clinical indexes that indicate the severity of disease, such as Gensini score, CK-MB, FFA, and UA.

In a word, we came up with a new concept of "alcohol modulation through gut microbiome" in human cardiovascular health. It was found that alcohol consumption impacted many diseases causally, including ischemic heart disease, hypertensive heart disease, and stroke (86). We speculated that a moderate amount of alcohol consumption may modulate the intestinal microecology and serum metabolites, which may play an important role in the amelioration of CAD. Alcohol consumption should be taken into considerations as an important modulator in the cardiovascular system. However, there is still a need for functional validation and exploration studies to verify the cause-and-effect relationship in the future.

Several limitations of the study need to be acknowledged. First, although the clinical features of our cohort were consistent with common patients with CAD, the sample size was relatively small. Second, the study population only included men; hence, it was uncertain whether the finding could apply to women with CAD as well. Third, untargeted metabolomics had limited accuracy in the annotation of serum metabolites. Future work needs to be done to establish the exact relationship between moderate drinking and these alterations in serum metabolites and gut microbiota.

## CONCLUSIONS

In general, our study provided a novel insight into the effect of moderate alcohol consumption on cardiovascular health by affecting gut microbiota and serum metabolome. The impact on metabolites and microbiota in patients with CAD with moderate drinking seems to be separated from those in patients with CAD with heavy drinking or non-drinking. Drinking moderately may have more positive effects on the metabolic profiles and commensal flora of patients with CAD, which may explain how moderate drinking affects cardiovascular health.

## DATA AVAILABILITY STATEMENT

The dataset supporting the results of this article has been deposited in the Sequence Read Archive under BioProject accession code SRP167862.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Review Board at the Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

XZ, RZ, and XH conceived and designed the study and wrote the manuscript. RZ and HL contributed to the bioinformatics analysis and made the tables and figures. YF and YS conducted the literature search. HL contributed to the collections of samples and data acquisition. XH and SZ critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.767692/full#supplementary-material

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# Assessment of the $CHA_2DS_2$-VASc Score for the Prediction of Death in Elderly Patients With Coronary Artery Disease and Atrial Fibrillation

*Yangxun Wu[1,2†], Guanyun Wang[1,2†], Lisha Dong[1†], Liu'an Qin[1,2], Jian Li[2], Hengming Yan[2], Wenjie Guo[2], Xiaodong Feng[2], Yuting Zou[1,2], Ziqian Wang[1,2], Rina Du[2], Yuxiao Zhang[2], Jing Ma[2*] and Tong Yin[1,2*]*

[1] Institute of Geriatrics, National Clinical Research Center for Geriatric Diseases, 2nd Medical Center, Medical School of Chinese People's Liberation Army and Chinese People's Liberation Army General Hospital, Beijing, China, [2] Department of Cardiology, 1st Medical Center, Medical School of Chinese PLA and Chinese PLA General Hospital, Beijing, China

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Yan Zhang,
Peking University, China

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Jingyi Ren,
China-Japan Friendship Hospital, China
Junmeng Zhang,
Tsinghua University, China

***Correspondence:**
Tong Yin
yintong301@163.com
Jing Ma
crystalma@126.com

[†] These authors have contributed equally to this work and share first authorship

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**Purpose:** Coronary artery disease (CAD) and atrial fibrillation (AF) often coexist and lead to a much higher risk of mortality in the elderly population. The aim of this study was to investigate whether the $CHA_2DS_2$-VASc score could predict the risk of death in elderly patients with CAD and AF.

**Methods:** Hospitalized patients aged ≥65 years with a diagnosis of CAD and AF were recruited consecutively. Patients were divided into 5 groups according to the $CHA_2DS_2$-VASc score (≤2, =3, =4, =5, and ≥6). At least a 1-year follow-up was carried out for the assessment of all-cause death.

**Results:** A total of 1,579 eligible patients were recruited, with 582 all-cause deaths (6.86 per 100 patient-years) occurring during a follow-up of at least 1 year. With the increase in the $CHA_2DS_2$-VASc score, the 1-year and 5-year survival rate decreased (96.4% vs. 95.7% vs. 94.0% vs. 86.5% vs. 85.7%, respectively, $P < 0.001$; 78.4% vs. 68.9% vs. 64.6% vs. 55.5% vs. 50.0%, respectively, $P < 0.001$). Compared with the patients with $CHA_2DS_2$-VASc score <5, for patients with $CHA_2DS_2$-VASc score ≥5, the adjusted hazard ratio for death was 1.78 (95% CI: 1.45–2.18, $P < 0.001$). The predictive values of the $CHA_2DS_2$-VASc score ≥5 for in-hospital (C-index = 0.66, 95% CI: 0.62–0.69, $P < 0.001$), 1-year (C-index = 0.65, 95% CI: 0.63–0.67, $P < 0.001$) and 5-year (C-index = 0.60, 95% CI: 0.59–0.61, $P < 0.001$) death were in comparable.

**Conclusion:** In elderly patients with concomitant CAD and AF, the $CHA_2DS_2$-VASc score can be used to predict death with moderate accuracy.

**Keywords: elderly, atrial fibrillation, coronary artery disease, death, $CHA_2DS_2$-VASc score**

## INTRODUCTION

Coronary artery disease (CAD) is the most common cardiovascular disease, while atrial fibrillation (AF) is the most common cardiac arrhythmia (1). The prevalence of both CAD and AF increases monotonically with age (2, 3). CAD and AF often coexist and interact with each other (4). CAD is a leading cause of morbidity and mortality in elderly adults (5). Elderly patients are more likely than

their younger counterparts to present with comorbidities (6–9), contributing to worse outcomes. Patients with AF are relatively older and have higher risk of stroke, which may contribute to increased mortality (10–16). Furthermore, AF is a well-established marker of poor short- and long-term prognosis in patients with acute myocardial infarction (AMI) (11, 12, 17, 18) and is associated with a large increase in overall mortality (15, 19). Therefore, the coexistence of CAD and AF might lead to a much higher risk of mortality in the elderly population.

The $CHA_2DS_2$-VASc score [congestive heart failure, hypertension, age ≥75 years (doubled), diabetes, stroke/transient ischemic attack/thromboembolism (doubled), vascular disease (prior myocardial infarction, peripheral artery disease, or aortic plaque), age 65–74 years, sex category (female)] has been used for the assessment of thromboembolic (TE) risk and the guidance of antithrombotic treatment in patients with AF (20). In addition, this simple and well-established scoring system has been shown to predict the risk for other conditions beyond its original designations (20–26).

In the presence of comorbidities of CAD and AF, advancing age further elevates the risk of TE complications and death. Evaluating the risk of death from TE in an elderly population with CAD and AF is important, because a competing-risk setting taking careful consideration of the interplay between the mortality of elderly individuals with CAD and AF, and mortality of ischemic stroke/TE is needed to provide meaningful risk assessments. However, how to assess the relationship between the mortality of ischemic stroke/TE and the high mortality of the elderly population is still unclear. Therefore, we aimed to evaluate whether the $CHA_2DS_2$-VASc score can predict the risk of death in elderly CAD and AF patients and be used as an indicator of treatment and prognosis.

# METHODS

## Patients

From January 2010 to December 2017, patients aged ≥65 years with a diagnosis of both CAD and AF who were hospitalized in the Department of Cardiology, Chinese PLA General Hospital, were recruited consecutively. This study complied with the Declaration of Helsinki and was approved by the institutional ethics committee of the General Hospital of the People's Liberation Army. CAD was defined as stable coronary artery disease (SCAD, including stable angina, previous myocardial infarction and ischemic cardiomyopathy) and acute coronary syndrome (ACS, including unstable angina and acute myocardial infarction). AF was defined as an irregular rhythm recorded in a standard 12-lead electrocardiogram, including discrete P waves and their replacement with irregular chaotic oscillatory atrial activity (F waves) in the setting of irregular QRS complexes. Body mass index (BMI) was categorized according to the distribution of BMI among the patients and the WHO criteria (27). In line with the epidemiological evidence, a BMI from 22 to <25 $kg/m^2$ was used as the reference group. On defining the different classification of HF, we summarized the left ventricular ejection fraction (LVEF) data of the included CAD and AF patients. Patients with HF were stratified into 3 groups according to the criteria that LVEF <40% represents heart failure with reduced ejection fraction (HFrEF), LVEF ≥50% represents heart failure with preserved ejection fraction (HFpEF), and LVEF ranges from 40 to 50 represents heart failure with mid-range ejection fraction (HFmrEF).

## Data Collection, Follow-Up and Death Definitions

Baseline demographics and clinical characteristics in the hospital were extracted from the electronic health records system. The components of the $CHA_2DS_2$-VASc score were collected to retrospectively assess the risk of mortality. The $CHA_2DS_2$-VASc score was calculated as congestive heart failure (1 point), aged 65–74 years (1 point), hypertension (1 point), diabetes (1 point), vascular disease (prior myocardial infarction, peripheral artery disease, or aortic plaque; 1 point), female sex (1 point), aged 75 years or older (2 points) and stroke/transient ischemic attack/thromboembolism (2 points) (28, 29).

Participants were followed-up until Dec 31st, 2019. The follow-up protocol included a combination of hospital medical record reviews, telephone contacts with patients or family members and death certificate reviews. All deaths were independently adjudicated in a blinded manner by 2 members of the event adjudication committee. All-cause death was classified using the tenth revision of the International Classification of Disease and confirmed through death certificates using personal identity card numbers. Cardiac death was defined as death attributable to fatal myocardial infarction, sudden cardiac death or stroke. Apart from cardiac death, non-cardiac death included deaths from malignancies, infections, respiratory, trauma/accidental or other non-vascular deaths. If the cause of death could not be determined from the available evidence, death was classified as undetermined.

## Statistical Analysis

The patients were divided into $CHA_2DS_2$-VASc score groups (≤2, =3, =4, =5, and ≥6) according to whether they had died by the end of follow-up. Baseline demographic and clinical characteristics were summarized using medians and interquartile ranges (IQRs) for continuous measures and percentages for categorical measures. The comparison of the data was performed using the chi-square test for categorical variables and Mann–Whitney *U*-test for continuous variables. Univariate and multivariate Cox regression models were used to explore the risk factors associated with mortality. According to the $CHA_2DS_2$-VASc score, Kaplan–Meier curves with the log-rank test were used to compare survival. The calibration of the $CHA_2DS_2$-VASc score was assessed with the Hosmer-Lemeshow goodness-of-fit test (HL), which may determine the degree of agreement between the observed event rate and the predicted one over a series of scores. A significant value of $P < 0.05$ represents a lack of fit. The concordance index (C-index) was conducted to determine the discrimination of and the diagnostic value of the $CHA_2DS_2$-VASc score for death. All analysis were performed with the R 4.0.1 Statistical Package (the R foundation for Statistical Computing, Vienna, Austria) and SPSS v.24.0 (Statistical Package for Social Science; IBM, Chicago, IL, USA).

# RESULTS

## Baseline Characteristics

Follow-up data were available for 1,579 patients (with a total of 1,579 patients for 1-year and 910 for 5-year follow-up). The baseline characteristics according to the $CHA_2DS_2$-VASc score are shown in **Table 1**. The mean $CHA_2DS_2$-VASc score was 4.3 ± 1.6 (median 4.0, interquartile range 3.0–5.0). We divided the cohort into five quintiles based on the $CHA_2DS_2$-VASc score: ≤2 ($n = 192$), 3 ($n = 327$), 4 ($n = 384$), 5 ($n = 326$), and ≥6 ($n = 350$) (**Figure 1**). Patients with a higher $CHA_2DS_2$-VASc score were more likely to be women, older and with cardiovascular diseases, such as AMI and heart failure (HF). The proportion of patients with HF was 38.3% (602/1579) in the whole study. Among the patients with HF, LVEF data were retrieved in 581 patients, with 114 of HFrEF (19.6%), 81 of HFmrEF (13.9%) and 386 of HFpEF (66.4%). The rate of comorbidities, such as hypertension, diabetes, prior transient ischemic attack (TIA)/stroke, peripheral arterial disease (PAD), chronic kidney disease (CKD) and HF, increased with the increasing $CHA_2DS_2$-VASc score. In terms of treatment, diuretics, calcium channel blockers (CCBs) and renin angiotensin system inhibitors (RASI) were used more frequently in elderly patients with AF and CAD with a higher $CHA_2DS_2$-VASc score (**Table 1**). The application of oral anticoagulants

**TABLE 1 |** Baseline characteristics of elderly patients with CAD and AF according to the $CHA_2DS_2$-VASc score.

| Characteristics | $CHA_2DS_2$-VASc ≤2 ($n = 192$) | $CHA_2DS_2$-VASc = 3 ($n = 327$) | $CHA_2DS_2$-VASc = 4 ($n = 384$) | $CHA_2DS_2$-VASc = 5 ($n = 326$) | $CHA_2DS_2$-VASc ≥6 ($n = 350$) | *P*-value |
|---|---|---|---|---|---|---|
| **Demographics** | | | | | | |
| Age, yrs, median (IQR) | 72 (67–78) | 76 (71–81) | 77 (73–83) | 79 (75–84) | 80 (77–84) | <0.001 |
| Male, *n* (%) | 179 (90.2) | 231 (70.6) | 204 (53.1) | 137 (42.0) | 145 (41.4) | <0.001 |
| BMI, kg/m$^2$, median (IQR) | 24 (22–27) | 25 (23–27) | 25 (23–27) | 25 (22–27) | 24 (22–27) | 0.143 |
| **Medical history, *n* (%)** | | | | | | |
| Hypertension | 65 (33.9) | 239 (73.1) | 289 (75.3) | 273 (83.7) | 318 (90.9) | <0.001 |
| Diabetes | 4 (2.1) | 47 (14.4) | 106 (27.6) | 131 (40.2) | 195 (55.7) | <0.001 |
| Previous myocardial infarction | 2 (1.0) | 23 (7.0) | 47 (12.2) | 54 (16.6) | 71 (20.3) | <0.001 |
| Prior TIA/stroke | 0 (0.0) | 5 (1.5) | 51 (13.3) | 103 (31.6) | 276 (78.9) | <0.001 |
| Peripheral arterial disease | 3 (1.6) | 32 (9.8) | 73 (19.0) | 88 (27.0) | 134(38.3) | <0.001 |
| COPD | 6 (3.1) | 10 (3.1) | 21 (5.5) | 11 (3.4) | 10 (2.9) | 0.314 |
| Hyperlipidemia | 46 (24.0) | 62 (19.0) | 74 (19.3) | 73 (22.4) | 90 (25.7) | 0.152 |
| Chronic kidney disease | 6 (3.1) | 26 (8.0) | 44 (11.5) | 42 (12.9) | 47 (13.4) | 0.001 |
| Liver disease | 16 (8.3) | 31 (9.5) | 35 (9.1) | 23 (7.1) | 31 (8.9) | 0.829 |
| Malignancy | 24 (12.5) | 30 (9.2) | 49 (12.8) | 41 (12.6) | 50 (14.3) | 0.361 |
| **Clinical presentation, *n* (%)** | | | | | | |
| SCAD | 104 (54.2) | 188 (57.5) | 217 (56.5) | 171 (52.5) | 177 (50.6) | 0.342 |
| ACS | 88 (45.8) | 139 (42.5) | 167 (43.5) | 155 (47.5) | 173 (49.4) | 0.342 |
| Unstable angina | 87 (45.3) | 130 (39.8) | 144 (37.5) | 125 (38.3) | 121 (34.6) | 0.170 |
| Acute myocardial infarction | 1 (0.5) | 11 (3.4) | 27 (7.0) | 31 (9.5) | 54 (15.4) | <0.001 |
| Heart failure | 15 (7.8) | 54 (16.5) | 152 (39.6) | 159 (48.8) | 222 (63.4) | <0.001 |
| **In-hospital treatment, *n* (%)** | | | | | | |
| Diuretic | 56 (29.2) | 150 (45.9) | 230 (59.9) | 217 (66.6) | 264 (75.4) | <0.001 |
| Statins | 162 (84.4) | 275 (84.1) | 315 (82.0) | 276 (84.7) | 297 (84.9) | 0.842 |
| CCB | 57 (29.7) | 192 (58.7) | 206 (53.6) | 187 (57.4) | 226 (64.6) | <0.001 |
| β-blockers | 147 (76.6) | 246 (75.2) | 298 (77.6) | 263 (80.7) | 272 (77.7) | 0.566 |
| RASI | 56 (29.2) | 187 (57.2) | 218 (56.8) | 207 (63.5) | 232 (66.3) | <0.001 |
| **Antiplatelet therapy** | | | | | | |
| Aspirin | 162 (84.4) | 249 (76.1) | 289 (75.3) | 242 (74.8) | 259 (74.0) | 0.074 |
| $P2Y_{12}$ receptor inhibitors | 126 (65.6) | 198 (60.6) | 237 (61.7) | 191 (58.6) | 224 (64.0) | 0.471 |
| **Anticoagulation** | | | | | | |
| Warfarin | 26 (13.5) | 59 (18.0) | 68 (17.7) | 59 (18.1) | 54 (15.4) | 0.575 |
| NOACs | 28 (14.6) | 42 (12.8) | 46 (12.0) | 40 (12.3) | 29 (8.3) | 0.197 |
| Amiodarone | 64 (33.3) | 96 (29.4) | 83 (21.6) | 79 (24.2) | 98 (28.0) | 0.740 |
| PCI with drug-eluting stent | 24 (12.5) | 36 (11.0) | 50 (13.0) | 30 (9.2) | 30 (8.6) | 0.264 |

*Data are presented as the median (IQR), n (%), or n/N (%). CAD, Coronary artery disease; AF, Atrial fibrillation; BMI, Body mass index; TIA, Transient ischemic attack; COPD, Chronic obstructive pulmonary disease; SCAD, Stable coronary artery disease; ACS, Acute coronary syndrome; CCB, Calcium channel blocker; RASI, Renin angiotensin system inhibitors; NOACs, Nonvitamin K antagonist oral anticoagulants; PCI, Percutaneous coronary intervention.*

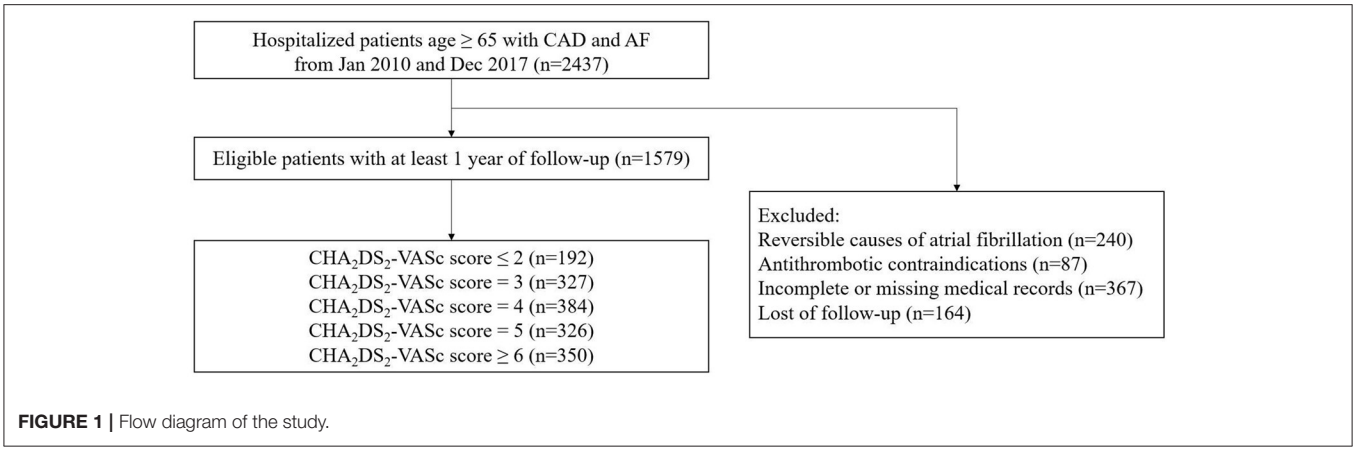


**FIGURE 1 |** Flow diagram of the study.

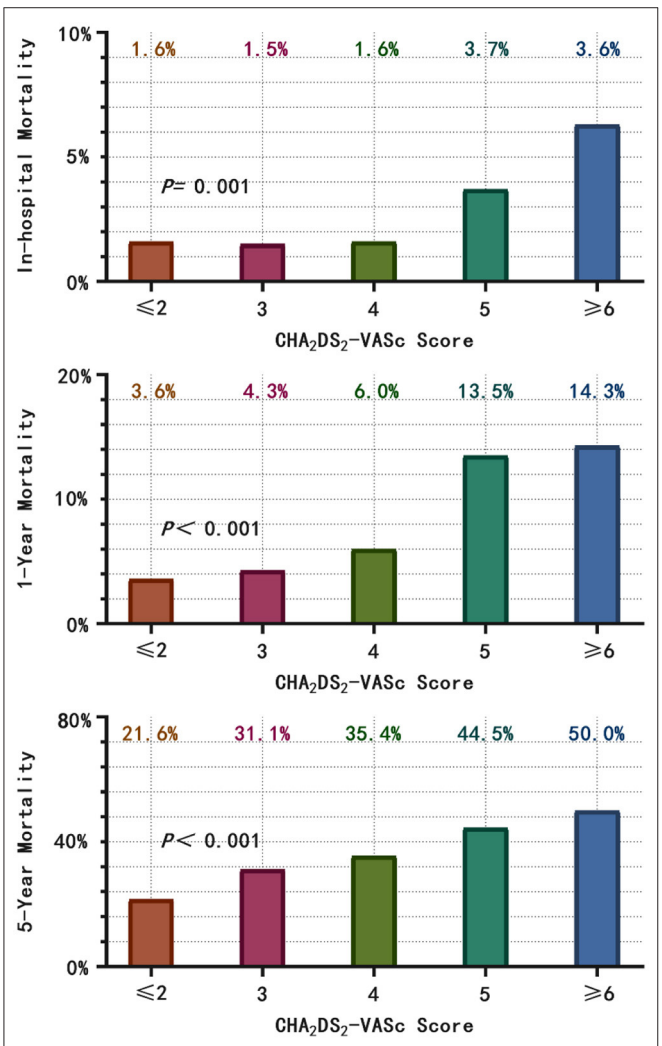


**FIGURE 2 |** According to the $CHA_2DS_2$-VASc score category, the in-hospital, 1- and 5-year all-cause mortality were calculated. With the increase in $CHA_2DS_2$-VASc score, the in-hospital, 1- and 5-year all-cause mortality increased.

(OACs) and oral antiplatelets (aspirin and $P2Y_{12}$ inhibitors) in all patients were 27.1% and 83.3%, respectively. With the increase of $CHA_2DS_2$-VASc score, no significant difference was found for the proportion of patients administrated with OACs ($CHA_2DS_2$-VASc score $\leq$2: 27.1%, score = 3: 28.4%, score = 4: 29.2%, score = 5: 27.3%, and score $\geq$6: 23.4%, $P$ = 0.475) or oral antiplatelet (score $\leq$2: 85.9%, score = 3: 81.3%, score = 4: 81.8%, score = 5: 82.8%, and score $\geq$6: 85.7%, $P = 0.404$).

## Association Between $CHA_2DS_2$-VASc Score and Mortality

A total of 582 patients died, with a mortality of 6.86 per 100 patient-years. The causes of death were cardiovascular in 152 patients (26.1%), non-cardiovascular in 247 patients (42.5%) and undetermined in 183 patients (31.4%). The in-hospital, 1-year and 5-year all-cause mortality were 3.0%, 8.7%, and 24.4%, respectively, and higher $CHA_2DS_2$-VASc scores were associated with a significantly higher mortality (**Figure 2**). The survival rate according to Kaplan–Meier analysis suggested that with the increase in the $CHA_2DS_2$-VASc score, the 1-year and 5-year survival rates decreased (96.4% vs. 95.7% vs. 94.0% vs. 86.5% vs. 85.7%, respectively, $P < 0.001$; 78.4% vs. 68.9% vs. 64.6% vs. 55.5% vs. 50.0%, respectively, $P < 0.001$) (**Figure 3**).

Compared with the patients with $CHA_2DS_2$-VASc scores <5, the patients with $CHA_2DS_2$-VASc scores $\geq$5 had higher risk of death (HR: 2.01, 95% CI: 1.65–2.45, $P < 0.001$). Multivariable Cox regression analysis demonstrated that $CHA_2DS_2$-VASc score $\geq$5 could independently predict mortality with the adjustment of the risk variables not included in the $CHA_2DS_2$-VASc score (adjusted HR: 1.78, 95% CI: 1.45–2.18, $P < 0.001$) (**Table 2**). Moreover, all types of HF could significantly predict the risk of death, with HFrEF contributing the most (adjusted HR for HFrEF: 2.06, 95%CI: 1.52–2.79, $P < 0.001$); for HFmrEF: 1.74, 95% CI: 1.24–2.45, $P = 0.001$; for HFpEF: 1.38, 95% CI: 1.13–1.69, $P$ = 0.002, respectively).

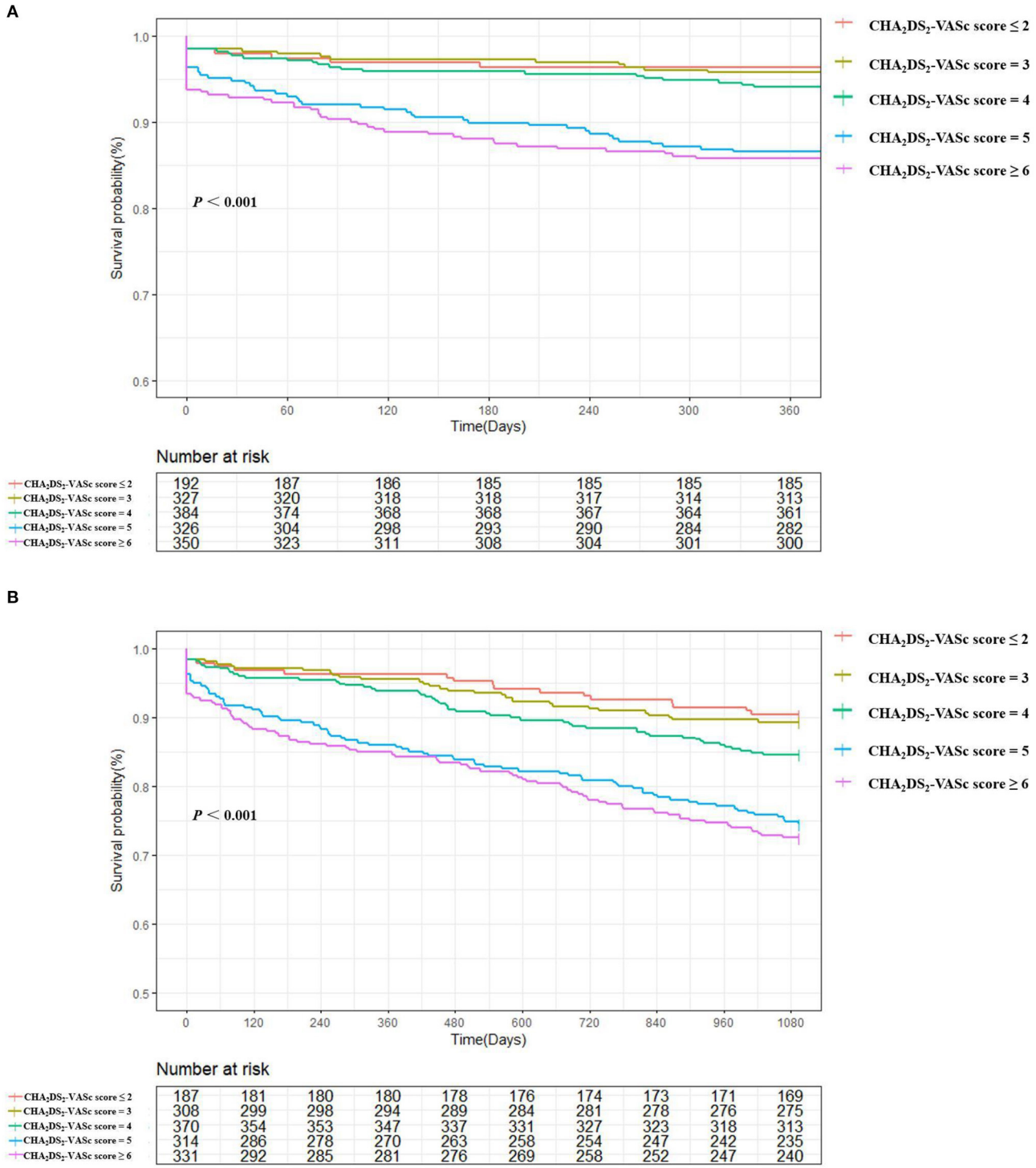


**FIGURE 3 |** 1-year **(A)** and 5-year **(B)** survival in based on Kaplan–Meier survival analysis according to $CHA_2DS_2$-VASc score: ≤2, =3, =4, =5, and ≥6. Increasing $CHA_2DS_2$-VASc scores were directly associated with reduced survival (by Log Rank Test).

## Prediction of the $CHA_2DS_2$-VASc Score for Mortality

In elderly patients with CAD and AF, $CHA_2DS_2$-VASc score ≥5 had a modest predictive ability for all-cause death in-hospital (C-index = 0.66, 95% CI: 0.62–0.69, $P < 0.001$), during 1-year (C-index = 0.65, 95% CI: 0.63–0.67, $P < 0.001$), and 5-year (C-index = 0.60, 95% CI: 0.59–0.61, $P < 0.001$) follow-ups. The diagnostic statistics for the $CHA_2DS_2$-VASc

**TABLE 2 |** Predictors of mortality in elderly patients with CAD and AF by Cox regression analysis.

| | Univariate analysis | | | #Multivariate analysis | | |
|---|---|---|---|---|---|---|
| **Variables** | **HR** | **95% CI** | ***P*** | **HR** | **95% CI** | ***P*** |
| Age | 1.09 | 1.07–1.10 | <0.001 | 1.08 | 1.06–1.09 | <0.001 |
| *BMI<18.5 | 1.84 | 1.34–2.51 | <0.001 | 1.72 | 1.25–2.35 | 0.001 |
| *BMI≥30 | 0.89 | 0.63–1.24 | 0.486 | 1.01 | 0.73–1.41 | 0.936 |
| Previous AMI | 1.62 | 1.31–1.99 | <0.001 | 1.33 | 1.08–1.65 | 0.008 |
| AMI | 2.44 | 1.90–3.13 | <0.001 | 2.08 | 1.62–2.67 | <0.001 |
| HF | 2.30 | 1.95–2.71 | <0.001 | 1.42 | 1.18–1.72 | <0.001 |
| HFrEF | 2.29 | 1.78–2.94 | <0.001 | 2.06 | 1.52–2.79 | <0.001 |
| HFmrEF | 2.13 | 1.57–2.89 | <0.001 | 1.74 | 1.24–2.45 | 0.001 |
| HFpEF | 1.54 | 1.29–1.84 | <0.001 | 1.38 | 1.13–1.69 | 0.002 |
| Diabetes | 1.27 | 1.07–1.51 | 0.006 | 1.11 | 0.92–1.33 | 0.296 |
| Prior TIA/stroke | 1.28 | 1.08–1.52 | 0.005 | 1.05 | 0.90–1.14 | 0.487 |
| PAD | 1.90 | 1.59–2.64 | <0.001 | 1.03 | 0.81–1.28 | 0.351 |
| CKD | 2.03 | 1.63–2.54 | <0.001 | 2.04 | 1.42–2.94 | <0.001 |
| COPD | 1.87 | 1.30–2.69 | 0.001 | 1.78 | 1.43–2.23 | <0.001 |
| Malignancy | 1.69 | 1.36–2.09 | <0.001 | 1.49 | 1.20–1.85 | <0.001 |
| $CHA_2DS_2$-VASc Score <5 | 1.0 (Reference) | 1.0 (Reference) | | | | |
| $CHA_2DS_2$-VASc Score ≥5 | 2.01 | 1.65–2.45 | <0.001 | †1.78 | 1.45–2.18 | <0.001 |

*Compared to the reference value of BMI 22 -<25 kg/m$^2$. #Adjusted by the risk factors with a statistically significant P-value <0.05 after the univariate analysis. †Adjusted by the risk factors not included in the $CHA_2DS_2$-VASc score with a statistically significant P value <0.05 after the univariate analysis. CI, Confidence interval; BMI, Body mass index; AMI, Acute myocardial infarction; HF, Heart failure; HFrEF, Heart failure with reduced ejection fraction; HFmrEF, Heart failure with mid-range ejection fraction; HFpEF, Heart failure with preserved ejection fraction; AF, Atrial fibrillation; TIA, Transient ischemic attack; PAD, Peripheral arterial disease; CKD, Chronic kidney disease; COPD, Chronic obstructive pulmonary disease.*

**TABLE 3 |** Statistics of the $CHA_2DS_2$-VASc Score for the prediction of death.

| | $CHA_2DS_2$-VASc score ≥ 5 | | | | | |
|---|---|---|---|---|---|---|
| | **HL-p** | **C-index (95% CI)** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **PPV (95% CI)** | **NPV (95% CI)** |
| All death | 0.28 | 0.60 (0.59–0.61) | 52.6 (48.4–56.7) | 67.1 (64.1–70.0) | 48.3 (44.3–52.2) | 70.8 (67.8–73.7) |
| In SCAD | 0.86 | 0.59 (0.57–0.60) | 48.2 (42.4–54.0) | 68.5 (64.4–72.3) | 45.0 (39.5–50.6) | 71.1 (67.1–74.9) |
| In ACS | 0.94 | 0.61 (0.60–0.63) | 57.2 (51.2–63.0) | 65.4 (60.7–70.0) | 51.6 (45.9–57.2) | 70.3 (65.6–74.7) |
| In hospital death | 0.57 | 0.66 (0.62–0.69) | 70.8 (55.7–82.6) | 58.1 (55.5–60.5) | 5.0 (3.6–7.0) | 98.4 (97.3–99.1) |
| In SCAD | 0.65 | 0.63 (0.58–0.68) | 62.5 (40.8–80.4) | 63.4 (60.0–66.6) | 4.7 (2.7–7.8) | 98.3 (96.7–99.2) |
| In ACS | 0.93 | 0.68 (0.64–0.73) | 79.2 (57.3–92.1) | 57.7 (54.0–61.4) | 6.1 (3.8–9.4) | 98.8 (97.0–99.5) |
| 1-year death | 0.97 | 0.65 (0.63–0.67) | 68.1 (59.6–75.6) | 59.6 (57.0–62.2) | 13.9 (11.5–16.8) | 95.1 (93.4–96.4) |
| In SCAD | 0.57 | 0.62 (0.58–0.65) | 59.4 (46.4–71.2) | 64.4 (61.0–67.8) | 11.9 (8.6–16.1) | 95.2 (92.9–96.8) |
| In ACS | 0.11 | 0.67 (0.64–0.70) | 75.3 (63.6–84.4) | 60.1 (56.2–63.9) | 17.5 (13.6–22.3) | 95.6 (93.0–97.3) |
| 5-year death | 0.51 | 0.60 (0.59–0.61) | 57.0 (51.9–62.0) | 60.0 (56.0–63.9) | 47.4 (42.8–52.1) | 68.8 (64.6–72.7) |
| In SCAD | 0.29 | 0.58 (0.57–0.60) | 51.1 (43.7–58.3) | 66.6 (62.8–70.1) | 30.3 (25.4–35.7) | 82.7 (79.2–85.7) |
| In ACS | 0.52 | 0.61 (0.60–0.63) | 60.7 (53.5–67.5) | 62.9 (58.6–67.0) | 37.9 (32.6–43.5) | 81.1 (76.9–84.7) |

*HL-p, Hosmer–Lemeshow goodness-of-fit test p-value; C-index, Concordance index; CI, Confidence interval; SCAD, Stable coronary artery disease; ACS, Acute coronary artery disease; PPV, Positive predictive value; NPV, Negative predictive value; CAD, Coronary heart disease; AF, Atrial fibrillation.*

score of in-hospital, 1-year death, 5-year death and all death are displayed in **Table 3**. We also performed the internal validation by dividing the patients into those with SCAD and those with ACS. The performance of the $CHA_2DS_2$-VASc score ≥5 was comparable between the patients with SCAD and ACS for predicting in-hospital death, 1-year or 5-year death, with a moderate higher C-index in ACS patients (**Table 3**).

## DISCUSSION

In this cohort study, the main findings were that (1) the $CHA_2DS_2$-VASc score was a significant predictor of death in elderly patients with CAD and AF, and the mortality generally increased with the increasing $CHA_2DS_2$-VASc score, exhibiting a clear dose-response relationship; (2) $CHA_2DS_2$-VASc score independently and strongly predicted the in-hospital, 1- and

5-year death in elderly patients with CAD and AF. To our knowledge, this is the first study to evaluate the predictive ability of the $CHA_2DS_2$-VASc score for death in elderly patients with CAD and AF. This study could facilitate risk stratification and improve the prevention of death associated with comorbid CAD and AF in elderly patients.

AF is the most common arrhythmia, with high incidence and prevalence, and is associated with an increased risk of all-cause death and stroke (30, 31). CAD, especially AMI, will also lead to other complications and increase the risk of death (32). Age is an obvious risk factor for patients with CAD and AF (31, 33), and elderly individuals are more likely to have coexisting CAD and AF, thus the risk of death in elderly patients with CAD and AF is higher. In addition to the assessment of thromboembolic risk in patients with AF, the $CHA_2DS_2$-VASc score has been shown to predict the adverse outcomes for other cardiovascular conditions, such as chest pain (25), ACS (21, 22), AMI (24), HF (20), pulmonary emboli (23), and ACS undergoing percutaneous coronary intervention (PCI) (26). Therefore, we believe that the $CHA_2DS_2$-VASc score is a feasible predictor of prognosis in elderly patients with CAD and AF. The sensitivity of the $CHA_2DS_2$-VASc score was higher than specificity for the prediction of death in-hospital or within 1-year. It indicated that the $CHA_2DS_2$-VASc score could effectively evaluate the mortality of elderly patients with CAD and AF in-hospital or within 1 year follow-up in the study. When the $CHA_2DS_2$-VASc score is $\geq$5, the probability of death within 1 year will increase significantly. Additionally, with the increase in the $CHA_2DS_2$-VASc score, the in-hospital, 1- and 5-year mortality also increased. These results indicate that the $CHA_2DS_2$-VASc score could predict the prognosis of elderly patients with CAD and AF.

The risk factors for death in elderly patients with CAD and AF were assessed for the first time in this cohort. We found that in addition to the $CHA_2DS_2$-VASc score, the independent risk factors for all-cause death in elderly patients with CAD and AF included BMI $<$18.5 kg/m$^2$, previous or current AMI, CKD, COPD, and malignancy. Similar risk factors for death were also found in AF patients in the ROCKET-AF study (34) and the GARFIELD-AF global prospective registry (35), suggesting that overall mortality due to AF is tightly linked to the same risk factors and comorbidities. Our results also emphasized the prognostic importance of underweight (BMI $<$18.5 kg/m$^2$) in the elderly population. We found that BMI $<$18.5 kg/m$^2$ was a significant predictor of overall mortality, which probably reflects the known association between a decrease in BMI and an increase in mortality in CAD patients, regardless of the baseline BMI value (36). Further studies are needed to better understand the impact of the combination of risk factors on mortality in the elderly population.

The combination of AF and CAD is a common and complex clinical condition in which to address anticoagulation therapy (37), especially in elderly patients. Taking OACs can reduce the risk of embolism, but it also increases the risk of bleeding. Therefore, whether to take OACs should be judged by the patient's health situation (38). If AF develops during the first year after ACS and there is an indication for thromboembolic prevention with anticoagulation, OACs should be started. In stable CAD patients with AF, oral anticoagulation is necessary when the $CHA_2DS_2$-VASc score is $\geq$2 (39). Elderly patients requiring anticoagulation for AF are at higher risk of adverse outcomes, but also have a higher absolute benefit from OAC (40). However, in our study, the application rate of OACs was only 27.1%, and it did not increase with the $CHA_2DS_2$-VASc score, suggesting that the application of OACs in elderly patients with CAD and AF was not sufficient, which might attribute to the increasing risk of death in patients with higher value of $CHA_2DS_2$-VASc score. Previous studies have found that the application of OACs in the elderly population was insufficient (41). Therefore, the use of OACs in elderly patients with CAD and AF should be increased, and further studies are needed to verify whether the application of OAC could decrease the mortality in these patients.

Several limitations of this study warrant consideration. The present study was an observational real-world study in which we did not exclude severely ill patients (who are typically excluded from clinical trials), thus, the mortality in elderly patients with CAD and AF might be higher than expected in clinical trials. Data on the clinical parameters and drug therapies were obtained from electronic health record. Although the data was validated and found to be highly accurate, not all clinical characteristics could be verified. While we tried to make adjustment for the clinically relevant parameters, it is impossible to adjust for all variables that may affect the outcomes. In addition, the study was based on a single-center cohort, and the findings should be validated in large multicenter cohorts.

## CONCLUSION

The $CHA_2DS_2$-VASc score could independently predict all-cause death in the elderly patients with concomitant CAD and AF.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethics Committee of the General Hospital of the People's Liberation Army. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

TY, JM, and YZha: study concept and design. YW, JL, LD, LQ, HY, WG, XF, GW, ZW, RD, and YZou: acquisition of data. GW, YW, LQ, LD, and TY: analysis and interpretation of data.

GW, YW, LD, LQ, and TY: drafting of the manuscript. TY and JM: critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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# Alterations of Gut Microbiome and Serum Metabolome in Coronary Artery Disease Patients Complicated With Non-alcoholic Fatty Liver Disease Are Associated With Adverse Cardiovascular Outcomes

*Xiaomin Hu[1*†], Ruilin Zhou[2†], Hanyu Li[2], Xinyue Zhao[2], Yueshen Sun[2], Yue Fan[2] and Shuyang Zhang[2*]*

[1] Department of Cardiology, Department of Medical Research Center, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China, [2] Department of Cardiology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

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**Edited by:**
Yan Zhang,
Peking University, China

**Reviewed by:**
Ying Song,
Peking University Third Hospital, China
Yuxuan Guo,
Peking University, China

***Correspondence:**
Xiaomin Hu
huxiaomin2015@163.com
Shuyang Zhang
shuyangzhang103@nrdrs.org

[†] These authors have contributed equally to this work

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**Rationale:** Patients suffering from coronary artery disease (CAD) complicated with nonalcoholic fatty liver disease (NAFLD) present worse cardiovascular outcomes than CAD patients without NAFLD. The progression of CAD is recently reported to be associated with gut microbiota and microbe-derived metabolites. However, it remains unclear how the complication of NAFLD will affect gut microbiota and microbe-derived metabolites in CAD patients, and whether or not this interplay is related to the worse cardiovascular outcomes in CAD-NAFLD patients.

**Methods:** We performed 16S rRNA sequencing and serum metabolomic analysis in 27 CAD patients with NAFLD, 81 CAD patients without NAFLD, and 24 matched healthy volunteers. Predicted functional profiling was achieved using PICRUSt2. The occurrence of cardiovascular events was assessed by a follow-up study. The association of alterations in the gut microbiome and metabolome with adverse cardiovascular events and clinical indicators was revealed by Spearman correlation analysis.

**Results:** We discovered that the complication of NAFLD was associated with worse clinical outcomes in CAD patients and critical serum metabolome shifts. We identified 25 metabolite modules that were correlated with poor clinical outcome in CAD-NAFLD patients compared with non-NAFLD patients, represented by increased cardiac-toxic metabolites including prochloraz, brofaromine, aristolochic acid, triethanolamine, and reduced potentially beneficial metabolites including estradiol, chitotriose, palmitelaidic acid, and moxisylyte. In addition, the gut microbiome of individuals with CAD-NAFLD was changed and characterized by increased abundances of *Oscillibacter ruminantium* and *Dialister invisus,* and decreased abundances of *Fusicatenibacter saccharivorans*, *Bacteroides ovatus* and *Prevotella copri*. PICRUSt2 further confirmed an increase of potential pathogenic bacteria in CAD-NAFLD. Moreover, we found that variations of gut

microbiota were critically correlated with changed circulating metabolites and clinical outcomes, which revealed that aberrant gut microbiota in CAD-NAFLD patients may sculpt a detrimental metabolome which results in adverse cardiovascular outcomes.

**Conclusions:** Our findings suggest that CAD patients complicated with NAFLD result in worse clinical outcomes possibly by modulating the features of the gut microbiota and circulating metabolites. We introduce "liver-gut microbiota-heart axis" as a possible mechanism underlying this interrelationship. Our study provides new insights on the contribution of gut microbiota heterogeneity to CAD-NAFLD progression and suggests novel strategies for disease therapy.

**Keywords: coronary heart disease, non-alcoholic fatty liver disease, microbiome, metabolome, cardiovascular outcomes**

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a leading contributor to the growing burden of chronic liver disease globally (1, 2). NAFLD begins with the aberrant accumulation of triglycerides in the liver, which in some individuals elicits an inflammatory response that can progress to cirrhosis and liver cancer (3). Moreover, NAFLD causes considerable liver-related and extrahepatic morbidity and mortality worldwide (4, 5). The increasing prevalence of NAFLD has been associated with obesity (6), type 2 diabetes mellitus (T2DM) (7), hyperlipidemia, hypertension (HTN) (7), and elevated liver enzymes, namely, alanine aminotransferase (ALT) (6) and γ-glutamyl transferase (GGT) (8). The strong association of fatty liver disease with metabolic syndrome has stimulated interest in the putative role of fatty liver disease in the progression of cardiovascular disease. Accumulating clinical and epidemiological evidence indicates that NAFLD is associated with an increased risk of cardiovascular disease and that NAFLD dictates the outcome of patients with coronary artery disease (CAD) more frequently and to a greater extent than does the progression of CAD itself (9–11). NAFLD detected with ultrasonography is strongly associated with increased intimal medial thickness of the carotid artery and an increased prevalence of carotid atherosclerotic plaques (12–14). Moreover, CAD patients complicated with NAFLD had a significantly higher 10-year risk of cardiovascular events than those with CAD alone (15). Recently, NAFLD has been proposed to be an independent risk factor for CAD (16). However, to our knowledge, the nature and extent of the associations between NAFLD and adverse outcomes in CAD patients is not clear.

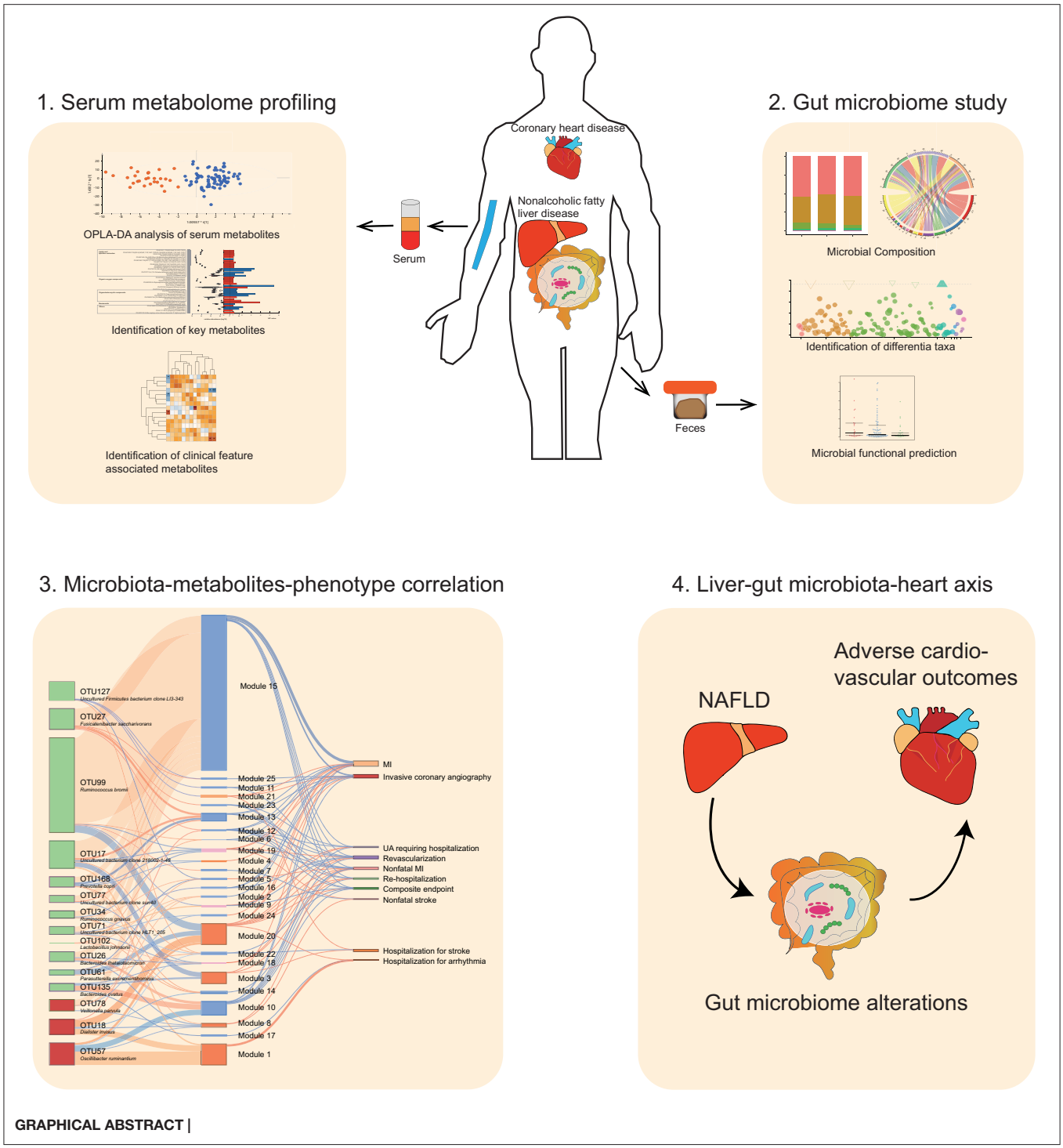
Recent work has begun to elucidate the potential contribution of NAFLD to CAD progression and outcome. Some evidence suggests that NAFLD might play a part in the pathogenesis of cardiovascular disease through the systemic release of several inflammatory (17), hemostatic, and oxidative stress mediators or by contributing to atherogenic dyslipidemia (11). Recent years, with the exploration of liver-gut microbiome axis (18), the potential role of gut microbiomes in liver disease was paid attention on. Recent studies showed that NAFLD and its severity were associated with a high abundance of inflammatory bacterial products (19). Species belonging to the family *Enterobacteriaceae* and the genera *Streptococcus* and *Gallibacterium* were enriched in NAFLD (17). One research showed significantly increased *Copococcus* and *Veillonella parvula* abundance and significantly decreased *Ruminococcus gnavus* and *Bacteroides dorei* abundance in CAD-NAFLD patients compared with CAD patients without NAFLD (20). These studies have provided evidence that gut microbiota might contribute to the progression of CAD-NAFLD. However, the relationship between gut microbiota, circulating metabolites and the prognosis of CAD-NAFLD patients remained ambiguous.

To address the questions above, we performed a multi-omics study on 108 CAD patients (CAD-NAFLD group $N = 27$ and CAD group $N = 81$) and 24 healthy controls. We conducted 16S rRNA sequencing to analyze the gut microbial characteristics, untargeted liquid chromatography-mass spectrometry (LC-MS) to analyze the serum metabolic profiles and a follow-up study to reveal the outcomes of the patients. The objectives of this study were to identify the specific features of the gut microbiota and host metabolite profiles in CAD-NAFLD patients, and reveal the interactions between gut microbiome, host circulating metabolites, and incidence of cardiovascular events.

## METHODS

### Study Participants and Sample Collection

We consecutively recruited 24 healthy volunteers and 108 CAD patients who were hospitalized for coronary angiography at Peking Union Medical College Hospital (PUMCH). Patients who exhibited $\geq$ 50% stenosis in at least one main coronary artery were diagnosed with CAD. The 108 CAD patients were further split into the following two subgroups basing on whether complicated with NAFLD or not: (1) CAD ($N = 81$) and (2) CAD-NAFLD ($N = 27$). CAD-NAFLD patients are defined as CAD patients who were also diagnosed with NAFLD. For the diagnosis of CAD and NAFLD, please refer to our previous study (21) and **Supplementary Materials**, respectively. For controls, we enrolled subjects who were identified as having no CAD-related clinical signs or symptoms. Subjects were excluded if they had gastrointestinal diseases, malignant tumors, autoimmune disorders, infectious diseases, renal dysfunction (creatinine $>$ 3.0 mg/dl), a history of gastrointestinal surgery in the previous year




**GRAPHICAL ABSTRACT |**

or were administered antibiotics for more than 3 days in the previous 3 months.

Peripheral venous blood was collected from the participants in the morning of the day after admission, and all clinical information was collected. Participants were given a stool sampler and provided detailed illustrated instructions for sample collection. Freshly collected stool samples from each participant were immediately transported to the laboratory and immediately frozen at−80°C. The detailed method for blood collection, stool collection and clinical information collection methods refer to

our previous study (21). The study was performed in accordance with the principles of the Declaration of Helsinki. Subjects provided written, informed consent for participation in the study.

## Follow-Up Study

Patient follow-up was conducted when they were reviewed in the clinic or through telephone interviews with patients/their close family members. The composite endpoint of this study consisted of all-cause mortality and/or reoccurrence of myocardial infarction (MI) and/or stroke and/or readmission for major adverse cardiac events (MACEs). New hospitalization and/or all-cause mortality was identified with the electronic medical record system of PUMCH or interviews with the patient (or a family member) in cases of events outside of PUMCH. Binary logistic regression analysis was utilized to explore the relationship between the complications of NAFLD and the outcome of CAD patients after adjusting for potential confounding factors. Statistical analysis was performed using SPSS statistics software (v24.0, SPSS Inc., Chicago, IL, USA). The results of binary logistic regression were visualized as forest plots using the R package ggplot2.

## Untargeted Metabolomics Study

Sample analysis was performed by a Waters ACQUITY ultra-high-performance liquid chromatography system (Milford, MA, USA) coupled with a Waters Q-TOF Micromass system (Manchester, UK). Sample analysis was performed in both positive and negative ionization modes. The detailed procedures for sample preparation, LC-MS (Liquid chromatography-mass spectrometry) experiments, and peak-ion intensity matrix preparation were described in our previous publication (21). The matrix was further reduced by removing peaks with missing values in more than 80% of the samples and those with isotope ions from each group to obtain consistent variables. The coefficient of variation (CV) of metabolites in the quality control (QC) samples was set at a threshold of 30% for the assessment of repeatability in the metabolomics datasets. Then, we used the Wilcoxon rank-sum test to identify peaks that differed between the CAD and CAD-NAFLD groups. Next, we performed orthogonal partial least squares discriminant analysis (OPLS-DA) by SIMCA software (v14.1, Umetrics, Sweden). Significant metabolites were selected on the basis of variable importance in the projection (VIP) value $> 1$ and $P < 0.05$. Annotation was achieved by online databases, including the online HMDB database (http://www.hmdb.ca)(version:4.0) (22). MetaboAnalyst (http://www.metaboanalyst.ca) (version 4.0) was used for the identification of metabolic pathways (23). Although 132 patients were recruited, the metabolite information of three people in the HC group was not collected. Thus, 129 samples were used in the metabolome analysis.

## Extraction of DNA and Sequencing of the 16S RRNA V3-V4 Region

Bacterial DNA was isolated from fecal samples by using the bead-beating method. The DNA extracted from each sample was used as the template to amplify the V3-V4 region of 16S rRNA gene by using PCR (Phusion High-Fidelity PCR Master Mix with GC Buffer, from New England Biolabs Company), which were illustrated in our previous publication (24). Raw data quality control was performed. A sequencing library of the V3-V4 regions of the 16S rRNA gene was prepared. The purified products were mixed at an equal ratio for sequencing using an Illumina MiSeq system (Illumina Inc., USA). The detailed method refers to our previous study (24).

## Sequencing Data Analysis

Operational taxonomic units (OTUs) were clustered at the cutoff of 97% by using USEARCH v.8.0. The protocol can be found on the website (http://drive5.com/usearch/manual/uparse_pipeline.html).

The taxonomic composition of each group was visualized as a stacked bar plot at the phylum level and as a chord plot at the genus level with the ggplot2 package. For comparisons among groups, edgeR was utilized to identify significantly differential features, and the Benjamini-Hochberg method was used to control the FDR. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was utilized to predict the metagenomic functional compositions. Pathways that were different in abundance between the CAD and the CAD-NAFLD groups were obtained using Welch's *t*-test by STAMP software (v2.1.3). The visualization of the identified pathways was obtained by using the pheatmap package. To obtain functional predictions based on the 16S rRNA sequences, the taxonomic classification of sequences based on the Greengenes database was performed using *usearch -otutab*. Then, Bugbase was used to predict the phenotypes of the bacterial community (25).

## Spearman Multiomic Correlation Analysis

Spearman correlations between important bacterial taxa, serum metabolites and clinical parameters were calculated by using SPSS 24.0. The correlations between features were visualized using the pheatmap package. Multiple omics correlations were visualized in a Sankey plot by utilizing the R package networkD3.

# RESULTS

## Participant Characteristics

The characteristics and traditional cardiovascular risk factors of the participants are summarized in **Table 1**. In terms of disease severity, the difference in Gesini score [an indicator for atherosclerotic burden quantification (26)] was not significant, although the CAD-NAFLD group presented a much higher percentage of 3 stenosed vessels (51.85%) than the CAD group (26.91%). Moreover, biomarkers of myocardial injury and inflammation such as cardiac troponin I (cTnI) (27) and high-sensitivity C-reactive protein (hsCRP) (28) presented no significant difference between CAD-NAFLD group and CAD group. As for medication, CAD-NAFLD group has a significantly higher percentage of patients taking statins (55.56%) and oral antidiabetic drugs (OAD) (29.63%) than CAD group (48.15% for statins and 19.75% for OAD). In aspect of NAFLD-related clinical indexes such as triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), CAD-NAFLD group and CAD

**TABLE 1 |** Characteristics of the study cohort.

| | Healthy control ($n = 24$) | CHD ($n = 81$) | CHD-NAFLD ($n = 27$) | *P*-value |
|---|---|---|---|---|
| Age, years[#] | 54.75 (48.25, 59.5) | 61.78 (52.5, 70) | 62.8 (60, 68) | <0.001[ac] |
| Female[$] | 7 (25.93) | 20 (24.69) | 15 (62.5) | <0.002[ac] |
| SBP, mmHg* | 119.17 ± 8.61 | 131.30 ± 18.27 | 129.15 ± 14.19 | <0.001[ac] |
| BMI, kg/m2* | 23.94 ± 2.59 | 26.18 ± 3.39 | 26.48 ± 3.18 | <0.004[ac] |
| Waistline, cm* | 80.58 ± 8.69 | 92.80 ± 9.13 | 95.48 ± 8.17 | <0.001[ac] |
| Current smoker[$] | 0 (0) | 28 (34.57) | 9 (33.33) | <0.001[ac] |
| Drinking history[$] | 1 (4.17) | 43 (53.09) | 16 (59.26) | <0.001[ac] |
| No. of stenosed vessels | | | | <0.05[abc] |
| NA | NA | 2 (2.47) | 2 (7.4) | |
| 1 | NA | 18 (22.22) | 7 (25.93) | |
| 2 | NA | 23 (28.39) | 4(14.81) | |
| 3 | NA | 38 (26.91) | 14 (51.85) | |
| Gesini score[#] | NA | 44.67 (19, 63) | 32.29 (15, 44) | |
| **Medication** | | | | |
| Statins[$] | 0 (0) | 24 (29.63) | 15 (55.56) | <0.05[abc] |
| Antihypertensive drugs[$] | 4 (16.67) | 54 (66.67) | 15 (55.56) | <0.001[ac] |
| Oral antidiabetic drugs[$] | 0 (0) | 16 (19.75) | 13 (48.15) | <0.05[abc] |
| **Laboratory data** | | | | |
| TG, mmol/l[#] | 1.49 (0.83, 1.87) | 1.73 (1.04, 1.88) | 1.73 (1.03, 2.58) | |
| TC, mmol/l[#] | 4.78 (4.1, 5.43) | 4.13 (3.22, 4.76) | 3.97 (3.25, 4.36) | <0.001[c] |
| HDL-C, mmol/l[#] | 1.22 (0.97, 1.37) | 0.98 (0.81, 1.1) | 0.93 (0.83, 1.08) | <0.01[ac] |
| LDL-C, mmol/l[#] | 2.84 (2.45, 3.24) | 2.40 (1.7, 2.75) | 2.19 (1.64, 2.44) | <0.001[c] |
| FBG, mmol/l[#] | 6.58 (6.63, 7.05) | 7.28 (5.75, 8.2) | 7.77 (6, 8.6) | <0.05[c] |
| CR, μmol/l[#] | 67.625 (62, 75.25) | 84.23 (71, 91) | 74 (60, 86) | <0.01[a] |
| cTnI, μg/l[#] | 0 (0, 0) | 0.69 (0, 0.03) | 0.29 (0, 0.05) | |
| IL-18 | 742.92 (570.89, 868.93) | 638.56 (247.47, 895.48) | 514.77 (248.99, 637.92) | <0.05[c] |
| IL-1β | 4.34 (2.82, 3.64) | 3.84 (2.49, 3.81) | 3.99 (2.59, 4.37) | |
| IL-6, pg/ml | 9.08 (2.37, 4.51) | 27.62 (2.77, 14.49) | 25.63 (2.85, 9.49) | <0.05[a] |
| hs-CRP, mg/l[#] | 2.08 (0.38, 1.21) | 5.19 (0.69, 3.845) | 3.79 (1.02, 3.93) | |
| TNF-α,pg/mL[#] | 11.77 (2.32, 17,97) | 36.72 (15.33, 51.54) | 35.03 (15.65, 35.35) | <0.001[ac] |

*[#]median (IQR), *mean ± SD, [$]n (%). Continuous, normally distributed variables among the four groups were analyzed by a one-way analysis of variance. The Kruskal-Wallis H- test was applied for data of this type that were not normally distributed. Continuous, normally distributed variables between two groups were analyzed by Student's t-test. The Mann Whitney U test was applied for data of this type that were not normally distributed. Categorical variables were compared by the χ2 test. NA, not available. Drinking history is defined as patients who consumed ≥ 50 g of alcohol per day. [a]P. < 0.05 for equality between HC vs. CHD. [b]P < 0.05 for equality between CHD vs. CHD-NAFLD. [c]P < 0.05 for equality between HC vs. CHD-NAFLD.*

group showed no significant difference, this may due to the medication of CAD-NAFLD patients. Overall, the difference in disease severity baseline information showed was inconspicuous in the diseased groups and clinical indexes were not significantly affected by the complication of NAFLD at baseline.

## CAD Patients Combined With NAFLD Had Worse Clinical Prognosis

Follow-up study was conducted for the 108 patients, and the median follow-up time was 2.16 (IQR: 2.04–2.24) years. A composite endpoint was observed in 10 out of 27 CAD-NAFLD patients (37.03%) and in 15 out of 81 CAD patients (18.52%). Binary logistic analyses demonstrated that complications of NAFLD were associated with an increased risk of composite endpoints [odds ratio (OR) = 2.059, 95% CI: 1.09–3.88, $P = 0.033$] after adjusting for confounding factors, including smoking history, male sex, age, drinking history, T2DM, HTN, abnormal thyroid function, OAD use and antihypertensive drug use **(Supplementary Figure 1C)**. These results indicated that the CAD-NAFLD group presented a relatively worse prognosis than the CAD group. It is worth mentioning that the difference in baseline information of diseased groups was inconspicuous but the prognosis of CAD-NAFLD patients was significantly worse than CAD patients without NAFLD. We speculated that there were driving forces for cardiovascular events concealed under the complication of NAFLD.

## Serum Metabolomic Features Are Changed in CAD Patients Combined With NAFLD

Serum metabolites were analyzed by untargeted mass spectrometry (LC-MS). Four different types of metabolomic profiling modes—polar positive mode, polar negative mode,

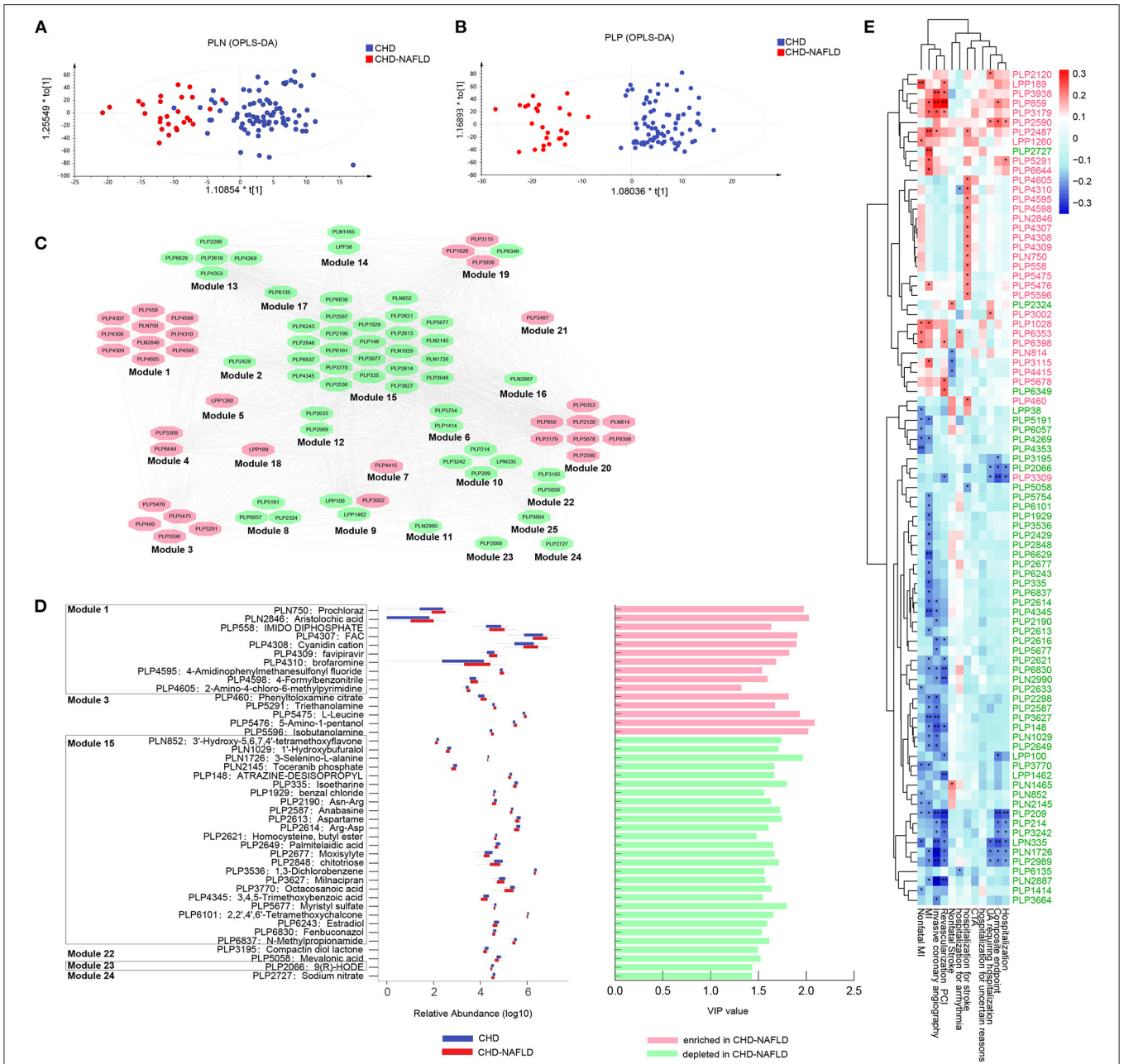



**FIGURE 1 |** Serum metabolites were significantly changed and correlated with poor clinical outcomes in CHD-NAFLD patients. **(A,B)** Separation of serum metabolome between patients with CHD-NAFLD and patients with CHD under polar ionic negative mode and polar ionic positive mode, revealed by orthogonal partial least squares discrimination analysis (OPLS-DA). **(C)** Distinct serum metabolites cluster analysis by Pearson correlation. Red ovals represent metabolic modules that contains metabolites that are enriched in CHD-NAFLDs. Green ovals are metabolic modules that contains metabolites depleted in CHD-NAFLD. **(D)** Relative abundance and variable importance in projection (VIP) value of several distinct serum metabolites annotated by Human Metabolites Data Base (HMDB). Blue box: relative abundance in CHD group. Red box: relative abundance in CHD-NAFLD group. The bars on the right show enrichment (highlighted in pink) or depletion (highlighted in green) of specific serum metabolites in CHD-NAFLD group when compared with CHD group. The VIP values are also presented. **(E)** Spearman analysis of the covariant relationship between key metabolome and adverse cardiovascular events. Pink font: serum metabolites enriched in CHD-NAFLD. Green font: serum metabolites depleted in CHD-NAFLD. $^{*}P < 0.05$, $^{**}P < 0.01$.

lipid positive mode, and lipid negative mode—yielded 14,585 (5,487 annotated), 7,394 (3,452 annotated), 5,193 (1,315 annotated), and 4,974 (701 annotated) metabolites, respectively, and the metabolites abundance profiles were obtained. We observed significantly different metabolomic profiles between CAD-NAFLD patients and CAD group under polar negative mode (**Figure 1A**) and polar positive mode (**Figure 1B**). Additional metabolite shifts were also discovered under

lipid mode (**Supplementary Figures 1A,B**). The OR of all patient's smoking history, drug (OAD and HTN drug) history, sex, age, HTN, drinking history, DM, NAFLD, abnormal thyroid function was calculated, and all the features were not distinct among the groups except for NAFLD ($P = 0.033$) (**Supplementary Figure 1C**). After thresholding metabolites with a VIP value $> 1$ and a Wilcoxon rank-sum $P$ value $< 0.05$, we filtered 87 annotated metabolites that significantly differed in abundance between CAD-NAFLD group and CAD group (**Supplementary Table 1**) and clustered them into 25 modules by Pearson correlation (**Figure 1C**). All serum metabolites contained in each module showed consistent alterations except for module 18 and module 19. Module 15 contained the largest number of metabolites and was a downregulated module. It is interesting to note that the metabolites contained in module 15, which was increased in CAD-NAFLD group, were mostly anti-inflammatory compounds (such as chitotriose, PLP2848, and estradiol, PLP6243). We also identified downregulated cardioprotective compounds in module 22 (e.g., mevalonic acid, PLP5058), module 23 [e.g., 9(R)-HODE, PLP2066], and module 24 (e.g., sodium nitrate, PLP2727) in CAD-NAFLD patients. Moreover, the enriched compounds in CAD-NAFLD group were mainly observed in three metabolite clusters including module 1, module 3, and module 20. To be noted that, these enriched compounds in module 1 and module 3 are known for their toxic effects (e.g., aristolochic acid, PLN2846, module 1; triethanolamine [TEA], PLP5291, module 3) or are considered allergic substances (isobutanolamine, PLP5596, module 3; phenyltoloxamine citrate, PLP460, module 3). The alteration of these crucial metabolites in different patient groups are presented in **Figure 1D**. In summary, CAD-NAFLD-associated serum metabolome alterations are characterized by the accumulation of toxic and proinflammatory compounds and scarcity of cardioprotective substances.

Furthermore, we analyzed the relationship between the alterations of serum metabolites and the clinical outcomes of patients. Spearman correlation showed that all the enriched serum metabolites were positively associated with non-fatal MI, MI events, invasive coronary angiography, revascularization percutaneous transluminal coronary intervention (PCI), and hospitalization for stroke (especially those in module 1), and the downregulated metabolites were negatively associated with hospitalization, composite endpoint, MI event, and non-fatal MI (**Figure 1E**). These results further confirmed that alterations in the serum metabolome are important driving factors in the prognosis of CAD-NAFLD patients. In addition, we further investigated the pathways these metabolites gathered on. Hypergeometric tests and topological data analysis (TDA) showed that terpenoid backbone biosynthesis was the most impacted pathway (**Supplementary Figure 1D**). Other impacted pathways included valine, leucine and isoleucine biosynthesis; valine, leucine and isoleucine degradation; aminoacyl-tRNA biosynthesis; and steroid hormone biosynthesis (**Supplementary Figure 1D**). In general, the accumulation of cardio-toxic and proinflammatory compounds and scarcity of cardioprotective substances in CAD-NAFLD patients are significantly associated with patient's prognosis and may affect clinical outcomes by regulating relevant pathways.

## Gut Microbiome Composition Is Changed in CAD Patients Combined With NAFLD and Associates With Serum Metabolome Alterations

Since some of the critical metabolites are associated with bacteria either by inhibiting bacterial enzymes (i.e., 4-Cyanobenzaldehyde, PLP4598, module1) or degraded by bacteria (i.e., sodium nitrate, PLP2727, module24), we speculate that the metabolome changes in patients with CAD-NAFLD may be mediated by the gut microbiota. We analyzed the gut microbiome by 16S rRNA sequencing. In the present microbiome investigation, a total of 2,830,519 high-quality 16S rRNA reads were obtained, with a median read count of 20,597 (range: 10,048 to 40,210) per 25 samples. A total of 626 OTUs were obtained after clustering. The rarefaction curves (**Supplementary Figure 2A**) of all samples supported the adequacy of the sequencing depth. In terms of alpha diversity, we observed no significant differences in the richness index (**Supplementary Figure 2B**) among the three groups. To assess the overall structures of the gut microbiota, a PCoA score plot based on the weighted UniFrac distances (**Supplementary Figure 2C**) was constructed. A Venn diagram was created to show the OTUs in each group (**Supplementary Figure 2D**).

Gut microbial analysis revealed that although the change in alpha diversity was inconspicuous, the taxonomic composition of the microbiome of CAD-NAFLD patients differed from those of the microbiomes of CAD patients and HCs. Among all the identified OTUs, *Firmicutes* and *Bacteroidetes* were the two most abundant phyla (**Figure 2A**), and *Bacteroides, Prevotella,* and *Faecalibacterium* were the main genera in the gut microbiota (**Figure 2B**). At the class level, *Actinobacteria, Bacteroidia, Bacilli, and Clostridia* were decreased in CAD-NAFLD patients compared with CAD patients (**Figure 2C**). A total of 15 species were significantly different between CAD and CAD-NAFLD patients (**Figure 2D**), and their relative abundances are presented in a boxplot (**Figure 2E**). *V. parvula* (OTU78, $P = 0.017$), *D. invisus* (OTU18, $P = 0.005$), and *O. ruminantium* (OTU57, $P = 0.045$) were significantly increased in CAD-NAFLD patients compared with CAD patients, and *V. parvula* (OTU78) and *D. invisus* (OTU18) were identified as driver species in cardiovascular disease (20, 29–31). We also noticed that *B. ovatus* (OTU135, $P = 0.004$) and *Ruminococcus bromii* (OTU99, $P = 0.005$) were decreased in the CAD-NAFLD group compared with the CAD group. *R. bromii* (OTU99) was reported to have a protective effect on the heart and vessels by inhibiting DCA biotransformation and to have a hypoglycemic effect (32). *B. ovatus* (OTU135) reduces trinitrobenzene sulfonic acid-driven colonic inflammation (33). Our observation was consistent with these studies, suggesting that inflammation-promoting species were enriched in CAD-NAFLD, while anti-inflammatory species were decreased in CAD-NAFLD patients when compared to CAD patients without NAFLD.

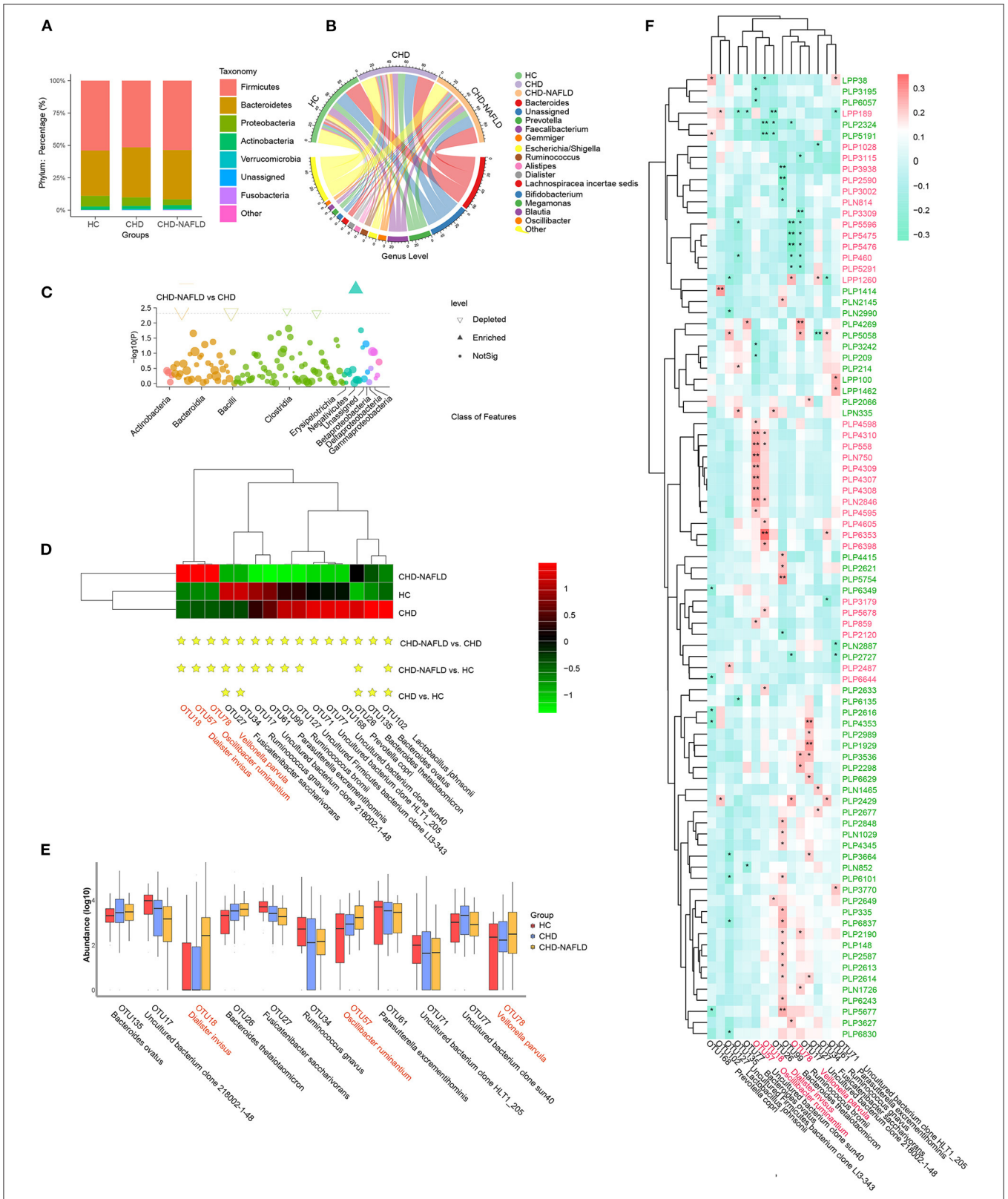



**FIGURE 2 |** Distinct gut microbiome in different groups. **(A,B)** Phylum-level and genus-level composition of gut microbiome in patients with CHD-NAFLD, patients with CHD, and healthy controls (HC). **(C)** Differential bacteria at class-level between CHD-NAFLD group and CHD group. **(D)** Species-level alterations in gut

*(Continued)*

**FIGURE 2 |** microbiome in CHD-NAFLD, CHD, HC groups. Stars are marked for significant enrichment or depletion either in the CHD-NAFLD group, CHD group, or in the HC group. **(E)** The summed relative abundances of gut microbes were compared between the CHD-NAFLD and CHD groups based on species-level. **(F)** Spearman correlation between key gut microbiomes and altered serum metabolites. Red font: gut microbiomes enriched in CHD-NAFLD. Black font: gut microbiomes depleted in CHD-NAFLD. Pink font: serum metabolites enriched in CHD-NAFLD. Green font: serum metabolites depleted in CHD-NAFLD. $^{*}P < 0.05$, $^{**}P < 0.01$.

We further correlated these microbiomes with key serum metabolites discovered previously, and the results were excellently consistent. The downregulated compounds were positively associated with the bacteria with decreased abundances, and the enriched compounds were positively associated with enriched taxa in the microbiomes (**Figure 2F**). In detail, enriched metabolites such as *prochloraz* (PLN750, module 1), *imido diphosphate* (PLP558, module 1), brofaromine (PLP4310, Module 1) were all significantly and positively correlated with *O. ruminantium* (OTU57, enriched in CAD-NAFLD). Other enriched metabolites, such as aristolochic acid, were significantly and positively correlated with *D. invisus* (OTU18, enriched in CAD-NAFLD). The remaining enriched metabolites, 5-amino-1-pentanol (PLP5476, module 3), TEA (PLP5291, module 3), isobutanolamine (PLP5596, module 3), and phenyltoloxamine citrate (PLP460, module 3), were all negatively correlated with uncultured bacterial clone 218002-1-48 (OTU17, decreased in CAD-NAFLD). For the downregulated metabolites, *estradiol* (PLP6243, module 15) and 2,2',4',6'-tetramethoxychalcone (PLP6101, module 15) were significantly and positively correlated with *R. bromii* (OTU99, decreased in CAD-NAFLD), and moxisylyte (PLP2677) was positively and significantly correlated with *R. gnavus* (OTU34, decreased in CAD-NAFLD). Additional details are shown in **Figure 2F**. It is not difficult to conclude that the accumulation of toxins was associated with increases in potentially pathogenic microbiomes and with decreases in potentially beneficial microbiomes, while reduction in cardioprotective compounds were associated with depletion of potentially beneficial microbiomes.

## Variations in Gut Microbiome Composition Lead to Altered Metabolic Pathway in CAD-NAFLD Patients

To further substantiate the potential role of gut microbes in the alteration of the serum metabolome, we focused on the microbial rRNAs predicted to correspond to microbes with known metabolic functions. A total of 626 OTU data points were functionally annotated with PICRUSt2 software. The proportion of potential pathogens showed a gradual increasing trend HCs, CAD patients, and CAD-NAFLD patients (**Figure 3A**). Although there isn't significant difference in HCs and CAD patients, the proportion of potential pathogens is significantly higher in CAD-NAFLD patients when compared with HCs ($P = 0.039$), representing a characteristic disorder of the gut microbiota. Furthermore, the abundances of stress-tolerant bacteria showed a decreasing trend in HCs, CAD patients, and CAD-NAFLD patients (**Figure 3B**). The enrichment of potential pathogens and decreases in stress-tolerant bacteria may be one of the reasons for the worse prognosis of CAD-NAFLD.

The impacted metagenomic pathways were predicted using the PICRUSt2 tool based on the MetaCyc database. A total of 8 pathways were found to differ in activity level between the CAD group and the CAD-NAFLD group (**Figure 3C**). We identified 7 upregulated pathways (lactose and galactose degradation I, formaldehyde assimilation I (serine pathway), L-lysine biosynthesis II, chorismate biosynthesis II from Archaea, L-glutamate degradation V via hydroxyglutarate, L-lysine fermentation to acetate and butanoate, succinate fermentation to butanoate). Only one pathway was downregulated. We speculate that the changes in these pathways may be the cause of the alterations in the metabolome. The gut microbiome may affect the metabolome by up- or downregulating these pathways. Moreover, these pathways are mainly amino acid-associated pathways and glucose-associated pathways, which is consistent with the pathways associated with the identified crucial metabolites (**Supplementary Figure 1D**). These differences in pathways active level further support the strong association between the gut microbiome and serum metabolome alterations in CAD-NAFLD patients.

Given that the gut microbiome is associated with prognosis-related serum metabolites, it is not hard to presume that these gut microbiomes also affect the prognosis of CAD-NAFLD patients. Correlations between CAD-NAFLD-associated OTUs and clinical indexes were calculated by Spearman correlation (**Figure 3D**). We noted that *V. parvula (OTU78),* a species enriched in CAD-NAFLD, was positively correlated with C-reactive protein (CRP), an inflammation factor. We also observed that species that were decreased in CAD-NAFLD, such as *uncultured bacterium clone 218002-1-48* (OTU17), *Parasutterella excrementihominis* (OTU61), and *Bacteroides thetaiotaomicron* (OTU26), were negatively correlated with inflammatory factors (e.g., IL-6, TNF-α, and IL-26). Moreover, the gut bacteria with decreased abundances (*B. ovatus*, OUT135; *F. saccharivorans*, OTU27; *uncultured Firmicutes bacterium clone LI3-343*, OTU127) were also negatively correlated with markers that represent the severity of the disease, such as Gesini score, CK-MB (creatine kinase MB), and creatine. These correlations further showed the association between the decreased beneficial species and higher risk of cardiovascular disease.

## Alterations of Bacterial Species and Associated Metabolome Changes Are Related to Adverse Clinical Outcomes in CAD Patients Combined With NAFLD

We subsequently analyzed the correlations of serum metabolites with the gut microbiome and with CAD-NAFLD patient

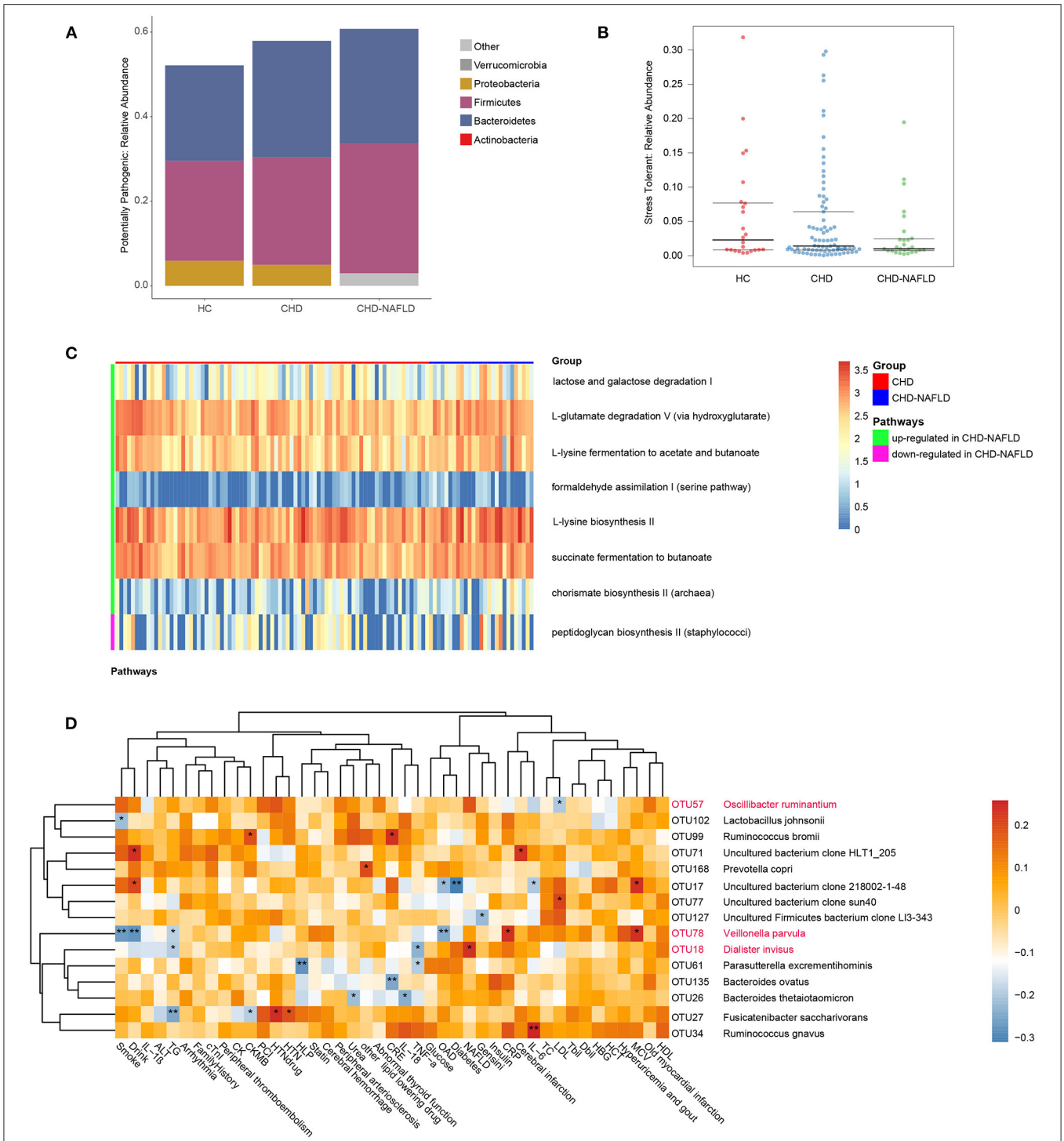



**FIGURE 3 |** Function prediction of gut microbiome among three groups at OTU level. **(A,B)** Predicted high-level phenotypes (potential pathogens and stress tolerant), calculated by BugBase. **(C)** Relative activity of pathways in CHD and CHD-NAFLD. **(D)** Correlation of the CHD-NAFLD featured gut microbes with clinical indexes. Rows: CHD-NAFLD-positive OTUs are highlighted in red, and CHD-NAFLD-negative OTUs are in black. Columns: clinical indexes. $^{*}P < 0.05$, $^{**}P < 0.01$.

prognosis, which were visualized in Sankey plots (**Figure 4**). In general, at the species level, *V. parvula* (OTU78), *D. invisus* (OTU18), and *O. ruminantium* (OTU57) were significantly enriched, while *P. excrementihominis* (OTU61), *B. ovatus* (OTU135), *F. saccharivorans* (OTU27), *R. gnavus* (OTU34), *uncultured bacterium clone 218002-1-48* (OTU17), *P. copri*

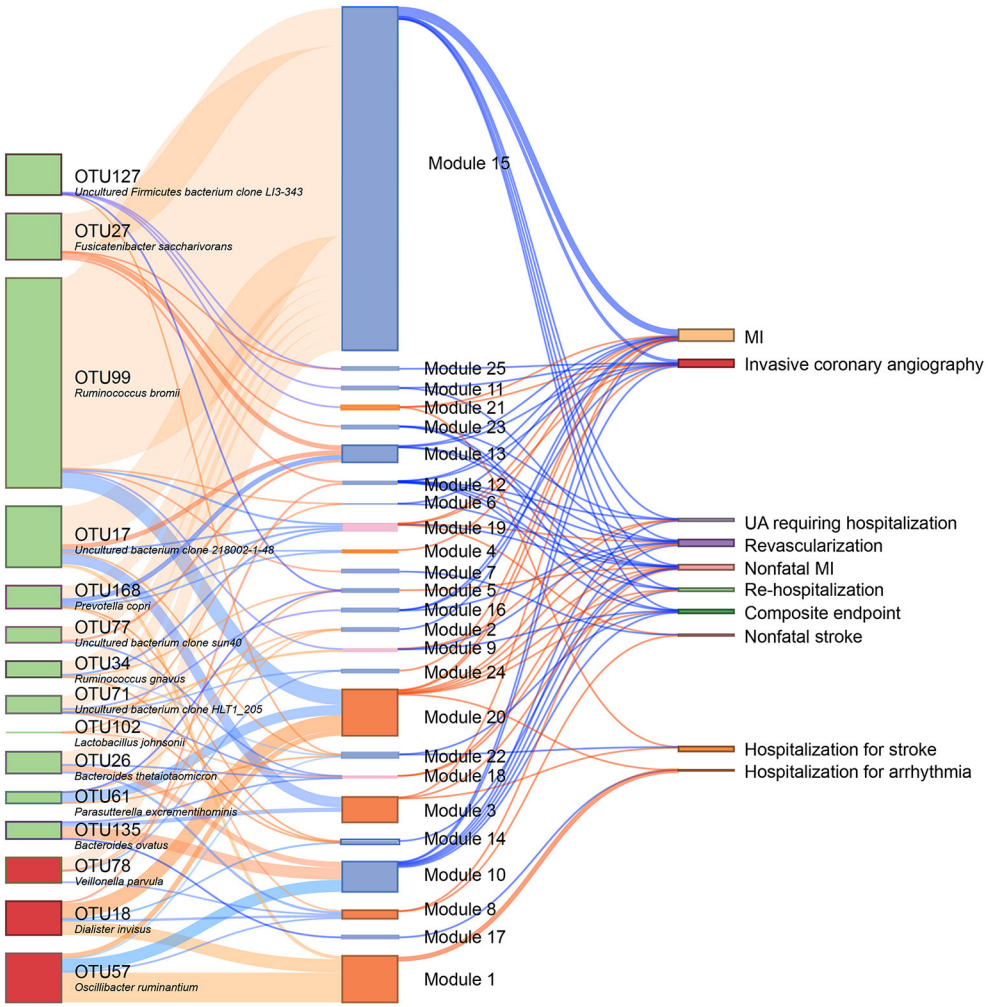



**FIGURE 4 |** Interrelationship between CHD-NAFLD-associated flora, serum metabolic features and clinical outcomes. Red connections indicate positive correlations, and blue connections indicate negative correlations (Spearman correlation analysis, $P < 0.05$). In the left first column, green boxes indicate CHD-NAFLD-negative OTUs, and red boxes indicate CHD-NAFLD-positive OTUs. In the middle column, light blue boxes indicate CHD-NAFLD-negative metabolic features, and orange boxes indicate CHD-NAFLD-positive metabolic features. A composite endpoint is defined as all-cause mortality and/or reoccurrence of ACS and/or readmission for cardiac causes.

(OTU168) and *R. bromii* (OTU99) were decreased. The serum metabolites shown in **Figure 4** were mostly decreased in CAD-NAFLD patients compared with CAD patients, while metabolites in module 1, 3, 8, 20, and 21 were enriched. Two metabolite clusters (module 10 and 15) were both composed of decreased metabolites and positively correlated with decreased bacteria (OTU17, OTU26, OTU77, OTU71, OTU127, OTU135, OTU61, OTU27, OTU34, OTU99, OTU102, and OTU168) or negatively correlated with enriched bacteria (*O. ruminantium*, OTU57). Additionally, another 2 metabolite clusters (module 3 and 20) were composed of enriched metabolites, which were either positively correlated with enriched bacteria (OTU18 and OTU57) or negatively associated with decreased bacteria (OTU135, OTU17, and OTU99). Moreover, the enriched metabolites were highly associated with a higher risk of MACEs, while the decreased metabolites were negatively associated with poor clinical outcome. This result systematically and clearly illustrated that the alteration of the serum metabolome in CAD-NAFLD is highly associated with poor clinical outcome through toxin accumulation and decreased protection. The relationship between NAFLD and CAD is illustrated in **Figure 5** in a more intuitive manner. This multiomics study highly suggests that complications of NAFLD are associated with gut microbiome alterations, such as the enrichment of *D. invisus* (OTU18) and *O. ruminantium* (OTU57) and the decrease in *P. copri* (OTU168). The change in gut microbes was further associated with serum metabolome alterations, and cardiac toxins (e.g., *aristolochic acid*, *prochloraz*, and *imido diphosphate*) and allergic compounds (e.g., *isobutanolamine* and *phenyltoloxamine citrate*) accumulated in the circulation,

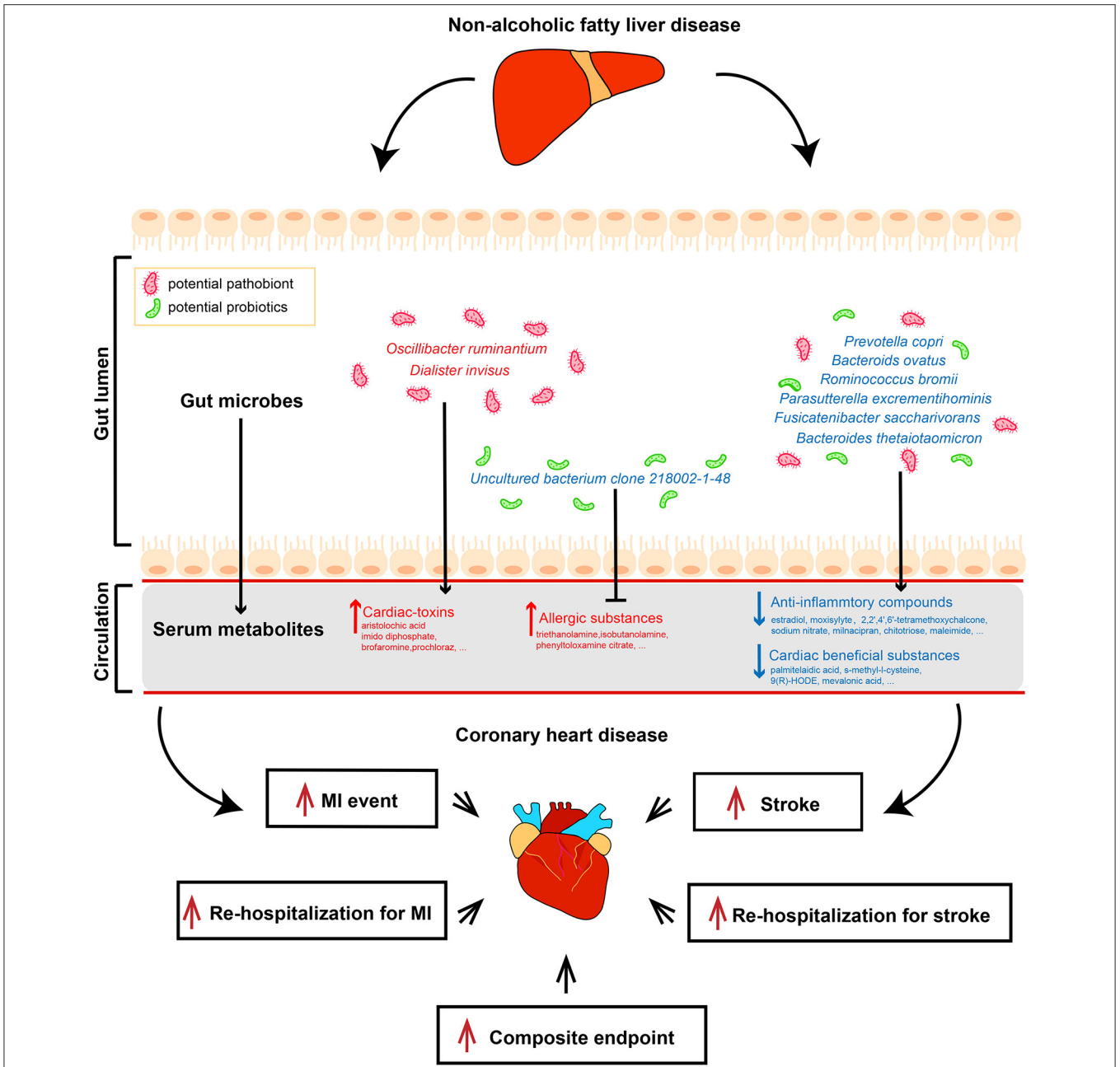



**FIGURE 5 |** Multi-omics analysis illustrates the interrelationship between non-alcoholic fatty liver disease and coronary heart disease. Features colored in red represent increase in corresponding gut microbiome or serum metabolites. Features colored in blue represent depletion in corresponding gut microbiome or serum metabolites. Arrows represent significant correlations between features.

while anti-inflammatory compounds (e.g., *estradiol, 2,2',4',6'-tetramethoxychalcone, moxisylyte, sodium nitrate, milnacipran,* and *chitotriose*) and cardioprotective substances (e.g., *mevalonic acid, 9(R)-HODE, S-methyl-L-cysteine,* and *palmitelaidic acid*) were decreased. These metabolome changes were highly associated with composite endpoints, MI events, and rehospitalization in CAD-NAFLD patients. In conclusion, our multiomics study presents associations among NAFLD, the gut microbiome, the serum metabolome, and CAD patient prognosis.

## DISCUSSION

In recent years, accumulating evidence has shown that changes in the microbiota are associated with CAD progression (34–36). NAFLD is considered a powerful driving force in CAD since fatty liver is strongly associated with systemic inflammation (37, 38). The regulation of the gut microbiota and its bacterial products plays an important role in the development of NAFLD (39–41). Intestinal bacterial species and their metabolites regulate adipose tissue and intestinal homeostasis and then contribute to the pathogenesis of NAFLD. Given the notably strong association between NAFLD and CAD, we demonstrate in this work that the gut microbiome in CAD individuals complicated with NAFLD has functional potential for accelerated biosynthesis of a number of inflammatory compounds, leading to elevated serum concentrations of proinflammatory metabolites and depleted potential cardiac-beneficial compounds, and finally resulting in aggravated adverse cardiovascular events.

We paid special attention to several microbiome associated-metabolites and modules that were significantly associated with poor clinical outcome. Prochloraz (PLN750, module 1) was significantly increased in CAD-NAFLD patients and was positively correlated ($P = 0.003$, Cor = 0.284) with *O. ruminantium* (OTU57). Prochloraz was documented as a gut microbiome-associated hepatotoxic metabolite and affects glucolipid metabolism (42, 43). Moreover, brofaromine (PLP4310, module 1), a MAO inhibitor, showed a positive correlation ($P = 0.008$, Cor = 0.253) with *O. ruminantium* (OTU57). Aristolochic acid (PLN2846, module 1), a well-known nephrotoxic phytochemical that was reported to induce heart failure in zebrafish embryos (44), was also significantly increased in NAFLD patients and was positively correlated ($P = 0.036$, Cor = 0.202) with *D. invisus* (OTU18). Additionally, *O. ruminantium* (OTU57) and *D. invisus* (OTU18) were shown to be greatly enriched in patients with atherosclerotic cardiovascular disease compared with healthy people (45, 46). Additionally, imido diphosphate (PLP558, module 1) was positively correlated ($P = 0.042$, Cor = 0.196) with *D. invisus* (OTU18). Our results suggest that potential pathogens such as *O. ruminantium* (OTU57) and *D. invisus* (OTU18) are significantly enriched in CAD-NAFLD and associated with the elevation in toxic serum metabolites (e.g., prochloraz and aristolochic acid), and the above-mentioned metabolites (prochloraz, brofaromine, aristolochic acid, imido diphosphate) are all positively associated ($P < 0.05$) with rehospitalization for stroke in CAD-NAFLD patients. Besides *O. ruminantium* (OTU57) and *D. invisus* (OTU18), *V. parvula* (OTU78) was also elevated in CAD-NAFLD, and it was documented to be enriched in rheumatoid arthritis (47) and Crohn's disease (48). *V. parvula* (OTU78), as a nitrate-reducing bacteria (49), was observed to be negatively associated ($P = 0.025$, Cor = −0.215) with sodium nitrate (PLP2727, module 24), which attenuates inflammation in metabolic syndrome patients (50) and is depleted in CAD-NAFLD patients. To sum up, our results suggested that the enrichment of potential pathogens (*O. ruminantium, D. invisus, V. parvula*) in the gut microbiome associates with increases in toxic metabolites (e.g., prochloraz and aristolochic acid) and decreases in anti-inflammatory metabolites (e.g., sodium nitrate), and this alteration in serum metabolome strongly associates with worse clinical outcomes in CAD-NAFLD patients. This association is suggestive to the mechanism of the progression of CAD-NAFLD. We speculate that accumulation of potential pathogenic gut microbiome in CAD-NAFLD is at least one of the important driving forces in disease progression.

TEA (PLP5291, module 3) caught our special attention, as it was enriched in CAD-NAFLD patients and significantly correlated with bacterial clone 218002-1-48 (OTU17, $P = 0.024$, Cor = −0.217) and shows negatively correlation trend with *P. excrementihominis* (OTU61, Cor = −0.028). TEA was documented to cause susceptibility to pathogenic infection by inducing apoptosis (51) and can be degraded by *Alcaligenes faecalis* (52). *Alcaligenes faecalis* belongs to the *Burkholderiales* order and is associated with atherosclerotic cardiovascular disease (45). Consistently, we observed that *P. excrementihominis* (OTU61), which also belongs to the *Burkholderiales* order, was depleted in CAD-NAFLD. Thus, the less harmful TEA underwent biodegradation and more accumulated in serum. Moreover, isobutanolamine (PLP5596, module 3) and phenyltoloxamine citrate (PLP460, module 3), both of which cause contact allergies (53), were also negatively correlated with uncultured bacterium clone 218002-1-48 (OTU17, $P < 0.05$). Consistently, these compounds were all positively associated ($P < 0.05$) with rehospitalization for stroke. In addition to these three compounds, the remaining two compounds in module 3 were also negatively associated with uncultured bacterium clone 218002-1-48 (OTU17, $P < 0.05$). These results suggested that the depletion of uncultured bacterium clone 218002-1-48 (OTU17) is associated with the accumulation of toxic metabolites (e.g., isobutanolamine and phenyltoloxamine citrate) in CAD-NAFLD patients, and we speculate that uncultured bacterium clone 218002-1-48 (OTU17) may play an inhibitory role in the biosynthesis of these toxic metabolites, just as *Alcaligenes faecalis does to TEA*. Combined with the results mentioned in the previous paragraph, it is worth mentioning that the increase of potential gut microbiome and decrease of potential beneficial gut microbiome modulate the serum metabolome to a more toxic status.

Not only did we identify the accumulation of toxic metabolites, we also found that a large number of protective metabolites were depleted in CAD-NAFLD patients, and these beneficial metabolites were mainly clustered in module 15. Estradiol (PLP6243, module 15) is one of the potentially beneficial substances that was decreased in CAD-NAFLD patients and was observed to be positively correlated ($P = 0.027$, Cor = 0.213) with *R. bromii* (OTU99, decreased in CAD-NAFLD). Estradiol (PLP6243, module 15) was shown to have protective effects against reperfusion injury (54) and to alleviate of cardiac aging caused by galactose (55). Additionally, 2,2',4',6'-tetramethoxychalcone (PLP6101, module 15) and chitotriose (PLP2848, module 15) are both anti-inflammatory metabolites (56, 57) that were decreased in CAD-NAFLD and associated with the decrease in *R. bromii* (OTU99). Moreover, other

metabolites in module 15 also have anti-inflammatory effects, such as milnacipran (58) (PLP3627, module 15), palmitelaidic acid (PLP 2649, module 15), and moxisylyte (PLP2677, module 15). Palmitelaidic acid prevents cardiac fibrosis (59) and was documented to be decreased in CAD-NAFLD patients (60), which is consistent with our observation. It was associated ($P = 0.038$, Cor $= 0.200$) with the depletion of *B. thetaiotaomicron* (OTU26). Moxisylyte (PLP2677, module 15) was associated ($P = 0.043$, Cor $= 0.195$) with *R. gnavus* (OTU34) and were reported to protect against inflammatory attacks (61). It is worth mentioning that these decreased serum metabolites all negatively correlated ($P < 0.05$) with MI events in CAD-NAFLD patients. The remaining metabolites in module 15 were highly suggested to be protective against cardiac events since they presented consistent alteration and microbiome association Combined with the accumulation of toxic compounds mentioned previously, the decreases in these potentially beneficial metabolites further strengthened the toxic serum status in CAD-NAFLD patients.

Notable changes in the gut microbiota in individuals with NAFLD had a functional potential to accelerate the biosynthesis of the above toxic compounds and the depletion of cardiac-protective compounds, leading to a higher risk of inflammatory exposure and aggravated CAD clinical outcome (**Figure 4**). *D. invisus* (OTU18), one of the species enriched in NAFLD, is predicted to be associated with the production of toxins such as imido diphosphate (PLP558, module 1) and aristolochic acid (PLN2846, module 1). Consistently, it has been reported that *D. invisus* (OTU18) s associated with intestinal inflammation, such as chronic apical abscess (62), is increased in human milk-fed piglets (63) and is positively associated with osteoarthritis (64). Another important potential pathogenic species, *V. parvula* (OTU78), was predicted to be associated with severe hepatitis (65). Consistently, *V. parvula* (OTU78) was significantly increased in CAD-NAFLD and negatively associated with an anti-inflammatory compound (sodium nitrate, PLP2727) due to its degradation (49). In general, *D. invisus* (OTU18) and *V. parvula* (OTU78) are two well-known pathogens and is also considered as potential pathogens in our study since they are associated with accumulation of serum toxins. This further suggested that *D. invisus* (OTU18) and *V. parvula* (OTU78) are important driving forces in the CAD-NAFLD pathogenesis.

We paid special attention to *R. gnavus* (OTU34), a controversial species (66), which showed a gradual decrease in HCs, CAD patients, and CAD-NAFLD patients. *R. gnavus* (OTU34) was negatively associated with moxisylyte, an anti-inflammatory compound. Consistently, *R. gnavus* was found to be decreased in CAD-NAFLD (20, 67), and our observation further confirmed that *R. gnavus* (OTU34) may be protective in CAD. Additionally, *F. saccharivorans* gradually decrease in HCs, CAD patients, and CAD-NAFLD patients. Previous studies indicated that *F. saccharivorans* (OTU27) was significantly decreased in inflammatory disease and that *F. saccharivorans* (OTU27) was involved in short-chain fatty acid- (SCFA) production (64, 68) and produced lactic acid, formic acid, acetic acid and succinic acid as fermentation end products from glucose (69). Therefore, it was speculated that *F. saccharivorans* (OTU27) might be related to lipid metabolism alterations in CAD patients and to the even worse lipid metabolism alterations in CAD-NAFLD patients. A newly found species, uncultured bacterium clone 218002-1-48 (OTU17), was shown to have an irreplaceable role in the downregulation of protective serum metabolites. The compounds in module 3 (PLP5475, PLP5476, PLP5291, PLP5596, and PLP460) were all decreased in CAD-NAFLD and have been shown to have cardiotoxic effects. Moreover, all metabolites in module 3 were significantly associated with uncultured bacterium clone 218002-1-48 (OTU17). We speculated that the decrease in OTU17 reduced the inhibition of these toxins. Although OTU17 may be a newly discovered species, it is worthy of further study and may be a potential treatment for CAD-NAFLD patients. To sum up, the gradual decrease of *R. gnavus* (OTU34) and *F. saccharivorans* (OTU27) highly suggest they are potential probiotics. Additionally, the down-regulation of these potential probiotics played supporting role in CAD-NAFLD disease progression by down-regulating cardiac-protective compounds.

To explain the above interrelationships, we introduce a new concept: liver-gut microbiota-heart axis, as a possible mechanism under these interactions. On the one hand, the concept of liver-gut microbiota axis has already been proposed and confirmed by many researches (70, 71). Because of the direct connection via portal vein between the intestines and the liver, gut microbiota and associated dysbiosis have been known as regulators in the pathophysiology of NAFLD (71). Thus, the alterations in gut microbiota in CAD-NAFLD patients can be explained by liver-gut microbiota axis. On the other hand, gut-heart axis has emerged as a novel concept to provide new insights into the complex mechanisms of cardiovascular disease and offer new therapeutic targets (72, 73). Moreover, multi-point axis is not unusual in disease development. For instance, recent research work has presented a liver-brain-gut axis in inflammatory bowel disease (74). Thus, we come up with a new concept of liver-gut microbiota-heart axis and we believe this axis may be the possible underlying mechanism in the prognosis of CAD-NAFLD. However, more experiments are needed to prove the existence of this novel axis, as well as its possible regulation in disease.

This work illustrated that CAD-NAFLD patients has worse clinical outcome than patients with CAD alone. We discovered that protective metabolites are decreased and proinflammatory toxins are increased in the serum of CAD-NAFLD patients compared with CAD patients, and the changes in metabolites were closely associated with changes in the gut microbiome. Therefore, we speculate that the increase in pathogenic gut bacteria (e.g., *V. parvula*, *O. ruminantium,* and *D. invisus*) and the decrease in beneficial gut bacteria (e.g., *R. gnavus* and *F. saccharivorans*) lead to the depletion or accumulation of different serum metabolites and finally lead to a higher risk of cardiovascular events. We speculate that it is the complication of NAFLD that impacted the serum metabolome and further accelerated the pathogenic process of CAD. Since liver is an organ known for breaking down harmful substances and

regulating most chemical levels in the blood (75, 76), the healthiness of liver strongly affects serum metabolome. NAFLD is the condition when too much fat is stored in liver cells, in other word, the liver is work-overload. In this case, the blood-regulating function of liver is significantly reduced and more harmful substances are accumulated in the blood (77). Moreover, research work has shown that hepatocyte affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism (78). Experiments done in mice have found that the hepatocyte deletion of MyD88, a crucial gene in obesity and diabetes, induces changes of specific gut microbes (78). All these evidences supported that NAFLD contributes to the alteration in serum metabolome, and gut microbiota may accelerate this process. Further, we found that the NAFLD-induced metabolic change could be summarized into the accumulation of cardio-toxic and proinflammatory compounds and scarcity of cardioprotective substances. This alteration definitely affects CAD in many ways, such as the accumulation of lipids (21), sclerosis of arteries (79), and exacerbates the inflammatory response (80). Our results provide a new insight to the understanding of disease mechanism and we highly speculate that gut microbiome plays at least one important role in the progression of CAD-NAFLD. To our knowledge, this is the first multiomics analysis on the serum metabolome, gut microbiome, clinical indexes and outcomes in CAD-NAFLD patients and CAD patients. Critical metabolome and microbial changes associated with adverse cardiovascular events in CAD-NAFLD were explored through a multiomics study. Our work is also the first to provide a schematic hypothesis that could be used as a framework for proof-of-concept studies that link the impact of serum metabolome and gut microbiome alterations with CAD-NAFLD patient outcomes. These findings are in line with the results from other NAFLD and CAD microbiome studies. Moreover, besides the several metabolites we discussed, the rest metabolites clustered into the same modules as the discussed metabolites are highly speculated to play the same role, since all the metabolites in one module are consistent in their spearman correlation with gut microbiome as well as their alteration in serum concentration. Not only did we identified currently known serum metabolites that are highly likely to participate in disease progression, but also we sorted out a list of potentially disease-related metabolites that we currently know little about. Our study provided potential targets for CAD-NAFLD treatment.

Our study is meaningful to current medication and treatment of CAD-NAFLD patients. Current treatment of CAD-NAFLD patients mainly focused on the treatment of CAD and blood lipid level and lipid-lowering drugs are the current mainstream treatment for NAFLD. But if the treatment of gut microbiomes is taken into consideration, the prognosis of CAD-NAFLD patients may be much better since our study showed that gut microbiome plays a driving force in disease progression. In addition, we provided detailed list of CAD-NAFLD related gut microbiomes and serum metabolites, which could all become potential targets for treatment. Last but not least, our newly proposed "liver-gut microbiota-heart axis" also indicates the importance of liver's health in CAD patients. The health status of liver may affect the clinical outcomes of CAD patients by the interrelationship with gut microbiome.

Notably, this study has some limitations. First, it is a correlation study and did not include animal experiments on the function of the key differential bacteria. As few reports have been made about the metabolic function of the differential bacteria, we need to undertake additional studies to confirm the function of these bacteria. Second, considering that our study was a single-center study, additional multicenter studies are needed to confirm the results. Third, the study had a small sample size. An enlarged study population would make the results more convincing.

## CONCLUSIONS

Taken together, our results indicate that NAFLD may drive the progression of CAD by altering the gut microbiome. The changes in the intestinal gut microbiota further modulate the serum metabolome of CAD-NAFLD patients toward a worse status, i.e., reducing cardioprotective compounds such as estradiol, sodium nitrate, chitotriose, and maleimide and increasing potentially toxic and proinflammatory compounds such as triethanolamine, isobutanolamine, aristolochic acid, prochloraz, and imido diphosphate, thus leading to a higher risk of MACEs. We came up with a novel concept of "liver-gut microbiota-heart axis" which could possible explain this interrelationship. Our study presents a wide perspective to develop new diagnostic and therapeutic approaches, and additional comprehensive studies are desired. We also suggested the importance of liver's health in CAD patients.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Review Board at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

SZ and XH: concept, design, and study supervision. XH and RZ: clinical research, analysis, and interpretation of data. RZ, XH, HL, YS, XZ, and YF: sample and data acquisition. RZ, XH, and SZ: writing, review, and/or revised the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.805812/full#supplementary-material

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

## GLOSARRY

HCs, Healthy controls; CAD, Coronary artery disease; CAD-NAFLD, Coronary artery disease complicated with fatty liver disease; BMI, Body mass index; BUN, Blood urea nitrogen; CK, Creatine kinase; CR, Creatinine; cTnI, Cardiac troponin I; HDL-C, High-density lipoprotein cholesterol; hsCRP, High-sensitivity C-reactive protein; LC-MS, Liquid chromatography-mass spectrometry; LDL-C, Low-density lipoprotein cholesterol; MI, Myocardial infarction; No. of SV, Number of stenosed vessels; OAD, Oral antidiabetic drugs; OPLS-DA, Orthogonal projection to latent structure-discriminant analysis; OUT, Operational taxonomic unit; PCI, Percutaneous transluminal coronary intervention; SBP, Systolic blood pressure; TG, Triglyceride; TNF-α, Tumor necrosis factor alpha; VIP, Variable importance in projection; T2DM, type 2 diabetes mellitus; HTN, hypertension; γ-GGT, γ-glutamyl transferase; MACEs, major adverse cardiac events; CV, coefficient of variation; QC, quality control; PICRUSt2, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; TEA, triethanolamine; TDA, topological data analysis; SCFA, short-chain fatty acid.

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# Effects of High-Intensity Interval vs. Moderate-Intensity Continuous Training on Cardiac Rehabilitation in Patients With Cardiovascular Disease: A Systematic Review and Meta-Analysis

*Tian Yue*[1], *Yan Wang*[1], *Hui Liu*[2], *Zhaowei Kong*[3] *and Fengxue Qi*[4]*

[1] School of Sports Medicine and Rehabilitation, Beijing Sport University, Beijing, China, [2] China Institute of Sport and Health Science, Beijing Sport University, Beijing, China, [3] Faculty of Education, University of Macau, Macau, China, [4] Sports, Exercise and Brain Sciences Laboratory, Beijing Sport University, Beijing, China

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***Correspondence:**
*Fengxue Qi*
*fengxue.qi@hotmail.com*

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**Background:** Studies have shown that high-intensity interval training (HIIT) is superior to moderate-intensity continuous training (MICT) for increasing peak oxygen uptake ($VO_{2peak}$) and reducing cardiovascular disease (CVD) and mortality. To our knowledge, previously published systematic reviews have neither compared different HIIT models with MICT nor investigated intervention frequencies of HIIT vs. MICT for purposes of improving cardiorespiratory fitness in patients with CVD.

**Objective:** The purpose of this meta-analysis was to compare the effects of different training models, intervention frequencies and weeks of HIIT vs. MICT on changes in cardiorespiratory fitness during cardiac rehabilitation (CR).

**Methods:** A systematic search was carried out for research articles on randomized controlled trials (RCTs) indexed in the PubMed, Cochrane Library, Web of Science, Embase and Scopus databases for the period up to December 2021. We searched for RCTs that compared the effect of HIIT vs. MICT on cardiorespiratory fitness in patients with CVD.

**Results:** Twenty-two studies with 949 participants (HIIT: 476, MICT: 473) met the inclusion criteria. Sensitivity analysis revealed that HIIT increased $VO_{2peak}$ more than MICT (MD = 1.35). In the training models and durations, there was a greater increase in $VO_{2peak}$ with medium-interval HIIT (MD = 4.02) and more than 12 weeks duration (MD = 2.35) than with MICT. There were significant improvements in $VO_{2peak}$ with a HIIT frequency of 3 times/week (MD = 1.28). Overall, one minor cardiovascular and four non-cardiovascular adverse events were reported in the HIIT group, while six non-cardiovascular adverse events were reported in the MICT group.

**Conclusion:** HIIT is safe and appears to be more effective than MICT for improving cardiorespiratory fitness in patients with CVD. Medium-interval HIIT 3 times/week for more than 12 weeks resulted in the largest improvement in cardiorespiratory fitness during CR.

**Systematic Review Registration:** https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021245810, identifier: CRD42021245810.

**Keywords: cardiovascular disease, cardiac rehabilitation, high-intensity interval training, peak oxygen uptake, cardiorespiratory fitness, moderate-intensity continuous training**

## INTRODUCTION

Cardiovascular disease (CVD) is responsible for more deaths than any other illness worldwide, and the past decade has witnessed a 12.5% increase in deaths, accounting for 1/3 of the global total (1). The increasing incidence of CVD has increased its financial burden (2). Cardiac rehabilitation (CR) is a promising therapeutic approach to secondary prevention of CVD (3). It includes health education, lifestyle changes, social-psychological support, and supervised exercise (4). Exercise-based CR not only reduces the traditional cardiovascular risk factors (hypertension, hyperlipidemia, diabetes, and obesity) (5), but also cardiovascular risk from conditions such as chronic systemic inflammation (6), which has gradually emerged as a risk factor for CVD (7). Exercise is associated with beneficial anti-inflammatory effects, reduced serum levels of C-reactive protein (CRP) in healthy individuals (8) and improved cardiac output (9), stroke volume (9), and vascular endothelial function (6) as well as reduced heart rate variability (10) in patients with CVD. Exercise-based CR improves cardiorespiratory fitness in patients with CVD (5). Peak oxygen uptake ($VO_{2peak}$), as the gold standard for evaluating cardiorespiratory fitness, has been identified as an important predictor of CVD and all-cause mortality (11). $VO_{2peak}$ is a basic element for controlling CVD all-cause risk factors such as diabetes, dyslipidemia and obesity. Some studies have shown that CVD all-cause mortality decreases by 8–17% when individual cardiorespiratory fitness increases by one metabolic equivalent (12, 13).

Moderate-intensity continuous training (MICT) is regarded as a successful approach to CR because of its efficacy and safety (14–16). Some studies found that MICT can reduce cardiovascular risk and cardiovascular mortality (17, 18). MICT entails longer durations of moderate-intensity continuous aerobic activity, maintaining an intensity between 60 and 80% ($VO_{2peak}$ or reserve heart rate). High-intensity interval training (HIIT) refers to physical activity characterized by relatively brief bursts of vigorous activity (85–100% of $VO_{2peak}$), interspersed with short periods of rest or low-intensity physical activity to allow recovery (19, 20). HIIT requires less time and yields benefits similar to MICT (21). HIIT is better than MICT for improving ventilation (22) in obese patients, and MICT can result in fatigue and respiratory restriction (23). Some studies showed that, compared with MICT, HIIT has good efficacy in improving motor performance, cardiovascular function and reducing cardiovascular risk factors in patients with CVD (3, 24, 25). However, other studies have shown that both HIIT and MICT can improve cardiorespiratory fitness in patients with CVD (26–28). This controversy might be attributed to different training models, frequencies, and intervention durations in the different studies, complicating interpretation of results and clinical applications (29).

HIIT has been divided into three models defined by exercise and recovery times. Long-interval HIIT involves 4 min of high-intensity exercise interspersed with 3 min of active or passive recovery. Medium-interval HIIT involves 1–2 min of high-intensity exercise interspersed with 1–4 min of low-intensity recovery. Short-interval HIIT involves 15–60 s of high-intensity training interspersed with 15–120 s of low-intensity recovery (22, 30). However, which model of HIIT is most effective in improving cardiorespiratory fitness in patients with CVD, and how the various models compare with MICT, remains unclear (22).

Some studies have shown that HIIT twice a week, and even at lower frequencies, can significantly improve cardiorespiratory fitness (31, 32). Chin et al. found that HIIT once a week can improve cardiorespiratory fitness compared with no intervention, and HIIT 2–3 times a week can improve cardiorespiratory fitness to a greater extent than MICT (33). However, the American College of Sports Medicine (ACSM) guidelines state that only moderate to high-intensity continuous training or intermittent training at least three times a week can effectively improve cardiorespiratory fitness, while training $<2$ times a week will not yield significant improvement in healthy adults (34). Stavrinou et al. reported that HIIT twice weekly increases $VO_{2peak}$ by 10.8%, while training three times a week increases $VO_{2peak}$ by 13.6% (35). It has been reported that there is a dose-response relationship between lactate threshold and the frequency of intermittent training (36). Considering the physical condition of CVD patients, it is important to explore an optimal frequency of HIIT in CR.

It has been shown that intervention duration is a key factor determining adaptive changes in body function and structure in response to exercise (37). A previous systematic review and meta-analysis reported that more than 6 weeks of HIIT was superior to MICT in improving cardiorespiratory fitness in patients with CVD, and 7–12 weeks of HIIT was the largest improvements in cardiorespiratory fitness (3). However, some studies have yielded contradictory results (38, 39). For these reasons, this systematic review and meta-analysis of randomized controlled trials (RCTs) aimed to explore the effects of MICT and different HIIT training models and intervention

frequencies and durations on cardiorespiratory fitness in patients with CVD.

## MATERIALS AND METHODS

This systematic review and meta-analysis was carried out in conformance with PRISMA guidelines (40). The literature search and screening plan were pre-established. The protocol for this systematic review has been registered on PROSPERO (CRD42021245810).

### Literature Search

Articles were systematically searched journals indexed in the PubMed, Web of Science, Cochrane Library, Embase and Scopus databases from inception to December 2021 using the following terms: [(High-intensity interval training) OR (High-intensity interval exercise) OR (High-Intensity Intermittent Exercise) OR (Sprint Interval Training) OR (High-Intensity Intermittent Exercises) OR (Anaerobic interval exercise) OR (Exercise, High-Intensity Intermittent) OR (HIIT) OR (HIT) OR (HIIE)] AND [(Cardiac rehabilitation) OR (Rehabilitation, Cardiac) OR (Cardiovascular Rehabilitation) OR (Rehabilitation, Cardiovascular)]. We also searched the literature in other ways, retrieving gray literature, printed materials in the library, and references cited in the articles.

### Study Selection

Two researchers selected articles in an unblinded manner. When there were differences in their selections, a third researcher participated in the discussion to reach a final decision. Inclusion criteria for this systematic review and meta-analysis included (1) randomized controlled trials written in English; (2) adult patients with CVD who had undergone cardiac rehabilitation; (3) HIIT and MICT exercise interventions, but not other training (e.g., HIIT combined with strength training, intervention based on aquatic HIIT programs, etc.); (4) a clear statement of the type, intensity, duration, intervention time, frequency, and interval of the exercise intervention; (5) $VO_{2peak}$ among the outcome measures; and (6) complete datasets with a report of the mean and standard deviation of $VO_{2peak}$ before and after the intervention.

Exclusion criteria included (1) duplicated articles; (2) abstract and conference articles; (3) outcome measures without $VO_{2peak}$; (4) incomplete reports of study data.

### Data Extraction

Two researchers independently read the full text of the literature in an unblinded manner and extracted outcomes. When there was disagreement, a third person participated in the discussion to reach a final decision. The extracted information included (1) citation (author and year of publication); (2) patient characteristics (sample size, age, gender and diagnosis); (3) intervention (exercise intervention type, duration, intensity and frequency); (4) outcome measures (pre- and post-$VO_{2peak}$ values and changes of $VO_{2peak}$); (5) adverse events.

### Study Quality

Study quality was assessed using the Cochrane Collaboration's tool (41) and the Physiotherapy Evidence Database (PEDro) Scale (42). Items of the Cochrane Collaboration's tool were evaluated in three categories: low risk of bias, unclear bias, and high risk of bias. The following characteristics were evaluated: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. The PEDro-scale included the following 11 items: eligibility criteria and source, random allocation, concealed allocation, baseline comparability, blinding of participants, blinding of therapists, blinding of assessors, adequate follow-up (>85%), intention-to-treat analysis, between-group statistical comparisons, reporting of point measures, and measures of variability (42). Eligibility criteria and source affected the external validity of the experiment without affecting internal and statistical validity; this item was therefore not used to calculate the PEDro score (42). The item "blinding of participants and blinding of therapists" did not apply to the intervention studies in CR (3). We removed these two items from the quality assessment, yielding a total score of eight.

### Statistical Analysis

Consistent with the purpose of this study, previous studies were collated according to the HIIT model (long-, medium-, or short-interval) (22, 30), HIIT intervention frequency (two, three, or five times a week) (43), and intervention duration (up to 6 weeks, 7–12 weeks, and more than 12 weeks) (3). The primary outcome was changes in $VO_{2peak}$ after intervention in CR. The secondary outcome was adverse events, including cardiovascular events among others. An adverse event was defined as an event that occurred during or up to 4 h after an intervention session (44).

Pooled-effect estimates were obtained from the random-effects model and the mean differences (MDs) of the pre- to post-intervention values, from which the corresponding 95% confidence intervals (95% CI) were calculated. If studies did not provide the standard deviation (SD) of change in $VO_{2peak}$, it was calculated using a correlation coefficient (r) of 0.5 and the following equation from the Cochrane Handbook (45):

$$SD_{change} = \sqrt{SD_{pre}^2 + SD_{post}^2 - \left(2r \times SD_{pre} \times SD_{post}\right)} \quad (1)$$

Heterogeneity was assessed by Cochrane's Q and $I^2$ static. $I^2 <$ 25% indicates no significant heterogeneity; 25% $< I^2 <$ 50%, low heterogeneity; 50% $< I^2 <$ 75%, medium heterogeneity; $I^2 >$ 75%, high heterogeneity. Sensitivity analysis was used to examine the possible effects of individual studies on heterogeneity and overall effect of an intervention. This systematic review and meta-analysis was conducted using Review Manager 5.4 and Stata. The threshold for statistical significance was $p < 0.05$.

Publication bias was assessed with a visual inspection of funnel plots. Additionally, funnel plot asymmetry was statistically tested by Egger's test and $p < 0.05$ was considered significant (46). If there was any publication bias, the stability of the results was evaluated using a trim and fill method (47).

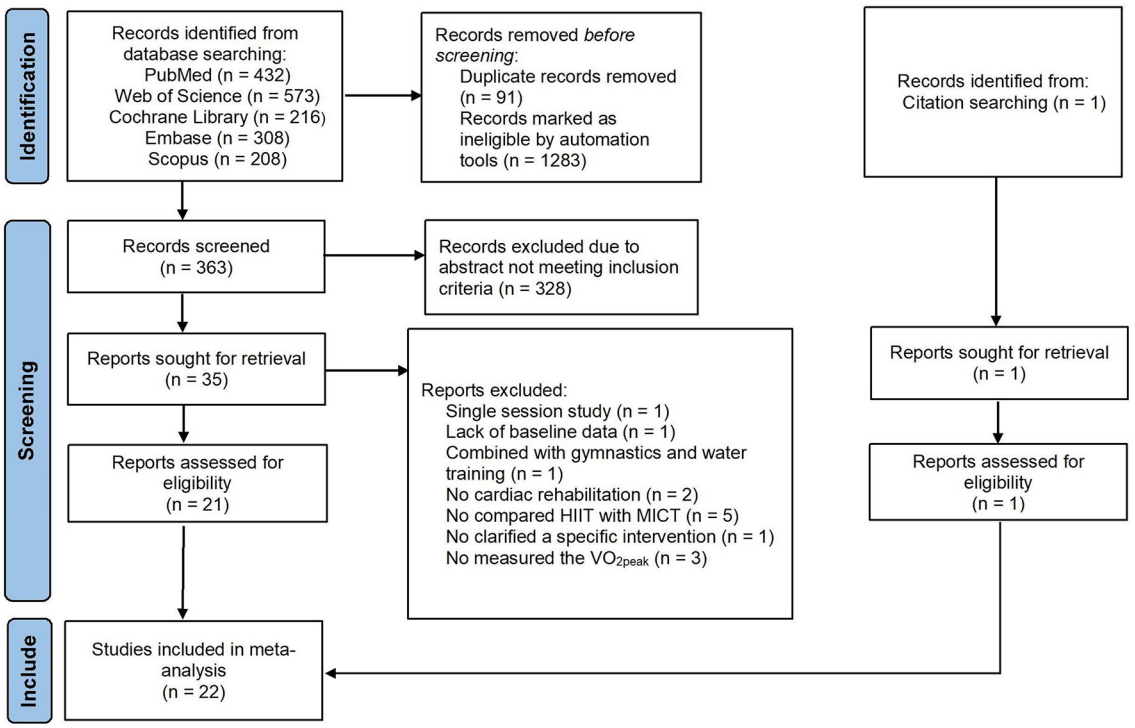


**FIGURE 1 |** PRISMA flow diagram of literature search strategies. HIIT, high-intensity interval training; MICT, moderate-intensity interval training; $VO_{2peak}$, peak oxygen uptake.

# RESULTS

## Literature Search

A PRISMA diagram of literature search and selection was presented in **Figure 1**. The initial search resulted in 1,738 articles from journals indexed in the PubMed, Web of Science, Cochrane Library, Embase, Scopus and other ways. The duplicated ($n$ = 91) and ineligible documents ($n$ = 1283) were excluded by automation tools. The remaining articles ($n$ = 364) were screened. Three hundred and twenty-eight articles did not meet the inclusion criteria and thus were excluded. The remaining articles ($n$ = 36) were read in full text and 22 articles were finally included in this study. Fourteen articles were excluded because of single-session intervention ($n$ = 1), the lack of baseline data ($n$ = 1), the study combined with gymnastics and underwater sports ($n$ = 1), no cardiac rehabilitation ($n$ = 2), without compared HIIT with MICT ($n$ = 5), no clarified a specific intervention ($n$ = 1) and no measured the $VO_{2peak}$ ($n$ = 3).

## Study Characteristics

The studies of RCTs were included from 2004 to 2020. There were 949 participants (age: 48 to 76 years), of which 476 participants were in the HIIT group, and 473 participants were in the MICT group. Not all studies reported gender, for those who did, 735 men and 155 women were reported. The studies reported the patients with coronary artery disease (25, 27, 48–56), heart failure (HF) (19, 28, 57–62), myocardial infarction (63, 64), and heart transplant patients (65) in CR.

In included studies, the intervention program included cycle ergometers and treadmill exercise except one study used a combination of a stair climber, treadmill, and arm/leg ergometer exercise (49). The HIIT models included short-interval training model in six studies (25, 50, 54, 57–59), medium-interval training model in two studies (49, 52), and long-interval training model in 14 studies (19, 27, 28, 48, 51, 53, 55, 56, 60–65). All studies based on maximum/peak test data to set exercise intensity, such as $VO_{2peak}$, $HR_{peak}$ (peak heart rate), $VO_2R$ (oxygen uptake reservation), HRR (heart rate reservation), PPO (peak power output), maximum workload, maximum effort, and respiratory compensation point. Intervention duration was from 3.5 weeks to 9 months, with five studies reporting for 0–6 weeks (55, 56, 59, 60, 63), 15 studies reporting for 7–12 weeks (19, 25, 27, 28, 48, 50, 51, 53, 54, 56–58, 61, 62, 64), and four studies reporting data more than 12 weeks (49, 52, 57, 65). The intervention frequency was between 2 and 5 times per week, with 16 studies for three times per week (19, 25, 27, 48, 51–57, 60–63, 65), three studies for two times per week (49, 50, 58), one study for five times per week (59) and two study performed dynamic frequency (28, 64). The duration of intervention sessions ranged from 25 to 50 min. Seventeen studies were supervised by professional therapists and five studies were unsupervised. The monitor control index incorporated the heart rate, blood pressure, electrocardiogram, and RPE (rating of perceived exertion). Descriptive characteristics of the included studies were shown in **Table 1**.

**TABLE 1 |** Descriptive characteristics of the included studies.

| Study | Participants | | | Duration, and frequency | Exercise intervention | |
|---|---|---|---|---|---|---|
| | Age | Gender (M/F) | Population | | HIIT | MICT |
| Rognmo et al. (48) | HIIT 62.9 ± 11.2 MICT 61.2 ± 7.3 | HIIT 6/2 MICT 8/1 | CAD | 10 wks; 3 times / wk | 4*4-min intervals at 85–95% $HR_{peak}$, interspersed by 3 min active recovery at 65–75% $HR_{peak}$ | 41 min at 65–75% $HR_{peak}$ |
| Warburton et al. (49) | HIIT 55 ± 7 MICT 57 ± 8 | HIIT 7/0 MICT 7/0 | CAD | 16 wks; 2 times / wk | 2 min at 85–95% HRR/$VO_2$R interspersed by 2 min active recovery at 35–45% HRR/$VO_2$R, a total of 30 min | 30 min at 60% HRR/$VO_2$R |
| Wisløff et al. (19) | HITT 76.5 ± 9 MICT 74.4 ± 12 | HIIT 7/2 MICT 7/2 | HF | 12 wks; 3 times / wk | 4*4-min intervals at 90–95% $HR_{peak}$, interspersed by 3 min active recovery at 50–70 % $HR_{peak}$ | 47 min at 70–75% $HR_{peak}$ |
| Iellamo et al. (28) | HIIT 62.2 ± 8 MICT 62.6 ± 9 | HIIT 8/0 MICT 8/0 | HF with reduced ejection fraction | 12 wks; 2–5 times / wk | 4*4-min intervals at 75–80% HRR, interspersed by 3 min active recovery at 45–50% HRR | 30–45 min at 45–60% HRR |
| Currie et al. (50) | HIIT 62 ± 11 MICT 68 ± 8 | HIIT 11 MICT 11 Total 20/2 | CAD | 12 wks; 2 times / wk | 10*1-min intervals at 80–104 % PPO, interspersed by 1 min active recovery at 10% PPO | 30–50 min at 51–65% PPO |
| Keteyian et al. (51) | HIIT 60 ± 7 MICT 58 ± 9 | HIIT 11/4 MICT 12/1 | CAD | 10 wks; 3 times / wk | 4*4-min intervals at 80–90% HRR, interspersed by 3 min active recovery at 60–70% HRR | 30 min at 60–80% HRR |
| Koufaki et al. (57) | Total:59.1 ± 8.6 | HIIT 8 MICT 9 Total 14/3 | HF with reduced ejection fraction | 12 wks; 3 times / wk | 2*15 min bouts,30 s at 50% of the maximum workload reached with the MSEC test (100% PPO), interspersed by 1 min recovery periods at 20–30% of peak power output (25–40 watts) | 40 min at 40–60% $VO_{2peak}$ |
| Koufaki et al. (57) | Total:59.1 ± 8.6 | HIIT 8 MICT 9 Total 14/3 | HF with reduced ejection fraction | 24 wks; 3 times / wk | 2*15 min bouts,30 s at 50% of the maximum workload reached with the MSEC test, interspersed by 1 min recovery periods at 20–30% of peak power output (25–40 watts) | 40 min at 40-60% $VO_{2peak}$ |
| Angadi et al. (60) | HIIT 69.0 ± 6.1 MICT 71.5 ± 11.7 | HIIT 8/1 MICT 4/2 | HF with preserved ejection fraction | 4 wks; 3 times / wk | 4*4-min intervals at 85–90% $HR_{peak}$, interspersed by 3 min active recovery at 50% $HR_{peak}$ | 30 min at 70% $HR_{peak}$ |
| Kim et al. (63) | HIIT 57 ± 11.58 MICT 60.2 ± 13.64 | HIIT 12/2 MICT 10/4 | Acute myocardial infarction patients with drug-eluting stent | 6 wks; 3 times / wk | 4*4-min intervals at 85–95% HRR, interspersed by 3 min active recovery at 50–70% HRR | 25 min at 70–85% HRR |
| Benda et al. (58) | HIIT 63 ± 8 MICT 64 ± 8 | HIIT 9/1 MICT 10/0 | HF with reduced ejection fraction | 12 wks; 2 times / wk | 10*1-min intervals at 60–75% of maximal workload and Borg score of 15–17, interspersed by 2.5 min active recovery at 30% of maximal workload | 30-min at 60–75% of maximal workload, Borg score of 12–14 |
| Cardozo et al. (52) | HIIT 56 ± 12 MICT 62 ± 12 | HIIT 14/9 MICT 16/8 | CAD | 16 wks; 3 times / wk | 2 min at 90% $HR_{peak}$, interspersed by 2 min active recovery at 60% $HR_{peak}$, a total of 30 min | 30 min at 70–75% $HR_{peak}$ |
| Jaureguizar et al. (25) | HIIT 58 ± 11 MICT 58 ± 11 | HIIT 28/8 MICT 33/3 | CAD | 8 wks; 3 times / wk | In the first month, 20 s at 50% of the maximum load reached with the SRT, interspersed by 40 s recovery periods at 10% of the maximum load, the total duration was 40 min. In the second month, the intensity of exercise was adjusted using the results of a new SRT | 40 min below the HR at $VT_1$ during the first month. During the second month, the intensity of the exercise was adjusted, increasing to a training HR that corresponded to $VT_1$ plus 10% |

*(Continued)*

**TABLE 1 |** Continued

| Study | Participants | | | Duration, and frequency | Exercise intervention | |
|---|---|---|---|---|---|---|
| | Age | Gender (M/F) | Population | | HIIT | MICT |
| Prado et al. (53) | HIIT 56.5 ± 2.7 MICT 61.3 ± 2.2 | HIIT 14/3 MICT 14/4 | CAD | 12 wks; 3 times / wk | 7*3-min intervals at the respiratory compensation point, interspersed by 3 min active recovery at VAT intensity | 50 min at VAT intensity. |
| Conraads et al. (56) | HIIT 57.8 ± 8.8 MICT 59.9 ± 9.2 | HIIT 91/9 MICT 89/11 | CAD | 6 wks; 3 times / wk | 4*4-min intervals at 85–95% $HR_{peak}$, interspersed by 3 min active recovery at 50–70% $HR_{peak}$ | 37 min at 70–75% $HR_{peak}$ |
| Conraads et al. (56) | HIIT 57.8 ± 8.8 MICT 59.9 ± 9.2 | HIIT 91/9 MICT 89/11 | CAD | 12 wks; 3 times / wk | 4*4-min intervals at 85–95% $HR_{peak}$, interspersed by 3 min active recovery at 50–70% $HR_{peak}$ | 37 min at 70–75% $HR_{peak}$ |
| Besnier et al. (59) | HIIT 59 ± 13 MICT 59.5 ± 12 | HIIT 11/5 MICT 11/4 | HF with reduced ejection fraction | 3.5 wks; 5 times / wk | 2*8 min blocks, 30 s at 100% peak power output, interspersed by 30 s passive recovery | 30 min at 60% peak power output |
| Jaureguizar et al. (54) | HIIT 57.6 ± 9.8 MICT 58.3 ± 9.5 | HIIT 50/7 MICT 42/11 | CAD | 8 wks; 3 times / wk | In the first month, 20 s at 50% of the maximum load reached with the SRT, interspersed by 40 s recovery periods at 10% of the maximum load, the total duration was 40 min. In the second month, the intensity of exercise was adjusted using the results of a new SRT | 40 min below the HR at $VT_1$ during the first month. During the second month, the intensity of the exercise was adjusted, increasing to a training HR that corresponded to $VT_1$ plus 10% |
| Rolid et al. (65) | HIIT 50 ± 12 MICT 48 ± 14 | HIIT 28/9 MICT 29/12 | Heart transplantation | 36 wks; 3 times / wk | 4*4-min intervals at 85–95% maximal effort (RPE 16–18), interspersed by 3 min active recovery at RPE 11-13 | 25 min at 60–80% maximal effort (RPE 12–15) |
| Choi et al. (64) | HIIT 53.00 ± 6.84 MICT 57.31 ± 12.62 | HIIT 21/2 MICT 18/3 | MI | 9-10 wks; 1-2 times / wk | 4*4-min intervals at 85–100% $HR_{max}$, interspersed by 3 min active recovery at 50–60% $HR_{max}$ | 28 min at 60–70% $HR_{max}$ |
| Anderson et al. (61) | HIIT 60 ± 10 MICT 60 ± 9 | HIIT 3/7 MICT 4/5 | HF with preserved ejection fraction | 12 wks; 3 times / wk | 4*4-min intervals at 85–95% $HR_{peak}$, interspersed by 3 min active recovery at 60–70% $HR_{peak}$ | 47 min at 60–70% $HR_{peak}$ |
| Rocco et al. (27) | HIIT 56.5 ± 3.0 MICT 62.5 ± 2.0 | HIIT 14/3 MICT 15/5 | CAD | 12 wks; 3 times / wk | 7*3-min intervals at the respiratory compensation point, interspersed by 3 min active recovery at VAT intensity | 50 min at VAT intensity |
| Ulbrich et al. (62) | HIIT 53.15 ± 7.0 MICT 54.02 ± 9.9 | HIIT 12/0 MICT 10/0 | HF | 12 wks; 3 times / wk | 3 min at 95% $HR_{peak}$, interspersed by 3 min active recovery at 70% $HR_{peak}$, a total of 40 min | 40 min at 75% $HR_{peak}$ |
| Taylor et al. (55) | HIIT 65 ± 7 MICT 65 ± 8 | HIIT 43 MICT 43 Total 86 | CAD | 4 wks; 3 times / wk | 4*4-min intervals at 15–18 RPE, interspersed by 3 min active recovery at 11–13 RPE | 40 min at 11–13 RPE |

*M, male; F, female; HR, heart rate; $HR_{peak}$, peak heart rate; HRR, heart rate reservation; $VO_2R$, oxygen uptake reservation; $VO_{2peak}$, peak oxygen uptake; PPO, peak power output; MSEC, maximum short exercise capacity; SRT, steep ramp test; VAT, ventilatory anaerobic threshold; $VT_1$, the first ventilatory thresholds; RPE, rating of perceived exertion, Wks; weeks; CAD, coronary artery disease; HF, heart failure; MI, Myocardial Infarction.*

## Quality Assessment

Two researchers independently assessed the quality of the included studies and discrepancies were resolved by consensus. The quality of the included studies was evaluated using the Cochrane Collaboration's tool and the result showed reasonably (**Figure 2**). The quality of rehabilitation trials was assessed by the PEDro scale and the score ranged from 4 to 7.

## Sensitivity Analysis

The total heterogeneity and the subgroup heterogeneity for long-interval HIIT, three times a week and 7–12 weeks were 13, 28, 22, and 35%, respectively. To verify the reliability of the findings, we excluded the literature one by one and examined whether each article had a significant effect on the pooled results. Sensitivity analysis showed that the study of Wisløff et al. had a significant effect on the combined results of this meta-analysis (19). After

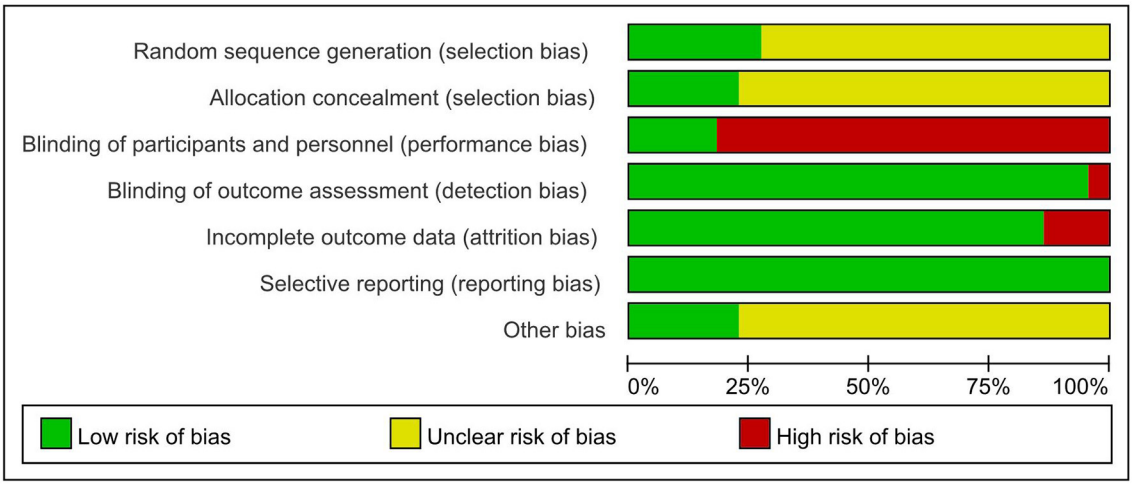


**FIGURE 2 |** Summary of risk of bias by domain.

removing this study, the total heterogeneity and intra subgroup heterogeneity of this meta-analysis dropped to 0%.

In the Wisløff et al. study, the participants were mainly diagnosed with heart failure and cardiac dysfunction (mean left ventricular ejection fraction 29%), and the baseline $VO_{2peak}$ was very low (19). This might be the reason for the large heterogeneity. Therefore, we excluded this literature and performed a meta-analysis of the remaining 21 articles (23 studies).

## Changes of $VO_{2peak}$: Meta-Analysis Results

The random-effect model showed that $VO_{2peak}$ of patients with CVD was significant improvement in HIIT group as compared with MICT group (MD = 1.35, 95% CI = 0.87–1.84, $I^2$ = 0%, $p < 0.00001$, **Figure 3**). In HIIT model, $VO_{2peak}$ was significant increasement in short-interval HIIT (MD = 1.14, 95% CI = 0.40–1.88, $I^2$ = 0%, $p = 0.003$), medium-interval HIIT (MD = 4.02, 95% CI = 1.29–6.76, $I^2$ = 0%, $p = 0.004$) and long-interval HIIT (MD = 1.36, 95% CI = 0.71–2.02, $I^2$ = 0%, $p < 0.0001$) in comparison with MICT group (see **Figure 4**). In intervention frequencies of HIIT, there was a significant improvement in $VO_{2peak}$ using HIIT three times a week (MD = 1.28, 95% CI = 0.77–1.79, $I^2$ = 0%, $p < 0.00001$, **Figure 5**). $VO_{2peak}$ showed a significant improvement in HIIT group with 0–6 weeks (MD = 1.42, 95% CI = 0.39–2.45, $I^2$ = 0%, $p = 0.007$), 7–12 weeks (MD = 1.12, 95% CI = 0.52–1.71, $I^2$ = 0%, $p = 0.0002$) and >12 weeks (MD = 2.35, 95% CI = 0.94–3.75, $I^2$ = 0%, $p = 0.001$) as compared with MICT group (see **Figure 6**).

## Adverse Events

Adverse events related to exercise intervention were reported for 17 of 21 studies (80.95%). Eleven adverse events were reported. There was only one minor cardiovascular event in the HIIT group and the patient had syncope during one session, but continued to participate in the study. The other ten adverse events were classified as non-cardiovascular. Four adverse events occurred in the HIIT group: knee pain, ankle injury and ankle fracture. The other six adverse events were in the MICT group: leg pain, knee injury, anxiety/panic attack, back pain, epilepsy, knee pain (prosthesis) and ankle injury.

## Publication Bias

Twenty-one articles (23 studies) were examined for publication bias. Visual inspection of the funnel plot (**Figure 7**) was asymmetry, but Egger's test ($p = 0.101$) revealed there was no significant publication bias. The trim and fill adjusted 26 studies, and the mean difference was 1.26 (95% CI = 0.78–1.74). The three imputed hypothetical studies produced a symmetrical funnel plot (**Figure 8**). Further research would include the three studies to guarantee the symmetry of the funnel chart and eliminate potential publication bias.

# DISCUSSION

This systematic review and meta-analysis carried out here identified different HIIT models for improving $VO_{2peak}$ in patients with CVD, and explored the most effective intervention frequency and duration to optimize HIIT. In contrast to previous meta-analyses (3, 43, 66), our study included new and large-sample trials as well as multicenter randomized controlled trials. To our knowledge, this is the first study to explore which model of HIIT provides the greatest benefits for cardiorespiratory fitness in CR when compared with MICT. The results revealed that HIIT is superior to MICT for improving cardiorespiratory fitness in patients with CVD. Medium-interval HIIT 3 times/week for more than 12 weeks resulted in the greatest improvement in cardiorespiratory fitness in CR.

The meta-analysis in this study showed that HIIT increased $VO_{2peak}$ much more than MICT. These results are consistent with the report of Liou et al. that HIIT improves $VO_{2peak}$ in patients with coronary artery disease (CAD) (29). The meta-analysis of Pattyn et al. also showed that HIIT elicits larger

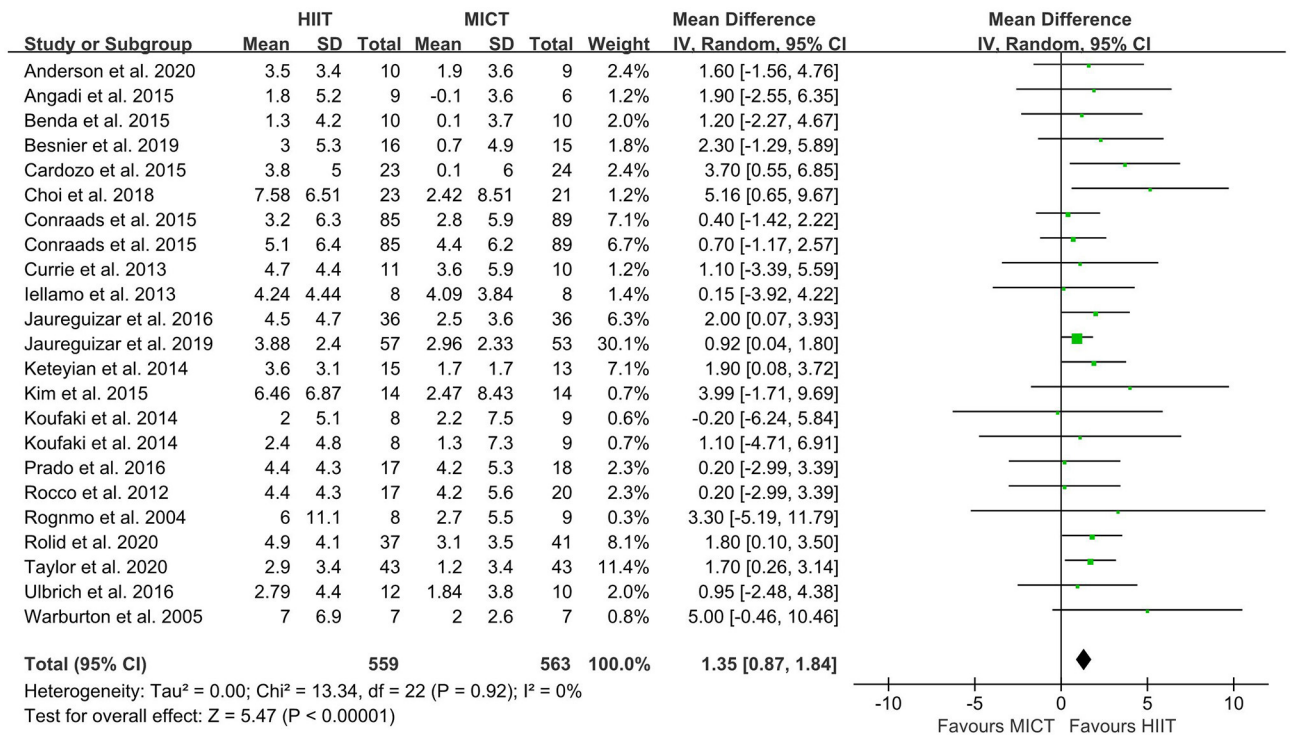


**FIGURE 3 |** Forest plot depicting cardiorespiratory fitness changes as a HIIT vs. MICT. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

increases in $VO_{2peak}$ than does MICT in patients with CAD (67). Studies have shown that cardiorespiratory fitness is a strong predictor of cardiovascular disease and mortality (12). Compared with MICT, our meta-analysis showed that HIIT intervention elicited a 1.35 mL/kg/min greater improvement in $VO_{2peak}$. This is of clinical significance because an increase in $VO_{2peak}$ reduces the risk of all-cause mortality in patients with CAD and HF (68, 69).

The improvement in $VO_{2peak}$ using HIIT occurred over periods of 0–6 weeks, 7–12 weeks and >12 weeks, with the maximum benefit observed at >12 weeks. Intervention duration plays an important role in the efficacy of HIIT (37). For patients with chronic heart failure, 16 weeks may be enough to achieve maximum improvement in function (> 15%), as suggested by a systematic review (70, 71). Moreover, unpublished data in the Smart and Steele review showed that $VO_{2peak}$ increased by 13% after 8 weeks of aerobic exercise and 21% after 16 weeks (71). Moholdt et al. trained patients who had undergone coronary artery bypass grafting and found that $VO_{2peak}$ was not significantly different in the HIIT and MICT groups at the fourth week, but was significantly higher in the HIIT group after 6 months (72). Jurio-iriarete and Maldonado-Mar-tin also reported that HIIT of <12 weeks did not improve cardiorespiratory fitness any more than MICT, but there did seem to be a greater increase with HIIT after 12 weeks (38). The study showed that long-term HIIT is significantly better than short-term HIIT or MICT in improving $VO_{2peak}$ in overweight/obese adults with hypertension (38). Guadalupe-Grau et al. showed that up to 6 months of HIIT of middle-aged patients with metabolic syndrome not only improved skeletal muscle deoxygenation and oxygen extraction, but also increased mitochondrial enzyme activity and $VO_{2peak}$ (73). Stroke volume, heart rate, cardiac output, and blood volume are core parameters that affect $VO_{2peak}$ (30). A previous study showed that long-term HIIT is significantly superior to MICT in improving cardiac output and stroke volume in CR (74). Long-term HIIT can increase stroke volume (75) and improve cardiac autonomic function (76) *via* baroreflex-mediated augmentation of sinoatrial node regulation, enhancing $VO_{2peak}$ as well as improving resting heart rate (67). Long-term HIIT resulted in greater adaptive changes in the musculoskeletal and cardiovascular systems in patients with CVD, and more than 12 weeks of HIIT was associated with a reduction in risk factors for CVD (38). The intensity-dependent improvements in the cardiovascular and musculoskeletal systems can account for HIIT being more effective than MICT in improving $VO_{2peak}$ (67). The type of skeletal muscle, number of muscle fibers, density of capillaries, and content of mitochondria all contribute to uptake and utilization of oxygen (77). Moreover, HIIT can increase PGC-1 α and the body's oxidative capacity, as well as glucose uptake (19, 78). Long-term HIIT can increase the number and density of mitochondria and improve maximum metabolic capacity (79).

With respect to the HIIT models, the present meta-analysis showed that $VO_{2peak}$ increased significantly in short-, medium-

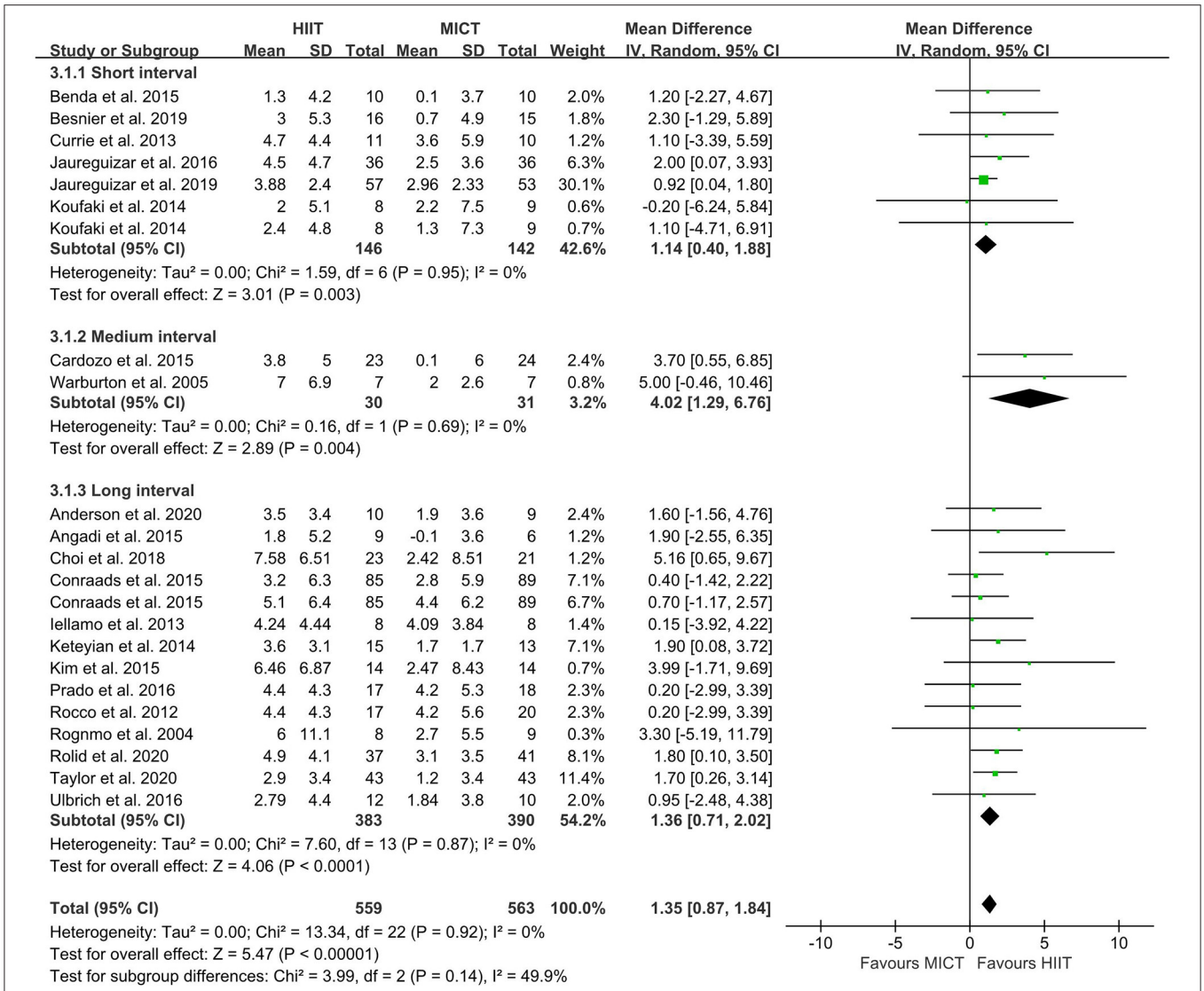


**FIGURE 4 |** Forest plot of subgroup analysis by a different model of HIIT (short-interval, medium-interval and long-interval HIIT). HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

and long-interval HIIT, but the medium-interval model had the greatest effect. In a previous study, a multicenter RCT showed that long-interval HIIT reduced ejection fraction in patients with heart failure to a greater extent than did MICT (39). This indicated that the long-interval HIIT model was superior to MICT in improving cardiac remodeling and increasing cardiorespiratory fitness. However, this study found that only 51% of patients in the long-interval HIIT group maintained their target heart rate throughout the exercise. This implied that many geriatric patients with CVD were unable to perform prolonged high-intensity exercise. The average intensity (%$VO_{2peak}$) of long-interval HIIT was higher, but there was lower tolerance and exercise compliance (22, 80, 81), which was presumably a result of long-interval HIIT being more burdensome than short- and medium-interval HIIT for patients with CVD (82). Conraads et al. found that the mean HR of patients with CAD did not reach the level required to sustain long-interval HIIT, and that training intensity had to be reduced for several patients to allow completion of the pedaling exercise or avoid extreme hyperventilation (56).

Patients in the long-interval HIIT group experienced more shortness of breath and had a higher Borg score than did those in the MICT group. Therefore, the study suggested that long-interval training at 90–95% of $HR_{peak}$ was not feasible for most of the CAD patients. In contrast, Valstad et al. showed that short-interval training of healthy college students tended to lower lactate acid (LA) concentration as well as RPE and was perceived to be easier than long-interval training (83). Ballesta et al. (43) and Ribeiro et al. (84) demonstrated that short-interval HIIT is beneficial for CVD patient compliance with

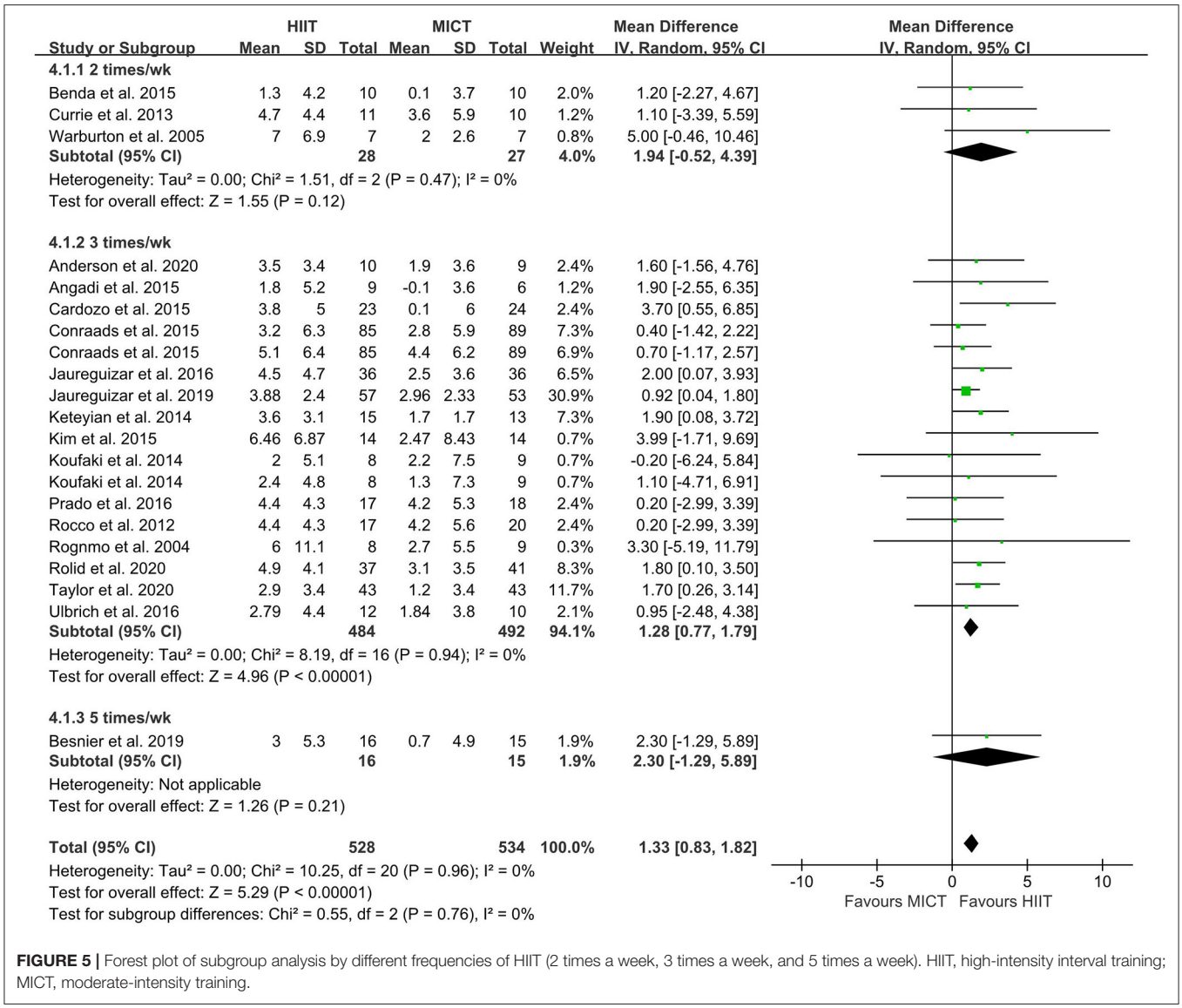


**FIGURE 5 |** Forest plot of subgroup analysis by different frequencies of HIIT (2 times a week, 3 times a week, and 5 times a week). HIIT, high-intensity interval training; MICT, moderate-intensity training.

long-term treatment. Some studies reported that short-interval HIIT improved cardiorespiratory fitness in patients with CVD (25, 58, 59). Short-interval HIIT has a shorter exercise time and more training sets compared with the medium- and long-interval models. Although short-interval HIIT saves time and is similar in training efficacy to long-interval HIIT, 15–60 s of high-intensity training is too short for patients to reach the target intensity (82). This would imply that this model might be not sufficient to produce superior benefits (85, 86). Some studies have also shown that short-interval HIIT is not superior to MICT in patients with CVD (50, 71, 87). In our study, the ability of long-interval HIIT to improve cardiorespiratory fitness in patients with CVD was shown to be greater than that of short-interval HIIT, but medium-interval HIIT was superior to both. Similarly, Cardozo et al. showed that medium-interval HIIT was superior to MICT in improving cardiorespiratory fitness in patients with CAD (52). This implies that medium-interval HIIT is more suitable for persuading patients with CVD to maintain high intensity training and to achieve the target intensity because it involves relatively moderate exercise and interval times.

Regarding HIIT frequency, three times per week increased $VO_{2peak}$. This result is consistent with the exercise frequency recommended by ACSM guidelines. One study used an intervention frequency of five times a week, so this result needs to be interpreted with caution. Similarly, Ballesta et al. in a meta-analysis of HIIT for patients with heart failure showed that HIIT three or four times a week has a significant effect on $VO_{2peak}$, while no significant change was observed when two times a week was used (43). Kavaliauskas et al. found that sprint interval training (SIT) twice a week did not improve cardiorespiratory fitness for untrained young healthy women (88). The intensity of SIT was higher than that of HIIT, but the $VO_{2peak}$ of participants did not improve. This implied that training frequency is an important variable in determining the physiological effects of

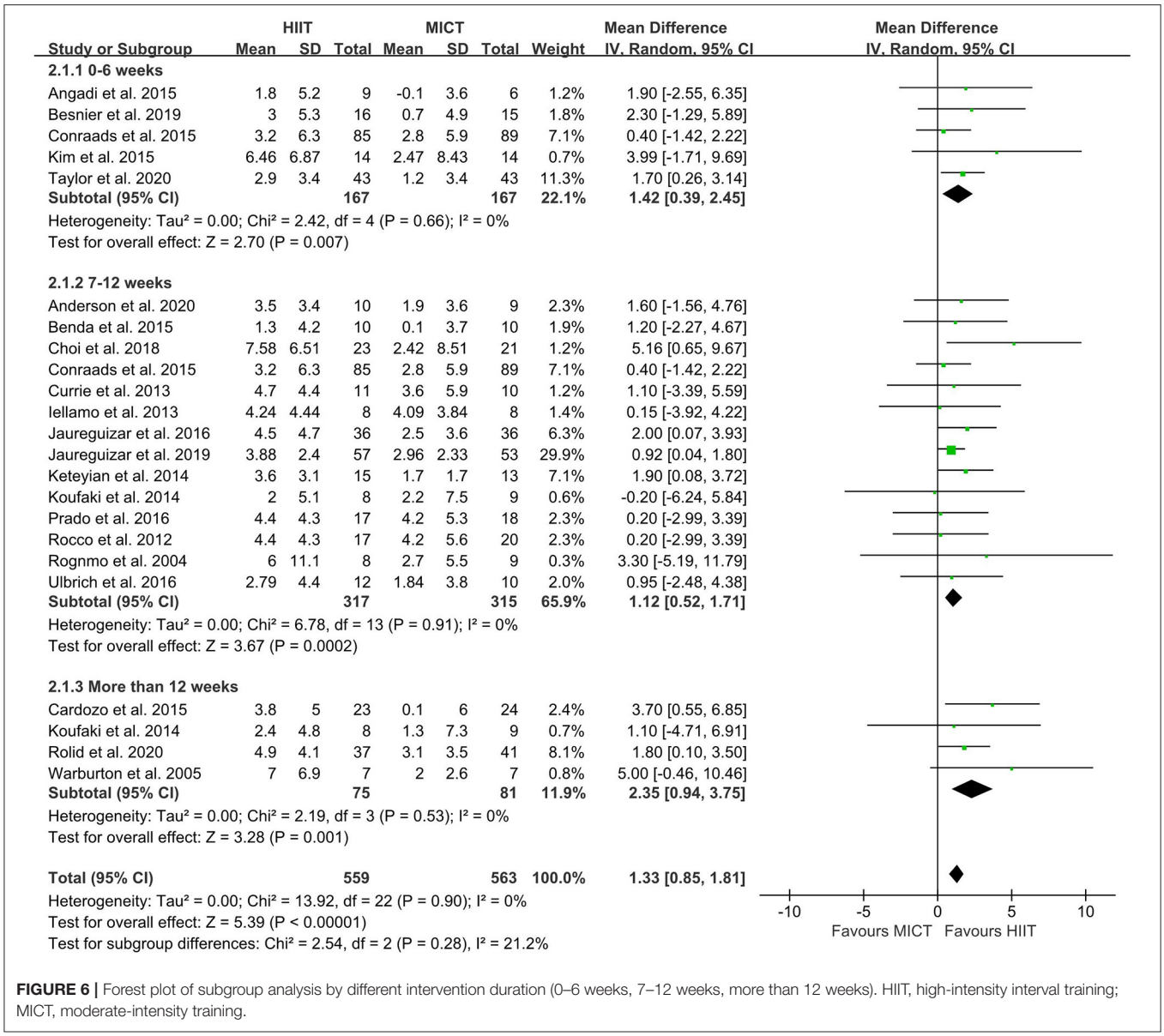


**FIGURE 6 |** Forest plot of subgroup analysis by different intervention duration (0–6 weeks, 7–12 weeks, more than 12 weeks). HIIT, high-intensity interval training; MICT, moderate-intensity training.

SIT. Some studies have shown that HIIT once or twice a week improves cardiorespiratory fitness, but the participants were healthy adults or athletes and their baseline exercise capacity and health status were generally higher than those of patients with CVD. As suggested in the ACSM guidelines, HIIT at least three times a week can increase $VO_{2peak}$ to achieve central and peripheral adaptive changes in CR. These studies indicated that HIIT three times a week might be the lowest training frequency sufficient to increase cardiorespiratory fitness in CR.

Our study found that one minor cardiovascular adverse event and four non-cardiovascular adverse events were reported in the HIIT group. Six non-cardiovascular adverse events were reported in the MICT group. Similarly, Wewege et al. (44) carried out a meta-analysis of 23 studies of CR (HIIT: 547 patients, MICT: 570 patients) and found one minor cardiovascular adverse event and three non-cardiovascular adverse events in the HIIT group and two non-cardiovascular events in the MICT group. A systematic review reported that no deaths or major cardiovascular events occurred in 17 studies of CR (HIIT: 465, MICT: 488) (3). Rognmo et al. (89) retrospectively analyzed cardiovascular adverse events in 4,846 patients with CAD and found that there was one case of fatal cardiac arrest per 129,456 patient-exercise hours for MICT and 1 per 23,182 h for HIIT. This indicated that both HIIT and MICT are at low risk of a cardiovascular event for patients with CAD in CR (89). The physical and rehabilitation medicine (PRM) physician is crucial in CR. The key responsibilities of PRM physicians are to develop and implement safe CR procedures (15) and to closely monitor patients during CR (90). Therefore, PRM physicians can help patients with CVD to reduce the incidence of adverse events.

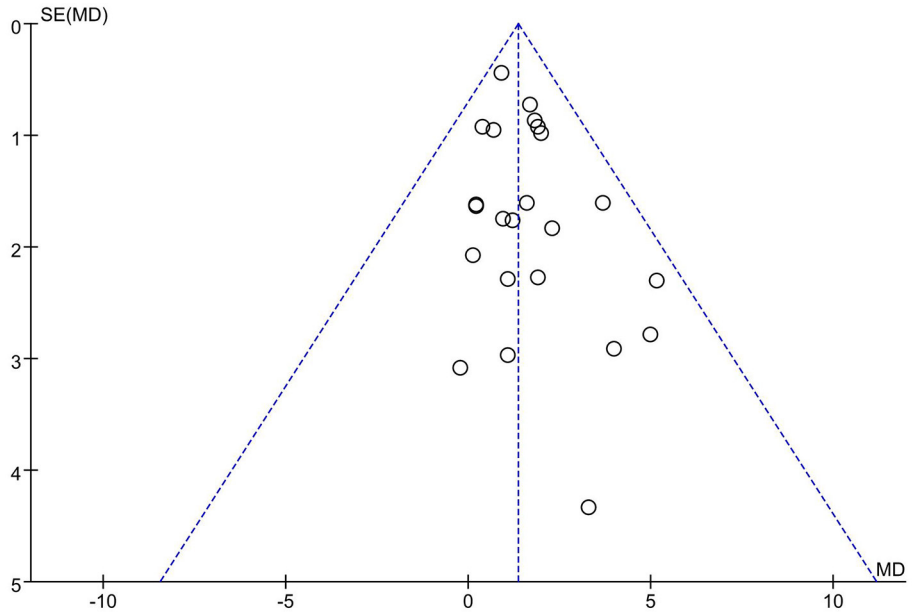


**FIGURE 7 |** Funnel plot of publication bias.

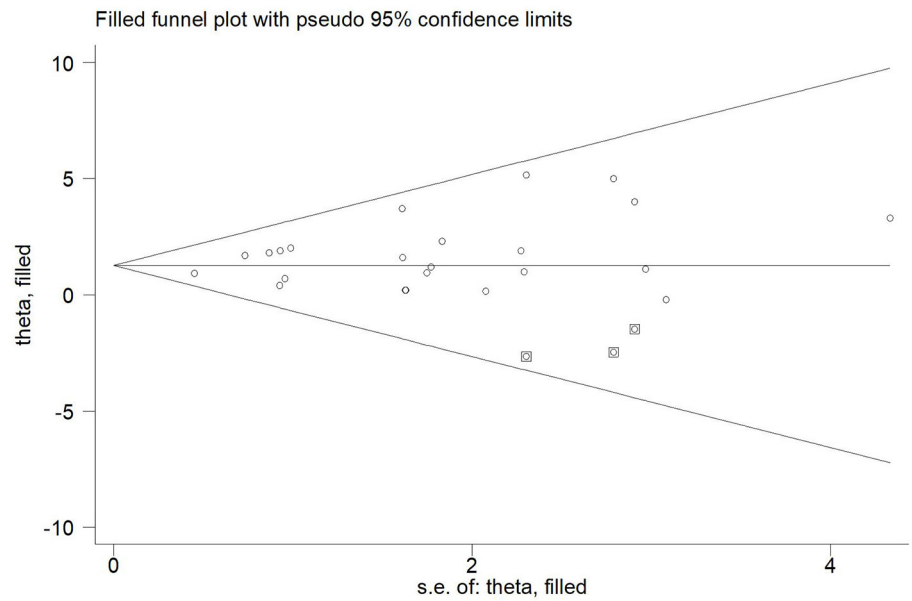


**FIGURE 8 |** The funnel plot showed the trim and fill method adjusted publication bias. ○, previous studies; ◙ filled studies.

## STRENGTHS AND LIMITATIONS

To our knowledge, this study included all literature prior to December 2021, and therefore has a large sample size. This is the first study of the effects of long-, medium- and short-interval HIIT on improvement of cardiorespiratory fitness in patients with CVD. The strengths of systematic reviews and meta-analyses include greater precision and statistical power of the estimates, but potential drawbacks include heterogeneity of the studies and publication bias (67). Imputed hypothetical studies accounted for potential publication bias in **Figure 8**, and the results are not meaningfully changed. Furthermore, the heterogeneity in similar earlier studies was large, while that of our study was low.

There were some limitations to this study. This study included many male participants, which may cause bias in the results. Only two studies in the medium-interval HIIT group were compared with MICT, and one study included HIIT five times a week, so the results from those meta-analyses have to be interpreted with some caution.

## CONCLUSION

This systematic review and meta-analysis found that HIIT is safe and appears superior to MICT for improving cardiorespiratory fitness in patients with CVD. To optimize these benefits, medium-interval HIIT three times/week for more than 12 weeks is recommended for improving cardiorespiratory fitness in patients with CVD.

## FUTURE DIRECTIONS

Future research should explore (1) the effects of medium-interval HIIT at least three times a week for more than 12 weeks in patients with CVD; (2) the long-term benefits of HIIT in patients with CVD and whether the exercise regiment is maintained. In addition, further research should recruit more female participants to examine whether HIIT is superior to MICT in a broader range of CVD patients in CR.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

TY and FQ contributed to the conception and design and drafted the manuscript. TY, YW, and FQ extracted the data and evaluated the quality. YW, HL, and ZK verified the data. TY, FQ, YW, HL, and ZK contributed to the analysis and interpretation of the data. TY, FQ, YW, HL, and ZK revised it critically for important intellectual content. All authors have read and approved the final version of the manuscript.

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# Predicting ICU Mortality in Rheumatic Heart Disease: Comparison of XGBoost and Logistic Regression

*Yixian Xu[1†], Didi Han[2†], Tao Huang[3†], Xiaoshen Zhang[4], Hua Lu[4], Si Shen[5], Jun Lyu[3*] and Hao Wang[1*]*

[1] Department of Anesthesiology, The First Affiliated Hospital of Jinan University, Guangzhou, China, [2] School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, China, [3] Department of Clinical Research, The First Affiliated Hospital of Jinan University, Guangzhou, China, [4] Department of Cardiovascular Surgery, The First Affiliated Hospital of Jinan University, Guangzhou, China, [5] Department of Radiology, Medical Imaging Center, The First Affiliated Hospital of Jinan University, Guangzhou, China

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*Yan Zhang,*
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*Linggen Gao,*
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*Marco Matteo Ciccone,*
*University of Bari Aldo Moro, Italy*

***Correspondence:**
*Jun Lyu*
*lyujun2020@jnu.edu.cn*
*Hao Wang*
*haowang@jnu.edu.cn*

*†These authors have contributed equally to this work and share first authorship*

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**Background:** Rheumatic heart disease (RHD) accounts for a large proportion of Intensive Care Unit (ICU) deaths. Early prediction of RHD can help with timely and appropriate treatment to improve survival outcomes, and the XGBoost machine learning technology can be used to identify predictive factors; however, its use has been limited in the past. We compared the performance of logistic regression and XGBoost in predicting hospital mortality among patients with RHD from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database.

**Methods:** The patients with RHD in the MIMIC-IV database were divided into two groups retrospectively according to the availability of data and its clinical significance based on whether they survived or died. Backward stepwise regression was used to analyze the independent factors influencing patients with RHD, and to compare the differences between the two groups. The XGBoost algorithm and logistic regression were used to establish two prediction models, and the areas under the receiver operating characteristic curves (AUCs) and decision-curve analysis (DCA) were used to test and compare the models. Finally, DCA and the clinical impact curve (CIC) were used to validate the model.

**Results:** Data on 1,634 patients with RHD were analyzed, comprising 207 who died during hospitalization and 1,427 survived. According to estimated results for the two models using AUCs [0.838 (95% confidence interval = 0.786–0.891) and 0.815 (95% confidence interval = 0.765–0.865)] and DCA, the logistic regression model performed better. DCA and CIC verified that the logistic regression model had convincing predictive value.

**Conclusions:** We used logistic regression analysis to establish a more meaningful prediction model for the final outcome of patients with RHD. This model might be clinically useful for patients with RHD and help clinicians to provide detailed treatments and precise management.

**Keywords: MIMIC-IV, rheumatic heart disease, XGBoost, logistic regression, intensive care unit, mortality, prediction**

# INTRODUCTION

Rheumatic heart disease (RHD) is a high priority in areas with restricted health systems (1–3), and causes approximately 250,000 deaths worldwide annually. RHD is heart damage caused by an abnormal immune response to group A streptococcal infections, which makes the morbidity and mortality of its complications a major burden for developing countries (4). Therefore, the early identification and diagnosis of RHD are very important for providing clinicians with meaningful information and allowing timely and appropriate treatment to improve survival outcomes. The pathogenesis of RHD is complicated and not yet fully understood. For this reason, an effective and reliable model for evaluating the prognosis of RHD is urgently needed to offer a basis for comprehensive diagnoses and treatments of the disease and the effective use of medical and health resources. Recent studies have indicated that serum-related markers, such as IL-1β, IL-8, IL-6, CXCL-1, tumor necrosis factor α, antistreptolysin, antideoxyribose, and nuclease B concentrations have been widely used in prognostic evaluations of RHD. However, their prognostic values are limited, for they lack sensitivity or specificity (5, 6). XGBoost is a machine learning technology with the noteworthy characteristics of assembling weak prediction models, and providing flexible and efficient missing data processing for establishing accurate prediction models (5). A logistic regression model is usually developed by the variables of each patient that predict the outcomes of future patients. Its accuracy is primarily based on the ability of the model to correctly assign patients as higher risk. The ability of the model to determine and assign the correct average absolute risk level is essential for judging the usefulness of any new predicting tool (7). Compared with machine learning technology, logistic regression has indicated better predictive performance (8–11).

In a word, this study has two purposes: (I) to use the XGBoost algorithm and logistic regression to compare the overall performance of the model in predicting mortality of patients from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database with RHD during hospitalization, and (II) to perform decision-curve analysis (DCA) and calculate clinical impact curves (CICs) to verify the logistic regression model.

# METHODS

## Database

This study was based on version 0.4 of the MIMIC-IV database and contained information on Acute Medical Unit (AMU) and ICU admissions at the Beth Israel Deaconess Medical Center from 2008 to 2019 (12). We extracted patient parameters, including demographic data, vital signs, and laboratory test data. GitHub was used to locate the codes for data extraction (https://github.com/mit-lcp/mimic-iv).

**Abbreviations:** ICU, Intensive care unit; RHD, Rheumatic heart disease; MIMIC-IV, Medical Information Mart for Intensive Care IV; AUCs, Areas-under-the-receiver operating characteristic curves; DCA, decision-curve analysis; CIC, Clinical impact curve; AMU, Acute Medical Unit; $SpO_2$, Oxyhemoglobin saturation; APSIII, Acute Physiology Score-III; CKD, chronic kidney disease; GCS, Glasgow Coma Scale; $PO_2$, partial pressure of oxygen; $PCO_2$, Partial Pressure of Carbon Dioxide; MELD, Model for End-stage Liver Disease; PT, prothrombin time; BUN, blood urea nitrogen; ANP, atrial natriuretic peptide; AF, atrial fibrillation; CCUs, coronary care units; ROC, The receiver operating characteristic curves; OR, Odds ratio; CI, Confidence interval.

## Study Population

This study included adult patients clinically diagnosed with RHD. The inclusion criteria included (I) patients were elder than 18 years and (II) diagnosed with heart tissue lesions caused by rheumatic fever activity that affected the heart structure. Only patients with first admission information showing RHD would be selected. We used the R packages "vim" (13) and "MICE" (14) for multiple imputation and visualization of <20% missing or randomly missing data in order to improve the accuracy of the review of the MIMIC-IV database, and deleted variables that were absent in more than 20% of observations.

## Data Extraction

The pgAdmin PostgreSQL (version 1.22.1) and Navicat Premium (version 12.0.28) tools were used to extract the raw data of patients diagnosed with RHD during their first hospital admission. R software (version 3.6.3) was used for the data processing (15). The following demographic data were extracted: age, sex, race, weight, length of hospital stay, and hospital death signs at their first admission. The vital signs of ICU patients were collected within 24 h of admission, including heart rate, blood pressure, body temperature, respiratory rate, Oxyhemoglobin saturation ($SpO_2$), heart rhythm, glucose, and urine output. Laboratory indicators were extracted, such as routine blood tests, liver function, kidney function, arterial blood gas analysis, Oxyhemoglobin saturation, blood electrolytes, and coagulation function. The following severity scoring systems were extracted: Charlson Comorbidity Index, systemic inflammatory response syndrome score, Simplified Acute Physiology Score-II, sequential organ failure assessment score, Acute Physiology Score-III (APSIII), Logical Evaluation System for Organ Dysfunction, sepsis, chronic kidney disease (CKD), and Glasgow Coma Scale (GCS) score (5). Information on whether the patients were using vasoactive drugs or antibiotics and taking the valve replacement/valvuloplasty, and their ventilation statuses, were all extracted at the first hospital admission.

## Statistical Analysis

Patients with RHD were divided into two groups according to survival or death during hospitalization, and the variables were compared between the groups. We eliminated the confounding variables and outliers with great impacts, and determined the influence of variables on the mortality of patients with RHD during hospitalization using correlation analysis. The Kolmogorov-Smirnov test was used to test continuous variables that conformed to a normal distribution. Student's *t*-test, one-way analysis of variance, Mann-Whitney U test, or Kruskal-Wallis H test were used to test and compare nonnormally distributed continuous data. Categorical variables were reported as numbers or percentages, and were evaluated using chi-square or Fisher's exact tests according to the number of patients.

We developed the logistic regression and XGBoost algorithm models during the model construction phase. First, through

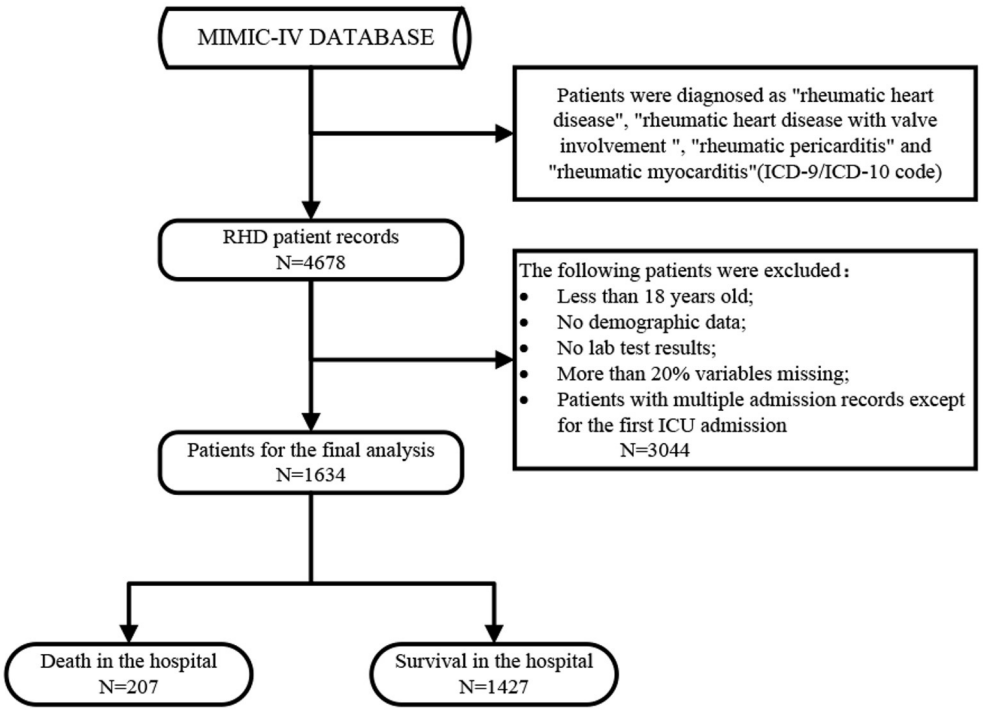


**FIGURE 1 |** The detailed process of data extraction.

backward stepwise analysis and identification using the chi-square test, the variables for which $p < 0.05$ were selected for the logistic regression model. Second, the XGBoost model (16, 17) was established to analyze the impact of each factor on mortality gain during the hospitalization period. Backward stepwise analysis was performed according to the Akaike information criterion (18), variables for which $p < 0.05$ were selected, and clinical symptoms, signs, and laboratory test variables were used to develop the XGBoost machine learning models. We tested and compared the overall performances of the two predictive models using the area under the receiver operating characteristic curve (AUC) and DCA, and selected and verified the model with the highest diagnostic value and prognostic evaluation. Finally, the CIC and DCA were drawn to clarify the clinical practicality and applicability of the model with the highest prognostic value.

Both models were analyzed using R software, and the criterion for statistically significance was set at $p < 0.05$.

# RESULTS

## Baseline Characteristics

This study analyzed the data of 1,634 patients with RHD, comprising 207 who died during hospitalization and 1,427 survived. Among the dead patients, admission age, norepinephrine, vasopressin, heart rhythm, sepsis, valve replacement, cefazolin, cefepime, mupirocin ointment, vancomycin, CKD, ventilation status, serum creatinine, urine volume, Partial Pressure of Oxygen ($PO_2$), total $CO_2$, $SpO_2$, Partial Pressure of Carbon Dioxide ($PCO_2$), blood gas analysis, routine blood test, blood biochemical indicators, scoring system, comorbidity index, and survival group were significant different. However, the differences were not statistically significant ($p > 0.05$) indicators between groups were sex, race, valvuloplasty, $PCO_2$, diastolic blood pressure, temperature, weight, bicarbonate, and hematocrit. **Figure 1** showed a flow chart of the measures for extracting research objective data. **Table 1** compared the baseline characteristics, laboratory data, and vital signs between the dead and surviving patients during the hospitalization periods. **Figure 2** was a pie chart showing racial characteristics and overall heart rhythm.

## Features Selected in Models

The most important features were included in the logistic regression and XGBoost models (**Tables 2**, **3**, respectively), which were determined by the results of backward stepwise regression analysis, and had strong correlations with mortality during hospitalization, with all $p < 0.05$. According to the analysis results for the logistic regression model regarding the contribution rate of each feature (**Table 2**; **Figure 3**), the 13 most important variables in the data set were APSIII, vasopressin, $Sp_2$, valve replacement, cefepime, GCS score, admission age, Model for End-stage Liver Disease (MELD), urine output, magnesium, prothrombin time (PT), norepinephrine, and red blood cells.

## Model Comparisons

The AUCs of the two models during the model development and verification stages were 0.838 (95% confidence interval = 0.786–0.891) and 0.815 (95% confidence interval = 0.765–0.865),

**TABLE 1 |** Baseline characteristics, vital signs, laboratory parameters and statistic results of mimic-IV patients with Rheumatic heart disease.

| Baseline variables and in-hospital factors | | Survival | Death | *P*-value |
|---|---|---|---|---|
| Number (sample size) | | 1,427 | 207 | |
| Day | | 9.0 [6.0, 15.0] | 9.0 [5.0, 17.0] | 0.422 |
| Sex (%) | | | | |
| | Man | 676 (47.4) | 107 (51.7) | 0.277 |
| | Female | 751 (52.6) | 100 (48.3) | |
| Race (%) | | | | |
| | White | 992 (69.5) | 126 (60.9) | 0.051 |
| | Black | 134 (9.4) | 23 (11.1) | |
| | Asian | 42 (2.9) | 8 (3.9) | |
| | Hispanic/Latino | 43 (3.0) | 4 (1.9) | |
| | Others | 216 (15.1) | 46 (22.2) | |
| Admission_age (year) | | 74.0 [64.0, 82.0] | 78.0 [68.0, 86.5] | <0.001 |
| Weight (kg) | | 76.6 [64.15, 90.8] | 75.5 [63.0, 91.6] | 0.396 |
| Vital signs | | | | |
| Heart_rhythm (%) | | | | |
| | AF | 267 (18.7) | 58 (28.0) | 0.002 |
| | SR | 544 (38.1) | 72 (34.8) | |
| | ST | 129 (9.0) | 26 (12.6) | |
| | SB | 38 (2.7) | 7 (3.4) | |
| | Others | 449 (31.5) | 44 (21.3) | |
| Heart_rate (bpm) | | 80.4 [72.8, 90.1] | 87.4 [75.6, 100.3] | <0.001 |
| Sbp (mmHg) | | 111.0 [104.0, 119.8] | 104.8 [97.9, 114.9] | <0.001 |
| Dbp (mmHg) | | 59.4 [53.5, 66.0] | 58.9 [52.9, 65.6] | 0.344 |
| Mbp (mmHg) | | 74.9 [69.7, 80.8] | 72.4 [66.4, 78.8] | <0.001 |
| Resp_rate (bpm) | | 18.8 [16.8, 21.3] | 21.2 [18.4, 24.0] | <0.001 |
| Temperature (°C) | | 36.7 [36.5, 36.9] | 36.7 [36.5, 37.0] | 0.61 |
| $SpO_2$ (%) | | 97.1 [95.9, 98.3] | 96.6 [95.1, 98.2] | 0.003 |
| Ventilation_status (%) | | | | |
| | No | 192 (13.5) | 19 (9.2) | 0.025 |
| | Oxygen | 612 (42.9) | 95 (45.9) | |
| | InvasiveVent | 568 (39.8) | 76 (36.7) | |
| | Non-InvasiveVent | 36 (2.5) | 11 (5.3) | |
| | HighFlow | 19 (1.3) | 6 (2.9) | |
| Urineoutput (L) | | 150.0 [60.0, 285.0] | 105.0 [37.5, 215.0] | <0.001 |
| Laboratory parameters | | | | |
| Scr | | 0.8 [0.6, 1.1] | 1.1 [0.7, 1.9] | <0.001 |
| $PO_2$ (mmHg) | | 143.0 [58.0, 347.0] | 68.0 [41.0, 133.0] | <0.001 |
| $PCO_2$ (mmHg) | | 41.0 [36.0, 46.0] | 41.0 [35.0, 49.0] | 0.552 |
| PH | | 7.4 [7.3, 7.5] | 7.4 [7.3, 7.5] | <0.001 |
| Baseexcess (mmol/L) | | 0 [−1.0, 2.0] | −1.0 [−5.0, 1.0] | <0.001 |
| Total $co_2$ (mmol/L) | | 26.0 [24.0, 29.0] | 24.0 [21.0, 29.0] | <0.001 |
| Glucose (mg/dl) | | 128.4 [115.0, 145.5] | 138.0 [114.8, 177.3] | <0.001 |
| Rdw | | 14.6 [13.5, 16.4] | 15.9 [14.9, 18.1] | <0.001 |
| White_Blood_Cells ($10^9$/L) | | 9.0 [6.8, 12.6] | 10.1 [7.15, 14.9] | 0.003 |
| Anion_Gap (mmHg) | | 26.0 [23.0, 30.0] | 29.0 [25.0, 32.0] | <0.001 |
| Bicarbonate (mmol/L) | | 38.0 [35.0, 41.0] | 39.0 [36.0, 42.0] | 0.050 |
| Calcium_Total (mmol/L) | | 10.7 [10.2, 11.3] | 10.8 [10.4, 11.6] | 0.008 |
| Chloride (mmol/L) | | 117.0 [115.0, 121.0] | 119.0 [116.0, 123.0] | 0.001 |
| Hematocrit (%) | | 51.2 [46.9, 56.1] | 52.6 [48.2, 56.8] | 0.062 |
| Hemoglobin (g/dl) | | 17.0 [15.6, 18.8] | 17.6 [16.1, 18.9] | 0.021 |
| INR_PT | | 4.4 [3.5, 6.0] | 4.8 [3.8, 6.9] | 0.013 |
| Magnesium (mmol/L) | | 3.2 [2.9, 3.7] | 3.3 [2.9, 3.8] | 0.029 |

*(Continued)*

**TABLE 1 |** Continued

| Baseline variables and in-hospital factors | | Survival | Death | *P*-value |
|---|---|---|---|---|
| MCH (pg) | | 37.5 [36.1, 39.0] | 38.1 [36.5, 40.0] | 0.001 |
| MCHC(g/L) | | 36.9 [36.3, 37.4] | 37.1 [36.4, 37.8] | 0.011 |
| MCV (fl) | | 113.0 [109.0,117.0] | 114.0 [109.0,120.0] | 0.016 |
| Phosphate (mmol/L) | | 7.7 [6.5, 9.2] | 8.6 [7.4, 10.3] | <0.001 |
| Platelet_Count ($10^9$/L) | | 702.0 [590.5, 856.0] | 739.0 [624.0, 884.0] | 0.035 |
| Potassium (mmol/L) | | 6.4 [5.8, 7.4] | 6.8 [6.0, 7.7] | 0.003 |
| PT (s) | | 46.2 [36.6, 62.7] | 49.8 [38.7, 68.9] | 0.030 |
| PTT (s) | | 150.0 [123.1, 150.0] | 150.0 [150.0, 150.1] | 0.006 |
| Red_Blood_Cells ($10^9$/L) | | 5.5 [5.2, 5.8] | 5.6 [5.2, 5.9] | 0.012 |
| Score system | | | | |
| GCS | | 14.0 [14.0, 15.0] | 12.0 [7.0, 14.0] | <0.001 |
| SOFA | | 5.0 [3.0, 8.0] | 9.0 [5.0, 12.0] | <0.001 |
| Charlson_Comorbidity_Index | | 6.0 [5.0, 8.0] | 8.0 [6.0, 10.0] | <0.001 |
| APSIII | | 41.0 [32.0, 53.0] | 71.0 [55.0, 92.5] | <0.001 |
| LODS | | 5.0 [3.0, 6.0] | 8.0 [6.0, 11.0] | <0.001 |
| MELD | | 10.0 [10.0, 20.0] | 21.32 [10.0, 30.3] | <0.001 |
| SAPSII | | 37.0 [30.0, 44.0] | 48.0 [39.0, 58.0] | <0.001 |
| Advanced life support | | | | |
| Valve_replacement (%) | | | | |
| | No | 853 (59.8) | 183 (88.4) | <0.001 |
| | Yes | 574 (40.2) | 24 (11.6) | |
| Valve_shaping (%) | | | | |
| | No | 1,379 (96.6) | 204 (98.6) | 0.205 |
| | Yes | 48 (3.4) | 3 (1.4) | |
| CeFAZolin (%) | | | | |
| | No | 889 (62.3) | 183 (88.4) | <0.001 |
| | Yes | 538 (37.7) | 24 (11.6) | |
| CefePIME (%) | | | | |
| | No | 1,249 (87.5) | 120 (58.0) | <0.001 |
| | Yes | 178 (12.5) | 87 (42.0) | |
| Mupirocin_Ointment (%) | | | | |
| | No | 1,158 (81.1) | 196 (94.7) | <0.001 |
| | Yes | 269 (18.9) | 11 (5.3) | |
| Vancomycin (%) | | | | |
| | No | 816 (57.2) | 76 (36.7) | <0.001 |
| | Yes | 611 (42.8) | 131 (63.3) | |
| Norepinephrine (%) | | | | |
| | No | 1,059 (74.2) | 70 (33.8) | <0.001 |
| | Yes | 368 (25.8) | 137 (66.2) | |
| Vasopressin (%) | | | | |
| | No | 1,275 (89.3) | 128 (61.8) | <0.001 |
| | Yes | 152 (10.7) | 79 (38.2) | |
| Accompanied diseases (comorbidity) | | | | |
| SIRS | | 2.0 [2.0, 3.0] | 3.0 [2.0, 3.0] | <0.001 |
| Sepsis (%) | | | | |
| | No | 797 (55.9) | 65 (31.4) | <0.001 |
| | Yes | 630 (44.1) | 142 (68.6) | |
| CKD (%) | | | | |
| | No | 943 (66.1) | 98 (47.3) | <0.001 |
| | Yes | 484 (33.9) | 109 (52.7) | |

*AF, Atrial Fibrillation; SR, Sinus Rhythm; ST, Sinus Tachycardia; SB, Sinus Bradycardia; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MBP, mean blood pressure; Resp_rate, respiratary rate; $SpO_2$, oxyhemoglobin saturation; SCR, serum creatinine; Rdw, Red blood cell distribution width; INR_PT, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; SAPSII, Simplified Acute Physiology Score II; GCS, Glasgow Coma Scale; APSIII, Acute Physiology Score III; LODS, Logistic Organ Dysfunction Score; SOFA, Sequential Organ Failure Assessment; MELD, Model for End-stage Liver Disease; SIRS, Systemic Inflammatory Response Syndrome; CKD, Chronic Kidney Disease.*

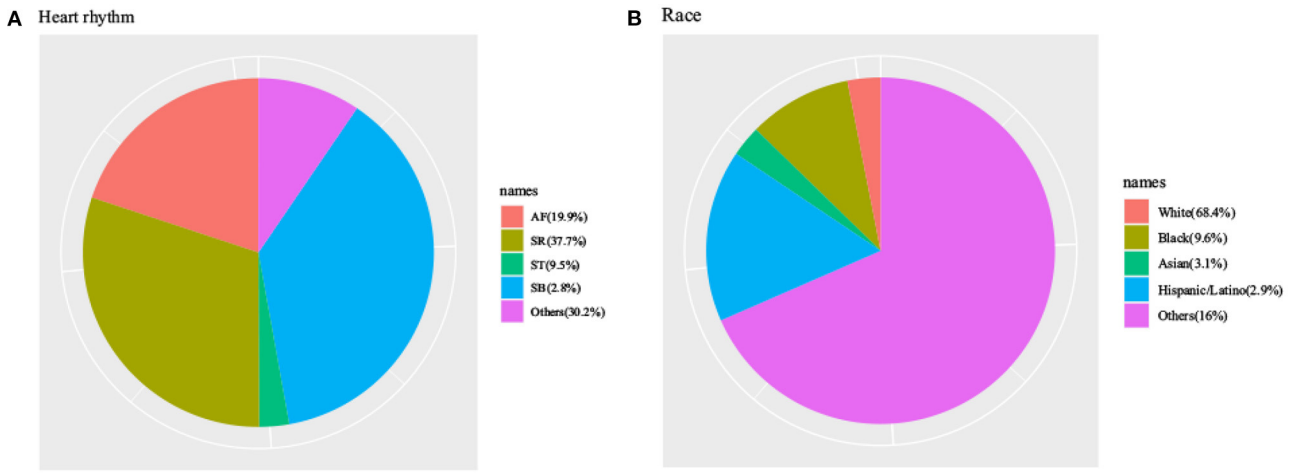


**FIGURE 2 |** Characteristics of MIMIC-IV patients with Rheumatic heart disease by heart rhythm **(A)** and characteristics of MIMIC-IV patients with Rheumatic heart disease by races **(B)**.

respectively, which indicated good distinguishing ability (**Figure 4**). The logistic regression model had a larger AUC than the XGBoost algorithm model. DCA of the two predictive models at the same time indicated that the logistic model had greater net benefit than the XGBoost algorithm model (**Figure 5**).

## Optimal Model Analysis

**Figure 6** visualizes the logistic regression model as a nomogram for calculating the risks of mortality and incidence during hospitalization using 13 selected variables for the logistic regression and 15 for the XGBoost algorithm model. The clinical applicability of the risk prediction nomogram in **Figure 7** was assessed using CIC analysis. CIC intuitively indicated that the clinical intervention guided by the nomogram scoring system had a superior overall net benefit within the actual range of the threshold probability and affected the prognoses of patients. This indicated that the logistic regression model had significant predictive value. In short, logistic regression analysis was the best model for the prognoses of patients with RHD.

**TABLE 2 |** Features selected in the logistic regression.

| Variables | OR | CI | *P*-value |
|---|---|---|---|
| Norepinephrine | 1.091 | 1.051–1.133 | <0.001 |
| Vasopressin | 1.104 | 1.052–1.158 | <0.001 |
| Valve_replacement | 0.942 | 0.910–0.975 | 0.001 |
| CefePIME | 1.092 | 1.048–1.138 | <0.001 |
| Mupirocin_Ointment | 0.968 | 0.929–1.008 | 0.114 |
| CKD | 0.970 | 0.934–1.008 | 0.116 |
| APSIII | 1.003 | 1.002–1.004 | <0.001 |
| Charlson_Comorbidity_Index | 1.006 | 0.999–1.014 | 0.102 |
| GCS | 0.989 | 0.983–0.996 | 0.001 |
| SBP | 0.999 | 0.998–1.000 | 0.109 |
| Resp_rate | 1.004 | 1.000–1.008 | 0.050 |
| $SpO_2$ | 0.990 | 0.983–0.997 | 0.004 |
| Admission_age | 1.001 | 1.000–1.002 | 0.029 |
| MELD | 1.005 | 1.002–1.007 | <0.001 |
| Urineoutput | 1.000 | 1.000–1.000 | 0.018 |
| Magnesium | 0.996 | 0.993–1.000 | 0.042 |
| PT | 0.999 | 0.999–1.000 | 0.016 |
| Red_Blood_Cells | 0.964 | 0.938–0.991 | 0.010 |

*OR, Odds Ratio; CI, Confidence Interval; CKD, Chronic Kidney Disease; APSIII, Acute Physiology Score III; GCS, Glasgow Coma Scale; SBP, Systolic Blood Pressure; Resp_rate, respiratary rate; $SpO_2$, oxyhemoglobin saturation; MELD, Model for End-Stage Liver Disease; PT, Prothrombin Time.*

# DISCUSSION

RHD causes severe mortality and huge medical economic burdens in developing countries worldwide (19). Previous reports have indicated that about 1% of over one million asylum seekers who immigrated to Europe in 2015 may have had RHD (20). Previous data suggested that the hospitalization rate of heart disease patients due to RHD increased from 20 to 50% in 1945 and 1963, respectively. According to the annual mortality rate of 1.5%, the number of worldwide deaths from RHD is estimated to be 233,000–294,000 (19).

It is currently difficult for ICU doctors to predict the adverse clinical consequences of patients with RHD, and it is also difficult to enhance the prognoses of these patients through timely interventions and treatments. Hence, the establishment of a reliable clinical prediction model is particularly important in clinical decision-making. The calculated AUCs and DCA suggested the benefits of using the logistic regression model very much more than XGBoost machine algorithm model analysis, which could be used for early predictions of patient mortality during ICU stay.

**TABLE 3 |** Features selected in the XGboost model.

| Variables | OR | CI | *P*-value |
|---|---|---|---|
| Day | 0.997 | 0.996–0.998 | <0.001 |
| Race | 1.010 | 1.001–1.019 | 0.036 |
| Norepinephrine | 1.091 | 1.051–1.132 | <0.001 |
| Vasopressin | 1.105 | 1.054–1.159 | <0.001 |
| Valve_replacement | 0.948 | 0.916–0.982 | 0.003 |
| CefePIME | 1.102 | 1.058–1.149 | <0.001 |
| Mupirocin_Ointment | 0.966 | 0.927–1.006 | 0.093 |
| CKD | 0.969 | 0.933–1.007 | 0.109 |
| APSIII | 1.003 | 1.001–1.004 | <0.001 |
| $PCO_2$ | 0.996 | 0.991–1.000 | 0.071 |
| PH | 0.476 | 0.219–1.034 | 0.061 |
| Baseexcess | 1.011 | 0.999–1.024 | 0.081 |
| Charlson_Comorbidity_Index | 1.009 | 1.002–1.017 | 0.017 |
| GCS | 0.989 | 0.982–0.995 | 0.001 |
| SBP | 0.999 | 0.998–1.000 | 0.035 |
| Resp_rate | 1.005 | 1.001–1.009 | 0.027 |
| $SpO_2$ | 0.990 | 0.983–0.997 | 0.005 |
| Admission_age | 1.001 | 1.000–1.002 | 0.129 |
| MELD | 1.005 | 1.002–1.007 | <0.001 |
| Urineoutput | 1.000 | 1.000–1.000 | 0.022 |
| Magnesium | 0.997 | 0.993–1.000 | 0.072 |
| PT | 0.999 | 0.999–1.000 | 0.056 |
| Red_Blood_Cells | 0.968 | 0.942–0.996 | 0.024 |

*OR, Odds Ratio; CI, Confidence Interval; CKD, Chronic Kidney Disease; APSIII, Acute Physiology Score III; GCS, Glasgow Coma Scale; SBP, Systolic Blood Pressure; Resp_rate, respiratary rate; $Spo_2$, oxyhemoglobin saturation; MELD, Model for End-stage Liver Disease; PT, Prothrombin Time.*

## XGBoost Model Performance

XGBoost is an efficient, flexible, and scalable machine learning algorithm classifier that improves the subsampling rate, learning rate, and maximum tree depth to control overfitting and enhance its performance. It has been extensively used to detect cardiovascular diseases and associations between prognoses (21). For example, Rong et al. (22) observed that T-wave repolarization synchronization was an important factor for determining the presence of ischemic heart disease using the non-invasive XGBoost machine learning algorithm, and found the correlation between magnetic pole characteristics and cardiac ischemia. Localized ischemia provided an opportunity, and Baskaran et al. (23) used machine learning to obtain insight into the role of images and clinical variables in predicting obstructive coronary artery disease and revascularization, while Tseng et al. (24) determined the risks after cardiac surgery, which could optimize postoperative treatment strategies and minimize postoperative complications.

## Logistic Regression Model Performance

Logistic regression technology has recently been used to analyze the unique benefits of utilizing quantified independent variables, and to determine the influence of a set of independent variables on the regression results (25). Moreover, a prediction model

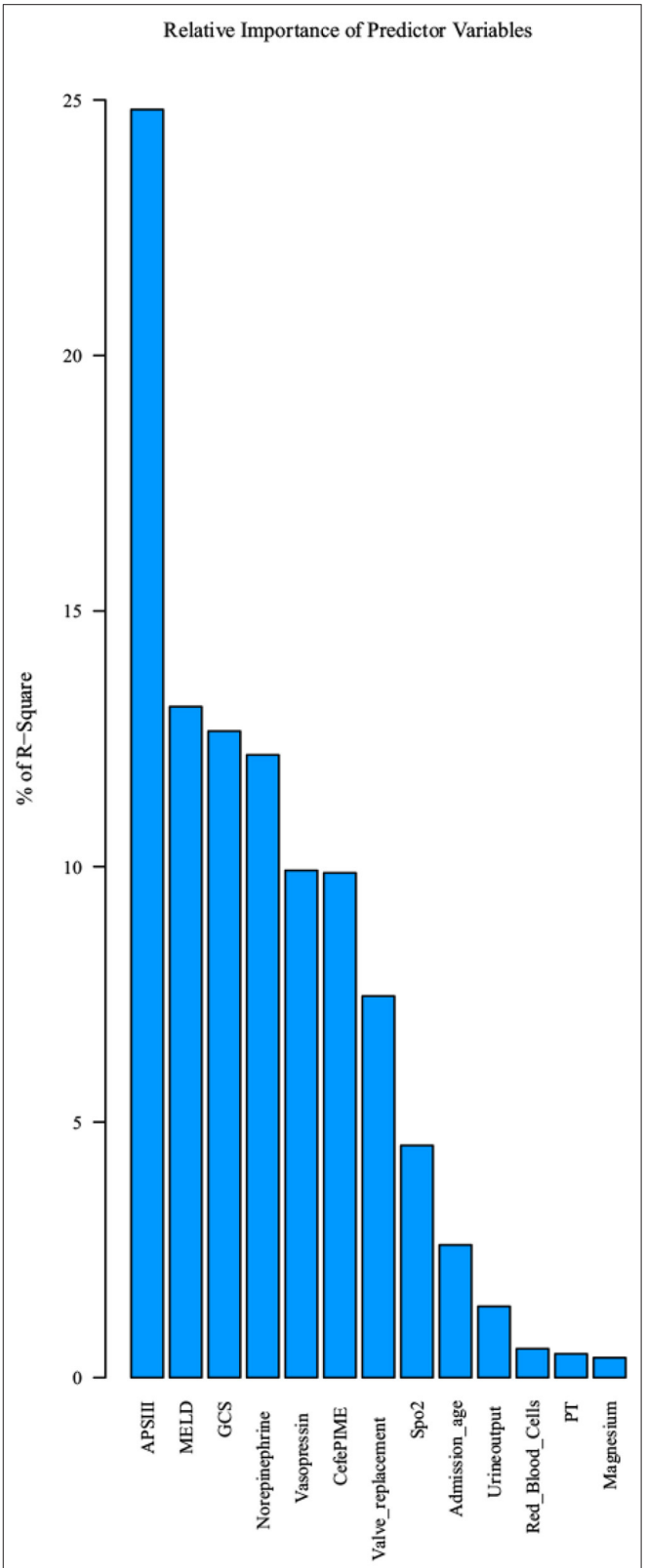


**FIGURE 3 |** Top 13 features selected using logistic regression and the corresponding variable importance score. X-axis indicates the top 13 weighted variables, Y-axis indicates the importance score which is the relative number of a variable that is used to distribute the data.

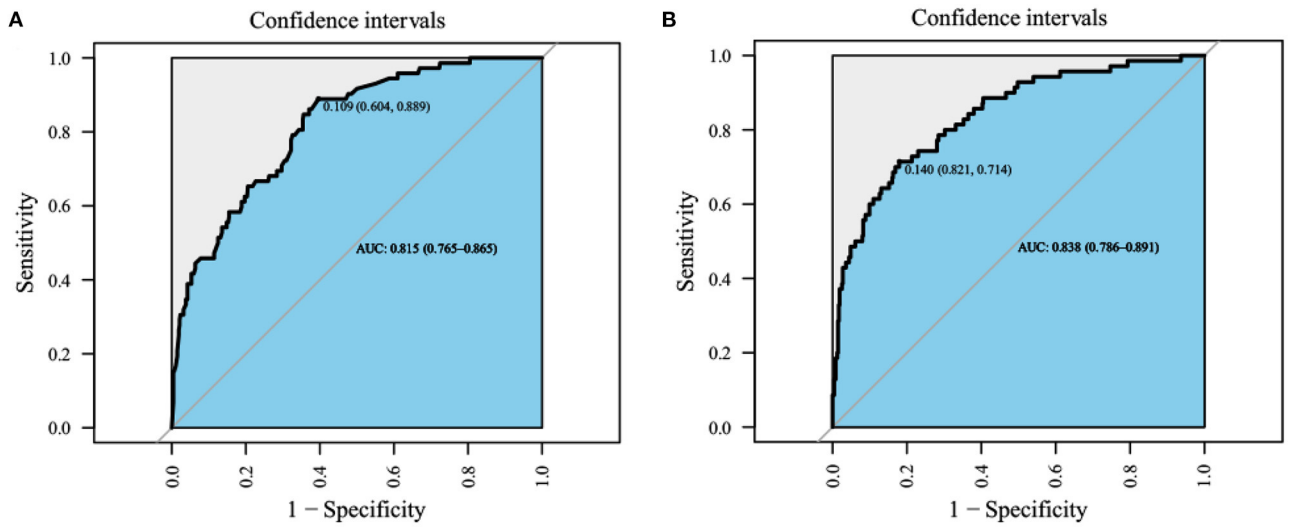


**FIGURE 4 |** The receiver operating characteristic (ROC) curves. **(A)** XGboost model, AUC is 0.815 [0.765–0.865], **(B)** logistic regression model, area under curves (AUC) is 0.838 [95% confidence interval (CI); 0.786–0.891], the best performance of the models was the logistic regression model.

derived from the data set of patient information can predict adverse clinical consequences (26). Sandfort et al. (27) found a significant correlation between long-term heart rate increase and the decrease in ICU survival rate based on logistic regression analysis. Liu et al. (28) investigated the relationship between blood urea nitrogen (BUN) and hospital mortality in patients with critical cardiogenic shock. Their use of a logistic regression algorithm indicated that higher BUN was associated with poorer clinical outcomes. Sun et al. (29) used a logistic regression algorithm to determine that the anion gap was an independent risk variable for the mortality of inpatients in coronary care units (CCUs) and was linked to the poor prognosis of CCU patients. Li et al. (30) used a logistic regression algorithm to estimate whether β2-agonist inhalation would increase the ICU mortality of patients with heart failure. However, compared with other types of predictive models, none of the above studies verified the superiority of the logistic regression model or analyzed it in-depth. More importantly, the main aims of these studies were to detect the poor prognosis of patients with cardiogenic shock and coronary heart disease, whereas logistic regression analysis had not been applied previously to the prognoses of patients with RHD.

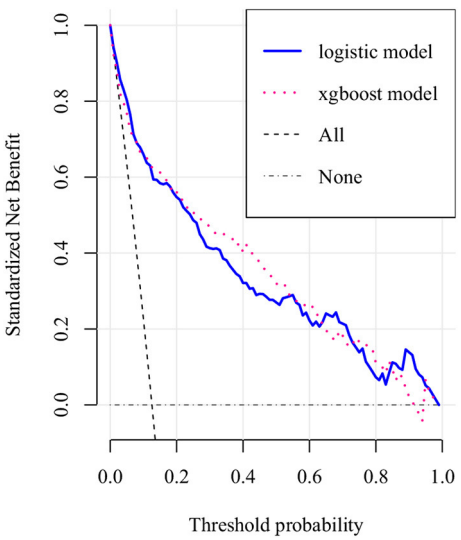


**FIGURE 5 |** Decision curve analysis (DCA) of the two prediction models. The net benefit curves for the two prognostic models are shown. X-axis indicates the threshold probability for critical care outcome and Y-axis indicates the net benefit. dashed purple line = XGboost model, dashed blue line = logistic model. The preferred model is the logistic model, the net benefit of which was larger over the range of XGboost model.

## Predictors of Logistic Regression Model Outcomes

The features in the logistic regression and XGBoost models were consistent, highlighting the excellent performance of the logistic regression model. However, the characteristics and adverse consequences of RHD had not yet been completely explained. Therefore, it is necessary to further investigate the effects of these characteristics on patients with RHD. Among these characteristics, ASPIII had the greatest weight, indicating that it was the most important predictor of the RHD mortality of patients in the MIMIC-IV. Meng et al. (31) reported that APSIII and GCS scores were validated disease severity and mortality prediction tools that did well in identifying high-risk patients in a timely manner and in formulating intervention strategies (32). The MELD score was a clinical predictor in the logistic regression analysis of hospital mortality patients with RHD in ICUs and was used to assess cardiovascular-disease-related secondary liver dysfunction (33), and also was taken as a measure of liver and

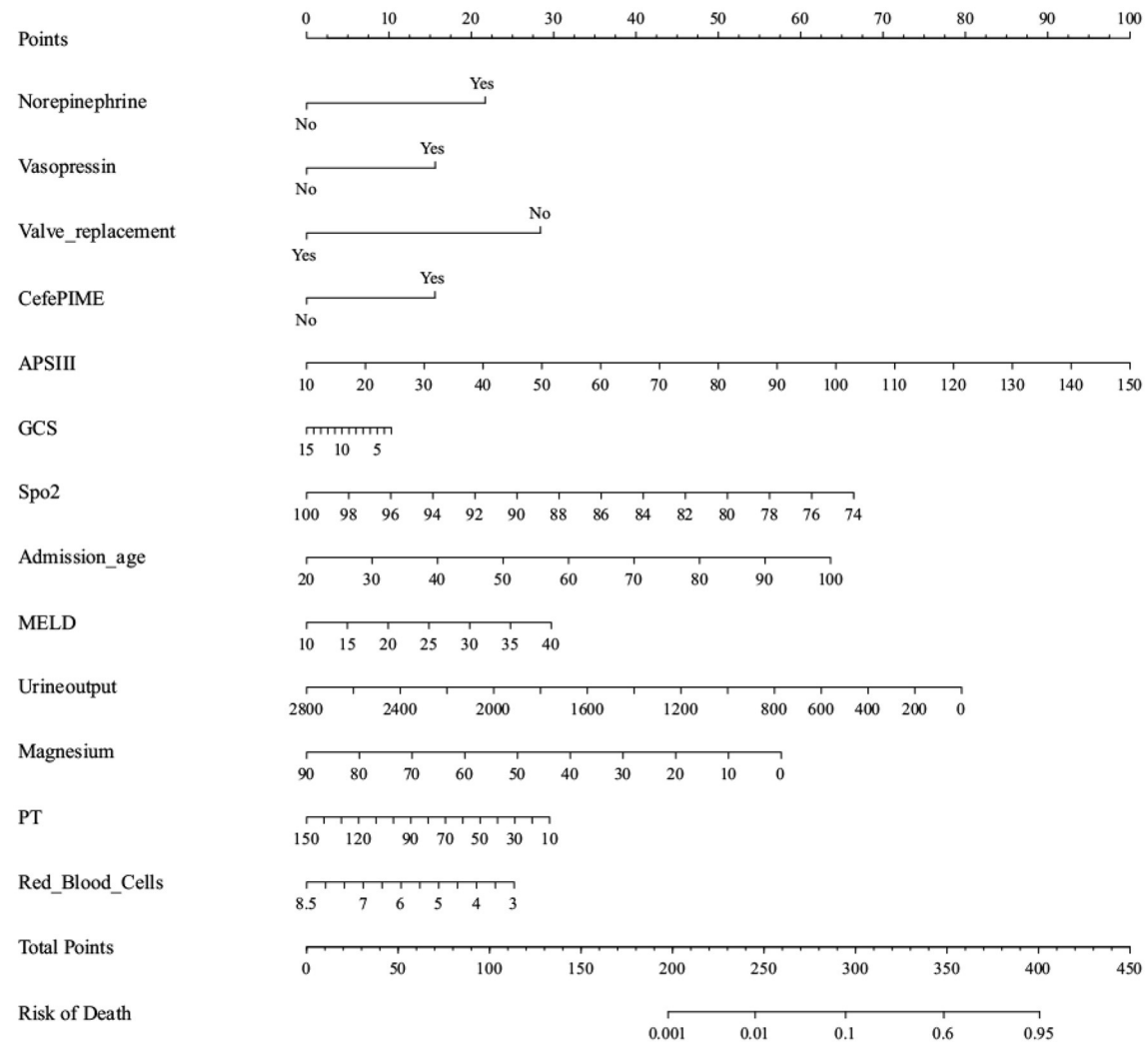


**FIGURE 6 |** Nomogram to estimate the risk of mortality in Rheumatic heart disease patients. To use the nomogram, we first draw a line from each parameter value to the score axis for the score, the points for all the parameters are then added, finally, a line from the total score axis is drawn to determine the risk of mortality on the lower line of the nomogram.

kidney dysfunction. Quantitative indicators had high specificity and sensitivity for predicting the operative mortality of RHD valve surgery (34).

Dzimiri et al. (35) reported that the density of lymphocyte β-adrenergic receptors was markedly decreased in patients with valvular RHD, and the significant decrease in blood oxygen saturation might directly stimulate atrial natriuretic peptide (ANP) release into the heart. There was a good evidence showing that ANP, norepinephrine, vasopressin, and the renin-angiotensin-aldosterone system were involved in controlling water and electrolyte balances. When the blood pressure was low, the elevation of ANP could inhibit the response of vasopressin and renin to arterial hypotension. Hence, the administration of norepinephrine and vasopressin affected the prognosis and mortality of patients in ICUs with RHD, and was essential for the prevention and treatment of complications (36). RHD was the leading cause of valve damage, and heart valve prosthesis implantation was one of the key predictors in the logistic regression model for RHD. Chen et al. (37) and other studies indicated that mitral valve replacement should be performed when patients with RHD had mitral valve damage. Valve replacement could be performed to strengthen driving the decisions making around the surgical treatment of RHD (37). Valve damage that occurring during atrial fibrillation (AF) might be persisting or be aggravated by repeated occurrences, leading to chronic RHD (6). Cefepime is a fourth-generation cephalosporin that is used for the infections of respiratory, urinary, skin, soft tissue and so on from bacteria. Long-term antibiotics use for secondary prevention is critical for preventing disease progression. Prospective studies have demonstrated that life-threatening allergic reactions are uncommon after intravenous antibiotics, and the long-term benefits of this preventive measure

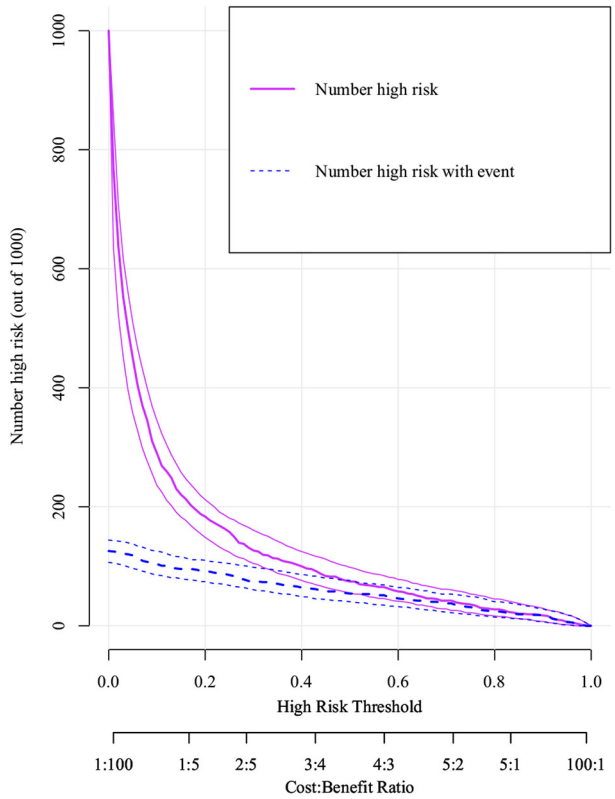


**FIGURE 7 |** Clinical impact curve (CIC) of logistic model. The purple curve (number of high-risk individuals) indicates the number of people who are classified as positive (high risk) by the model at each threshold probability; the blue curve (number of high-risk individuals with outcome) is the number of true positives at each threshold probability. CIC visually indicated that nomogram conferred high clinical net benefit and confirmed the clinical value of the logistic model.

outweigh its risks (38). The prevention and treatment of AF recurrence with long-term antibiotics can contribute to reducing the progression and severity of RHD (6, 39).

Age was included in our model as a demographic parameter. Agenson et al. (40) indicated that advanced age had an adverse effect on mortality. In sub-Saharan Africa, RHD is thought to be responsible for up to 32% of heart failure cases (41). Patients with RHD, like those with congestive heart failure, show a significantly improved condition after treatment with cardiac drugs and diuretics, which can be relieved by treating pulmonary vein congestion that can prolong diastolic time and improve cardiac output (6). For this reason, recording urine volume is of great significance to ICU patients (42).

PT was another key predictor in the logistic regression model. Increased fibrinogen levels in patients with RHD were linked to accidental ischemic stroke (43). Arvind and Ramakrishnan (6) suggested that patients with RHD accompanied by AF, a medical history of thromboembolism, or left atrial thrombosis require anticoagulant therapy when testing their coagulation function indexes, including PT. The most common persistent arrhythmia is AF, which can increase the likelihood of stroke about 5 fold and the risk of death from all causes by 2 fold in patients with RHD.

Serum magnesium is a particularly interesting parameter. With serum magnesium levels >3.8 mg/dl, sinus rhythm of patients had a conversion rate of 88.89%, which far exceeded that of 16.67% in patients with serum magnesium levels <3.8 mg/dl. Intravenous magnesium supplementation can significantly improve the effect of converting AF and improve sinus rhythm in patients with RHD (44). A recent study by Deora et al. (45) found that the main cause of heart failure was diastolic heart failure, and the most common cause was RHD. Anemia has a huge impact on the course and prognosis of patients with heart failure. Appropriate interventions and prognoses should be provided early in the course of the disease. Treatment will improve the survival rate of patients, and the red blood cell count has also been included in the model (45). Our research indicated that in addition to the unknown heart rhythm and normal heart rhythm, AF accounted for the highest proportion of cases. To prevent adverse complications such as cardiovascular or cerebrovascular embolism, some studies have indicated that socioeconomic and environmental factors related to race were also the reasons for the increase in RHD prevalence (46).

## Strengths

The main advantage of this study was that it was the first one to use a logistic regression model to predict hospital mortality of patients with RHD from the MIMIC-IV database. According to the variables selected by the backward stepwise regression analysis, the accuracy and representativeness were improved over XGBoost. The models were compared and verified using DCA and CIC, and certain important parameters such as the body weight and heart rhythm were not missing.

## Limitations

Our research inevitably has some limitations: Firstly, it was a retrospective observational study, not a randomized study, and the data were only obtained from the MIMIC-IV database, which the majority of patients were white, and there may be unobserved confounding factors that might lead to potential biases in the results. Secondly, the MIMIC-IV database did not provide patient history and long-term follow-up events. The inherent limitations in the data extraction technology meant that some crucial influencing variables were overlooked. Thirdly, RHD patients might also have complications such as cardiovascular and cerebrovascular accidents during ICU admission. Fourthly, despite the very high quality of the MIMIC-IV database, there were still some underlying variables and missing data that prevented the availability of certain clinical variables and surgical operation data, including left ventricular ejection fraction, functional classification of heart failure, echocardiographic data, variables such as C-reactive protein, lactate and D-dimer, implying that the inability to accurately identified the severity of RHD is another limitation of this study. Finally, as a retrospective observational single-center study of electronic health record data, the earliest cases were taken from nearly 14 years ago, when care might have been inconsistent with currently accepted standards. The single-center nature of the study might also make our

findings limiting the applicability of other sites. Nonetheless, single-center studies have increased the likelihood that patients will be treated uniformly, alleviating concerns that observed differences in mortality may be due to differences in practice across centers.

Despite owning these limitations, it can currently provide comprehensive and high-quality data on critical illnesses, and has provided a large number of scientific researchers with good research ideas and data, and published many high-quality scientific articles. And some studies using retrospective studies are sufficient, such as when rofecoxib was withdrawn from the market or we convinced the public that smoking was associated with a risk of cancer. In this paper, we aimed to utilize secondary analysis of electronic health record (EHR) data to evaluate practical application-related tests and treatments based on limited benefit or theory, and we considered that the proposed model could help further the understanding of the prognosis of patients with RHD during ICU hospitalization. However, further prospective multicenter studies are needed to validate our findings.

## CONCLUSIONS

In summary, this research has indicated that machine learning based on logistic regression analysis algorithm is better than using the XGBoost algorithm. Our simple and efficient nomogram based on logistic regression analysis is indicated to be clinically useful. It can help clinicians to tailor precise management and treatments for patients with RHD, which is conducive to maximizing the survival probability of these patients.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

YX, DH, and TH performed statistical analysis and data interpretation. HW and JL contributed to the study concept and study design and contributed equally. XZ performed literature research and data extraction. HL and SS were responsible for the quality control of data and algorithms. All authors contributed to writing of the manuscript and approved the final version.

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REVIEW
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# Clinical Characterization and Possible Pathological Mechanism of Acute Myocardial Injury in COVID-19

*Siyi Li*[1,2], *Jinan Wang*[3], *Yan Yan*[1,2], *Zekun Zhang*[1,2], *Wei Gong*[1,2]* *and Shaoping Nie*[1,2]*

[1] Coronary Heart Disease Center, Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China, [2] Beijing Institute of Heart, Lung, and Blood Vessel Diseases, Beijing, China, [3] The Affiliated Rehabilitation Hospital of Chongqing Medical University, Chongqing, China

COVID-19 is a respiratory disease that can cause damage to multiple organs throughout the body. Cardiovascular complications related to COVID-19 mainly include acute myocardial injury, heart failure, acute coronary syndrome, arrhythmia, myocarditis. Among them, myocardial injury is the most common complication in COVID-19 hospitalized patients, and is associated with poor prognosis such as death and arrhythmias. There is a continuous relationship between myocardial injury and the severity of COVID-19. The incidence of myocardial injury is higher in critically ill patients and dead patients, and myocardial injury is more likely to occur in the elderly critically ill patients with comorbidities. Myocardial injury is usually accompanied by more electrocardiogram abnormalities, higher inflammation markers and more obvious echocardiographic abnormalities. According to reports, COVID-19 patients with a history of cardiovascular disease have a higher in-hospital mortality, especially in the elder patients. At present, the mechanism of myocardial injury in COVID-19 is still unclear. There may be direct injury of myocardial cells, systemic inflammatory response, hypoxia, prethrombotic and procoagulant state, myocardial interstitial fibrosis, interferon-mediated immune response and coronary artery plaque instability and other related factors, and angiotensin-converting enzyme-2 receptor may play a key role in the myocardial injury in COVID-19.

**Keywords: COVID-19, myocardial injury, inflammation markers, angiotensin-converting enzyme-2 receptor, prognosis**

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*Yan Zhang,*
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*Liu Wanjun,*
*Huazhong University of Science and Technology, China*
*Shenglan Yang,*
*First Affiliated Hospital of Chongqing Medical University, China*

***Correspondence:**
*Shaoping Nie*
*spnie@ccmu.edu.cn*
*Wei Gong*
*gongwei@ccmu.edu.cn*

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## INTRODUCTION

Coronavirus disease 2019 (COVID-19), a severe acute respiratory infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has caused widespread epidemics worldwide since its outbreak in 2019. According to real-time data, as of February 2022, the cumulative number of confirmed cases of COVID-19 worldwide exceeded 393 million, and the number of deaths exceeded 5 million, especially among the elderly and those with other complications such as diabetes, hypertension, and cardiovascular disease (1). The current epidemic continues to exist and is in a recurring stage. Although the use rate of vaccines is getting higher and higher, the number of infections continues to rise.

COVID-19 mainly affects the respiratory system. The most common early symptoms are fever (80–90%), dry cough (60–70%) and shortness of breath (53–80%) (2). With the increase in the number of infected people, more and more clinical evidence shows that COVID-19 can damage

various systems of the human body to varying degrees, and the damage to the cardiovascular system is particularly serious. Previous literature found that cardiovascular manifestations related to COVID-19 include acute myocardial injury, myocarditis, cardiomyopathy, acute coronary syndrome and myocardial infarction, arrhythmia, cardiac arrest, cardiac tamponade, Kawasaki disease, cardiovascular thromboembolism (including venous thromboembolism, stroke) and so on (3–8). In addition, doctors from many countries have reported that compared with the general population, patients with cardiovascular disease have a worse prognosis for SARS-CoV-2 infection. Previous studies suggested that COVID-19 patients with cardiovascular disease are often more severely ill and have a higher risk of death, especially in elderly patients (9). It can be seen that COVID-19 and cardiovascular diseases affect each other and together lead to acute and malignant adverse events. Among the cardiovascular manifestations related to COVID-19, arrhythmia is the most common, with an incidence rate of up to 44%. The mortality rate of cardiac injury is the highest, about 50% (10). Therefore, it is particularly important to pay attention to the pathological mechanism, clinical manifestations, disease process, and prognosis of heart damage caused by COVID-19.

At present, the mechanisms of cardiac injury caused by COVID-19 are still unclear. Viruses directly damage cardiomyocytes, systemic inflammatory storms, immune response disorders, endothelial injury and thrombotic inflammation, and functional maladaptation of angiotensin-converting enzyme-2 (ACE-2) receptor-related pathways may all contribute to COVID-19's serious damage to myocardium (11). This review focused on the myocardial injury caused by COVID-19 and summarized the relevant clinical features and pathogenesis.

## CLINICAL CHARACTERIZATION OF MYOCARDIAL INJURY IN COVID-19

Acute myocardial injury is the most common extrapulmonary manifestation of COVID-19. The definition of acute myocardial injury in COVID-19 is blood levels of cardiac biomarkers [high-sensitivity troponin I (hs-TnI)] above the 99th percentile upper reference limit. The existing literature reports that the incidence of acute myocardial injury ranges from 12 to 100%. Almost all patients with severe illness have severe myocardial damage, which is close to 100% in critically ill patients and 70 to 80% in severely ill patients. Critically ill patients requiring intensive care unit (ICU) admission are approximately 13 times more likely to have acute cardiac injury than non-critically ill patients (12).

Patients suspected of cardiac injury caused by COVID-19 often experience chest pain alongside other viral systemic symptoms, including fever, cough, and/or dyspnea (13). Patients with acute myocardial injury tend to be older, have more comorbidities and more severe illness. In addition, the symptoms of respiratory system of patients with acute myocardial injury caused by COVID-19 is relatively more severe. Studies showed that 46.3% of patients with acute myocardial injury required non-invasive mechanical ventilation, compared with 3.9% of patients without myocardial injury. Similarly, the demand for invasive mechanical ventilation in patients with and without heart injury increased by 22 and 4.2%, respectively, and the incidence of acute respiratory distress syndrome (ARDS) in patients with myocardial injury also increased significantly (58.5 vs 14.7%, $P < 0.001$) (14).

In COVID-19 patients, cardiovascular markers such as creatine kinase (CK), creatinine, TnI (heart), brain natriuretic peptide (BNP), lactate dehydrogenase (LDH), alanine aminotransferase, aspartate aminotransferase and D-dimer concentration significantly increased, which may indicate the occurrence of acute myocardial injury (15). Guo et al. found that among 187 COVID-19 patients with an average age of 58.5 years, 52 patients suffered from acute myocardial injury, with a mean CK-MB fraction of 3.34 ng/ml; mean myoglobin of 128.7 μg/ml (16). Shi et al. found that among 416 COVID-19 infected patients, 19.7% of the patients had myocardial injury, which was defined as increased hs-cTnI and NT-proBNP (14). In a retrospective cohort study, Zhou et al. found that 33 out of 191 COVID-19 patients (17%) had acute myocardial injury, of which 24 patients showed high-sensitivity cardiac troponin levels higher than 28 pg/ml (4).

In addition, the abnormal results of other laboratory and imaging examination also indicated the occurrence of acute myocardial injury. The 12-lead electrocardiogram showed low voltage, diffuse ST-segment elevation, T-wave inversion, PR segment depression and new Q waves (13, 14), and echocardiogram showed diffuse myocardial dyskinesia and decreased ejection fraction always suggest that myocardial injury occur (13, 17). Besides, cardiovascular magnetic resonance (CMR) revealed 78% German patients who recovered from COVID-19 recently had myocardial involvement. The abnormal CMR findings included increased myocardial native T1 (73%), raised myocardial native T2 (60%), myocardial late gadolinium enhancement (32%), or pericardial enhancement (22%) (18). It was reported in the previous literature that in severe cases, direct acute myocardial injury caused by COVID-19 was characterized by concentric left ventricular hypertrophy, right ventricular dilatation, severe hypokinesia and cardiac amyloidosis (19). Right ventricular dysfunction is a powerful indicator of the incidence and mortality of COVID-19-related heart injury (20, 21). In a systematic echocardiography study of COVID-19 patients with different ill severity, elevated troponin levels were associated with right ventricular dysfunction (22). Interestingly, clinical deterioration, including hemodynamic instability and a further increase in cardiac troponin, seems to rarely lead to left ventricular pump failure, and in most cases, it is accompanied by further dilatation of right ventricle and further deterioration of right ventricular function (22, 23).

COVID-19 patients with acute cardiac injury have a poor prognosis. The in-hospital complications of COVID-19 patients with acute myocardial injury increased significantly, and the clinical outcome deteriorated significantly (14, 16, 24). Complications such as acute kidney injury (8.5 vs. 0.3%), electrolyte imbalance (15.9 vs. 5.1%), and coagulation dysfunction (7.3 vs. 1.8%) were significantly higher in COVID-19 patients with heart injury (14). Life-threatening arrhythmias,

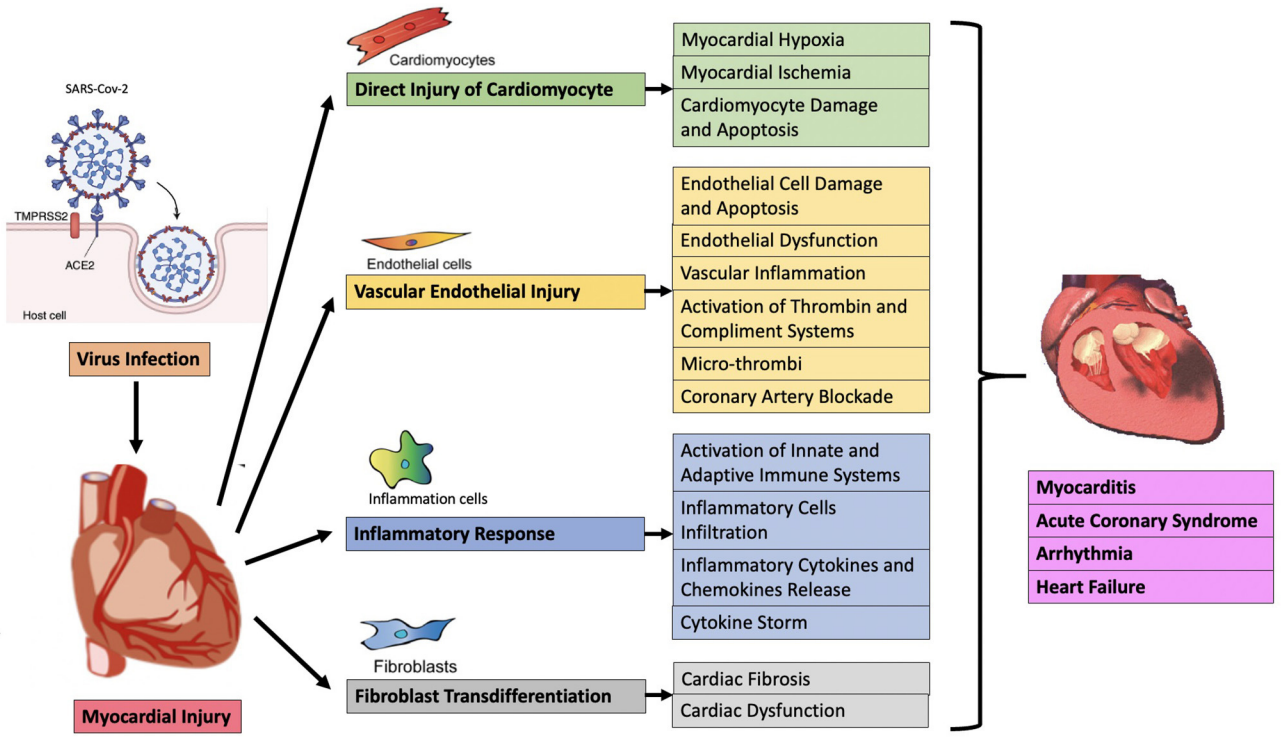


**FIGURE 1 |** Possible pathological mechanism of acute myocardial injury in COVID-19.

including ventricular tachycardia and ventricular fibrillation (17.3 vs. 2%) were also significantly higher in COVID-19 patients with cardiac injury (16). More importantly, COVID-19-related acute myocardial injury was significantly associated with an increased mortality. Compared with patients without heart injury, patients with heart injury had a significantly higher mortality rate (51 vs. 4.5% and 59.6 vs. 8.9%) (14, 16). The increase of cardiac biomarkers may indicate a poor prognosis (16, 25, 26). In a study involving 1,099 COVID-19 patients from 552 hospitals, the expression of cardiac biomarkers was found to be significantly elevated in critically ill patients (7). Compared with mild non-ICU patients, CK-MB, LDH and hs-cTnI levels of severely ill patients admitted to ICU increased significantly (27). Guo et al. observed that the TnT and hsNT-proBNP levels significantly increased during hospitalization of deceased patients (16). The mortality of patients with elevated cardiac injury biomarkers was significantly higher than that of patients without cardiac injury (14). Therefore, the detection of cardiac biomarkers has certain clinical significance for judging the prognosis of COVID-19 hospitalized patients (28).

## POSSIBLE PATHOLOGICAL MECHANISM OF ACUTE MYOCARDIAL INJURY IN COVID-19

Coronavirus is an enveloped, positive-stranded, single-stranded and highly diverse RNA virus family. The pathogenic and lethal SARS-CoV, Middle East respiratory syndrome coronavirus and SARS-CoV-2 all belong to this family (29). SARS-CoV-2 is a new type of β-coronavirus (large RNA virus) with a genome homology of up to 79.5% with SARS-CoV (30). The SARS-CoV-2 virus envelope is covered with spike S glycoprotein, which consists of two subunits S1 and S2. Subunit S1 has affinity for the ACE2 receptor on the cell surface, and subunit S2 fuses with the cell membrane. These two proteins work together to help the endocytosis of virus particles (31, 32). Different from SARS-CoV, the three-dimensional structure of the SARS-CoV-2 binding site is more compact, which not only improves the stability of the binding, but also enhances the binding affinity to the ACE2 receptor (33). In addition, SARS-CoV-2 contains a polyacid (furin) cleavage site inserted at the boundary of the S1/S2 subunits of the spike S protein (34). This furin binding site is unique and can enhance the ability of viruses to enter cells. It is a common feature of several highly pathogenic viruses including avian influenza.

At present, the mechanisms of acute myocardial injury in COVID-19 patients are unclear. Heart injury may be caused by direct or indirect mechanisms. The direct mechanism includes the virus invading the myocardial tissue, that is, direct infection, leading to the death and inflammation of myocardial cells. The indirect mechanism is secondary to respiratory failure and hypoxemia, leading to cardiac stress and hypoxia-related cardiomyocyte damage. Cardiac inflammation caused by severe systemic congestion is also included in the indirect mechanism (35), but it should be the third mechanism itself,

because it may involve sepsis, toll-like receptor 4 (TLR4) activation and/or cytokine storm (also called "immune mediated cytokine release syndrome") (36). In general, myocardial injury may be related to direct viral damage, inflammatory cell infiltration and pro-inflammatory cytokine release, oxidative stress, coronary vascular damage, endothelial damage with microthrombosis, myocardial interstitial fibrosis, and interferon-mediated immunity response, the excessive cytokine response of type 1 and type 2 helper T cells, the instability of coronary plaques and hypoxia (**Figure 1**) (37). Clarifying the underlying mechanisms of myocardial injury in COVID-19 are extremely important for finding therapeutic targets, reducing the severity of the disease, and reducing mortality.

## Angiotensin-Converting Enzyme-2 (ACE2) Receptor and Transmembrane Protease Serine 2 (TMPRSS2) Play a Key Role in COVID-19 Patients With Myocardial Injury

ACE2 is a type 1 transmembrane protein whose enzyme domain is located on the outer surface of the cell, where it can convert angiotensin II into angiotensin 1–7 (38). ACE2 is mainly expressed in the vascular endothelium of a variety of tissue structures, such as type I and type II lung cells, smooth muscle cells in the pulmonary vascular system, bronchial epithelium, epithelial cells in the lung, heart, intestine, blood vessels, testes and kidneys (39). ACE2 has an important immunomodulatory effect. On the one hand, ACE2 can directly interact with macrophages in the inflammatory environment of blood vessels and lungs to exert anti-inflammatory effects (40). On the other hand, ACE2 can reduce the pro-inflammatory and pro-oxidant effects of angiotensin II. Therefore, under pathological conditions, ACE2 has a positive effect on controlling excessive inflammation (41).

The main mechanism for SARS-CoV-2 to enter the host cell is through the ACE2 receptor (42). SARS-CoV-2 enters the lungs through the respiratory tract, and its spike S protein first binds to the ACE2 receptor on the surface of lung cells to mediate virus entry into the cell. On the one hand, the presence of TMPRSS2 can start the viral spike S protein to assist the virus to enter the cell and enhance the infectivity of the virus (43). On the other hand, after the virus binds to the ACE2 receptor and enters the cell, TMPRSS2 can help the virus to replicate in a large amount, so that the replicated virus further binds to other ACE2 receptors and promotes virus invasion. When the virus load in the lung overflows, it may attack multiple organs throughout the body, causing extensive tissue and organ damage.

ACE2 is widely expressed in the heart and lungs. It has been reported that compared with the lungs, the heart is the second major organ attacked by SARS-CoV-2 (44). Evaluation of the expression of ACE2 and TMPRSS2 by RNA sequencing of the hearts in normal people and COVID-19 patients found that ACE2 was most highly expressed in pericytes and was also clearly expressed in other cells such as cardiomyocytes, indicating that the heart may be an important target for SARS-CoV-2 infection (45, 46). The expression of ACE2 in the heart of COVID-19 patients is significantly increased, and direct cardiac injury is prone to occur (44, 47). Studies found that TLR4 played an important role in the pathogenesis of SARS-CoV-2. SARS-CoV-2 may bind and activate TLR4 to increase the expression of ACE2, promote virus entry and cause excessive or long-term inflammation (48). Once SARS-CoV-2 targets ACE2, it may increase the level of angiotensin II through the action of NADPH oxidase activity, and may cause endothelial dysfunction and chronic myocardial hypoxia (49).

The internalization of the virus and ACE2 causes the loss of ACE2 on the cell surface, leading to increased levels of angiotensin II and decreased levels of angiotensin 1-7. Sakamoto et al. (50) performed ACE2 and TMPRSS2 immunostaining on the hearts of 15 COVID-19 patients from Bergamo, Italy. They observed that ACE2 was mainly located in the cell membrane, TMPRSS2 was mainly located in the nucleus and cytoplasm, and ACE2 and TMPRSS2 were extremely low in the heart of COVID-19 patients (50). There was also evidence in preclinical models using SARS-CoV-2 that due to the binding of the virus to the ACE2 receptor during infection, ACE2 in the heart was significantly down-regulated (51). Oudit et al. found that the expression of ACE2 in the myocardium of mice infected with SARS-CoV-2 was significantly reduced, and SARS-CoV-2 could reduce the expression of ACE2 due to internalization (51). The reduction of ACE2 expression has a dual effect. On the one hand, the host can defend against infection and limit the continuous proliferation of the virus. On the other hand, the biological effect of ACE2 is also significantly weakened, and it cannot limit the pro-inflammatory, pro-thrombotic and pro-oxidant effects of angiotensin II, the cardioprotective effect is weakened, and the risk of heart failure increases. Although this virus proliferates at a low level in the heart of the host, it may cause potential danger signals to be further released to the immune system, triggering an excessive downstream inflammatory response. When there is a slight increase in troponin, it indicates the appearance of virus or immune-mediated myocardial injury. If the immune response continues to increase, it will further aggravate the heart injury. When the heart biomarkers continue to increase, it often indicates an inflammatory storm and a poor prognosis. Hydroxychloroquine has been confirmed to bind cell surface sialic acid and gangliosides with high affinity, thereby impairing SARS-CoV-2 spike protein recognition and binding to host cell ACE2 receptors (52).

## The Direct Injury of SARS-CoV-2 to Heart in COVID-19

Previous literature analysis of the hearts of patients who died of SARS showed that SARS-CoV virus RNA was detected in 35% of the hearts (51). After SARS-CoV-2 infects human body, combining with ACE2 on the surface of cardiac epithelial cells directly damages myocardium and causes its dysfunction is a possible damage mechanism. The high expression of ACE2 in cardiomyocytes, pericytes, fibroblasts, endothelial cells, epicardial adipocytes and smooth muscle cells supports the mechanism of direct viral damage (39, 53). After the virus invades the heart, it can induce inflammatory cells to infiltrate the pericardium, myocardium and intima. Immune cells

such as neutrophils, pro-inflammatory monocytes/macrophages and lymphocytes gather around the area of the myocardium infiltrated by the virus. Many cases reported myocarditis induced by COVID-19. These findings support the hypothesis that SARS-CoV-2 directly damages the myocardium (13, 54, 55). Lindner et al. studied the heart tissues of 39 patients whose throat swabs tested positive for SARS-CoV-2 through autopsy and found that SARS-CoV-2 virus was present in the hearts of 24 cases (61.5%), of which 16 cases (41 %) the viral load copy number was greater than 1000 copies/μg RNA (56). However, there are few studies to detect the virus carried by cardiomyocytes. Sakamoto et al. used real-time polymerase chain reaction to detect the expression of SARS-CoV-2 in the hearts of 15 patients with COVID-19, and found that the virus was only present in the left atrium of one patient, and there was no virus in any of the heart chambers of the remaining patients. In addition, *in-situ* hybridization (ISH) and indirect immunofluorescence showed that SARS-CoV-2 was localized in the myocytes in the left atrium (50). Electron microscopy detected virus particles in the cardiac myocytes, endothelial cells and fibroblasts of an 11-year-old boy who developed multiple system inflammatory syndrome and died of heart failure (57). In a patient with a rapid onset of myocardial injury caused by SARS-CoV-2 infection, myocardial biopsy showed that there were virus particles in the interstitial tissue, but there were no virus particles in the cardiomyocytes (58). Nevertheless, the direct invasion of the myocardium by the virus is still a possible damage mechanism. Antiviral therapy can effectively inhibit the invasion of SARS-CoV-2, including chloroquine and hydroxychloroquine, type 1 interferons (IFN–I), lopinavir/ritonavir (Kaletra) and remdesivir (59).

## Activation of Immune System and Imbalance of Immune Response in COVID-19 With Myocardial Injury

The human immune system is a multi-stage network that protects the body from harmful bacteria, viruses, and other organisms. The occurrence and development of COVID-19 is mediated by viruses and individual immune systems. To successfully defend against viral infection, the body relies on innate and adaptive immune systems. The innate immune system is the first line of defense and is responsible for identifying certain structurally conserved components of the virus: early production of interferons, inflammatory cytokines and chemokines. Interferons and other cytokines recruit immune cells (such as natural killer cells, neutrophils, and monocytes) for subsequent cytotoxic and adaptive immune responses. Specific B lymphocytes and T lymphocytes are part of the adaptive immune system. Their role is to act as a barrier to delay the replication of the virus, to produce specific neutralizing antibodies against the virus to avoid further infection, and to form a persistent memory against the virus (60). However, when the infected cells proliferate beyond the range of T cells, the patient's condition may rapidly deteriorate. The endothelium plays a role in controlling the immune system through various receptors and cytoplasmic proteins (61). Endothelial cells secrete cytokines to regulate innate and adaptive immune responses, and recruit immune cells at their sites of action (62). Endothelial activation increases the permeability of blood vessels to plasma proteins, releases pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), chemokines, adhesion molecules (from activated white blood cells) and induces inflammation (63). Endothelial dysfunction stimulates a series of signaling molecules to release nuclear factor-κB (NF-κB) to limit the innate and adaptive immune response (64). If the innate immunity cannot eliminate the threat, it will activate the adaptive immune response, turning acute inflammation into chronic inflammation (65). The innate immune response (such as increased neutrophils, pro-inflammatory macrophages, and lymphopenia) and adaptive immune responses (activation of $CD4^+$ T cells and $CD8^+$ T cells) play important roles in autoimmunity or anti-inflammation of COVID-19 patients (66). When the immune regulation dysfunction, the human body cannot cope with the virus infection and enters a critical stage, and a strong and harmful inflammatory reaction occurs.

## Systemic Inflammatory Response Syndrome and Cytokine Storm Leads to Myocardial Injury in COVID-19

Excessive systemic inflammation can occur after infected with COVID-19, including cytokine storm-like results with extremely high mortality. Such COVID-19 patients with a strong immune response may present with acute myocarditis, heart failure or cardiogenic shock, accompanied by hypercytokineemia and cardiac inflammatory cell infiltration (67). Studies showed that leukocytosis (especially neutrophilia) was a high inflammatory response to SARS-CoV-2 infection and/or secondary bacterial infection, which can aggravate the severity of the disease and promote myocardial injury (68). In addition, more than 80% of patients had lymphopenia after infected with SARS-CoV-2. The degree of lymphopenia was a very important prognostic indicator in the early stage of infection. In the early analysis of patients who died of COVID-19, the most notable findings were the significant decrease in circulating $CD4^+$ and $CD8^+$ T lymphocyte levels, and monocytes (monocytes and macrophages) were relatively dominant in the damaged myocardium (69, 70).

With the consumption of $CD4^+$ T cells, the decrease of regulatory T cell function, the decrease of progressive lymphocytes, and the aging immune system lead to the proliferation of steady-state lymphocytes, which has the characteristics of autoimmunity and excessive inflammation (71). The ability of senescent macrophages to swallow apoptotic cells decreases, leading to a general pro-inflammatory state. When the elderly population is infected with SARS-CoV-2, the unbalanced immune system of the elderly will worsen, which will further aggravate the exhaustion of $CD4^+$ T cells and the inflammatory macrophage response, and expand the systemic inflammatory response. This is also one of the reasons why the incidence of critical illness and mortality in the elderly are more frequent after being infected with COVID-19. In addition, epicardial adipose tissue may be directly related to myocardial injury. It is a high inflammatory response

reservoir with a large number of macrophage infiltration and rich pro-inflammatory cytokines. This may be the reason why obese patients have higher mortality and complication rates (72).

The autopsy results of the myocardial tissue of patients who died from COVID-19 support the hypothesis that systemic inflammation may be the driving factor of heart damage. Analysis of myocardial tissue showed that there was a small amount of inflammatory infiltration of monocytes in the myocardial interstitium without substantial myocardial damage (73). In addition, a study of 112 hospitalized COVID-19 patients also showed that the cardiac injury was attributed to systemic cytokines rather than the direct damage to the heart by the virus (26).

Another main hypothetical mechanism of myocardial injury may be the release of various pro-inflammatory cytokines during inflammation, such as interleukin-1 (IL-1), beta interferon-gamma (IFN-γ), macrophage inflammatory protein (MIP)-1A, TNF-α as well as IL-6 (74). SARS-CoV-2 mainly invades the lungs through ACE2 receptors and progresses to pneumonia and acute respiratory distress syndrome (ARDS). According to the viral load, the infection can be further spread through the ACE2 receptor to various organs, such as the heart, liver, kidney, brain, endothelium, gastrointestinal tract, immune cells. When the virus invades the human body and damages the organs, systemic pro-inflammatory factors are activated, the inflammation self-amplifies and triggers an inflammatory storm, leading to systemic inflammation and toxicity. Mcp-1 is one of the significantly increased cytokines, and it is also the main regulator of the migration and infiltration of the monocyte/macrophage system to the SARS-CoV-2 infection site. IL-1β is a key regulator of inflammatory response, which can stimulate the release of other cytokines (such as IL-17 and IL-21), and can also increase cell proliferation and differentiation (75). Studies showed that the inflammatory response level of IL-1β elevated in COVID-19 patients, especially in patients with poor prognosis. Subsequently, on the basis of the increase in IL-1β, the level of IL-6 also increased, which may herald the upcoming cytokine storm. Cytokine storm will further aggravate myocardial injury, and the widespread and malignant combination of cytokine and organ crosstalk leads to systemic excessive inflammation and ultimately to cardiac dysfunction (35). In addition, the continuous increase of inflammatory cytokines after COVID-19 infection may eventually lead to the reduction of coronary blood flow, oxygen supply, microthrombosis and the degeneration of coronary plaques (16). Anti-inflammatory agents, such as glucocorticoids and Tocilizumab, a recombinant-humanized monoclonal antibody targeting both soluble and membrane-bound forms of IL-6 receptor, could effectively reduce the release of inflammatory factors (59). Besides, as a potential antithrombotic drug, although the overall beneficial effect of chloroquine is not clear in COVID-19, it has been confirmed can reduce neutrophil extracellular trap formation, platelet aggregation and circulating tissue factor in mice (52).

## Vascular Endothelial Injury, Microthrombosis and Myocardial Ischemia Are Associated With Myocardial Injury in COVID-19

Another potential mechanism of cardiac injury is that the virus directly enters the heart endothelial cells instead of the cardiomyocytes. There are also ACE2 receptors on the surface of endothelial cells. After SARS-CoV-2 invades these epithelial cell membranes, it damages the endothelium, triggers the overproduction of thrombin, inhibits fibrinolysis, activates the complement pathway, triggers thrombus inflammation, and ultimately leads to microthrombus deposition and microvascular dysfunction (76, 77). Through electron microscopy and virus particle identification, evidence of direct endothelial infection has been recorded in cardiac endothelial cells at autopsy (76, 78). Excessive immune system activation, activation of inflammatory cells (such as macrophages) and the release of inflammatory mediators (such as IL-1β, IL-6, TNF-α) can further promote endothelial cell activation and release more pro-inflammatory cytokines, promote the expression of adhesion molecules (such as ICAM-1, VCAM-1), and recruit more leukocyte infiltration and platelet activation and aggregation. In addition, circulating cytokines can stimulate the expression of macrophages and leukocyte adhesion molecules on endothelial cells with potential atherosclerotic lesions, making them more susceptible to destruction and increasing the possibility of clinically obvious acute coronary syndromes (79, 80). Systemic cytokines may also activate microvascular endothelial cells, leading to coronary microvascular dysfunction, myocardial ischemia and myocardial damage (79). The inflammation and subsequent dysfunction of endothelial cells in the heart are the result of the direct effect of SARS-CoV-2 infection of endothelial cells and the indirect effect of host inflammatory response. Dysfunctional endothelial cells become adhesion promoters and coagulants, further accelerating vascular inflammation, enhancing the prethrombotic state, increasing the levels of D-dimers and fibrin degradation products (FDPs), and even appearing microthrombus (81). The formation of microthrombus can make patients prone to cardiac micro-infarction, further aggravating the state of myocardial injury and heart failure. Changes in the coagulation and fibrinolytic system are important in COVID-19 patients, and diffuse intravascular coagulation (DIC) has been observed in most patients who died (82). Microthrombosis caused by damaged endothelium or hypercoagulable state can make existing coronary plaques unstable, leading to type I myocardial ischemia (83). The right ventricle is the most prone to ischemia and dysfunction. This is due to the sudden increase of the right ventricular afterload caused by microthrombosis and the mismatch between the supply and demand of the coronary arteries. In addition, endothelial and microvascular damage in COVID-19 leads to increased inflammation, increased coronary capillary permeability, vasospasm, decreased myocardial perfusion and myocardial ischemia, and increased oxygen supply/demand leading to vascular homeostasis damage are all incentives for right ventricular dysfunction (84). More than 50%

of patients with right ventricular dysfunction are associated with moderate to severe ARDS and are a recognized determinant of mortality (84). In addition to coagulation disorders, endothelial damage may also lead to increased vascular permeability and decreased levels of nitric oxide in the lining of capillaries (85). All these factors can cause severe cardiac injury and eventually heart failure.

## Myocardial Injury Related to Myocardial Hypoxia in COVID-19

SARS-CoV-2 infection may be related to myocardial injury by increasing myocardial oxygen demand and reducing myocardial oxygen supply. Myocardial injury is secondary to the imbalance of myocardial oxygen supply and demand without rupture of atherosclerotic plaque, which is called type 2 myocardial infarction. Hypoxia caused by severe respiratory-related complications (such as ARDS/respiratory failure) is common in myocardial injury in COVID-19 patients (4, 7, 86). Hypoxia caused by respiratory failure may put an additional burden on the heart, and is associated with increased biomarkers of myocardial injury, leading to a worse prognosis (4). Pulmonary vasculitis with extensive vascular thrombosis, microangiopathy and alveolar capillary occlusion, combined with the overall prethrombotic state, can promote pulmonary embolism and lead to hypoxemia (87, 88). Increased myocardial oxygen demand in severe COVID-19 patients secondary to tachycardia, increased cardiac output, and increased right ventricular afterload. Decreased oxygen supply will reduce the hydrolysis of adenosine triphosphate (ATP), significantly reduce the energy supply for cell metabolism, increase anaerobic fermentation, and cause intracellular acidosis. Oxygen free radicals damage the phospholipid layer of the cell membrane, leading to cell membrane damage and mitochondrial damage, thereby further reducing ATP synthesis. The imbalance between increased oxygen demand and decreased supply is likely to cause subendocardial ischemia. At the same time, the influx of calcium ions induced by hypoxia also lead to cardiomyocyte damage and apoptosis (12). Hypotension is a common clinical feature of sepsis and cytokine storm syndrome, and can also reduce myocardial oxygen supply (79). In addition, systemic infection and fever increase the metabolic demands of peripheral tissues and end-organs, thereby increasing the metabolic demands of cardiomyocytes (89).

## Other Potential Mechanisms of Myocardial Injury in COVID-19

Myocardial interstitial fibrosis may be another mode of myocardial injury. The infiltration of neutrophils, macrophages and $CD4^+$ T lymphocytes in patients with COVID-19 can promote the activation of fibroblasts to myocardial fibroblasts, causing pathological heart remodeling and fibrosis, leading to the development of heart failure and early death of infected patients. Zhao et al. found that SARS virus activated TGF-β signaling through the Smad pathway to induce pulmonary fibrosis, which is also a common pathway for myocardial interstitial fibrosis (90). It was also suggested that in COVID-19 patients, a strong interferon-mediated response may contribute to myocardial function. Specifically, it is mainly the transition of interferon from overactive innate immunity to protective adaptive immunity (91, 92). Secondly, pro-inflammatory cytokines can promote the formation of reactive oxygen species and free radicals, cause oxidative stress, and ultimately lead to the depletion of $NAD^+$ and ATP, resulting in apoptosis and necrosis of cardiomyocytes. Finally, another proposed mechanism is the overreaction of type 1 and type 2 helper T cells to cytokines (93). The above are all possible mechanisms of myocardial injury, but the specific targets and pathways of action need to be further verified.

# CONCLUSION

Myocardial injury in COVID-19 is usually accompanied by abnormal electrocardiogram and echocardiography, and increased inflammation markers. The mechanisms of myocardial injury in COVID-19 maybe include direct injury of myocardial cells, systemic inflammatory response, hypoxia, prethrombotic and procoagulant state, myocardial interstitial fibrosis, interferon-mediated immune response, coronary artery plaque instability and other related factors. And angiotensin-converting enzyme-2 receptor may play a key role in the myocardial injury in COVID-19. A correct understanding of COVID-19's damage to the heart is of positive significance for early recognition, rapid diagnosis, and drug intervention, and has an important impact on reducing the incidence and mortality of myocardial injury in COVID-19 patients.

# AUTHOR CONTRIBUTIONS

SL completed and revised the manuscript. JW searched literature and completed partial writing. WG reviewed and revised the article. YY and ZZ searched literature. SN revised the article. All authors contributed to the article and approved the submitted version.

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# Cost Utility Analysis of Multidisciplinary Postacute Care for Stroke: A Prospective Six-Hospital Cohort Study

*Yu-Ching Chen[1,2], Yu-Jo Yeh[1], Chung-Yuan Wang[3,4], Hsiu-Fen Lin[5,6], Ching-Huang Lin[7], Hong-Hsi Hsien[8], Kuo-Wei Hung[9], Jung-Der Wang[2†] and Hon-Yi Shi[1,10,11,12*†]*

[1] Department of Healthcare Administration and Medical Informatics, Kaohsiung Medical University, Kaohsiung, Taiwan, [2] Department of Public Health, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, [3] Department of Physical Medicine and Rehabilitation, Pingtung Christian Hospital, Pingtung, Taiwan, [4] Department of Nursing, Meiho University, Pingtung, Taiwan, [5] Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [6] Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan, [7] Division of Neurology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, [8] Department of Internal Medicine, St. Joseph Hospital, Kaohsiung, Taiwan, [9] Division of Neurology, Department of Internal Medicine, Yuan's General Hospital, Kaohsiung, Taiwan, [10] Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [11] Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan, [12] Department of Business Management, National Sun Yat-sen University, Kaohsiung, Taiwan

**Background:** Few studies have compared the optimal duration and intensity of organized multidisciplinary neurological/rehabilitative care delivered in a regional/district hospital with the standard rehabilitative care delivered in the general neurology/rehabilitation ward of a medical center. This study measured functional outcomes and conducted cost-utility analysis of an organized multidisciplinary postacute care (PAC) project in secondary care compared with standard rehabilitative care delivered in tertiary care.

**Methods:** This prospective cohort study enrolled 1,476 patients who had a stroke between March 2014 and March 2018 and had a modified Rankin scale score of 2–4. After exact matching for age ± 1 year, sex, year of stroke diagnosis, nasogastric tube, and Foley catheter and propensity score matching for the other covariates, we obtained 120 patients receiving PAC (the PAC group) from four regional/district hospitals and 120 patients not receiving PAC (the non-PAC group) from two medical centers.

**Results:** At baseline, the non-PAC group showed significantly better functional outcomes than the PAC group, including EuroQol-5 dimensions (EQ-5D), Mini-Mental State Examination (MMSE) and Barthel index (BI). During weeks 7–12 of rehabilitation, improvements in all functional outcomes were significantly larger in the PAC group ($P<0.001$) except for Functional Oral Intake Scale (FOIS). Cost-utility analysis revealed that the PAC group had a significantly lower mean (± standard deviation) of direct medical costs (US$3,480 ± $1,758 vs. US$3,785 ± $3,840, $P<0.001$) and a significantly higher average gain of quality-adjusted life years (0.1993 vs. 0.1233, $P<0.001$). The PAC project was an economically "dominant" strategy.

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*Yan Zhang,*
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*National Clinical Research Center for Geriatric Diseases, China*
*Justin Sattin,*
*University of Wisconsin-Madison, United States*

***Correspondence:**
*Hon-Yi Shi*
*hshi@kmu.edu.tw*

*†These authors have contributed equally to this work*

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**Conclusions:** The PAC project saved costs and significantly improved the functional outcomes of patients with stroke with slight to moderately severe disabilities. Randomized control trials are required to corroborate these results.

**Keywords: postacute care, cost-utility, stroke, incremental cost-utility ratios, cost saving**

## INTRODUCTION

Stroke is the second leading cause of death and also the second highest burden estimated with disability-adjusted life-years worldwide (1). In Taiwan, which has a population of approximately 23 million people, stroke is the third leading cause of death and most common cause of complex disability (2). The percentage of patients with disability at 1 and 6 months after the first incident of stroke was 61.2 and 51.72%, respectively (3). In addition, 10.4% of Taiwanese patients with acute stroke had a prolonged hospital stay, which accounted for 47.8% of the total in-hospital medical expenses for stroke (4). In the United States, 59.1% to 82.1% patients hospitalized for stroke required post-acute care within 30 days after discharge (5). Stroke patients treated in an inpatient rehabilitation facility experienced shorter length of rehabilitation stay, less emergency room utilization and lower mortality, but incurred higher cost, than those receiving rehabilitation in a skilled nursing facility (6).

Based on measures of EQ-5D (7), MMSE (8), BI (9), Instrumental Activities of Daily Living Scale (IADL) (10), rehabilitation to improve quality of life emphasizes training/re-training on functional and daily activities including self-care, mobility, cognitive skills, and psychosocial skills, which could actually be accomplished by a multidisciplinary PAC project. To our knowledge, cost-utility analysis of PAC has rarely been prospectively investigated (11). In addition, few studies seem to have compared the optimal duration and intensity of organized multidisciplinary neurological/rehabilitative care delivered in a regional/district hospital (secondary care) vs. standard rehabilitative care delivered in the general neurology/rehabilitation ward of a medical center (tertiary care). Launched in 2014 by the National Health Insurance (NHI) of Taiwan to contain PAC cost without compromising functional outcomes, the PAC project was executed to enroll stroke patients with slight to moderately severe disability and potential for active rehabilitation.

Therefore, the objective of this prospective cohort study is to measure functional outcomes and conduct cost-utility analysis of an organized multidisciplinary PAC project in secondary care compared with standard rehabilitative care delivered in tertiary care.

## MATERIALS AND METHODS

### The Post-acute Care-Cerebrovascular Diseases Project

A PAC-CVD project was launched in Taiwan in 2014 to contain PAC cost by two approaches: (I) across-level transfer of patients in post-acute phase of stroke from neurology wards in medical centers (tertiary care) to neurology/rehabilitation wards in regional and district hospitals (secondary care); (II) within-level transfer of patients from neurology wards in regional/district hospitals (secondary care) to neurology/rehabilitation wards in regional/district hospitals (secondary care). The PAC-CVD project was designed to improve functional outcomes by organizing a multidisciplinary team, which included neurologists, physiatrists, physiotherapists, occupational therapists, speech therapists and registered nurses. The average number of days from stroke onset to PAC ward admission was significantly shorter in patients transferred within level (9.88 days) compared to those transferred across level (17.11 days) (12). The 12-week PAC-CVD project delivered in regional/district hospitals (secondary care) featured more reimbursement and higher intensity of rehabilitation compared to non-PAC care delivered in medical centers (tertiary care). The reimbursement schedule of the PAC project (per diem) in this study is summarized as follows: NT$3,486 (US$113) per day if 3–5 sessions a day within 12 weeks; physical therapy: 1–2 sessions per weekday, 30–60 min each session, and 1 session per weekend, 30–60 min each session; occupational therapy: 1–2 sessions per weekday, 30–60 min each session and 1 session per weekend, 30–60 min each session; speech therapy: at least 5 sessions a week, depending on how well a patient can communicate and/or swallow. The reimbursement of standard rehabilitation for non-PAC care (fee for service): NT$600 (US$19.4) per session; 1 session per weekday for physical therapy, occupational therapy and/or speech therapy, respectively, with 30–60 min each session. The longest duration of non-PAC care usually allows for hospitalization of 28–42 days after acute stroke by National Health Insurance. Details of the PAC-CVD project are described in the **Supplementary Material**.

### Study Design and Sample

This is a prospective cohort study to evaluate the cost-utility of multidisciplinary post-acute care project (vs. standard rehabilitation care) for patients with stroke, which is defined as ICD-9-CM codes 433.xx, 434.xx, and 436 for ischemic stroke and codes 430 and 431 for hemorrhagic stroke; for their counterparts in ICD-10-CM, please see **Supplementary Table 1**. Patients were admitted to a PAC ward at one of four hospitals (three regional hospitals and a district hospital) or to a non-PAC ward at two medical centers in southern Taiwan between March 2014 and March 2018. The inclusion criteria were: (I) diagnosis of acute stroke; (II) stroke onset day within 30 days; and (III) modified Rankin Scale (MRS) scores of 2, 3, and 4, corresponding to slight, moderate, and moderately severe disability, respectively (13). The PAC project was a national health policy and stroke patients were

allocated into either group at the discretion of the physician-in-charge after shared decision-making. **Figure 1** is a flow diagram of the study procedure, which features enrollment, allocation, repeated measures of functional status outcomes, and analysis.

## Economic Evaluation

The costs of both treatment options over 1 year were compared, using the direct-cost approach.

## Outcome Measures

The EuroQol-5 dimensions (EQ-5D) questionnaire is a preference-based, generic and self-reported instrument that can help to understand the impact of stroke, and provides a utility value based upon mobility, self-care, usual activities, pain/discomfort and anxiety/depression, each of which has three levels of severity (7, 14). The Mini-Mental State Examination (MMSE) is a widely used cognitive function test for the elderly, which includes tests of orientation, attention, memory, language and visual-spatial skills (8). The Barthel Index (BI) score is used to measure functional disability in daily self-care activities (e.g., bowels, bladder, grooming, toilet use, feeding, transfer, mobility, dressing, stairs and bathing) (9). The Lawton-Brody Instrumental Activities of Daily Living Scale (IADL) involves eight tasks: telephone use, shopping, meal preparation (C), housekeeping (D), laundry (E), use of transportation, responsibility for medication intake and handling finances (10). Tasks C, D and E are excluded when assessing men. The Functional Oral Intake Scale (FOIS) is used to assess functional oral intake in stroke patients with dysphagia (15). The FOIS classifies swallowing function from level 1 (nothing by mouth) to level 7 (total oral diet with no restrictions). The Berg Balance Scale (BBS) is a scale of functional balance, including static and dynamic balance (16). Each item on this 14-item scale is rated from 0 (poor balance) to 4 (good balance) with maximum score of 56. When overall utility, based on EQ-5D, serves as the dependent variable, all the variance inflation factors (VIFs) are $<5$, indicating negligible or acceptable multicollinearity (17). Therefore, we allow BI and BBS to show the robustness of our results. All enrolled patients were scheduled to complete repeated measures of the EuroQol-5 dimensions (EQ-5D) questionnaire and the other five functional outcomes at four time points: at baseline, at the end of the 6th week and 12th week of rehabilitation, and at the end of one year.

## Estimation of Cost

In accordance with the reimbursement criteria established by the National Health Insurance Administration (NHI), direct costs included fees for physician, laboratory, pharmacy, procedures, and rehabilitative therapy, etc. All cost inputs were adjusted to 2019 U.S. dollars and discounted annually by 3%.

## Estimation of Utility

To estimate quality-adjusted life-years (QALYs), cost-utility analysis often uses "utility scores" (health state valuations) anchored by 0 and 1, where 0 indicates death and 1 indicates full health. This study used the time trade-off valuation procedure to convert EQ-5D total scores to utility. The cost-utility of PAC for stroke patients was estimated using QALYs (14, 18).

The two components used to calculate QALYs are the gain in quality of life and the number of life years over which the gain has been sustained. In this study, because the number of year remained at 1, QALYs were calculated based on a measure of utility derived from EQ-5D. The utilities reported by each participant were multiplied by the assumed duration of sustained benefit after intervention (summed up to the end of 1 year) to estimate the number of QALYs. To maintain consistency with the QALYs calculation, this study assumed that the only resources used by patients were those captured during the 1 year of follow-up. That is, the analysis assumed that patient did not incur any other healthcare costs during the remainder of the year.

## Statistical Analysis

To minimize the potential selection bias, firstly, the patients were selected through exact matching for the following variables: age $\pm$ 1 year, gender, year of stroke diagnosis, nasogastric tube and Foley catheter. Next, the propensity score matching (PSM) approach was used to minimize baseline differences in education, body mass index, stroke type, hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, and previous stroke. The choice of matching algorithm was greedy nearest neighbor matching; within a matched pair, we chose a caliper width of within 0.2 of the standard deviation of the logit score; matching ratio of PAC-to-non-PAC patients was 1 to 1; and the matching was run without replacement (19). Finally, 120 patients each in the PAC and non-PAC group were obtained (**Table 1**).

For repeated assessments within individual subjects, a linear mixed effects model was constructed for EQ-5D, MMSE, BI, IADL, FOIS, and BBS, respectively, with major determinants as fixed effects. The utility values estimated by EQ-5D and the scores for MMSE, BI, IADL, FOIS and BBS served as the dependent variables. Predictor/confounder variables used in this statistical model included PAC (PAC vs. non-PAC), measures of functional outcomes at four time points, year of stroke diagnosis, age, gender (male vs. female), nasogastric tube (yes vs. no), Foley catheter (yes vs. no), education, body mass index, stroke type (ischemic type vs. hemorrhagic type), and comorbidities (yes vs. no). A negative coefficient denoted that the variable predicted a worse functional status score, with the magnitude representing the effect. Effect size was obtained by using the Cohen d statistic, i.e., the difference in the mean post-intervention value minus the mean pre-intervention value divided by the pooled standard deviation (**Supplementary Table 4**) (20). Given the large number of patients lost to follow up in the non-PAC group, the robustness of the results was evaluated by another PSM of 116 PAC subjects to 69 non-PAC subjects at the end of 1 year. Sixty two patients each in the PAC and non-PAC group were obtained.

After converting EQ-5D scores into utility values, the number of QALYs over a period of 1 year was calculated for each participant using the area under the curve approach with control for imbalances in baseline utility scores (21). A *t*-test was performed to compare mean direct medical costs between the two groups. The incremental cost utility ratio (ICUR) was calculated as the ratio of the difference in mean costs per patient

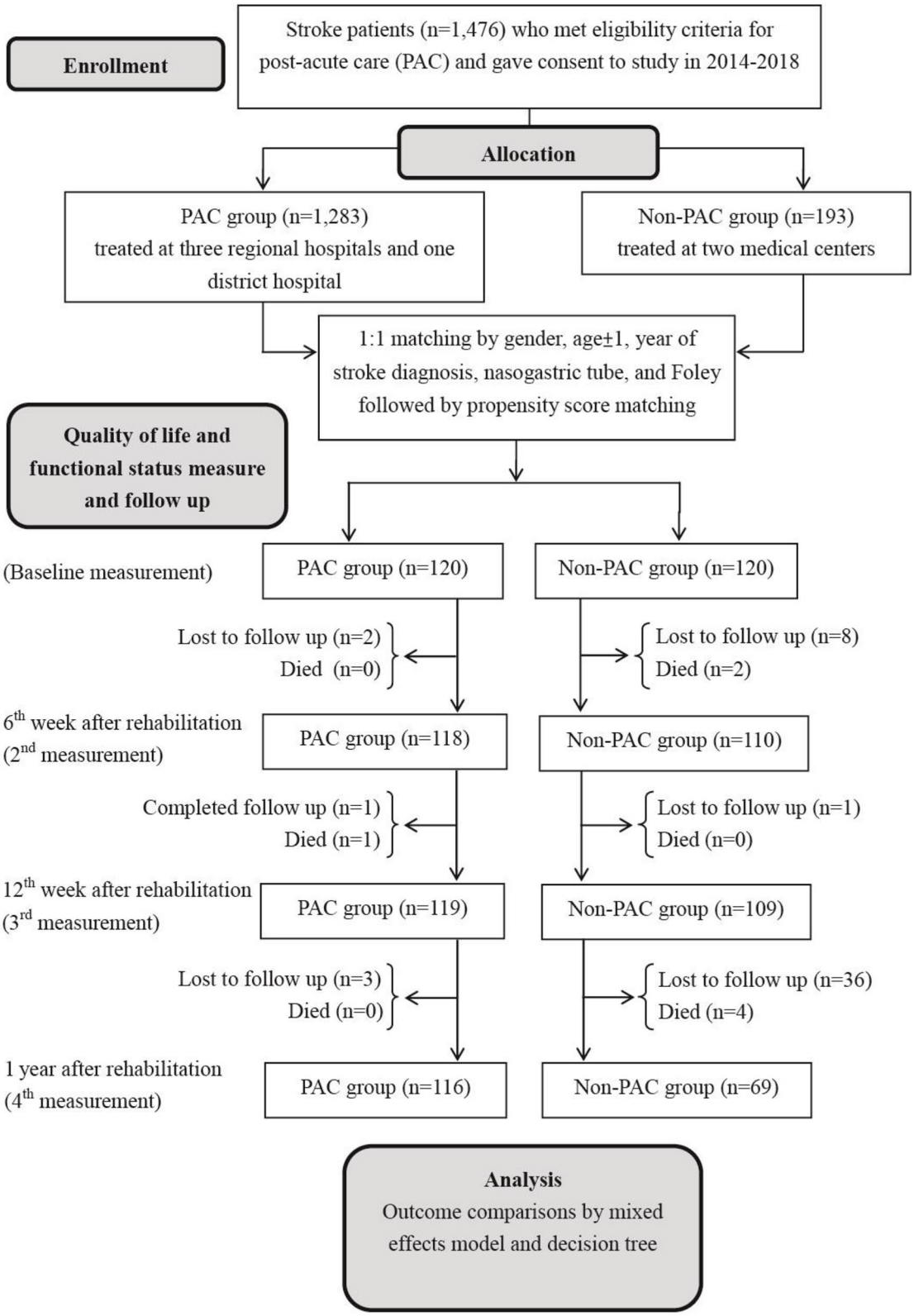


**FIGURE 1 |** Flowchart of sample selection for prospective cohort analysis.

to the difference in mean QALYs per patient between PAC and non-PAC groups. A willingness-to-pay (WTP) threshold of gross domestic product (GDP) US$26,263.5 per QALY was used to assess cost-effectiveness. A project is termed an economically dominant strategy when it is both clinically superior and cost saving. To derive the cost-effectiveness acceptability curve, this study performed nonparametric bootstrapping on the incremental cost and effectiveness with 1,000 replications and **Supplementary Figure 4** presents the results (22–24). The Statistical Analysis System® software version 9.4 (SAS Institute, Cary, NC, USA) was used for statistical analyses. All *P* values reported were two-sided, and a *P* value of <0.05 was considered statistically significant.

## Sensitivity Analysis

For sensitivity testing, 164 patients in the PAC group and 82 in the non-PAC group were matched successfully. Comparisons of the two groups over 1 year revealed that the PAC group showed significantly greater improvement in EQ-5D and all functional outcomes, except for FOIS, where *p*-value for trend was 0.78 (**Supplementary Table 7**, **Supplementary Figure 5**). This study also conducted cost-utility analysis of PAC ($n = 164$) and non-PAC ($n = 82$) at the end of 1 year after stroke rehabilitation (**Supplementary Table 8**).

# RESULTS

**Table 1** compares the baseline characteristics of patients receiving PAC project with those of matched patients receiving standard rehabilitation. As presented in **Table 2**, at baseline, compared with the non-PAC group, the PAC group had significantly lower mean scores for EQ-5D utility (0.23 vs. 0.40; $P < 0.0001$), for cognitive function measured by MMSE (11.72 vs. 13.92; $P = 0.020$) and for self-care activities measured by BI (15.49 vs. 25.05; $P = 0.001$). The scores for IADL, FOIS, or BBS did not significantly differ between the two groups. When $T_0$ values were used as reference values, the non-PAC group had larger improvements in BI, IADL and BBS than the PAC group at the end of the 6$^{th}$ week (**Supplementary Table 2**). When T1 values were used as reference values, the PAC group achieved significantly larger improvements in the EQ-5D and in all functional outcomes ($P < 0.001$) except for the FOIS score during weeks 7–12 of rehabilitation than the non-PAC group (**Supplementary Table 2**). When T2 values were used as reference values, the two groups did not significantly differ in any functional outcome measures obtained at T3 when rehabilitation had ended at T2. During weeks 1–12, the PAC group had larger improvements in the EQ-5D and in MMSE, BI, and BBS scores than did the non-PAC group after controlling for baseline values (**Supplementary Figure 1** and **Supplementary Table 3**). Overall, the PAC group exhibited a significantly better trend of improvement over the non-PAC group in the least squares mean scores of functional outcome measures, except for the FOIS score, over the 1-year duration of the study ($P < 0.001$; **Table 2** and **Figure 2**). **Supplementary Tables 5, 6** present the results for 62 patients each in the PAC and non-PAC group. Furthermore, compared with the non-PAC group ($n = 62$), the PAC group ($n = 62$) had significantly larger improvements in all outcomes, including the FOIS score (**Supplementary Figure 2**).

The mean direct medical cost per patient was US$3,480 in the PAC group and US$3,785 in the non-PAC group (**Table 3**). Cost-utility analysis revealed that the PAC treatment had a higher effectiveness (QALYs gain of 0.076) and a lower cost (cost reduction of US$305 ± US$2,986) than the non-PAC treatment. The ICUR was cost saving at US$ −4,013 per QALY, demonstrating that the PAC project was an economically dominant strategy. At a WTP threshold of US$26,263.5 per QALY, the PAC project had a 100% likelihood of being cost-effective compared to standard rehabilitation. For each set of 1,000 bootstrap resamples, **Supplementary Figure 3** presents the corresponding cost-effectiveness plane, with incremental mean total direct medical cost on the y-axis, and incremental mean QALYs on the x-axis. All bootstrap observations were located in the southeast quadrant of the cost-effectiveness plane. **Supplementary Figure 4** illustrates the cost-utility acceptability curve. The stochastic uncertainty associated with the mean incremental cost-effectiveness ratio indicated that our findings were robust.

# DISCUSSION

Although PAC has been promoted for the past 3 decades (25), evidence of its cost-effectiveness has been limited (11). To address this issue, we used exact and propensity score matching in this study. Although we observed that the PAC project saved cost compared with standard rehabilitation among stroke patients with an MRS scores 2–4, it did not necessarily imply that such an association was causal. However, we have the following arguments to corroborate this hypothesis: First, since we have controlled potential confounding factors including age, gender, year of stroke diagnosis, nasogastric tube, Foley catheter, and other covariates, such as hypertension (26), diabetes and atrial fibrillation etc. in the mixed effect model, the above factors cannot be explicable for the difference between PAC group and non-PAC group. Second, at the end of the one-year follow-up, only 69 subjects stayed in non-PAC group, while 116 subjects in PAC group remained, indicating a lower rate of adherence to standard rehabilitation. In further analysis, 69 non-PAC patients were matched by propensity score with 116 PAC patients, which resulted in 62 patients in each group (**Supplementary Table 5**). Compared with patients receiving standard rehabilitation, the patients receiving PAC consistently achieved significant improvement in all functional outcomes, including the FOIS score ($P = 0.020$, **Supplementary Table 6**). This further increased the robustness of positive outcomes shown in patients receiving PAC. Third, at baseline, the non-PAC group had statistically significant higher scores in EQ-5D utility, the MMSE, and the BI ($P < 0.05$); at 1 year, the outcome measures including EQ-5D utility, MMSE, BI, IADL, and BBS, showed a consistent better improvement in patients under PAC project than those receiving standard rehabilitation ($P < 0.001$) (**Table 2**). Finally, direct medical costs were lower for patients receiving PAC project than for those receiving standard

**TABLE 1 |** Distributions of patient characteristics before and after matching by demographic characteristics and by propensity scores.

| Variables | | Before matching | | | After matching | | |
|---|---|---|---|---|---|---|---|
| | | PAC ($n$ = 1,283) | Non-PAC ($n$ = 193) | $P$ value | PAC ($n$ = 120) | Non-PAC ($n$ = 120) | $P$ value |
| Cerebrovascular accident, year of diagnosis | 2014 | 221 (17.2%) | 0 (0%) | | 0 (0%) | 0 (0%) | |
| | 2015 | 302 (23.5%) | 15 (7.8%) | | 11 (9.17%) | 11 (9.17%) | |
| | 2016 | 392 (30.6%) | 107 (55.7%) | <0.001 | 66 (55%) | 66 (55%) | 1 |
| | 2017 | 363 (28.3%) | 70 (36.5%) | | 43 (35.83%) | 43 (35.83%) | |
| | 2018 | 5 (0.4%) | 0 (0%) | | 0 (0%) | 0 (0%) | |
| Age, years† | | 65.16 ± 12.84 | 68.28 ± 13.95 | 0.002 | 67.45 ± 12.15 | 67.5 ± 12.27 | 0.975 |
| Stroke patients, No. (%) | | 1,283 (100%) | 193 (100%) | <0.001 | 120 (100%) | 120 (100%) | 1 |
| Gender (% male) | | 800 (62.4%) | 122 (63.2%) | 0.880 | 81 (67.5%) | 81 (67.5%) | 1 |
| Nasogastric tube, No. (%) | | 233 (18.2%) | 56 (29.0%) | 0.001 | 11 (9.17%) | 11 (9.17%) | 1 |
| Foley catheter, No. (%) | | 96 (7.5%) | 38 (19.7%) | <0.001 | 8 (6.67%) | 8 (6.67%) | 1 |
| Education, years† | | 8.95 ± 1.30 | 8.64 ± 4.85 | 0.370 | 8.84 ± 1.73 | 8.96 ± 4.85 | 0.804 |
| BMI, kg/m† | | 24.03 ± 2.53 | 24.16 ± 3.46 | 0.630 | 24.22 ± 2.26 | 24.13 ± 3.4 | 0.815 |
| Stroke type, Ischemic (%) | | 1,048 (81.7%) | 176 (91.2%) | 0.003 | 110 (91.67%) | 109 (90.83%) | 0.819 |
| Hemorrhagic (%) | | 235 (18.3%) | 17 (8.8%) | | 10 (8.33%) | 11 (9.17%) | |
| Hypertension, No. (%) | | 890 (69.4%) | 137 (71.0%) | 0.710 | 78 (65%) | 80 (66.67%) | 0.785 |
| Diabetes mellitus, No. (%) | | 499 (38.9%) | 71 (36.8%) | 0.630 | 48 (40%) | 44 (36.67%) | 0.595 |
| Hyperlipidemia, No. (%) | | 463 (36.1%) | 46 (23.8%) | 0.001 | 37 (30.83%) | 30 (25%) | 0.314 |
| Atrial fibrillation, No. (%) | | 106 (8.3%) | 16 (8.3%) | 0.990 | 8 (6.67%) | 8 (6.67%) | 1 |
| Previous stroke, No. (%) | | 178 (13.9%) | 48 (24.9%) | <0.001 | 17 (14.17%) | 17 (14.17%) | 1 |

*PAC, post-acute care; BMI, body mass index.*
*†Values are expressed as mean ± standard deviation.*

**TABLE 2 |** Comparison of functional status trends between PAC and non-PAC groups after matching (120:120).

| Outcomes | | Baseline (T0) | | 6th week after rehabilitatio (T1) | | 12th week after rehabilitation (T2) | | 1st year after rehabilitatio (T3) | | $P$ value for trend¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LS-mean ± S E | $P$ value† | LS-mean ± SE | $P$ value† | LS-mean ± SE | $P$ value† | LS-mean ± SE | $P$ value† | |
| Utility_TW | PAC | 0.23 ± 0.04 | <0.001 | 0.27 ± 0.05 | <0.001 | 0.47 ± 0.05 | 0.260 | 0.47 ± 0.05 | 0.260 | <0.001 |
| | Non-PAC | 0.40 ± 0.05 | | 0.48 ± 0.05 | | 0.51 ± 0.05 | | 0.51 ± 0.05 | | |
| Utility_UK | PAC | −0.07 ± 0.07 | <0.001 | −0.01 ± 0.07 | <0.001 | 0.24 ± 0.07 | 0.300 | 0.24 ± 0.07 | 0.310 | <0.001 |
| | Non-PAC | 0.16 ± 0.07 | | 0.25 ± 0.07 | | 0.28 ± 0.07 | | 0.28 ± 0.07 | | |
| MMSE | PAC | 11.72 ± 1.66 | 0.020 | 12.05 ± 1.67 | 0.010 | 14.44 ± 1.68 | 0.420 | 14.50 ± 1.67 | 0.240 | <0.001 |
| | Non-PAC | 13.92 ± 1.67 | | 14.48 ± 1.68 | | 13.62 ± 1.70 | | 13.31 ± 1.71 | | |
| BI | PAC | 15.49 ± 4.69 | 0.001 | 21.76 ± 4.69 | <0.001 | 38.02 ± 4.79 | 0.450 | 37.94 ± 4.81 | 0.930 | <0.001 |
| | Non-PAC | 25.05 ± 4.72 | | 34.49 ± 4.73 | | 40.26 ± 4.84 | | 38.22 ± 4.91 | | |
| IADL | PAC | 0.62 ± 0.27 | 0.070 | 0.74 ± 0.28 | 0.130 | 1.81 ± 0.29 | 0.010 | 1.84 ± 0.29 | 0.010 | 0.001 |
| | Non-PAC | 0.35 ± 0.27 | | 1.05 ± 0.29 | | 1.26 ± 0.29 | | 1.27 ± 0.30 | | |
| FOIS | PAC | 5.11 ± 0.47 | 0.470 | 5.16 ± 0.47 | 0.720 | 5.00 ± 0.29 | 0.400 | 5.00 ± 0.29 | 0.280 | 0.510 |
| | Non-PAC | 4.70 ± 0.48 | | 4.96 ± 0.48 | | 4.86 ± 0.30 | | 4.81 ± 0.30 | | |
| BBS | PAC | 2.48 ± 3.34 | 0.140 | 4.29 ± 3.37 | 0.001 | 18.08 ± 3.40 | 0.020 | 18.23 ± 3.40 | 0.010 | <0.001 |
| | Non-PAC | 5.22 ± 3.37 | | 11.37 ± 3.4 | | 13.09 ± 3.44 | | 12.53 ± 3.46 | | |

*PAC, post-acute care; Utility_TW, utility (Taiwan); Utility_UK, utility (United Kingdom); MMSE, Mini-Mental State Examination; BI, Barthel index; IADL, Instrumental Activities of Daily Living; FOIS, Functional Oral Intake Scale; BBS, Berg Balance Scale; LS-mean, least squares mean; SE, standard error;*
*†Each functional status measure was compared btween the PAC and non-PAC groups at baseline and after 6, 12, and 52 weeks.*
*¶Trends in differences between PAC and non-PAC groups for each functional status measure during the study period.*

rehabilitation (**Table 3**). Therefore, we tentatively concluded that PAC project saved cost compared with standard rehabilitation for mild to moderate stroke patients, and the difference could not be attributed to any known alternative cause.

Another major issue to be addressed is whether the sampled 120 patients receiving standard rehabilitation accurately represented all 193 non-PAC patients. Although this cohort was enrolled from stroke patients with modified Rankin scale

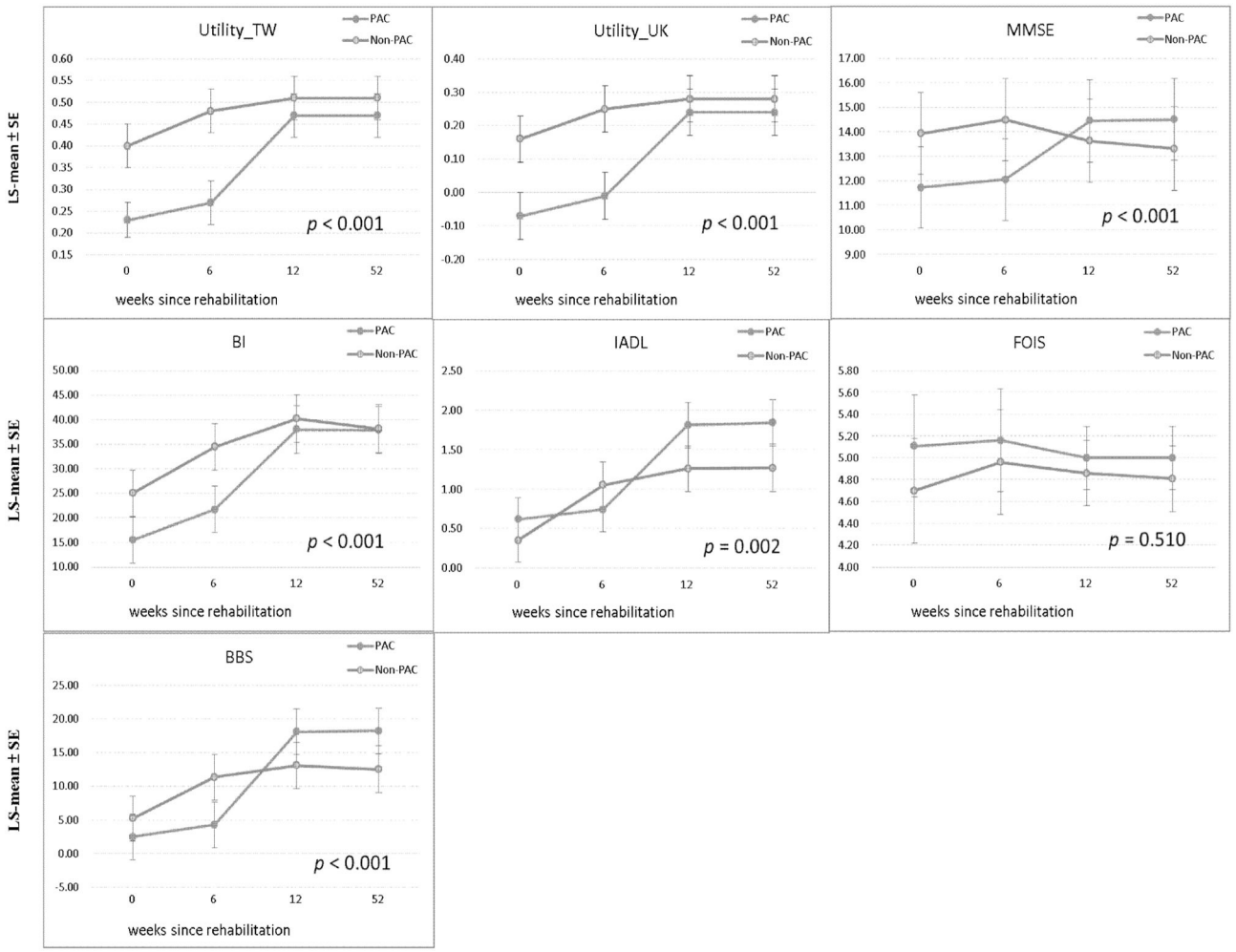


**FIGURE 2 |** Comparison of LS-mean ± SE of each functional status measure between PAC and non-PAC groups at baseline, weeks 6 and 12, and 1 year and comparison of trend in each functional status measure between the groups. Utility_TW, utility (Taiwan); Utility_UK, utility (United Kingdom); MMSE, Mini-Mental State Examination; BI, Barthel Index; IADL, Instrumental Activities of Daily Living Scale; FOIS, Functional Oral Intake Scale; BBS, Berg Balance Scale; PAC, postacute care; LS-mean, least squares mean; SE, standard error. *P* values for trend in each functional status measure between the groups (120:120).

2–4, the PAC project has been a national policy, and only 13% (193/1476) of them were assigned to standard rehabilitation, particularly those with moderate severity. Among stroke patients, having a nasogastric tube, a retained Foley catheter, or a previous stroke was associated with impaired cognition and resilience, which was demanding in rehabilitation, and these conditions were present in approximately 29, 20, and 25%, respectively, of the non-PAC group, making these patients less likely to be matched with those in the PAC group (**Table 1**). To improve the comparability for rehabilitation at initial stage, we only found 120 pairs. Thus, the selectivity of our final matched samples may limit the generalizability of our findings to stroke patients with better rehabilitation potential, but still demonstrates the causal validity of cost-effectiveness of PAC project.

Lacking measure of functional outcomes in the multifaceted quality improvement intervention of stroke care, Pan et al. reported that the intervention gained 0.013 QALYs at an additional cost of US $140 in the first year, yielding an ICER of US $11,120 per QALY gained (11). The intervention was cost-effective in the first year, and more so in the second year at US$ 9,200 per QALY gained; our PAC project gained 0.076 QALYs at a negative cost of US $305 in the first year, yielding an ICUR of US-$4,013 per QALY gained (**Table 3**), demonstrating that the PAC project was cost saving.

## Clinical Implications for Health Policy

This study corroborated a previous series of Taiwan studies reporting that a PAC project for stroke patients improved quality of life and functional status at the time of hospital discharge (12, 27–29). In these studies, the largest improvements seemed to be achieved after 3 months of rehabilitation. In contrast, our repeated measures of multiple functional disabilities found although the non-PAC group performed better at baseline and first 6 weeks, PAC yielded significantly larger improvements in

**TABLE 3 |** Cost-utility analysis of PAC and non-PAC groups within 1 year after stroke rehabilitation (120:120).

| | PAC group ($n$ = 120) | Non-PAC group ($n$ = 120) | Incremental difference |
|---|---|---|---|
| | mean ± SD (%) | mean ± SD (%) | (PAC-non-PAC)[¶] mean ± SD (%) |
| **Baseline** | | | |
| Utility score | 0.40 ± 0.18 | 0.57 ± 0.24 | –(0.17 ± 0.21)[c] |
| **1 year after stroke rehabilitation** | | | |
| NHI total direct medical cost[†] | 3,480 ± 1,758 | 3,785 ± 3,840 | –(305 ± 2,986) |
| Utility score | 0.63 ± 0.26 | 0.74 ± 0.26 | –(0.10 ± 0.26)[#] |
| QALYs gained[§] | 0.1993 | 0.1233 | 0.0760 |
| ICUR (PAC – non-PAC) | dominant | | −4,013 |

*PAC, post-acute care; mean, arithmetic mean; SD, standard deviation; NHI, national health insurance; QALYs, quality adjusted life years; ICUR, incremental cost-utility ratio.*
*[†]Mean direct cost for the PAC group. Per diem reimbursement packages received by hospitals varied by intensity of rehabilitation, e.g., per diem reimbursement for high-intensity rehabilitative care was the maximal packaged reimbursement of NT$3,587; per diem reimbursement for usual rehabilitative care was NT$2,411 (2019 exchange rate, NT $30.5 = US $1). Reimbursement included fees for physician, ward service, nursing, laboratory, rehabilitation therapy, and medication/pharmacy service fee, etc.*
*[§]Area under the curve with control for baseline utility.*
*[¶]$P < 0.001$ for independent t test of the two groups.*
*[#]$P = 0.01$ for independent t test of the two groups.*

EQ-5D and all functional outcomes, except for FOIS, during weeks 7–12 of rehabilitation (**Figure 2** and the difference in differences in **Supplementary Table 2**). And these scores did not significantly differ between the two groups from the end of the 12th week to 1 year. Moreover, the above improvements were accomplished under cost-saving condition.

Although inpatient stroke care by an organized multidisciplinary healthcare team could reduce mortality (30–32), treatment in an inpatient rehabilitation facility (IRF) was more expensive than treatment in a skilled nursing facility (SNF) (6, 33). Moreover, co-locating acute care and rehabilitation care for stroke in a district hospital was associated with reduced mortality and decreased length of hospital stay (34). As **Table 3** presents, the average PAC cost of standard rehabilitative care in a single medical center in Taiwan where acute care and rehabilitative care are delivered in neurology ward, and rehabilitation ward, of tertiary-care hospital (US$3,785 per person) is higher than that of an intensive PAC project delivered in a secondary-care hospital (US$3,480 per person), where the improvement in functional status and reduced mortality were achieved at a lower cost. Additionally, the high maximum age in the PAC group (89 years old) suggests that advanced age alone should not be excluded from criteria for admission to a neurorehabilitation unit following acute stroke treatment (28).

## Limitations of This Study

The following limitations of this study must be acknowledged. First, being not a randomized trial, this study exercised rigorous matching to secure comparability at the expense of generalizability. Second, at baseline, the non-PAC group had higher average scores for EQ-5D, MMSE and BI compared to the PAC group. Therefore, the magnitude of functional improvements obtained by PAC may have been underestimated. Nonetheless, the potential bias would not change our conclusion that PAC seemed more cost-effective than non-PAC. Third, we did not include time to rehabilitation, distance to hospital, and costs (including productivity loss and out-of-pocket expenses) involved in the non-health care sector or societal perspective (35). Further research is required to explore stroke onset to rehabilitation, geographic location, and those costs. Fourth, this study did not collect data of detailed emergency intervention and co-medications, including thrombectomy, tissue plasminogen activator treatment, novel oral anticoagulants, antiplatelet drugs and medications for hypertension, diabetes and hyperlipidemia. Thus, their impacts on functional outcomes could not be ascertained. However, since all management of stroke patients must follow the guideline recommended by the Taiwan Stroke Society (36) to achieve the target values (37) and avoid rejection of reimbursement by Taiwan NHI, the likelihood of potential confounding by different emergency treatments and co-medications would not be too large. Fifth, repeated measures of functional outcomes were limited to 1 year. Further longitudinal follow-up studies are needed to assess long-term effect of a PAC project on functional outcomes, morbidity and mortality.

# CONCLUSIONS

The PAC group had lower direct medical costs and higher QALY gains compared to the non-PAC group during 1-year follow-up period. Thus, enrolling stroke patients into an organized multidisciplinary PAC project could significantly improve their functional status and saved medical costs. The improved effectiveness of PAC was corroborated by evidence of significant improvements in at least 4 functional outcomes during weeks 7-12 of rehabilitation. Further long-term research is required to validate its benefit on clinical outcomes, and should include survival and overall societal impact.

# DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# ETHICS STATEMENT

The studies involving human participants were reviewed and approved by before study commencement, we obtained formal approval from the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-20140308). The patients/participants provided their written informed consent to participate in this study.

# AUTHOR CONTRIBUTIONS

Y-CC, J-DW, and H-YS collated, analyzed, interpreted the data and wrote the manuscript, designed the study,

provided statistical expertise, and wrote the first draft of the manuscript. Y-JY, H-FL, C-HL, H-HH, and K-WH collected the data. All authors contributed to the interpretation of the results, critical revision of the manuscript for important intellectual content, and have approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no authors who met the criteria for authorship have been omitted. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.826898/full#supplementary-material

**Supplementary Figure 1 |** Comparison by LS-mean ± SE of each functional status measure between PAC and non-PAC groups, after controlling for baseline, week 6 and 12, and 1 year data and comparison of trend in each functional status measure between the groups. Utility_TW, Utility (Taiwan); Utility_UK, Utility (United Kingdom); MMSE, Mini-Mental State Examination; BI, Barthel index; IADL, Instrumental Activities of Daily Living; FOIS, Functional Oral Intake Scale; BBS, Berg Balance Scale; PAC, postacute care; LS-mean, least squares mean; SE, standard error. *P* values for trend in each functional status measure between the groups (120:120).

**Supplementary Figure 2 |** Comparison by LS-mean ± SE for each functional status measure between PAC and non-PAC groups, after PSM of 116 vs. 69, at baseline, weeks 6 and 12, and 1 year, respectively, and trend for each functional status measure between the groups (62:62). Utility_TW, Utility (Taiwan); Utility_UK, Utility (United Kingdom); MMSE, Mini-Mental State Examination; BI, Barthel Index; IADL, Instrumental Activities of Daily Living; FOIS, Functional Oral Intake Scale; BBS, Berg Balance Scale; PAC, postacute care; LS-mean, least squares mean; SE, standard error. P values for trend for each functional status measure between the groups.

**Supplementary Figure 3 |** Incremental cost effectiveness [postacute care (PAC) vs. non-PAC]. Scatter plots of incremental effectiveness (quality-adjusted life years) vs. incremental costs from 1,000 resamplings in the probabilistic sensitivity analysis with variation limited to cost and effectiveness assumptions and with transition-probabilities constant. This probabilistic sensitivity analysis demonstrated that PAC has a 100% probability of achieving cost savings relative to non-PAC in patients with stroke. Each plotted point is the result of an incremental cost divided by incremental quality-adjusted life years. The elliptic circle represents the 95% confidence interval.

**Supplementary Figure 4 |** Cost-effectiveness acceptability curves for the probabilistic sensitivity analysis. Results from 1,000 resamplings in the probabilistic sensitivity analysis created synthetic populations of patients from the trial using bootstrapping. The lines represent the fraction of simulation iterations in which the postacute care (PAC) project was cost effective compared with standard rehabilitation (y-axis) at various levels of willingness to pay for quality-adjusted life years (QALYs) gains (x-axis).

**Supplementary Figure 5 |** Comparison of LS-mean ± SE of each functional status measure between PAC and non-PAC groups, after 2:1 matching, at baseline, weeks 6 and 12, and 1 year and comparison of trend in each functional status measure between the groups. Utility_TW, utility (Taiwan); Utility_UK, utility (United Kingdom); MMSE, Mini-Mental State Examination; BI, Barthel Index; IADL, Instrumental Activities of Daily Living; FOIS, Functional Oral Intake Scale; BBS, Berg Balance Scale; PAC, postacute care; LS-mean, least squares mean; SE, standard error. P values for trend in each functional status measure between the PAC and non-PAC groups (164:82).

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# Efficacy of a 6-Week Home-Based Online Supervised Exercise Program Conducted During COVID-19 in Patients With Post Percutaneous Coronary Intervention: A Single-Blind Randomized Controlled Trial

*Jiajia Li[1†], Bo Liu[2†], Zheng Wang[1†], Doa El-Ansary[1,3], Roger Adams[4], Jia Han[5*] and Shu Meng[2*]*

[1] Department of Sport Rehabilitation, School of Kinesiology, Shanghai University of Sport, Shanghai, China, [2] Department of Cardiology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China, [3] Department of Health Professions, Faculty of Art, Health and Design, Swinburne University of Technology, Melbourne, VIC, Australia, [4] Research Institute for Sport and Exercise, University of Canberra, Canberra, ACT, Australia, [5] College of Rehabilitation Sciences, Shanghai University of Medicine and Health Sciences, Shanghai, China

**Objective:** The aim of this study was to assess the efficacy of a 6-week cardiac rehabilitation (CR) program designed for patients with coronary artery disease (CAD) after percutaneous coronary intervention (PCI) that involved an online supervised exercise program that they could access during COVID-19.

**Methods:** One hundred patients were randomly allocated into control group (CG) and supervision group (SG). CG accepted conventional health education with a home exercise program booklet delivered before discharge, SG had an additional home-based online supervised exercise program (HOSEP). Questionnaires, motor function and lipid profile were administered at baseline. Questionnaires included the Godin-Shephard Leisure-Time Physical Activity questionnaire (GSLTPAQ) and Bandura's Exercise Self-efficacy (ESE). Motor function included: 6-min walk test (6 MWT), timed up and go test (TUG), 30-s sit to stand (30-s STS), and Hand Grip Strength (HG). Lipid profile included: low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG). The questionnaires were re-administered after 2-weeks, all tests were re-evaluated after 6-weeks.

**Results:** ①the questionnaire results showed that scores on GSLTPAQ and ESE were significantly improved in the SG. The changes in GSLTPAQ scores from baseline to 2- and 6-weeks in the SG were significantly higher than in the CG (2-week: 6.9 ± 13.0 for SG and 0.2 ± 10.2 for CG, $p = 0.005$; 6-week: 9.4 ± 18.1 for SG and 0.2 ± 11.8 for CG, $p = 0.003$). ②in terms of motor function, both the CG and SG improved TUG and 6 MWT performance, with the 6 MWT improvement being significantly greater in the SG than CG (43.7 ± 39.2 m for SG and 16.6 ± 39.1 m for CG, $p = 0.001$). Improvement in the 30-s STS was significantly greater in the SG than CG (2.4 ± 3.6 repetitions for SG

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Anna Pieniazek,
University of Łódz, Poland

***Correspondence:**
Jia Han
jia.han@canberra.edu.au
Shu Meng
msdoctor@126.com

[†]These authors have contributed equally to this work

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and 0.4 ± 3.5 repetitions for CG, $p = 0.007$). ③the lipid profile level significantly improved over baseline in both SG and CG after 6-week intervention, and these changes were not statistically different between groups.

**Conclusion:** This pilot randomized control study demonstrated that a 6-week HOSEP, when added to education delivered pre-hospital discharge for CAD patients following PCI, was beneficial with respect to exercise self-efficacy, exercise behavior, motor function and lipid profile. Supervised exercise programs delivered online in addition to education providing effective and accessible CR during COVID-19.

**Keywords: coronary artery disease, percutaneous coronary intervention, cardiac rehabilitation, home-based, education, exercise, COVID-19**

## INTRODUCTION

According to the *Report on Cardiovascular Health and Diseases in China: an Updated Summary of 2020*, the prevalence of cardiovascular disease in China is 330 million, of which 11 million are coronary artery disease (CAD) (1). Percutaneous coronary intervention (PCI) is a clinical intervention to dilate and maintain patency for narrowing of the coronary arteries with significant therapeutic efficacy (2).

Cardiac rehabilitation (CR) has been recommended as an effective treatment that reduces mortality and morbidity in CAD patients after PCI (3, 4), and is supported as secondary prevention for CAD (5). CR should commence as soon as possible after hospital admission, and continue into the community upon hospital discharge (3, 6). Although the majority of patients may commence CR after PCI whilst in hospital, many cannot continue when discharged due to a shortage of programs and health professionals to support CR programs in the community (7, 8).

Home-based CR with online supervision has shown its merit in overcoming barriers of time and distance, and thus has become successful in increasing CR participation rates (7). A systematic review has demonstrated that long term (over 3 months) supervised home-based CR is not significantly different from center-based CR with respect to improving exercise capacity, risk factors and psychosocial state (8). However, the effect of short-term supervised home-based CR has not been studied and its effect on both physical and psychological measures remains unclear. Therefore, the purpose of this study was to evaluate the safety and efficacy of a 6-week home-based online supervised exercise program (HOSEP) as CR for CAD patients after PCI. The results will inform CR practice by providing evidence regarding low-cost, more convenient home-based CR, especially during the COVID-19 pandemic.

## METHODS

### Participants

This study was approved by the Human Subjects Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University school of Medicine (No. XHEC-C-2020-078-1) and registered in the Chinese Clinical Registration Center (clinical trial website: http://www.chictr.org.cn/enIndex.aspx; clinical trial registry number: Chi CTR2000037435). All Participants completed informed consent before commencement of data collection. Participants included all patients with CAD after PCI in the Department of Cardiology from Xinhua Hospital during January to December 2020. Inclusion criteria were: ①clinically diagnosed CAD with PCI; ②Mini Mental State Examination (MMSE) ≥24 (9); ③native language is Chinese; ④age ≥18 years old; ⑤able to evaluate and sign informed consent on time. Exclusion criteria were: ①left ventricular ejection fraction <40% or heart function-New York Heart Association (NYHA) grade above level III (10); ②severe cardiac arrhythmia and cardiogenic shock; ③combined with severe hypertension, hypertrophic cardiomyopathy, moderate to severe valvular disease, acute pericarditis or myocarditis; ④combined with any other diseases that affect activity, such as severe liver, kidney and respiratory dysfunction, nervous system diseases, musculoskeletal system diseases, visual or auditory dysfunction; ⑤had or is having CR.

### Procedure

Participants were tested at the Department of Cardiology of Xinhua Hospital during January to December 2020. All participants were blind to the intervention and randomly allocated into the Supervision group (SG) or the Control group (CG).

Baseline information, including questionnaires, motor function and lipid profile, were collected on the day before discharge.

Questionnaires were administered under the supervision of a CR physical therapist, motor function was assessed by another CR physical therapist, and lipid profile tests were done in a certified medical laboratory.

All participants had conventional face-to-face health education and the home exercise program booklet was given to the patients by a CR physical therapist before discharge. The home exercise program included walking for 30–60 min, and STS exercise for 2–3 sets every day, with scoring at 4–5 on RPE 0–10, and at 60–80% of maximum heart rate (HRmax) (HRmax = 220-age) calculated for intensity (11–13) (see **Supplementary Material** for an example of home exercise program). The SG had additional HOSEP, where patients were added into a WeChat virtual community and reminded every

day, at 8 a.m., to 10 a.m., by CR physical therapist, to complete the walking and STS exercise as per their home exercise program.

Participants were followed up as outpatients for a 6-week period. Questionnaires were administered again at 2- and 6-weeks, motor function and lipid profile were evaluated again after the 6-week intervention. All tests were done with the same estimator and place as baseline in order to minimize sources of variability.

The study design and experiment flowchart are presented in **Figure 1**.

## Outcome Measures

Questionnaires administered included Bandura's Exercise Self-efficacy (ESE) instrument (14) and the Godin-Shephard Leisure-Time Physical Activity Questionnaire (GSLTPAQ) (15).

Motor function tests included the 6-min Walk Test (6 MWT) (16), 30-s sit-to-stand test (30-s STS test) (17), Timed Up and Go test (TUG test) (17) and Hand Grip Strength (HG) (18). HG and TUG were assessed at first, then the 6 MWT and 30-s STS were conducted in random order. There was a 1-min interval between HG, TUG and 6 MWT, and 30 min between 6 MWT and the 30-s STS test to ensure Ratings of Perceived Exertion (RPE), heart rate and blood pressure returned to normal (18, 19). 6 MWT was conducted as per the American Thoracic Society statement (16). It was tested in a straight, unobstructed, flat corridor that was over 30 meters long. A start line was marked at one end of the corridor and the 30 m point was marked with a traffic cone as the turnaround point. For safety during the test, a chair and a source of oxygen station in the corridor were prepared. Before 6 MWT, a CR physical therapist measured patients' pulse, blood pressure, and RPE, and gave standardized instructions and encouragement. Walking distance and Post-test RPE, pulse and blood pressure were recorded.

Lipid profile included low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG).

## Data Analysis

SPSS 25.0 software (IBM Corp., Armonk, NY) was used for data analysis, with intention-to-treat analysis (20). Demographic characteristics were analyzed via descriptive statistical methods. The intragroup comparison was conducted via repeated measures analysis of variance (ANOVA) and intergroup comparison of value changes by independent sample $t$-test. All results were expressed as mean ± standard deviation ($\bar{x} \pm s$) and $p < 0.05$ indicated a statistically significant difference.

# RESULTS

## Basic Information

At the commencement of this study, 50 patients were randomly assigned for each SG and CG. At 2-week follow-up, two patients in the SG and one patient in CG dropped out for family reasons. At 6-week follow-up, one patient in each group decined to continue participation in the experiment. Therefore, 47 and 48 patients in the SG and CG, respectively completed the 6-week intervention. There were no adverse events reported during the intervention period. All patients were treated with dual antiplatelet therapy (Aspirin + Clopidogrel/Ticagrelor), β-Blocker and Stain. There were no differences between the two groups on antihypertensive (34:40, $p = 0.17$) and hypoglycemic treatments (18:15, $p = 0.52$). There were no significant intergroup differences in the demographic data at baseline (**Table 1**).

## Questionnaires

The intergroup comparison showed no statistically significant differences in the baseline questionnaire results (**Table 2**).

Obtained $p$ in the SG showed that, compared with baseline, the ESE scores significantly improved at 6-weeks ($p = 0.001$, 95% *CI*: 2.8–13.8), and the GSLTPAQ scores significantly improved at 2- and 6-weeks (2-week: $p < 0.01$, 95% *CI*: 2.9–10.9; 6–week: $p < 0.01$, 95% *CI*: 4.2–14.6). The intragroup comparison in the CG showed no significant differences in ESE and GSLTPAQ scores across the 3 testing points.

The intergroup comparison for the score change in GSLTPAQ showed significantly greater progress at 2- and 6-week in the SG compared with CG (2-week: 6.9 ± 13.0 vs. 0.2 ± 10.2, $p = 0.005$, 95% *CI*: 2.1–11.4. 6-week: 9.4 ± 18.1 vs. 0.2 ± 11.8, $p = 0.003$, 95% *CI*: 3.1–15.2).

## Motor Function Tests

The intergroup motor function tests comparisons showed no statistically significant differences at baseline (**Table 3**).

Results of 30-s STS tests improved significantly only in the SG ($p < 0.01$, 95% *CI*: 1.4–3.4). In addition, the repetitions changes in the SG were significantly better than those in CG (SG: 2.4 ± 3.6 repetitions; CG: 0.4 ± 3.5 repetitions, $p = 0.007$, 95% *CI*: 0.5–3.3). The 6 MWT performance increased significantly after 6-weeks in both the SG and CG (SG: $p < 0.01$, 95% *CI*: 32.7–54.7; CG: $p = 0.003$, 95% *CI*: 5.6–27.6), and the distance changes of 6 MWT in SG were significantly better than those in CG (SG: 43.7 ± 39.2 m, CG: 16.6 ± 39.1 m, $p = 0.001$, 95% *CI*: 11.5–42.6). After 6-weeks, the RPE scores for 6 MWT decreased significantly in the SG (RPE for respiration: $p = 0.002$, 95% *CI*: 0.2–0.9; RPE for leg fatigue: $p = 0.01$, 95% *CI*: 0.1–0.7), while in the CG only lower extremity fatigue RPE decreased significantly ($p = 0.03$, 95% *CI*: 0.04–0.64). Results for the TUG after the 6-week intervention improved significantly in both groups (SG: $p = 0.001$, 95% *CI*: 0.3–1.2; CG: $p < 0.01$, 95% *CI*: 0.4–1.3). There was no significant change in HG in either group.

## Lipid Profile

The intergroup analysis showed no statistically significant differences at baseline for lipid profile (**Table 4**). Both LDL and HDL changed significantly compared with baseline at 6-weeks in both groups, and the value changes did not differ significantly between groups.

# DISCUSSION

This randomized controlled pilot study showed that both conventional education with a home exercise program booklet

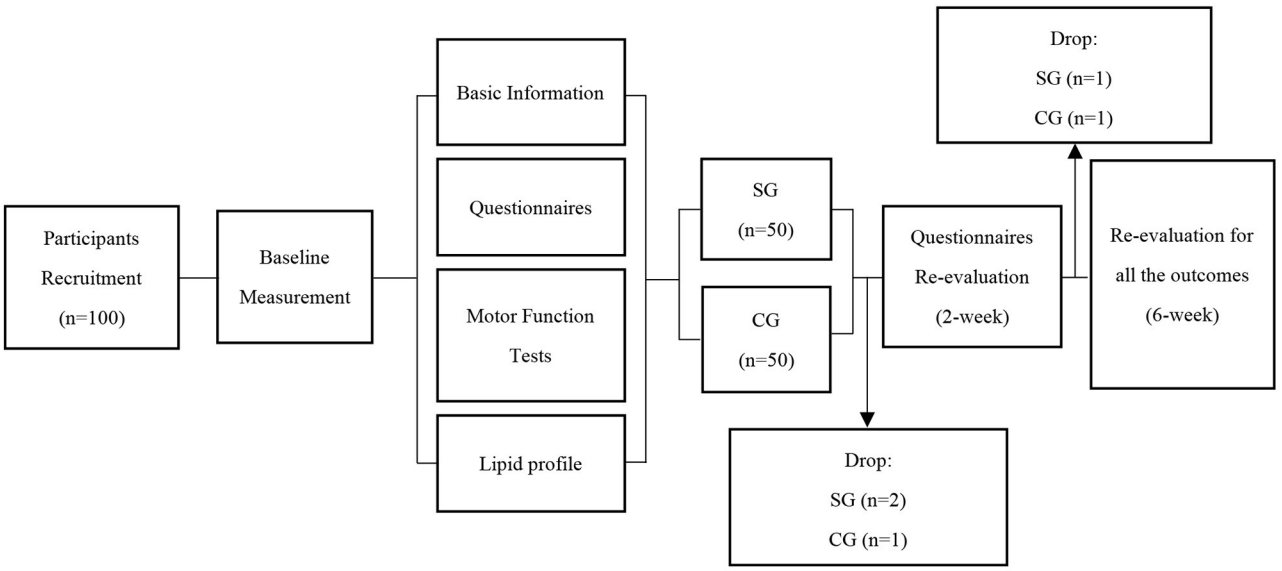


**FIGURE 1 |** Study design and experiment flowchart. SG, Supervision group; CG, Control group.

**TABLE 1 |** Demographic characteristics of the patients.

| | SG | CG | *p* |
|---|---|---|---|
| Age ($\bar{x} \pm s$, years) | 65.3 ± 8.7 | 67.7 ± 7.6 | 0.13 |
| Gender (male/female, cases) | 35:15 | 39:11 | 0.82 |
| BMI (kg/m$^2$) | 24.6 ± 3.5 | 24.6 ± 2.8 | 0.96 |
| Risk Factor (items) | 1.8 ± 1.0 | 2.0 ± 0.8 | 0.33 |
| Occupation (full time job/retirement, cases) | 12:38 | 8:42 | 0.45 |
| Diploma (lower than high school/high school/with bachelor' s degree and above, cases) | 23:16:11 | 19:19:12 | 0.71 |
| Coronary artery lesions (single/double and above, cases) | 18:32 | 20:30 | 0.68 |

*BMI, Body Mass Index; SG, Supervision group; CG, Control group.*

**TABLE 2 |** Results of questionnaires ($\bar{x} \pm s$).

| Group / Questionnaire | SG | | | CG | | |
|---|---|---|---|---|---|---|
| | Baseline | 2-week | 6-week | Baseline | 2-week | 6-week |
| ESE | 55.2 ± 15.3 | 60.8 ± 11.8 | 63.6 ± 9.5** | 55.6 ± 12.9 | 59.0 ± 14.0 | 59.4 ± 12.3 |
| GSLTPAQ | 14.3 ± 11.8 | 21.2 ± 7.4** | 23.7 ± 15.8** | 16.8 ± 9.6 | 17.0 ± 5.7☆☆ | 17.0 ± 8.8☆☆ |

***Intragroup comparing with baseline $p < 0.01$.*

*☆☆Intergroup comparison the scores change with baseline $p < 0.01$.*

*ESE, Bandura's Exercise Self-efficacy; GSLTPAQ, Godin-Shephard Leisure-Time Physical Activity questionnaires; SG, Supervision group; CG, Control group.*

delivered before hospital discharge and an additional 6-week HOSEP were safe and effective for improving motor function and lipid profile in early Phase II community CR for CAD patients after PCI. More importantly, compared to conventional intervention, the patients who received additional HOSEP showed further improvements in their exercise self-efficacy, exercise behavior and motor function, suggesting that 6-week HOSEP, as a short-term intervention, was a feasible and valid home-based CR. These findings provide evidence regarding a low-cost, more convenient, short-term, supervised home-based CR program, especially during the COVID-19 pandemic.

In this study, WeChat (a messaging and calling app for smartphones) was used as the online supervision platform. WeChat-based CR intervention for CAD has been shown to be promising in China, with advantages such as convenience, popularity and flexibility (21). More importantly, the WeChat app has been found to be client-friendly and easy to use, even

**TABLE 3 |** Results of motor function test ($\bar{x} \pm s$).

| Test \ Group | | SG | | CG | |
|---|---|---|---|---|---|
| | | Baseline | 6-week | Baseline | 6-week |
| HG (kg) | | 27.2 ± 8.4 | 28.0 ± 8.1 | 26.8 ± 8.5 | 27.1 ± 7.9 |
| TUG (s) | | 10.7 ± 3.5 | 9.9 ± 3.0** | 10.6 ± 2.0 | 9.7 ± 1.9** |
| 30-s STS (repetitions) | | 11.9 ± 3.5 | 14.3 ± 4.7** | 10.9 ± 3.4 | 11.4 ± 3.5‖ |
| 6 MWT | B-HR (bpm) | 74.9 ± 12.7 | 74.2 ± 10.5 | 76.1 ± 10.7 | 75.9 ± 11.3 |
| | B-SBP (mmHg) | 123.2 ± 15.2 | 123.6 ± 13.2 | 125.2 ± 19.0 | 127.7 ± 16.6 |
| | B-DBP (mmHg) | 71.4 ± 9.5 | 71.7 ± 8.3 | 71.5 ± 10.0 | 73.2 ± 10.7 |
| | B-b-RPE | 0.2 ± 0.6 | 0.0 ± 0.1 | 0.2 ± 0.6 | 0.1 ± 0.3 |
| | B-l-RPE | 0.1 ± 0.4 | 0.0 ± 0.1 | 0.2 ± 0.7 | 0.1 ± 0.3 |
| | WD (m) | 445.6 ± 36.6 | 489.2 ± 48.8** | 436.8 ± 43.6 | 453.4 ± 50.7☆☆ |
| | A-HR (bpm) | 81.4 ± 12.9 | 82.2 ± 11.5 | 81.0 ± 12.9 | 82.1 ± 13.3 |
| | A-SBP (mmHg) | 146.3 ± 16.1 | 147.8 ± 17.3 | 142.3 ± 21.9 | 145.8 ± 18.2 |
| | A-DBP (mmHg) | 74.4 ± 10.0 | 75.0 ± 11.2 | 71.9 ± 12.6 | 75.5 ± 10.5 |
| | A-b-RPE | 4.1 ± 1.2 | 3.5 ± 0.8** | 3.8 ± 1.2 | 3.6 ± 0.8 |
| | A-l-RPE | 3.8 ± 1.1 | 3.4 ± 0.7* | 3.7 ± 1.1 | 3.4 ± 0.7* |

**Intragroup comparison with baseline $p < 0.05$. **Intragroup comparing with baseline $p < 0.01$.*

*☆☆Intergroup comparison the value change with baseline $p < 0.01$.*

*SG, Supervision group; CG: Control group; HG, Hand Grip Strength, TUG: Timed Up and Go Test, 30-s STS: 30-second Sit to Stand; HR, Heart Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; B, before test; A, after test; b, breath; l, leg; RPE, Ratings of Perceived Exertion; WD, walking distance.*

**TABLE 4 |** Results of lipid profile ($\bar{x} \pm s$).

| Test \ Group | SG | | CG | |
|---|---|---|---|---|
| | Baseline | 6-week | Baseline | 6-week |
| LDL (mmol/L) | 2.2 ± 0.9 | 1.8 ± 0.6** | 2.3 ± 0.9 | 1.8 ± 0.7** |
| HDL (mmol/L) | 1.1 ± 0.3 | 1.2 ± 0.4** | 1.1 ± 0.2 | 1.2 ± 0.4** |
| TC (mmol/L) | 3.9 ± 1.0 | 3.7 ± 0.8 | 4.0 ± 1.0 | 4.0 ± 1.0 |
| TG (mmol/L) | 1.8 ± 1.4 | 1.5 ± 1.0 | 1.5 ± 0.8 | 1.3 ± 0.8 |

***Intragroup comparing with baseline $p < 0.01$.*

*SG, Supervision group; CG, Control group; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; TC, Total Cholesterol; TG, Triglycerides.*

for older people (8, 22, 23). With the video call function, patients could have immediate access to medical support through their WeChat group or use one-to-one chat.

The motor function performance of both groups improved significantly. Specifically, the 6 MWT and TUG improved significantly in both groups, and the SG also improved their performance on the 30-s STS tests.

The 6 MWT is an important indicator of cardiopulmonary function in patients with CAD (16). After a 6-week intervention, both groups improved their walking distance over 450 m, indicating that their cardiopulmonary fitness may be close to normal (24). Further, the distance change in the SG was more than the minimal clinically important difference (MCID) (22), suggesting that the beneficial effect from the additional HOSEP was clinically significant. In addition, the RPE of lower extremity fatigue after 6 MWT was significantly reduced in both groups, whereas the RPE for respiratory fatigue was significantly reduced only in the SG. These findings suggest that the additional HOSEP had benefits in both musculoskeletal and respiratory systems. This is consistent with previous research, where the researchers found that aerobic exercise combined with resistance exercise improved musculoskeletal and cardiopulmonary fitness of patients with CAD (23, 25).

TUG is a test that reflects the quality of dynamic balance function (17), which involves both proprioceptive input and motor output (26). In the current study, both groups showed significant improvement in TUG but lower limb strength, i.e., motor output, only improved in the SG, suggesting that the change in observed TUG performance may be also related to proprioceptive change through exercise intervention. Future study is needed to explore the effect of CR on proprioceptive function, and how it contributes to dynamic balance control after PCI.

Lower limb muscle strength, measured by using STS, is an important contributor to CAD patients' daily living, because it has been found to be strongly associated with exercise capacity and risk of all-cause and cardiovascular mortality (27, 28). Studies by Fujita (29) found that regular STS can be used as an effective

resistance exercise to improve lower extremity strength. In this study, although we prescribed for both groups to perform regular STS, only patients in the SG improved their STS performance significantly. This is likely because the HOSEP improved patients' motivation to perform the hop exercise regularly.

For the lipid profile, the blood results showed that HDL and LDL were more ideal in both groups after 6-weeks of CR. The results were comparable to other long-term HOSEP studies (30, 31), suggesting that short-term HOSEP can also benefit participants' lipid profile. Additionally, research has shown that long-term (more than 3 months intervention) HOSEP can also improve TC and TG (32). However, we did not find any significant change in TC and TG in this short-term (6 week) study. Further work is needed to determine how long the intervention needs to be to achieve effective change in TC and TG. In addition, there is a need to consider including more biochemical/physiological parameters, such as blood morphology, electrocardiogram, or respiratory fitness tests, in that this information feedback may contribute to greater patient motivation to continue physical activity.

The questionnaire results showed that the score change on GSLTPAQ in the SG was significantly higher than the CG. Scores for ESE significantly improved between baseline and 6 weeks in the SG. These findings suggest that patients in the HOSEP group may have developed a better sense of self-efficacy and exercised more regularly at home, a result which is also similar to the effects found in a long-term online exercise study (33).

## CONCLUSION

Both conventional education and the additional 6-week HOSEP were safe and beneficial with respect to motor function and lipid profile. HOSEP can further improve patients' exercise behavior and motor function over conventional treatment. Therefore, a 6-week HOSEP in early phase II CR should be considered as an effective intervention as it can be conducted at home, especially during the COVID-19 pandemic, and it provides safe access to all patients following PCI in the community, thereby reducing demand on services.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Subjects Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JL, ZW, BL, JH, and SM designed the study and had primary responsibility for this work. JL, ZW, and BL responsible for the execution of the trial and data collection. JL and ZW analyzed the data and discussed with JH and SM. JL wrote the first draft, which was improved by SM and JH. DE-A and RA critically reviewed and improved the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

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# Exploring the Causal Effects of Circulating ST2 and Galectin-3 on Heart Failure Risk: A Mendelian Randomization Study

*Xizhi Wang[1†], Xingchen Wang[1†], Jun Zhu[1], Yu Liu[2], Lenan Zhuang[1], Zhe Zhang[1], Danfeng Zhong[1], Wenbin Zhang[1*] and Dongwu Lai[1*]*

[1] Key Laboratory of Cardiovascular Intervention and Regenerative Medicine of Zhejiang Province, Department of Cardiology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Department of Cardiac Surgery, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

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Renmin Hospital of Wuhan University, China

***Correspondence:**
Dongwu Lai
laidw@zju.edu.cn
Wenbin Zhang
3313011@zju.edu.cn

[†] These authors have contributed equally to this work

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**Background:** Heart failure (HF), primarily caused by conditions such as coronary heart disease or cardiomyopathy, is a global health problem with poor prognosis and heavy burden on healthcare systems. As biomarkers of myocardial injury and fibrosis, suppression of tumorigenicity 2 (ST2) and galectin-3 were recommended for prognosis stratification in HF guidelines. However, the causality between these two mediators and HF remains obscure. This study aimed to explore the causal relationship of genetically determined ST2 and galectin-3 with the risk of HF.

**Methods:** We used the two-sample Mendelian randomization (MR) method, incorporating available genome-wide association summary statistics, to investigate the causal association of ST2 and galectin-3 with HF risk. We applied inverse-variance weighted analysis as the main method of analysis.

**Results:** In our final MR analysis, 4 single-nucleotide polymorphisms (SNPs) of ST2 and galectin-3, respectively, were identified as valid instrumental variables. Fixed-effect inverse variance weighted (IVW) analysis indicated that genetically predicted ST2 and galectin-3 were not causally associated with HF risk 3. [odds ratio (OR) = 0.9999, 95% confidence interval [CI] = 0.9994–1.0004, $p$ = 0.73; OR = 1.0002, 95% CI = 0.9994–1.0010, $p$ = 0.60, respectively]. These findings were robust in sensitivity analyses, including MR-Egger regression and leave-one-out analysis.

**Conclusion:** This MR study provided no evidence for the causal effects of ST2 and galectin-3 on HF risk.

**Keywords: ST2, galectin-3, heart failure, Mendelian randomization, causal association**

**Abbreviations:** HF, heart failure; MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IV, instrumental variable; GWAS, genome-wide association study; IL, interleukin.

## INTRODUCTION

Heart failure (HF) remains a primary public health issue with high rates of hospitalization and mortality (1, 2). HF was caused by many cardiovascular diseases, such as coronary heart disease or cardiomyopathy as a consequence of leading to adverse cardiac remodeling (3, 4). Despite ongoing advances in medications and biomedical devices, pathophysiological progress of HF put patients on a trajectory of poor prognosis (5).

Recent HF guideline recommended biomarkers, such as natriuretic peptides, ST2 and galectin-3, for diagnosis and prognosis in management of HF patients (6). As classical HF biomarkers, BNP (B-type natriuretic peptide) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) are widely used to establish the presence and severity of HF in clinical practice (6). Further, causality assessment of natriuretic peptides and HF indicated that HF therapy targeting natriuretic peptides may work through indirect mechanisms (7).

It is well known that inflammation plays a crucial role in the pathogenesis of HF. As predictive biomarkers for prognosis of HF patients, both soluble ST2 and galectin-3 are extensively involved in inflammatory mechanisms resulting in myocardial fibrosis and adverse cardiac remodeling (8). However, the causal effects of these two mediators on the risk of HF are still not fully elucidated (9).

Mendelian randomization (MR) is a statistical method used to detect and quantify causality using genetic instrumental variables (IVs) as proxies (10). It is not confounded by environmental factors, lifestyle factors, or reverse causation owing to randomly allocated genetic variants (10). The ascendancy of MR is only valid if three core assumptions, as follows, are held: (1) the genetic variant must be associated with the exposure, (2) the genetic variant must not be directly associated with the outcome, and (3) the genetic variant must not be associated with any confounding factor (10).

Thus far, the question of whether ST2 and galectin-3 are involved in the pathogenesis of HF in a causative manner remains obscure. Hence, we aimed to investigate the causality between these two novel biomarkers and HF risk by applying a two-sample MR analysis.

## MATERIALS AND METHODS

### Study Design

We selected genetic instruments for circulating ST2 and galectin-3. A two-sample MR study was designed to determine the causal association between these two biomarkers and HF risk based on the available summary-level data from the genome-wide association study (GWAS). The single nucleotide polymorphisms (SNPs) that were selected as IVs were supposed to fulfill the three aforementioned key assumptions; the schematic diagram is presented in **Figure 1**.

### Data Source

Summary statistics data of SNPs related to circulating ST2 and galectin-3 were extracted from the GWAS with a sample size of 30,931 European individuals (11). HF data were obtained by Neale lab analysis of UK Biobank phenotypes,[1] with a sample size of 361,194 Europeans (1,405 HF cases and 359,789 controls). Adequate patient consent and ethical approval were acquired in the original studies from which data for this study were obtained. A flowchart of the MR analysis performed in the present study is shown in **Figure 2**.

### Single Nucleotide Polymorphism Validation

First, we selected SNPs associated with ST2 and galectin-3 at a genome-wide significance threshold ($p < 5 \times 10^{-8}$) from the corresponding datasets to ensure a close relationship existed between the IVs and these two mediator levels. Second, we checked for the, respectively, ST2 and galectin-3-associated SNPs on PhenoScanner[2] to evaluate whether these SNPs were associated with potential confounders. Next, we excluded those SNPs with confounding traits that may influence the results. Third, given that linkage disequilibrium (LD) is a population-based parameter that measures the non-random association of alleles at different loci, we can apply measures in the MR analysis to explain any correlations of LD. Herein, we clustered the reference GWAS datasets of samples with European, and a similar ancestry is an effective way to assure the independence of all instruments. We also used LD-Link based on the European population to ensure the independence of the selected SNPs by calculating the pairwise-LD. The R software program's clumping procedure with the "TwoSampleMR" package was performed to automatically drop SNPs with linkage dependence, and for our study, we set the LD threshold at $r^2 < 0.001$ and kb $> 10{,}000$. Moreover, because of the same letters on the forward and reverse strands, palindromic SNPs were deleted to prevent unexpected biases. Subsequently, we extracted information on the genetic associations between the remaining SNPs and HF from the GWAS dataset on HF. When the specified SNP was not available in the HF dataset, a highly correlated SNP ($r^2 > 0.8$) was selected as proxy. Any SNP directly associated with HF at a genome-wide significance level was excluded. Finally, the estimated variance in ST2 and galectin-3 explained by each SNP and the corresponding F statistics were calculated to evaluate the strength of the IVs (12). To minimize potentially weak instrument bias, we considered an F-statistic of at least 10, as appropriate, for performing a two-sample MR approach.

### Mendelian Randomization Estimates

Primary MR analysis was conducted using the fixed-effect inverse variance weighted (IVW) method, which assumes the absence of invalid genetic instruments (e.g., no heterogeneity, no pleiotropy, and no outlier). Specifically, Wald ratios were estimated for each IV, and the mean IVW of these ratio estimates was calculated as the effect estimate. Finally, results were expressed as odds ratios (ORs) on HF risk for corresponding ST2 and galectin-3. When the MR assumptions were met, the ORs were used as an

[1] http://www.nealelab.is/uk-biobank

[2] http://www.phenoscanner.medschl.cam.ac.uk/

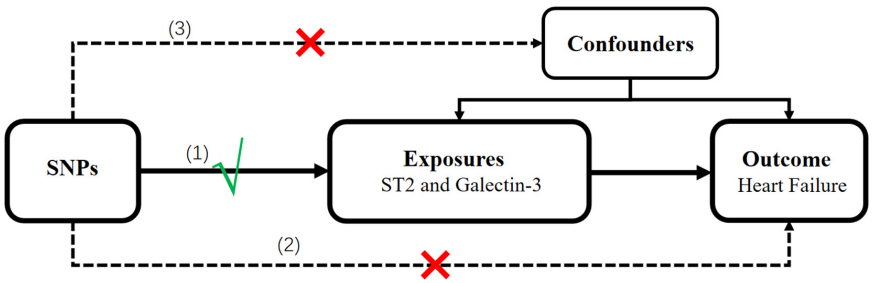


**FIGURE 1 |** Schematic representation of the MR analysis in this study. Three assumptions of MR analysis are as follows: **(1)** SNPs must be associated with the ST2 and galectin-3, **(2)** SNPs not directly be associated with heart failure, and **(3)** SNPs must not be associated with any confounding factor. MR, Mendelian randomization; SNPs, single-nucleotide polymorphisms.

estimate of the causal effect of the exposure (ST2 and galectin-3) on the outcome (HF).

## Sensitivity Analysis

To further examine the robustness of the effect estimate, the MR-Egger regression and weighted-median sensitivity analysis were performed. In MR-Egger, the intercept estimated the potential horizontal pleiotropic effects in genetic IVs; a value that deviates from the origin may indicate that the IVW estimate is biased. Besides, funnel plots were generated to visually inspect symmetry, which indicated that the causal estimates of weaker variants tended to tilt in one direction, and any deviation may imply potential pleiotropic effects. A weighted-median estimator analysis can provide a consistent valid estimate if more than one-half of the information for the analysis is derived from valid IVs. In addition, we conducted the leave-one-out analysis to assess whether one SNP affected the result. Furthermore, we used Cochran's Q test to estimate the heterogeneity among the Wald ratios estimated from different genetic variants.

All the analyses were performed in the "TwoSampleMR" package (version 0.5.5) in the software R (version 4.0.3). A $p$-value of $< 0.05$ was considered statistically significant.

# RESULTS

## Single Nucleotide Polymorphism Selection and Validation

Initially, 9 SNPs of ST2 and 7 SNPs of galectin-3, respectively, were extracted at a genome-wide significant threshold from the corresponding datasets. Then, the SNPs were scanned on PhenoScanner. Three SNPs of ST2 and galectin-3 individually were removed because of their associations with confounding traits, including high cholesterol, hypertension, coronary artery disease and so forth. The detailed information of removed SNPs is presented in **Supplementary Table 1**. Among them, additional SNPs (rs7604529, rs4311080, and rs6725806) of ST2 and SNPs (rs6650508, rs3742564, rs4363781, rs3825615 and rs62143199) of galectin-3 were dropped due to the LD ($r^2 > 0.001$, kb $< 10000$). Moreover, no SNPs were detected with palindromes and the F-statistic value of all SNPs was greater than 10. After the exclusion of these SNPs, the remaining 4 SNPs of ST2 and galectin-3, respectively, were identified as the IVs in our two-sample MR analysis. The detailed information about SNPs for these two exposures included as IVs in our analysis is shown in **Table 1**.

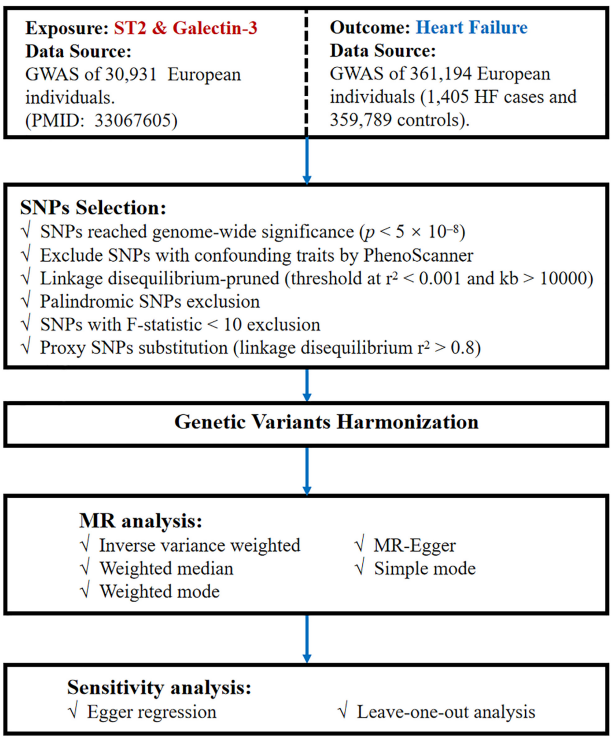


**FIGURE 2 |** Flow chart of our MR study. MR, Mendelian randomization; GWAS, genome-wide association study; SNPs, single-nucleotide polymorphisms.

## Analysis Using the Two-Sample Mendelian Randomization

As presented in **Figure 3**, the results of the MR analysis were expressed as odds ratios (ORs) of HF per standard deviation (SD) increase in circulating ST2 and galectin-3, indicating that neither

**TABLE 1** | The characteristics of SNPs and their associations with exposures and outcome.

| SNP | Nearest gene | Chr | Position | EA | OA | EAF | F | SNP-exposures association | | | SNP-HF association | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | SE | *p*-value | Beta | SE | *p*-value |
| **ST2** | | | | | | | | | | | | | |
| rs11603123 | KIRREL3 | 11 | 126,305,495 | A | G | 0.032 | 266 | 0.3712 | 0.032 | 2.61E-47 | 5.05E-05 | 0.000399938 | 0.89949 |
| rs13020553 | IL1RL1 | 2 | 102,931,826 | C | G | 0.63 | 7235 | 0.6377 | 0.0088 | 6.89E-1635 | −8.08E-05 | 0.000147981 | 0.585092 |
| rs2460382 | MGAT5 | 2 | 135,014,116 | A | G | 0.77 | 56 | −0.0714 | 0.011 | 1.31E-14 | 0.00027079 | 0.000172428 | 0.116312 |
| rs672806 | DCPS | 11 | 126,188,405 | A | G | 0.4 | 149 | 0.0998 | 0.0098 | 1.43E-34 | 0.000210482 | 0.000147057 | 0.152346 |
| **Galectin-3** | | | | | | | | | | | | | |
| rs3735080 | GIMAP7 | 7 | 150,217,309 | T | C | 0.24 | 86 | 0.087 | 0.011 | 8.41E-21 | −3.48E-05 | 0.00017118 | 0.839049 |
| rs62143206 | NLRP12 | 19 | 54,326,212 | T | G | 0.21 | 74 | 0.0849 | 0.011 | 1.19E-19 | −0.000162714 | 0.000176406 | 0.35633 |
| rs76480089 | ATG14 | 14 | 55,832,612 | A | G | 0.92 | 1885 | 0.6247 | 0.015 | 1.28E-512 | 0.000229768 | 0.000259548 | 0.376016 |
| rs812936 | FUT3 | 19 | 5,844,649 | A | G | 0.8 | 85 | 0.0925 | 0.012 | 2.59E-20 | −9.77E-05 | 0.000188528 | 0.604367 |

*SNPs, single-nucleotide polymorphisms; HF, heart failure; Chr, chromosome; EA, effect allele; OA, other allele; EAF, effect allele frequency; SE, standard error.*

genetically predicted ST2 nor galectin-3 were causally associated with the risk of HF ($p > 0.05$). Remarkably, the fixed-IVW method and other statistical methods of MR analysis showed similar results (**Figure 4** and **Supplementary Tables 2**, **3**). Scatter plots of the potential effects of both ST2 and galectin-3-associated SNPs on HF are shown in **Supplementary Figure 1**.

## Sensitivity Analysis

Results of heterogeneity and directional horizontal pleiotropy bias are shown in **Supplementary Tables 4**, **5**, respectively. There was no heterogeneity in ST2 and galectin-3 (All $p > 0.05$) (**Supplementary Table 4**). The MR-Egger regression and the appearance of the funnel plots showed that there was a low likelihood of horizontal pleiotropy for our estimations (Both $p$ for MR-Egger intercept $> 0.05$) (**Supplementary Table 5** and **Supplementary Figure 2**). No outlier was detected in the leave-one-out analysis, and the results illustrated that the overall estimate was not driven by any SNPs (**Figure 5**). Additionally, we performed MR analysis of all SNPs that included SNPs dropped due to LD or potential pleiotropy. The results were also negative, which showed no significant causal association of circulating ST2 and galectin-3 on HF (**Supplementary Table 6**).

# DISCUSSION

In this study, we examined the causal effects of circulating ST2 and galectin-3 on HF risk using MR analysis. Collectively, we did not find evidence that genetically determined ST2 and galectin-3 are causally associated with the risk of HF. To the best of our knowledge, this is the first study concerning the issue by MR analysis.

It is well known that inflammation plays a critical role in the pathophysiological processes of HF (3, 13). As inflammatory mediators involved in myocardial injury and fibrosis, soluble ST2 and galectin-3 were recommended for additive risk stratification of HF patients in the latest guideline (class II b, evidence level B) (6).

ST2 is a member of the interleukin (IL)-1 receptor family. Two isoforms of ST2, transmembrane receptor (ST2L) and soluble decoy receptor (sST2), are both bound to IL-33 (14). IL-33 could be activated by mechanical stress or inflammatory signals and released from cells (15). It acts as an "alarmin" on neighboring or immune cells expressing the ST2 receptor (16). Once released, IL-33 would activate the ST2L on nearby cells. sST2 could be secreted into the circulation by endothelial and various immune cells upon stimulation like IL-33 (17). IL-33 was thought to exert beneficial actions via ST2L that are related to cardiac repair or attenuation of adverse cardiac remodeling (15). Acting as a soluble truncated form of ST2L, sST2 functions as a decoy receptor for IL-33 and thereby attenuates beneficial effects of IL-33/ST2L signaling in the context of the heart (18). Previous clinical and experimental studies showed a strong correlation between ST2 and HF (19). Clinical evidence suggested that elevation of baseline sST2 was significantly associated with worsening HF (20, 21). In pressure-overload murine model, deletion of ST2 enhanced myocardial fibrosis

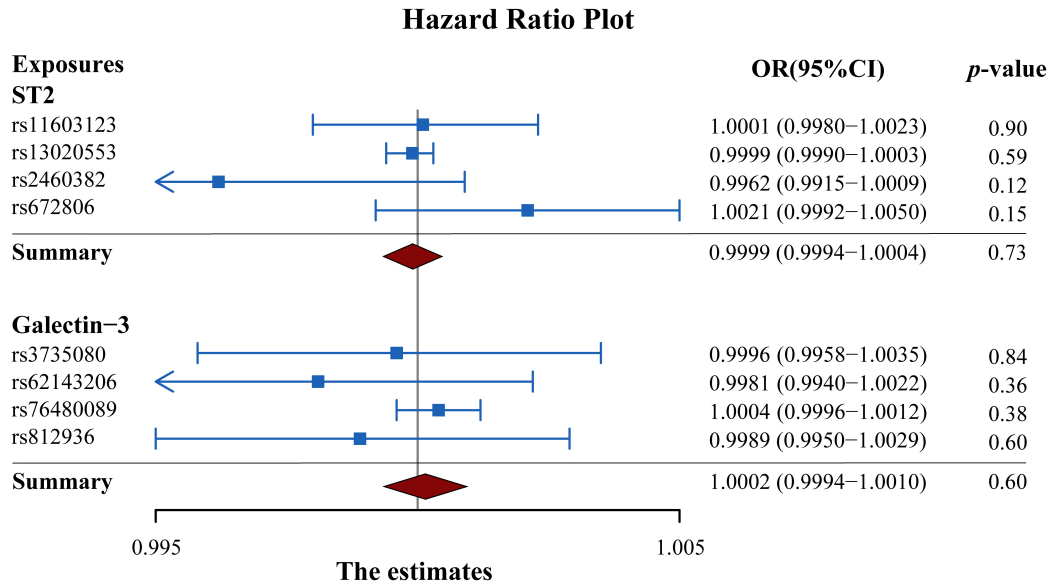


**FIGURE 3 |** Forest plot summarizing the overall MR estimates of SNP specificity and the causal effect on heart failure. Estimated odds ratios represent the effect per standard deviation increase in circulating ST2 and galectin-3 levels on heart failure, respectively. Results were obtained from the fix-effect IVW method. Squares represent the estimated ORs, and horizontal lines represent the 95% CI of the ORs. MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IVW, inverse variance weighted; OR, odds ratio; CI, confidence interval.

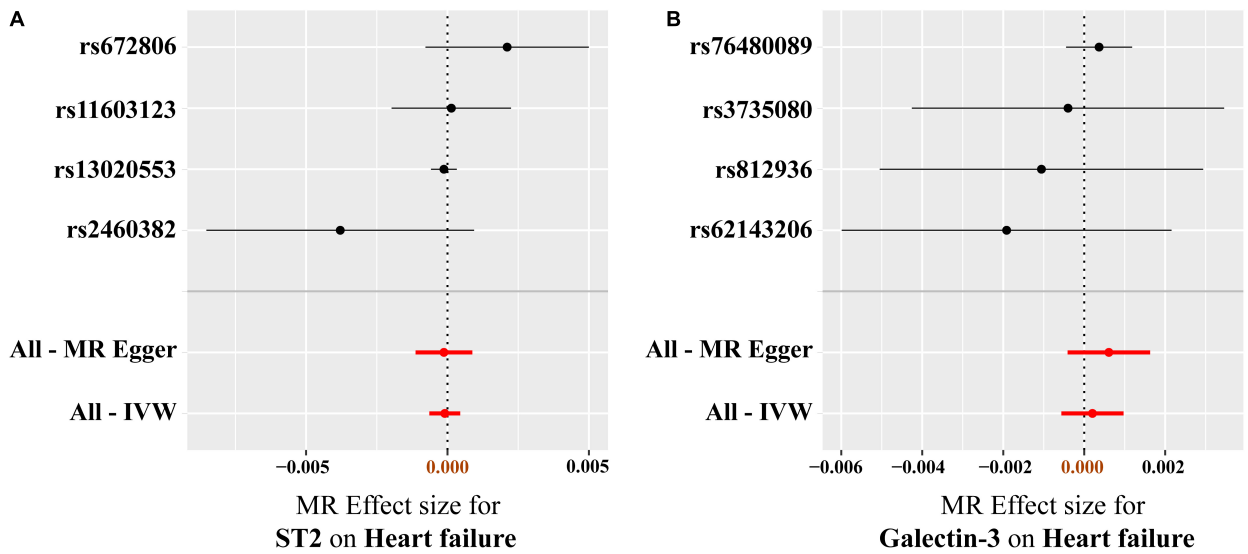


**FIGURE 4 |** MR-Egger and fixed-effect IVW analysis of the causal association between circulating ST2 and galectin-3 levels and heart failure risk. **(A)** ST2, **(B)** galectin-3. Forest plots reflect the causal effect of individual SNPs on heart failure. Black dots represent the estimated betas, and horizontal lines represent the 95% CI of the betas. IVW, inverse variance weighted; SNPs, single-nucleotide polymorphisms; CI, confidence interval; MR, Mendelian randomization.

and cardiac hypertrophy, suggesting a potential role for IL-33/ST2 signaling axis in cardiac remodeling under mechanical overload (22).

As a member of the β-galactoside-binding protein family, galectin-3 stimulates myofibroblasts proliferation and procollagen deposition, which lead to cardiac fibrosis and hypertrophy (23). In past years, a growing body of literature emerged on galectin-3 in HF patients. However, the causal relationship between galectin-3 and HF risk remains obscure. In clinical studies, such as Framingham Offspring Cohort study and Val-HeFT study, higher level of serum galectin-3 was associated with increased risk for incidence, hospitalization and all-cause mortality of HF (24, 25). In rat HF model, elevated galectin-3 level marked macrophages activation and contributed to cardiac dysfunction (26). Besides, attenuated cardiac fibrosis, left-ventricular

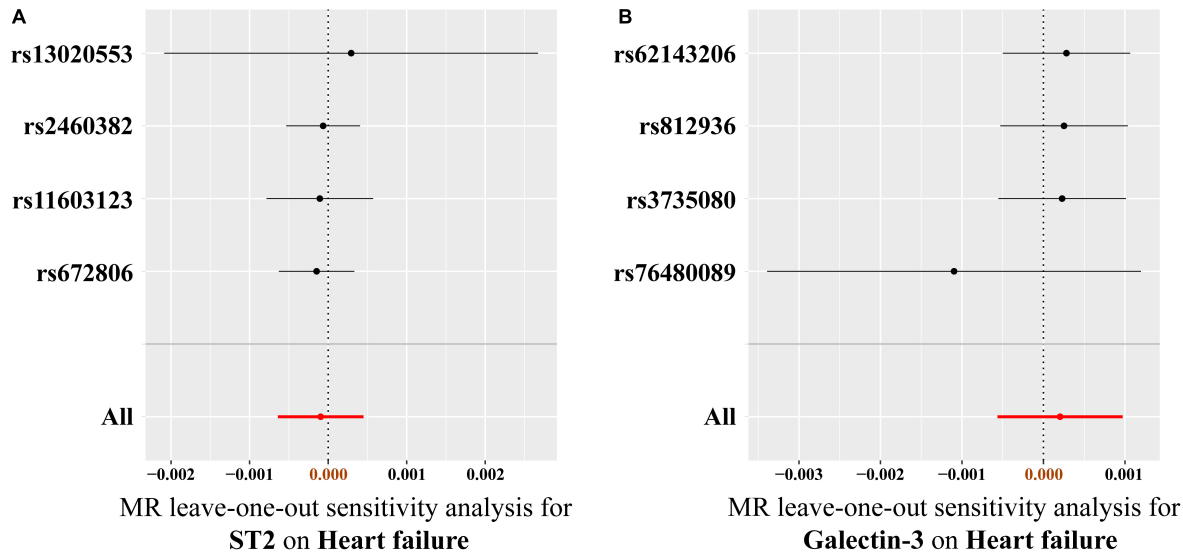


**FIGURE 5 |** Leave-one-out analysis of the causal association between circulating ST2 and galectin-3 levels and heart failure risk. **(A)** ST2, **(B)** galectin-3. Each SNP was excluded sequentially and the MR estimate effect was recalculated. Visually, the leave-one-out analysis plot illustrated that the results were not driven by any SNPs. SNP, single-nucleotide polymorphism; MR, Mendelian randomization.

dysfunction were also observed in genetic galectin-3 knockout mouse (27).

Nevertheless, some other studies showed different results. In RELAX-AHF study, enrolled 1,161 acute HF patients, galectin-3 levels remained stable over time and presented no independent relationship with cardiovascular mortality at 180 days (28). Likewise, no relationship was found between galectin-3 values and mortality events or heart transplantation in a study of chronic HF patients (29). In the mice myocardial infarction model, genetic deletion of galectin-3 altered the temporal evolution of macrophage infiltration and wound healing, which affected cardiac remodeling and function (30).

Considering to the aspect of HF risk in general population, our findings are not contradictory with previous studies. To some extent, circulating ST2 and galectin-3 are not cardiac-specific biomarkers (9). Concentration variation of these two mediators in serum is not parallel with that in myocardium. Furthermore, observational results are usually subjected to confounding factors (31). In the present study, from a genetic perspective, we found no causal effect of circulating ST2 and galectin-3 on HF risk, indicating that increased ST2 and galectin-3 in HF patients may be an epiphenomenon, rather than major driving factors in the pathogenesis of HF.

Randomized controlled trial is a powerful approach for proving hypothesis in epidemiological research. However, it requires rigorous design, strictly enroll/exclude standards and expensive cost. By using genetic variants as instruments of exposure, MR analysis mimics the randomization in clinical trials. MR analysis could overcome the potential impact of confounding factors and minimize the bias in causal effect estimates as much as possible (32). Additionally, sensitivity analysis appeared that the causal estimates were robust in our study.

There are some limitations in our study. First, the utilized datasets were obtained from individuals of European ancestry and were not stratified by the severity of HF. Further work is warranted to investigate whether similar findings are displayed in different ethnicities, subtypes and severity of HF patients. Second, because of a lack of data on exposure and outcomes, bidirectional MR analysis was not performed to study the reverse causation of HF on circulating ST2 and galectin-3. Third, we did not explore the causality between IL-33 and HF due to the lack of available data from the GWAS. Therefore, these questions deserve further study. Fourth, given the lifetime effect of SNPs, our research assumes that these mediators act on HF for a long time, which differs from the situation of clinical practice. Moreover, we appraised the causal association without consideration of environmental factors. Nevertheless, this study delivered some valuable clues into the pathophysiologic progress of HF, and we hope that our findings would promote the therapeutic strategy exploration of HF.

## CONCLUSION

This study provided no evidence for causative effects of circulating ST2 and galectin-3 on the risk of HF incidence, indicating that these two mediators may not be major driving factors in the pathogenesis of HF. However, further studies are needed to validate our findings.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

XZW, JZ, YL, and ZZ collected and interpreted the data. XZW and XCW analyzed the data. XZW, XCW, LZ, and DZ generated the figures and wrote the manuscript. DL and WZ designed the study and revised the manuscript. All authors approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.868749/full#supplementary-material

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REVIEW
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# Historical Context of Cardiac Rehabilitation: Learning From the Past to Move to the Future

*Julie Redfern[1]*, Robyn Gallagher[1], Adrienne O'Neil[2], Sherry L. Grace[3,4], Adrian Bauman[5], Garry Jennings[6], David Brieger[7] and Tom Briffa[8]*

*[1] Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia, [2] School of Medicine, IMPACT Institute, Deakin University, Geelong, VIC, Australia, [3] Faculty of Health, York University, Toronto, ON, Canada, [4] KITE Toronto Rehabilitation Institute and Peter Munk Cardiac Centre, University Health Network, University of Toronto, Toronto, ON, Canada, [5] School of Public Health, University of Sydney, Sydney, NSW, Australia, [6] National Heart Foundation of Australia, Melbourne, VIC, Australia, [7] Department of Cardiology, Concord Hospital, Sydney, NSW, Australia, [8] School of Population and Global Health, University of Western Australia, Perth, WA, Australia*

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**Edited by:**
*Yan Zhang,*
*Peking University, China*

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*Scientific Clinical Institute Maugeri (ICS Maugeri), Italy*

***Correspondence:**
*Julie Redfern*
*julie.redfern@sydney.edu.au*

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Contemporary myocardial infarction (MI) care and management has evolved dramatically since the 1950's; yet outpatient rehabilitation remains underutilized. Deepening our understanding of the origins and history of cardiac rehabilitation highlights a contemporary shift required for policy and practice related to secondary prevention of coronary disease in light of societal changes as well as medical, digital and surgical advancements. Contemporary "cardiac rehabilitation" began when bed rest and physical inactivity was recommended and commonplace for MI survivors. Today, most patients who survive an MI, undergo reperfusion therapy, a short inpatient stay and are discharged with minimal physical morbidity. Despite this, the majority of modern day programs continue to be structured in the same way they have been for the past 50 years and this model has become incongruent with the contemporary context, especially in the COVID-19 era. This review aims to describe the historical foundations of cardiac rehabilitation to inform solutions and meet the demands of contemporary MI management. Delivering health systems reform to address modernization is current healthcare challenge where a united and interdisciplinary effort is needed.

**Keywords: cardiac rehabilitation, secondary prevention, digital health, data, heart**

## INTRODUCTION

Cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke, is the leading cause of death and disease burden globally (1). It is estimated that 32% of deaths internationally in 2019 were due to CVD (approximately 17.9 million deaths) (1). Based on analysis of epidemiological data from the Global Burden of Disease dataset, ischemic heart disease (IHD) affects around 126 million individuals globally, which is approximately 1.72% of the world's population (2). In 2017, IHD was identified as the leading international cause of death (estimated 9 million) (2). People with a previous diagnosis of CVD are at the greatest risk of repeat events and data suggests that around one quarter will have another CVD event requiring admission to

hospital within in the first year of an acute coronary event (3, 4). The good news is, over the past 75 years, there have been major developments in management in terms of how a diagnosis is made, how coronary arteries are revascularized and what medications are available to patients. These advancements have resulted in more patients surviving initial events, reduced length of stay in hospital which in turn means there are escalating numbers of people requiring ongoing and lifelong cardiovascular risk management (5). As such, international groups and organizations have identified improved secondary prevention as an international priority (6, 7).

Understanding the historical context can inform our understanding of cardiac rehabilitation and its potential in the future. This includes deepening understanding of why current programs are formatted as they are and how this has failed to adapt with changed needs of societies where there has been major changes in culture, language and diversity coupled with a rapid expansion in availability of technology along with major changes in medical and surgical management of CVD in recent years. The aim of this review is to summarize the historical context of cardiac rehabilitation in order to highlight areas for modernization and reform. That is to put in context the timing of changes in acute care and the lack of change in cardiac rehabilitation during the same time period highlighting the subsequent gaps in health services and systems at the present time. The overall timeline is summarized in **Figure 1**.

## HISTORICAL APPROACH TO MYOCARDIAL INFARCTION: IDENTIFICATION TO 1950s

The early descriptions of myocardial infarction (MI) and its associated treatment evolved dramatically around the mid-twentieth century. The coronary circulation was first recognized in the early 17th century and angina pectoris first described in the 18th century, but it was not until the late 19th century that further research identified the link between coronary artery occlusion and MI (8). In a landmark paper published in 1912, James Herrick was the first to claim that MI was not necessarily fatal and documented the importance of total rest as treatment (9). This early work led to a new treatment paradigm for patients who experienced MI in the first half of the 18th century that recommended "*absolute rest in bed for not less than a month is imperative to allow healing of the infarct and to reduce the risk of embolism. . ..convalescence will therefore be prolonged and the return to ordinary life postponed as long as possible*" (10). However, while a 1938 paper described the importance of bed rest for congestive heart failure it also acknowledged that understanding of the amount of bed rest required for patients was unclear (11). However, up until the mid 20th century, after MI, patients who survived were required to stay confined to bed for over 6 weeks including being prohibited from walking to the bathroom independently (12). Once discharged from hospital, severely limited physical activity was prescribed, with functional tasks (including walking up stairs) being forbidden for 12 months in some cases (13). Therefore, for the whole first half of the 20th century, management of MI focused almost exclusively on complete *physical inactivity*.

## QUESTIONING BED-REST POST-MI: 1950s AND 1960s

By the mid 20th century, MI had become widely understood as a common cause of death and a significant health concern (14). However, bed-rest recommendations were also known to be associated with the sequelae of immobility including muscle atrophy, functional deconditioning and fear of activity coupled with increased risk of deep venous thrombosis and pulmonary embolism (15). In the early 1950s, two American physicians (Levine and Lown) began to probe the need for prescribed bed-rest and prolonged inactivity after MI and explored the possibility of what became know as "armchair" treatment (16). This treatment allowed patients to sit in a chair for 1–2 h a day (16). The treatment was pervasive and unconventional but Levine argued it also improved "mental state"(17). Following these developments, supportive evidence progressively emerged and eventually led to a changes in post-MI management where patients were allowed to progressively increase walking and function (12). By the 1960s, several studies had reported that light activity after MI was safe and indeed beneficial in terms of preventing the negative effects of extended immobility (12). This aligned with emerging research in other areas of health where researchers were beginning to report that regular and supervised exercise programs (two times a day for 3 months) could help overcome the deleterious effects of immobility and associated deconditioning (18).

## THE BIRTH OF CARDIAC REHABILITATION, ALONGSIDE ACUTE CARDIAC TREATMENTS: 1960s/1970s

By the 1970s, a model of structured "rehabilitation" for patients with CHD was progressively introduced around the world. A new area of research and clinical practice had emerged with numerous groups commencing research investigating potential benefits and safety of the group-based approach (13). Availability of medicines and the use of oxygen during exercise also evolved (19). One controversial study at the time, published in 1968, found that MI survivors benefited from participating in an exercise program both physically and physiologically without increasing risk of death or further events (20). The benefits of supervised exercise programs provided a new approach to post-discharge care and eventually evolved into what we know today as outpatient or traditional "cardiac rehabilitation." This concept of "rehabilitation" was a logical progression, where patients who survived a MI, required a period of supervised exercise to overcome the deconditioning associated with previously recommended treatment.

By the mid-1970s, cardiac rehabilitation programs had emerged in approximately 25 countries (21). These programs started primarily as an inpatient model but eventually progressed

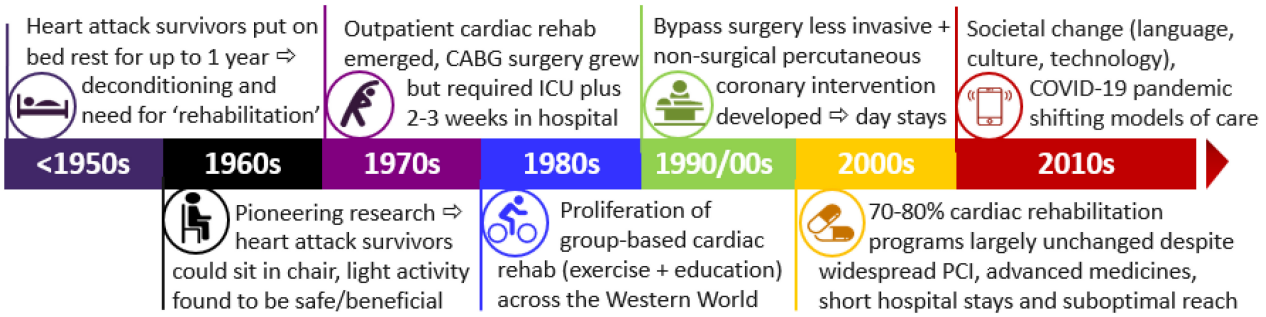


**FIGURE 1 |** Timeline for cardiac rehabilitation in the context of acute care and transformation and societal change. PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; ICU, intensive care unit.

**BOX 1 |** Quotes from an interview about the emergence of cardiac rehabilitation in the 1970s with Sister Doreen Hennesy who was "Sister-in-Charge" of a Coronary Care Unit in Sydney, Australia (26).

"In 1972 when I became the Sister-in-Charge of the Coronary Care Unit at Parramatta, cardiac monitoring and this type of thing was very new and exciting and we so conquered the cardiac arrest situation. . .. So a lot more patients were living that would have died. However, they weren't living. They were wrapped in cotton wool, they were scared, they were terrified. So I started an education program of the patient while they were in hospital, explaining what the heart attack was to them in lay terms."

"I started early ambulation in 1975 where we were getting patients out of bed within 2 days of their admission. This was unheard of."

"In 1978 we exercised the first patient, ten days after an 'infa' (infarction). It was exciting. It was everything that I ever wished to do. It was also very frightening. Although, I had seen it all working in Canada and knew it was safe, the first patient was exercised in front of doctors from the Heart Foundation, the medical directors and physicians from Parramatta Hospital and I was just there with one bike and a little machine."

"Within two months, I had about forty patients and I was just one staff. Then it grew and then I got more staff, more patients. At some stage we had 65 patients a day, just in a session in the evening where we used run the cardiac gymnasium. It was also really a lot of fun; the nursing staff did it in their own time and we used the hospital's equipment. Sometimes, we had up to 80 patients in an evening, just coming in skipping rope, bench stepping using some of the equipment, calisthenics; all this was done by these cardiac patients."

to outpatient programs that involved supervised physical activity sessions requiring a low-level of oxygen demand (13). As identified by Buckley, the standard approach to "rehabilitation" at this time focused on exercise with only a few some programs addressing psycho-social care (22). In Canada, early research found that men with CHD could safely participate in supervised exercise programs after MI with a small group training for and completing the Boston Marathon (23, 24). Recommendations at the time were focused on exercise and included stating that "the physician is responsible for both the safety and effectiveness of the exercise prescription" and that "all exercise should be supervised. . .with sessions once to twice per week for one year"(25). **Box 1** highlights the feeling of Australian health professionals about the emergence of cardiac rehabilitation in the 1970s (26). At the same time a survey in Britain indicated that there were no specific cardiac rehabilitation facilities although 8% (nine hospitals) of respondents reported there was some form of exercise program but on further investigation this seemed focused on early mobilization and/or physiotherapy and exercise regimens during hospital stay (27). Overall, although 74% of respondent cardiologists were in favor of a service there was a strong focus on the need for "*individual instruction by the physician*" and the focus was on exercise although several noted the importance of "*psychotherapy*" and "*individual advice*" although funding was identified as a barrier (27).

Also in the 1970s, the Framingham Heart Study had identified risk factors for CVD and their role in prevention and management was becoming widely acknowledged (28). The Framingham study had followed a large cohort of participants over a long period and eventually identified a variety of modifiable risk factors for CVD (28). The identified risk factors included high blood pressure, high blood cholesterol, tobacco use, obesity, diabetes, and physical inactivity, psychosocial issues along with non-modifiable factors including age, gender and genetic disposition (29). Theses factors subsequently became an integral part of primary and secondary prevention of CVD (29). This focus also increased emphasis on the importance of physical activity and exercise in addressing multiple risk factors and hence the evolving rehabilitation programs initially were mostly exclusively exercise-only but over time they progressively included multidisciplinary education and psychosocial support for patients.

At this time, advances and developments were also made in terms of medications with the potential benefits of thrombolytic agents, statins and antiplatelet medicines amongst others (29, 30). Major developments were also underway in the area of coronary artery bypass surgery (CABG). CABG was clearly a breakthrough in the care of patients with coronary disease, but it was and remains an invasive surgical procedure that requires cardiopulmonary bypass during surgery, along with sternotomy, mechanical ventilation and an intensive care stay (31). These requirements of course prolonged post-operative recovery as well as advice regarding return to physical activity and function (31). Early CABG required lengthy stays in intensive care units and hospital stays of several weeks and an ongoing need for inpatient ambulation and outpatient prescriptive exercise for recovery

(32). Further medical advancements saw coronary angioplasty first used in humans in Switzerland in 1977, which progressed to current routine use of percutaneous coronary intervention (PCI) that was minimally invasive with only a brief hospital stay and rapid return to normal activity and work (33). As such, "rehabilitation" needs post-PCI were (and remain) vastly different to the needs of patients who underwent CABG.

## PROLIFERATION OF GROUP-BASED CARDIAC REHABILITATION: 1980s AND 1990s

By the late 20th century, group-based, outpatient cardiac rehabilitation had become commonplace in many developed countries (21). By 1980, cardiac rehabilitation was reported to be running in an estimated 30 countries and by 2000 this had increased to almost 60 countries covering all continents (21). In 1993, a World Health Organization Expert Committee on rehabilitation after CVD identified that "rehabilitation is considered to be an essential part of the care that should be available to all cardiac patients. . .to improve functional capacity, alleviate or lesson activity-related symptoms, reduce unwarranted invalidism, an enable the cardiac patient to return to a useful and personally satisfying role in society"(34).

Cardiac rehabilitation was generally accepted as being made up of sequential phases: Phase 1 focused on inpatient mobilization and introductory information; Phase 2 was an outpatient hospital-based program that was run in groups attending for approximately 6–12 weeks; and Phase 3 was known as a maintenance phase of 4–6 months duration when patients continued their exercise and risk factor modification routine while returning to their regular life and work (35). Each of these phases also included multidisciplinary education component that provided information about risk factors such as smoking cessation, healthy diet, medication adherence and psychosocial support (36). Programs varied slightly in terms of session frequency per week and duration, likely based on funding availability, given rehabilitation has not been funded in the same direct manner as acute cardiac care (21). In the United States, and many other countries, private health insurance funding systems have facilitated this model where most companies provide coverage for a program of several sessions per week for 8–12 weeks (rather than life-long prevention) (21).

Numerous systematic reviews have since found these exercise-based cardiac rehabilitation is beneficial for those who attend (37–39). These benefits for people with CHD include reduced risk of MI, a modest reduction in all-cause mortality, and a considerable reduction in all-cause hospital admissions along with associated healthcare costs and improved quality of life up to 12 months (37–39). However, despite international (40–42) guidelines now universally recommending cardiac rehabilitation and secondary prevention, rates of referral, access to programs and adherence to recommendations remained problematic (43, 44). Use of evidence-based medications and lifestyle change typically started to regress within the first 6 months and was rarely sustained (45, 46). Research has since consistently found only 30–50% of those eligible are referred, around 10% of those eligible actually attend structured programs and less than 5% of those initially eligible complete a full program of traditional cardiac rehabilitation (43, 44, 47). Reasons for these suboptimal attendance and completion figures are widely reported and include issues with transport, lack of flexibility and lack of perceived need balanced with work and social commitments of patients (47, 48). Further, certain groups are less likely to attend including women, those from culturally/linguistically diverse or low socioeconomic backgrounds (49). Further, if one takes a health systems view, the financial burden and practical requirements of providing a traditional program to all who are potentially eligible remains a formidable challenge (6).

## CHANGING LIFESTYLE, MEDICAL, AND SURGICAL MANAGEMENT: 2000s

In more recent years, lifestyle factors including cigarette smoking, poor diet, inactivity and sedentary behavior have become widely accepted as contributing to increased likelihood of events (50, 51). Emergence of technology and fast food availability have influenced societal behaviors, resulting in increasing sedentary behavior and poor diet (52). Further, the importance of psychosocial factors gained attention, with many programs expanding to include psychological support and stress management (21).

During the 2000s, cardiac rehabilitation programs tended to continue as they had been in the decades prior. Importantly, a global study published in 2019 sought to gather data about all phase 2 cardiac rehabilitation programs offered worldwide (21). Data was collected by an online survey shared by local leaders and stakeholder organizations. Results found that the majority (83%) offered exercise training, but few programs reported offering an alternative model: 12% of programs offered a home-based service, and/or 10% offered a community-based (21). Sessions included a mean of 9 patients (i.e., most being group-based with a mean of 5 patients per staff member). Further, the majority (83%) offered exercise training but only 26% of programs reported offering an alternative model (21). These findings are similar to those of a 2009 Australian policy statement that found 72% of programs follow the traditional cardiac rehabilitation model based on approximately 2 months of supervised group exercise and education (53). This lack of flexibility has been identified as a barrier to participation in cardiac rehabilitation (54). Research exploring barriers and enablers to participation and completion was expanding at this time, with increasing recognition that suboptimal proportions of eligible patients were attending (48). Achieving health systems reform to address this growing gap remains a key challenge for the healthcare community.

## THE SOCIETAL TRANSFORMATION AND THE DIGITAL ERA: 2010s

Globalization has resulted in economic development raising many countries out of poverty as well as enormous

interdependence of the world's cultures and populations (55). Of course this includes major impacts on health at individual, population and systems levels. For CVD and cardiac rehabilitation, the associated challenges include a greater need to manage equity and diversity both within and between countries. For example, individuals who do not speak the language of the country in which they live, those who live in rural and remote geographical areas those with socioeconomic disadvantage and women remain under-represented in cardiac rehabilitation. Between countries we also see enormous disparity; up to 90% of the worldwide CVD burden is carried by low- and middle-income countries (LMICs), while these countries often have very large populations coupled with a lack of resources (56). Ultimately, it is not feasible to offer traditional, group-based and in-person cardiac rehabilitation at scale to all people who are eligible. As such, inequity and poor reach of cardiac rehabilitation has become a major challenge for clinicians and policy-makers.

The so-called Digital Era in the 21st century has elicited enormous transformation in the way people communicate, behave and interact on all levels. As of January 2021, it is estimated that around 60% of the world's population have internet access and 80% own a smartphone (57, 58). This has subsequently transformed health management with increased use of electronic devices to support health, now often referred to as digital health or eHealth. This technology affords new strategies for communication with patients. Examples in the literature where technology has supported patients with CVD include telephone coaching (59, 60), text message programs (61, 62), interactive online programs (63), smartphone apps (64), and the use of sensors and personal trackers to automatically monitor behavior (65). Digital health strategies also include electronic prescribing of medications, remote monitoring *via* Bluetooth devices and use of artificial intelligence linked to implantable devices to enable remote feedback and support in real-time (65). Such strategies can support tobacco cessation, diet, physical activity, mental health etc. However, despite promising developments from a technological perspective, there remains a lack of scientific evidence for effectiveness of some approaches, thus this is an increasingly active area of research.

In the early 2020s, the global acute respiratory syndrome (COVID-19) pandemic had a major impact on cardiac rehabilitation delivery around the world. Human transmission of infection with the novel coronavirus was first detected in late 2019 and rapidly spread. At the time of writing, there have been approximately 5.2 million deaths globally from the pandemic (66). COVID-19 has been responsible for enormous pressure on healthcare services and systems (67). In an effort to curb spread of the virus, hundreds of countries have enforced full or partial lockdown of their citizens which has of course impacted the lives and wellbeing of billions of people across the world (67). For patients with established CHD, the pandemic has resulted in enormous changes in access to the health care system such as reduced in-person medical appointments and closed cardiac rehabilitation services (68, 69). For cardiac rehabilitation, survey data suggest that approximately 4,400 programs (estimated 75% of programs around the world) ceased or were temporarily stopped due to COVID-19 (70). This has necessitated a dramatic shift from in-person models to home-based programs necessitating more widespread implementation of virtual and digital models of care (70). Many are now speculating about the future of cardiac rehabilitation and what format it should take, with calls from the International Council of Cardiovascular Prevention and Rehabilitation for ongoing availability of unsupervised delivery formats with associated reimbursement advocacy.

## LEARNINGS FROM HISTORY TO INFORM CONTEMPORARY RECOMMENDATIONS

Since cardiac rehabilitation programs emerged, there have been enormous changes to both the medical and surgical care of patients with CVD coupled with transformation of societies and technology. Very few patients now need a period of "rehabilitation," but rather life-long multifaceted prevention is needed to reduce the CVD burden. At the same time, even when offered, only the minority of eligible patients attend (traditional) cardiac rehabilitation programs and to meet current and projected expanding need within financial limits, contemporary models of cardiac rehabilitation are being modified so as to better align with other treatments, changing societies and technological advancements. Below, we suggest key recommendations that emerge with consideration of the history of cardiac rehabilitation as preventive cardiology moves forward into the new millennium:

1. Implementation of lifelong preventive strategies, rather than time-limited programs, would optimize continuous management and care for patients.
2. Building flexibility into cardiac rehabilitation delivery models to improve program reach and equity through for example, home-based programs, cultural and language tailoring, ensuring inclusivity with regard to diversity, cognitive impairment, geographical access etc. (54).
3. Systematic incorporation of cardiac rehabilitation into hospital performance measures, with digital integration such as automatic referral and standardized benchmarking (71).
4. Ensuring programs are focused on comprehensive risk factor management (not only exercise-based) based on individual patient need to optimize personalization of care across all relevant risk factors including psychosocial issues to optimize potential benefit of preventing new events (72).
5. Scientific evaluation of evidence for and implementation (where effective) of digital health interventions to support secondary prevention. Such strategies include communication *via* the telephone and internet which are now widely available, as well as use of mobile applications (apps), tracking sensors, text messaging and so on. These strategies have been evolving but have accelerated in availability as a result of the COVID-19 pandemic. However, robust trial/registry research is needed to continue to ensure effectiveness and usefulness for patients.

6. Focus on implementation of approaches that are tailored to the needs of LMICs where the CHD burden is greatest to improve access to and engagement with effective secondary prevention. Widespread availability of mobile technology offers a promising pathway to achieving this implementation although evidence-based strategies are needed (73).
7. Universal definition and classification of preventive "rehabilitation"; including cardiology, nursing, allied health, primary care, consumers, policy-makers is needed to demonstrate leadership and champion access and implementation of evidence-based care.
8. Advocacy for suitable reimbursement and funding of flexible models of cardiac rehabilitation.
9. Although controversial and potentially challenging, organizations and leading stakeholder groups could consider revisiting the term "rehabilitation" and revising to a more inclusive term such as "secondary prevention" or "preventive cardiology." While this particular term was relevant in the 1970s, it may not be reflective of the full potential of secondary prevention programs in the 21st century.

## CONCLUSION

During the last 75 years there has been a reversal of inpatient and post-discharge care and treatment guidelines for patients with CHD. This historical overview highlights how modern-day cardiac rehabilitation was born over 50 years ago at a time when *bed rest and physical inactivity were commonplace*. Despite undergoing some reform, this traditional model is still followed by the majority of programs around the world which in itself is a major barrier to change. This is despite major changes in medical management and surgical approaches to CHD coupled with different sociocultural norms and technological development globally. Understanding this history enables consideration of opportunities for reform that include greater flexibility, the need for life-long prevention and the potential value of digital health in improving reach and sustainability of programs.

## AUTHOR CONTRIBUTIONS

JR conceived and draft the manuscript. RG, AO'N, SG, and TB provided the rehabilitation expertise. AB, GJ, and DB gave policy, public health and cardiology input. All authors reviewed multiple versions and the manuscript and approval the final version.

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# Two-Minute Step Test as a Complement to Six-Minute Walk Test in Subjects With Treated Coronary Artery Disease

*María José Oliveros[1], Pamela Seron[1]*, Claudia Román[2], Manuel Gálvez[3], Rocío Navarro[4], Gonzalo Latin[5], Tania Marileo[6], Juan Pablo Molina[7], Pablo Sepúlveda[1], Gabriel Nasri Marzuca-Nassr[1] and Sergio Muñoz[1]*

[1] Facultad de Medicina, Universidad de La Frontera, Temuco, Chile, [2] Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Complejo Hospitalario San José, Santiago, Chile, [4] Hospital Clínico Universidad de Chile, Santiago, Chile, [5] Hospital Clínico San Borja-Arriarán, Santiago, Chile, [6] Hospital Regional de Antofagasta, Antofagasta, Chile, [7] Hospital San Juan de Dios, Santiago, Chile

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**Edited by:**
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***Correspondence:**
Pamela Serón
pamela.seron@ufrontera.cl

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The 2-Minute Step Test (2MST) has been presented as an alternative to the 6-Minute Walk Test (6MWT) based on the association between the two tests in older adults; however, some authors propose that it should not be a substitute but rather a complement to the latter in the fitness evaluation. Specifically, in coronary disease, despite the potential and clinical utility of 2MST, the relationship of both tests in this population is unknown. This study aimed to determine the relationship between 6MWT and 2MST and to explore the relationship of biodemographic factors for both tests in subjects with treated coronary artery disease. For this, the 6MWT and the 2MST were applied to patients with coronary artery disease treated in 6 hospitals in Chile between May 2019 and February 2020. Additionally, lower limb strength was assessed by a chair-stand test, grip strength was assessed by a dynamometer, and physical measurements were applied. In total, 163 participants underwent both tests (average age = 58.7 ± 9.8 years; 73.6% men; 64.4% revascularized by angioplasty; 28.2% revascularized by surgery, and 7.4% treated by drugs or thrombolysis). Heart rate was higher at the end of the 6MWT, while the perception of effort was greater at the end of the 2MST. There was a weak positive correlation between the 6MWT and the 2MST in subjects with treated coronary disease ($r$ = 0.28, $p$ = 0.0003). While age ($r$ = –0.27), weight ($r$ = 0.25), height ($r$ = 0.49), and strength of both lower limbs ($r$ = 0.41) and grip strength ($r$ = 0.53) correlated weakly or moderately to the covered distance in 6MWT, the number of steps by the 2MST correlated only weakly to height ($r$ = 0.23), lower limb strength ($r$ = 0.34), and grip strength ($r$ = 0.34). Age, weight, height, lower limb strength, and grip strength would explain better the meters walked in the 6MWT than the steps achieved in the 2MST. With these findings, we can conclude that, in patients with treated

coronary artery disease, it does not seem advisable to replace 6MWT with 2MST when it is possible to do so. Additionally, the 2MST may provide additional information in the fitness evaluation. However, the usefulness of 2MST in this population needs to be further studied.

**Keywords: step test, 6-minutes walking test, fitness assessment, exercise test [MeSH], functional capacity, coronary artery disease**

## INTRODUCTION

The assessment of fitness components is vital for the correct prescription of exercise. Specifically, cardiorespiratory fitness generates great interest as it is directly related to cardiovascular events and all-cause mortality in adult populations (1, 2). Furthermore, it is known that its protective effect on mortality is independent of health conditions and biodemographic variables (3).

In clinical practice, different tests are used to evaluate cardiorespiratory fitness, generally requiring sophisticated equipment and treadmills or cycloergometers that are not always readily available, especially in resource-constrained settings (4). For this reason, tests based on the ability to perform daily living tasks, such as walking, are becoming more and more widespread and studied. Among them, the 6-Minute Walk Test (6MWT) (5–7) is considered simple, safe (5–7), and has been shown to be useful in candidates for cardiac rehabilitation, people with pulmonary disease, people with heart failure, those with peripheral arterial disease, and post-intensive care unit (ICU) patients (6, 8–11). Specifically, in people with coronary artery disease, a moderate to high correlation between 6MWT and maximal oxygen consumption has been described ($r = 0.56–0.93$) (11). The Heart and Soul Study conducted in a population with stable coronary heart disease reported that the distance walked on the 6MWT predicted cardiovascular events, having 4 times the rate of events in people who walked less than 420 m as compared with those who walked 545 m or more (12).

On the other hand, the 2-Minute Step Test (2MST) has been used as an alternative to the 6MWT based on the association between both tests and the time on the treadmill to 85% max heart rate reported by Rikli and Jones for an older adult population (8). This test is proposed as an option, generally when the 6MWT cannot be used, either because of structural limitations or when it is necessary to prescribe exercise to people who do not have the physical capacity or ability to ambulate. In clinical practice, the 2MWT has been gaining popularity and its usefulness has been studied in different populations, such as older adults without reference to health status or adults with heart failure, chronic kidney disease, osteoporosis, Parkinson's disease, stroke, hypertension, depression, or Alzheimer's disease (13). However, since the motor activity of climbing stairs may be considered more physically challenging than walking, some authors propose that it should be used as a complement rather than a substitute for other fitness assessments (14).

In this context, and given the potential and clinical utility of the 2MST in people with coronary artery disease, our study aimed to determine the relationship between 6MWT and 2MST, as well as to explore the relationship of biodemographic factors for both tests in subjects with treated coronary artery disease.

## MATERIALS AND METHODS

### Study, Design, and Participants

In the context of a randomized, multi-center, non-inferiority clinical trial conducted in Chile (Hybrid Cardiac Rehabilitation Trial, HYCARET) (15), the relationship between 6MWT and 2MST was analyzed in subjects with coronary artery disease treated by medication only, thrombolysis, angioplasty, or revascularization surgery between May 2019 and February 2020. Subjects were recruited from 6 hospitals in Chile and were entered into the Cardiac Rehabilitation program of the HYCARET study, between 2 weeks and 2 months from their cardiovascular event.

The HYCARET study was approved by the corresponding Ethics Committee at the Sponsor Institution: Comité Ético Científico (CEC) of Universidad de La Frontera. This approval was considered for the study implementation in two centers. In addition, four more Ethics Committees approved the protocol and a specific informed consent for implementation in their centers was provided: CEC of Servicio de Salud Metropolitano Central, CEC of Servicio de Salud Metropolitano Norte, CEC of Hospital Clínico Universidad de Chile, and CEC of Servicio de Salud Araucanía Sur.

### Variables and Measurements

Assessments were performed prior to the start of CR by trained personnel using standardized protocols for all measurements. The main evaluations are described below, however, further details of the procedures can be found in the HYCARET study protocol (15).

The 6MWT was performed in accordance with the American Thoracic Society Statement (7). Blood pressure, heart rate, oxygen saturation, and perception of exertion were evaluated before and immediately after the test. Patients were instructed to walk as much as possible for 6 min. In case the patients required detentions during the test, they were allowed as many times as necessary, but they were encouraged to resume walking as soon as possible. The total distance covered during the test was recorded.

At least 1 day after the 6MWT was performed (7), the 2MST was performed in accordance with the Senior Fitness Test (16). All parameters were evaluated before and after the test was completed, in the same manner as for the 6MWT. To determine the height of limb elevation, the midpoint between the femorotibial joint and the anterosuperior iliac crest of

each participant was identified. Instructions were delivered in a standardized manner for all participants, and upper limb support was offered to those with balance problems. To initiate the test, participants were asked to raise the left limb and then the number of times the right knee reached the mark during the 2 min was counted.

Additionally, upper and lower limb strength, waist circumference, weight, height, and other sociodemographic variables were assessed to characterize the participants and explore potential associations with fitness as measured by the 6MWT and the 2MST.

Upper and lower limb strengths were evaluated by dynamometry and by standing up from a chair test, respectively. By using a Jamar dynamometer, patients were instructed to sit on a chair with armrests with the shoulder adducted, elbow articulation flexed at a 90 degree angle, forearm in a neutral position, and wrist between 0 and 30 degrees of dorsiflexion and to perform three maximal effort trials with each hand; the highest value achieved on each limb was considered. For the standing up from chair test, participants were asked to stand up from the chair from a seated position with their arms crossed at chest height, achieving full standing, and then to sit down again as many times as possible for 30 s (16).

Weight and height were measured with the subject in a bipedal position, with little clothing, using a non-automatic mechanical column scale, SECA Model 700. Waist circumference was measured at the midpoint between the lower edge of the last rib and the iliac crest at the end of a normal exhalation, using a 7-cm non-elastic flexible tape measure. For this measurement, 2 measurements were recorded; if they differed by more than 1 cm, a third measurement was requested.

Additionally, to evaluate the hemodynamic response to both tests, blood pressure, perceived exertion, heart rate, and oxygen saturation were measured before and at the end of the 6MWT and the 2MST using automatic devices, with the subject in a seated position with the upper extremity uncovered and resting on a table at heart level (17).

## Statistical Analysis

Univariate descriptive analyses were performed to examine the distribution and frequency of meters walked during the 6MWT and steps taken during the 2MST. Measures of central tendency and dispersion were used to present continuous variables, while proportions were used to describe categorical data. Differences by sex and type of treatment received were tested with a *t*-test.

The correlation coefficient and its *p*-values were estimated to answer the main study goal of determining the relationship between 6MWT and 2MST. Sensitivity analyses were carried out to explore tendencies between men and women, between those who were treated surgically or percutaneously, and according to the performance on the 6MWT.

To quantify the level of concordance between the tests, both were equally scaled to a scale ranging from 0 to 100, and then the Bland-Altman plot was performed by plotting the difference between the two paired measurements against the mean of the two measurements.

The relationships between both tests and age, weight, height, and strength were analyzed by correlation analysis.

# RESULTS

In total, 163 participants with treated coronary artery disease performed both tests and were therefore included in this report. The average age was 58.7 ± 9.8 years, and 76.6% of the sample were men. In terms of treatment received, 64.4% were revascularized by angioplasty, 28.2% by surgery, and 7.4% were treated by drugs or thrombolysis. More details on the characteristics of the population are available in **Table 1**.

The mean 6MWT distance was 478 ± 116 m. Men walked 105 ± 20 m more than women ($p < 0.05$) and patients undergoing cardiac surgery walked on average 42 ± 20 m less than those undergoing minimally invasive revascularization ($p < 0.05$).

The mean number of steps reached during the 2MST was 72 ± 34 in 30 s, and women had a lower performance than men (women, 63 ± 23 steps; men, 75 ± 24 steps; $p < 0.05$). Regarding the treatment received, there was no difference in the number of steps achieved between the patients who underwent cardiac surgery and those who underwent minimally invasive revascularization.

As shown in **Figure 1**, the distance covered during the 6MWT and the number of steps during the 2MST were weakly correlated

**TABLE 1 |** Baseline characteristics, clinical presentation, and treatment.

| Characteristics | |
|---|---|
| Age (years, mean ± SD) | 58.7 ± 9.8 |
| Sex (% male) | 76.7 |
| **Educational level (%)** | |
| Primary education | 37.4 |
| Secondary – Upper secondary | 33.1 |
| Trade or College/University | 29.5 |
| **Occupation (%)** | |
| Current paid work | 55.8 |
| Homemaker | 6.8 |
| Retired | 13.5 |
| Unemployed | 14.7 |
| Other | 9.2 |
| **Cause of hospital admisión (%)** | |
| Unstable Angina | 7.4 |
| Acute myocardial infarction | 78.5 |
| Intervention for stable coronary artery disease | 14.1 |
| **Treatment received (%)** | |
| Drugs or thrombolysis | 7.4 |
| Revascularization by angioplasty | 64.4 |
| Revascularization by surgery | 28.2 |
| **Physical measures** | |
| Weight (kg, mean ± SD) | 76.3 ± 13.1 |
| Height (cm, mean ± SD) | 162.7 ± 8.2 |
| Lower limb strength (number, mean ± SD) | 14.1 ± 4.8 |
| Grip strength (kilos, mean ± SD) | 29.8 ± 9.3 |

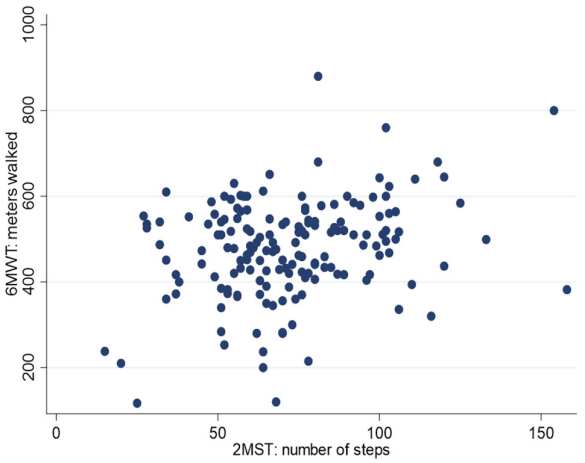


**FIGURE 1 |** Correlation between the 2-Minute Step Test (2MST) and the 6-Minute Walk Test (6MWT).

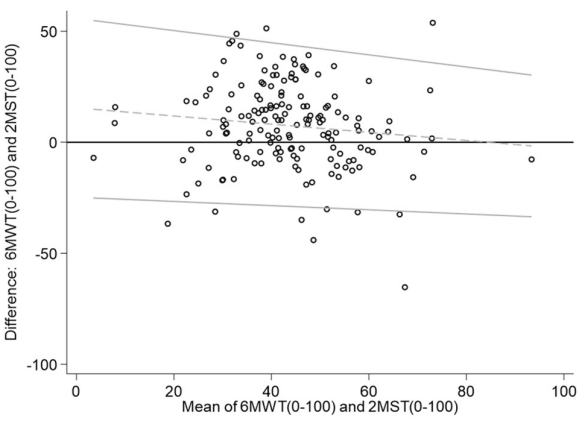


**FIGURE 2 |** Bland–Altman plot.

in subjects with treated coronary disease ($r = 0.28$, $p = 0.0003$). The sensitivity analysis showed that this correlation remained weak according to the type of treatment received (angioplasty, $r = 0.29$; cardiac surgery, $r = 0.22$) and among different sex (men, $r = 0.2$; women, $r = 0.31$). Finally, we discovered that the correlation between subjects who performed better during the 6MWT by walking 529 m or more was higher than those who walked fewer meters during the test ($\leq$436 m $r = 0.2903$; $>$436 m and $\leq$529 m $r = 0.11$; $>$529 $r = 0.45$).

**TABLE 3 |** Correlation between both tests and biodemographic variables.

| | 6MWT Overall | 2MST Overall |
|---|---|---|
| Age (years) | −0.27* | −0.13 |
| Weight (kg) | 0.25* | 0.09 |
| Height (cm) | 0.49* | 0.23* |
| Lower limb strength | 0.41* | 0.34* |
| Grip strength (kilos) | 0.53* | 0.34* |

*(*) $p < 0.05$.*

**Figure 2** shows that the scaled 6MWT 0–100 achieved a higher mean than the scaled 2MST, meaning that the walking test consistently achieved a higher mean than the 2MST. This difference was 21.27 in the scaled unit equivalent to 187 m walked in 6 min, which is well above a clinically significant change, indicating a possible bias in the measurements. This tendency was greater among subjects with lower functional capacity and approached zero in subjects with better performance.

On the other hand, the concordance between both tests was high, since only 6.75% of the observations lie outside the 95% *CI* limits of agreement.

Regarding the physical response to both tests, heart rate (bpm), systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), and Rating of Perceived Exertion (Borg 6–20) were higher at the end of both tests. However, this difference was greater for heart rate at the end of the 6MWT, while the perception of effort was greater at the end of the 2MST, as shown in **Table 2**.

In addition, we examined the association with other variables that have been shown to be a source of variability for the meters run in the walking test, such as age, weight, height, lower limb strength, and grip strength. For the 6MWT, age ($r = -0.27$) and weight ($r = 0.25$) correlated weakly with meters walked, while height ($r = 0.49$) and strength of both lower limbs ($r = 0.41$) and grip strength ($r = 0.53$) were moderately correlated to the meters walked in this test. On the other hand, the number of steps by 2MST correlated weakly with height ($r = 0.23$), lower limb strength ($r = 0.34$), and grip strength ($r = 0.34$), and unlike what was observed in the 6MWT, weight was not correlated to the steps achieved, which makes this test more suitable for overweight and obese patients, as shown in **Table 3**.

**TABLE 2 |** Heart rate, rating perceived exertion, and blood pressure pretests and posttests.

| (mean ± SD) | 6MWT | | | 2MST | | | *p*-value for the difference between the changes |
|---|---|---|---|---|---|---|---|
| | | | Δ (mean ± SD) | (mean ± SD) | | Δ (mean ± SD) | |
| | INITIAL | FINAL | | INITIAL | FINAL | | |
| Heart rate (bpm) | 69.3 ± 11.1 | 100 ± 14.9 | 30.9 ± 13.1* | 69.8 ± 10.4 | 94.4 ± 16.9 | 24.2 ± 15.9* | $p = 0.0000$ |
| Systolic blood pressure (mm Hg) | 121.5 ± 17.1 | 135.9 ± 20.2 | 14.6 ± 15.7* | 116.9 ± 16.3 | 133.6 ± 19.8 | 17.6 ± 15.9* | $p = 0.0875$ |
| Diastolic blood pressure (mm Hg) | 73.5 ± 10.9 | 75.9 ± 11.4 | 2.6 ± 8.2* | 72.1 ± 10.8 | 73.8 ± 10.9 | 2 ± 8.7* | $p = 0.5221$ |
| Rating of Perceived Exertion (Borg 6–20) | 8.1 ± 2.6 | 12.4 ± 2.8 | 4.4 ± 2.7* | 7.4 ± 2.1 | 13 ± 2.8 | 5.6 ± 3.1* | $p = 0.0002$ |

*(*) $p < 0.05$; (Δ) difference between initial and final evaluation; (SD) standard deviation.*

## DISCUSSION

The current study examined the relationship between 6MWT and 2MST and explored the relationship of biodemographic factors for both tests in subjects with treated cardiovascular disease. Our results show that, although there is a positive relationship between the two tests, it is weak, so the 2MST should still be considered as a complement to the 6MWT. However, in cases where the 6MWT cannot be performed, the 2MST seems to emerge as a valid option due to its high level of concordance.

Regarding the hemodynamic response to the effort of each test, our results showed that, although both tests generate physical effort, the increase in heart rate was greater at the end of the 6MWT, while the perception of effort was greater at the end of the 2MST, which is consistent with what has been found in other populations (14). This may be mainly because walking, although a habitual activity, involves displacement, requires balance, and is more global, while on the other hand, stair climbing is a stationary activity but more demanding for the lower limbs (18).

Similar experiences in other populations have reported contradictory results. Amaral et al. reported that there was no correlation between the steps achieved in the 2MST and the meters walked in the 6MWT ($r = 0.26$; $p = 0.23$) in patients with symptomatic peripheral artery disease, but that these did correlate with the number of steps in 6MWT ($r = 0.55$, $p < 0.01$) (19). In contrast, Haas et al. reported a strong correlation ($r = 0.93$) between the steps achieved in the 2MST and the meters run in the 6MWT in patients who were participating in cardiopulmonary rehabilitation (20). Along the same lines, the study by Wegrzynowska-Teodorczyk et al. performed on adults with heart failure also reported a positive but moderate correlation between both tests ($r = 0.44$; $p = 0.0001$) (14).

In this context, our results support the positive correlation between the two tests, even though it was not as strong as that reported in other populations. This could be explained by the fact that although the assessors were trained in the performance of both tests, the 2MST was considered a new assessment by the evaluator as it was not used in the context of cardiac rehabilitation programs unlike the 6MST. Additionally, the variability in the strength of the correlation between tests may be due to their submaximal nature and time limitation, which was also shown to influence the correlation with maximal oxygen consumption compared with maximal tests in patients with heart failure (21).

Our results also showed that the correlation between both tests was higher among those who walked a longer distance in the 6MWT, which could be explained by the fact that a test that provides an assessment of the patient's ability to perform submaximal activities of daily living, such as the 6MWT, achieves a strong predictive ability of peak oxygen consumption in subjects who walked more distance (22). At this point, we consider it important to highlight that the participants included in our study were evaluated at enrollment in a cardiac rehabilitation program, therefore their poor performance could also affect the strength of the estimate.

To the best of our knowledge, our study is the first to examine the relationship between the two tests in a population with treated coronary artery disease, so we believe that our results would further our knowledge about the usefulness of both tests and will serve as a basis for future studies. However, in terms of the clinical application of these findings, we consider that we should be cautious in the interpretation and not discourage the usefulness that the 2MST can have, especially in cases where the 6MWT cannot be performed.

A limitation of our study that future studies should consider is the direct assessment of maximal oxygen consumption and the oxygen consumption during the execution of both tests, which would allow us to establish both the predictive capacity of VO2 max and explain the observed and unexplained variabilities between the two tests in this population.

Whereas our findings showed that in patients with treated coronary artery disease, the distance walked in the 6MWT and the steps performed in the 2MST are weakly correlated and that both tests correlate differently to age, weight, height, and muscle strength, we can conclude that, in patients with treated coronary artery disease, it does not seem advisable to replace 6MWT with 2MST when it is possible to do so. Additionally, the 2MST may provide additional information in the fitness evaluation. However, the usefulness of 2MST in this population needs to be further studied.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, to those who submit a justified request.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité Ético Científico (CEC) of Universidad de La Frontera, CEC of Servicio de Salud Metropolitano Central, CEC of Servicio de Salud Metropolitano Norte, CEC of Hospital Clínico Universidad de Chile, and CEC of Servicio de Salud Araucanía Sur. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MO conceived and designed the study, performed the statistical analysis, interpreted data, and wrote the manuscript. PSer conceived and designed the study, interpreted the data, and thoroughly reviewed the writing of the article. MG, RN, GL, TM, JM, and PSep performed the measurements and collected the data. SM reviewed the statistical analysis. All authors read and approved the final version of the manuscript.

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