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Cardioneurology: Basic, translational and clinical research

Edited by
Leonardo Roever, Andre Rodrigues Duraes and Octavio Marques Pontes-Neto

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Cardioneurology: Basic, translational and clinical research

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Editorial: CardioNeurology: Basic, translational and clinical research

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Editorial on the Research Topic
CardioNeurology: Basic, translational and clinical research

Recent advances are allowing a better understanding of the interaction between the heart and the brain. Many acute cardiovascular events require a detailed neurological evaluation, and vice versa. With the advancement of knowledge, studies have allowed us to verify more points of connection between stroke, dementia, cerebral small vessel disease and its relationship with congestive heart failure, myocardial infarction, atrial fibrillation (AF), and other cardiovascular conditions (1).

In this Frontiers Research Topic, an international selection of high-quality studies has contributed to advancing our understanding of CardioNeurology, both in the field of diagnosis, treatment and imaging technologies. We believe that this edition about such a hot topic will become a valuable source of information for clinicians and researcher in the field.

To illustrate this view, Ernst et al. presented a study on whether low heart rate variability could predict stroke and other complications after hip surgery. Follow-up was 6 months and findings were related to mortality, pneumonia, stroke, and myocardial infarction (Ernst et al.). Zhou et al. studied about gender and age-specific associations and visit-to-visit blood pressure variability with anxiety. In their studies that included 48,023 patients, women were more likely to develop anxiety and among the predictors advanced age, cardiovascular, metabolic, and gastrointestinal diseases stood out (Zhou et al.). Naranjo et al. in a cohort of 245 ischemic strokes evaluated the presence of 28% of antiphospholipid antibodies (aPL) with an OR 2.40. Other risk factors such as arterial hypertension, atrial fibrillation, and active smoking have also been identified (Naranjo et al.).

In addition, Risseeuw et al. presented a case of a 69-year-old woman with a history of cigarette smoking, arterial hypertension, type 2 diabetes mellitus, hypercholesterolemia, right subcapital femur fracture, with repetitive episodes of

Takotsubo syndrome (TTS). In this case the authors observed the link between neurological disease and TTS, especially with involvement of the medulla oblongata (Risseeuw et al.). Teodoro et al. evaluated whether the percentage of patients with undetermined etiology according to the TOAST classification decreased after transthoracic echocardiography. In a total of 1,100 patients, echocardiography reduced the odds of an indeterminate TOAST score and the risk of in-hospital mortality (Teodoro et al.). In the retrospective study by Long et al. with 7,528 septic patients. The presence of a coagulation disorder in the first 24 h after admission to the ICU was found to be an independent risk factor for AF and its 90-day mortality varies with the severity of the clotting disorder (Long et al.).

Another study by Lu et al. with 296 patients the incidence of bleeding events in AF patients treated with ticagrelor was comparable to that in patients treated with clopidogrel plus aspirin at 6 month. In another study in Hong Kong, Mui et al. demonstrated that the use of sodium-glucose cotransporter 2 inhibitors is associated with lower risks of dementia, Parkinson's disease, and cerebrovascular mortality compared with dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes mellitus.

In a Bayesian Network Meta-Analysis, Duan et al. analyzed adequate time from collapse to return of spontaneous circulation (ROSC), which is an ideal indication for targeted temperature management (TTM) to improve survival and neurological outcome. The authors found that survival and good neurological outcome are closely associated with the time to collapse for ROSC (Duan et al.). In another study Zhang et al. investigated the acute hemodynamics of the lower extremities during enhanced external counterpulsation. Counterpulsation acutely improved blood flow, blood flow velocity, and maximum systolic accelerations of the anterior tibial artery. As well as significantly increased blood flow velocity and peripheral resistance of the inferior knee artery, while markedly reducing blood flow in the posterior tibial artery (Zhang et al.).

In an experimental model Park et al. found that reducing expression of the huntingtin gene mutation in the heart benefits cardiovascular function in the BACHD Mouse Model of Huntington's Disease. In the study by Primessnig et al. found that B-type natriuretic peptide (BNP) improves diastolic tension during adrenergic stress in human atrial myocardium and may have long-term positive effects on inotropic reserve. And that BNP and Sacubitril + valsartan can reduce atrial arrhythmogeneity during adrenergic stress *in vitro* (Primessnig et al.).

In the case study Moreno et al. demonstrated that non-compaction cardiomyopathy (NCCM) is associated with neuromuscular disorders. Patients with NCCM with a phenotype of dilated left ventricle with reduced left ventricular ejection fraction must be treated promptly to avoid the progression of heart failure (Moreno et al.). Another study by Zhu et al. demonstrated an expert consensus on the use of coated stents to treat complex cerebrovascular diseases. Other authors have shown that covered stents are an effective treatment option for complex cerebrovascular diseases, such as complex aneurysms, direct carotid-cavernous fistulas and internal carotid artery injuries (Zhu et al.).

In conclusion, the innovations presented in this valuable topic were fundamental to further strengthen and clarify the interrelationship between the cardiovascular and cerebral segments. Undoubtedly, they are extremely complex and intertwined systems. A holistic and complementary view of cardiology and neurology is essential for a more complete management of patients affected by such pathologies.

Author contributions

LR, AD, and OP-N contributed to the conception, design, and drafting of the work. All authors contributed to the article and approved the submitted version.

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.

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References

1. Qiu Q, Song W, Zhou X, Yu Z, Wang M, Hao H, et al. Heart rate variability is associated with cerebral small vessel disease in patients with diabetes. *Front Neurol.* (2022) 13:989064. doi: 10.3389/fneur.2022.989064

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ORIGINAL RESEARCH
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Low Heart Rate Variability Predicts Stroke and Other Complications in the First Six Postoperative Months After a Hip Fracture Operation

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Background: One-year mortality after hip fractures is underestimated and is reported as 25%. An improved risk stratifying could contribute to a better follow up of these patients. Heart Rate Variability (HRV) is an easy point-of-care investigation and is been used in cardiology, endocrinology, and perioperative care. This observational study intended to explore relevant associations between HRV parameters and 6-months mortality and morbidity after a hip fracture.

Methods: One hundred and sixty-five patients admitted to two hospitals were included, and short-time HRV measurements (5 min, and 10 min at the two hospitals, respectively) were obtained. Mortality data were gathered by means of the Norwegian central address register. Patients, close relatives of patients, and in some cases their general physicians or nursery home physicians were interviewed 6 months postoperatively regarding the incidence of pneumonia, cardiac events, or stroke.

Results: One and hundred fifty-seven (95.2%) patients were followed up after 6 months post-surgery. Twenty-one (13%) died during this period. Twenty patients (13%) developed pneumonia, eight (5 %) stroke, and four (2%) myocardial infarction. No HRV parameter was associated with 6-month general mortality. However, patients who developed stroke had significantly lower High Frequency Power (HF, $p < 0.001$) and lower Very Low Frequency Power (VLF, $p = 0.003$) at inclusion compared to patients without complications. Patients who developed pneumonia had at the inclusion lower root mean square of successive differences (RMSSD, $p = 0.044$). Patients with a history of coronary heart disease ($n = 41$) showed a mortality of 7%. Mortality in this group was associated with standard deviation of beat-to-beat intervals (SDNN, $p = 0.006$), Total Power (TP, $p = 0.009$), HF ($p = 0.026$), and Low Frequency Power (LF, $p = 0.012$). Beta-blocker intake was associated with lower heart rate, but not with differences in HRV parameters.

Conclusion: In this exploratory study, we present for the first-time significant associations between different preoperative HRV parameters and stroke, myocardial infarction, and pneumonia during a 6-month period after hip fracture. HRV might be a simple and effective tool to identify patients at risk that would warrant better follow-up.

Keywords: heart rate variability, stroke, hip fracture, prediction, pneumonia, myocardial infarction

BACKGROUND

Hip fractures occur frequently in older people, and the incidence is increasing (1). The normal therapeutic approach is surgery. This is associated with a significant proportion of complications both perioperatively and during the hospital stay until discharge (2). Moreover, a hip fracture may also have effects on many aspects of the patient's well-being after they are discharged from the hospital (3, 4). Long-term mortality after hip fractures has to a large extent been neglected for many years. Some studies report a 5-year mortality of 55–68% (compared to 12% in population-based controls adjusted for age and previous hospitalization for serious disease). The highest mortality is reported during the first 6 months (5–7). One-year mortality rates of hip fractures in Norway are 25% according to the Norwegian Hip Fracture Register (8). The fact that one third of all postoperative complications and 50% of postoperative mortality are due to cardiac events, underlines the importance of risk estimation (9). Besides cardiac events, the risk for stroke after operations might be increased up to six times in patients over 80 (10), and anesthesia and surgery by themselves might increase the odds ratio to 2.9 (11). Currently, no ideal risk estimation tool to predict long term mortality exists (9, 12). Ischemic stroke risk models have still only moderate predictive value in different patient groups (13). Thus, simple risk estimation tools to identify low-risk and high-risk groups are needed.

Short-term heart rate variability (HRV) is a simple point-of-care investigation. Patient's heart rhythm is evaluated by an ECG measurement over 5–10 min. The beat-to-beat variance measured by QRS distances shows variability in different frequency areas. This time series can be analyzed with several mostly simple algorithms which can be differentiated between time domain, frequency domain, fractal analysis, or measures of entropy (14). Since 1996, a general accepted standard procedure has been used which makes studies comparable (15). In *time domain* the standard recommends standard deviation of beat-to-beat intervals (SDNN), and root mean square of successive differences (rMSSD). In *frequency domain,* one determines the frequency bands Total Power (TP), Very Low Frequency (VLF), Low Frequency (LF), High Frequency (HF), and the ratio of LF/HF. After this standard was established, HRV has been investigated as risk estimator in cardiology, perioperative care, and diabetes, among others (16). Short-term heart rate variability has been tested perioperatively in unselected patients (17, 18) and patients at risk of coronary artery disease (19), but not in patients undergoing surgery after hip fractures during a long-time follow up.

This prospective observational study intended to explore relevant associations between linear and non-linear HRV parameters and mortality and morbidity 6 months after hip fracture surgery. We hypothesized that decreased heart rate variability parameters might be associated with 6-months mortality, and 6-months incidence of pneumonia, stroke and myocardial infarction. We anticipated that such associations would be especially prominent in patients with known coronary heart disease. Since Beta-blockers might influence HRV results (20, 21), we also planned to look specifically into possible effects of these drugs.

METHODS

Patients with hip fractures admitted to Oslo University Hospital (OUS) and Kongsberg Hospital between 2008 and 2013 and with sinus rhythm in ECG were eligible for inclusion in the study. The patients at OUS were at the same time participating in another randomized study investigating the effect of geriatric care on cognitive function (22). Exclusion criteria were technical problems to take a short-time ECG (e.g., due to delirium), patients with unstable circulation, patients with operations the last month before admission, neoplasms, high energy trauma, and patients with short life expectancy. Patients signed written informed consent. In Oslo, substitute decision-makers were allowed to consent if the patients were not capable, evaluated by the including physician. An ECG signal was obtained within the 1st day after admission before operation and digitalized. We used Biocom ECG recorders (Biocom 3000 in Kongsberg, Biocom 4000 in Oslo), equipped with dry silver/ silver chloride ECG electrodes being mounted on the index fingers of the right and the left hand, respectively. After a relaxing period of 5 min an ECG signal was recorded over 5 min (Oslo) or 10 min (Kongsberg).

Signal measurement and processing were done according to recommendations the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (15), and the methods are described in detail in recent publications (16, 23). Briefly, we used a sampling rate of 1,024 Hz (>512 Hz recommended for 5 min recordings). All ECGs were visually inspected and manually edited. Ectopic beats and noisy events were removed, and mean values was interpolated based on preceding and successive beats. Patients with more than 10% of ectopic beats or noise events were also excluded at this stage. Linear parameters (time domain: SDNN, rMSSD; frequency domain: HF, LF, VLF, LF/HF) were calculated by a Heart Rhythm Scanner - Version 2.0 – (Biocom

TABLE 1 | Preoperatively identified illnesses according to the case notes ($n = 165$).

Illness	Number	%
Coronary heart disease	41	25
Hypertension	62	38
Diabetes (insulin)	7	4
Diabetes (tablets)	15	9
COPD	31	19

TABLE 2 | Preoperative drug treatment ($n = 165$).

Drug treatment	Number	%
Beta blocking agents	47	29
AT2 antagonists	23	14
ACE inhibitors	27	16
Calcium channel blockers	22	13
Diuretics	37	22
Glucocorticoids	13	8
Lipid lowering drugs	37	22
Sedatives	25	15
Antidepressive drugs	35	21
Antiepileptic drugs	7	4

Technologies – U.S.A). The frequency domain calculations were conducted non-parametrically with Fast Fourier Transform.

The sample size was estimated according to reference values reported earlier (24). The calculation was based on mortality. Assuming a 6-months mortality between 3 and 8% according to Dahl (25), 150 patients were expected to be sufficient to test the hypothesis of a significant association between mortality and HRV.

Normal distribution of data was tested with the Kolmogorov-Smirnov test. To test univariate associations, the independent samples *T*-test was applied. The Chi-Square test or Fisher's exact test were used in case of nominal data, as appropriate. Statistical analyses were conducted by the Statistical Package for Social Sciences (SPSS), release 18.0.3 (September 2010). Values are given in mean +/− SEM.

Every person in Norway is identified by a unique number in the Central Personal Register. Deceased patients were identified by the Norwegian central address register which provides exact data for the time of death. In addition, patients, close relatives of patients and in some cases their general physicians or nursing home physicians were interviewed 6 months postoperatively in the Kongsberg group regarding pneumonia, cardiac events, and stroke. In the Oslo group patients, close relatives of patients and in some cases their general physicians or nursing home physicians were interviewed regarding pneumonia, cardiac events and stroke within the first 6 months postoperatively. In both groups the results of the interviews were cross-validated by the hospital journals and—if relevant—nursery home journals regarding new hospital admissions within 6 months after the operation date.

The study protocol was reviewed and approved of the Regional Committee for Medical and Health Research Ethics of Southern Norway (11.1.2008, S-07307b) and the Data Protection Officer of Oslo University Hospital.

RESULTS

One hundred sixty-five patients (123 females and 42 males, mean age 80, 9 ± 9.9 Std. dev.) were included. All HRV data were normally distributed. At admission, one in four patients had an established diagnosis of coronary heart disease, one in three patients had hypertension and nearly one fifth COPD. Other patient details (other illnesses, medication,) are reported in **Tables 1**, **2**.

One hundred fifty-seven (95%) patients were followed up at 6 months (with a similar proportion of illnesses, medication, ECG abnormalities and blood sample results compared to the original group).

Patients taking beta-blockers had a slightly lower heart rate (76 vs. 80, $p = 0.04$). There were no significant associations between intake of beta-blockers and HRV parameters. Thus, we did not include beta blockers as a variable in the statistical models.

Twenty-one patients (13%) died during the study period of 6 months after operation. Twenty-one patients (13%) developed pneumonia, eight (5%) stroke, and four (3%) myocardial infarction. Before discharge from the hospital, four (2.4%) patients deceased, 14 (8.5%) developed pneumonia, two (1.2%) patients developed stroke, and three (1.8%) myocardial infarction.

No HRV parameter was associated with 6-month mortality. Patients who developed stroke had at inclusion lower HF ($p < 0.001$), and lower VLF ($p = 0.003$, **Figure 1**), compared patients without complications. There were no statistical differences regarding sex, age, or other prevalent illnesses at inclusion. Patients who developed pneumonia had at inclusion lower RMSSD ($p = 0.044$, **Figure 2**) compared to patients without complications. Patients developing pneumonia had significantly more often COPD ($p = 0.004$) and depression ($p = 0.048$). We also found that patients with coronary heart disease ($n = 41$) had a mortality of 7%. Mortality within this patient group was associated with SDNN ($p = 0.006$), TP ($p = 0.009$), HF ($p = 0.026$), and LF ($p = 0.012$, **Figure 3**). There were no statistical differences regarding sex, age, or other prevalent illnesses at inclusion in this group. All HRV results are presented in **Table 3**.

DISCUSSION

This prospective study showed strong associations between the incident of stroke and lower HF and VLF at time of admission. Pneumonia was associated with lower RMSSD. Mortality in patients with coronary heart disease was associated with lower SDNN, TP, LF, and HF. We found no association between HRV parameters and 6 months mortality.

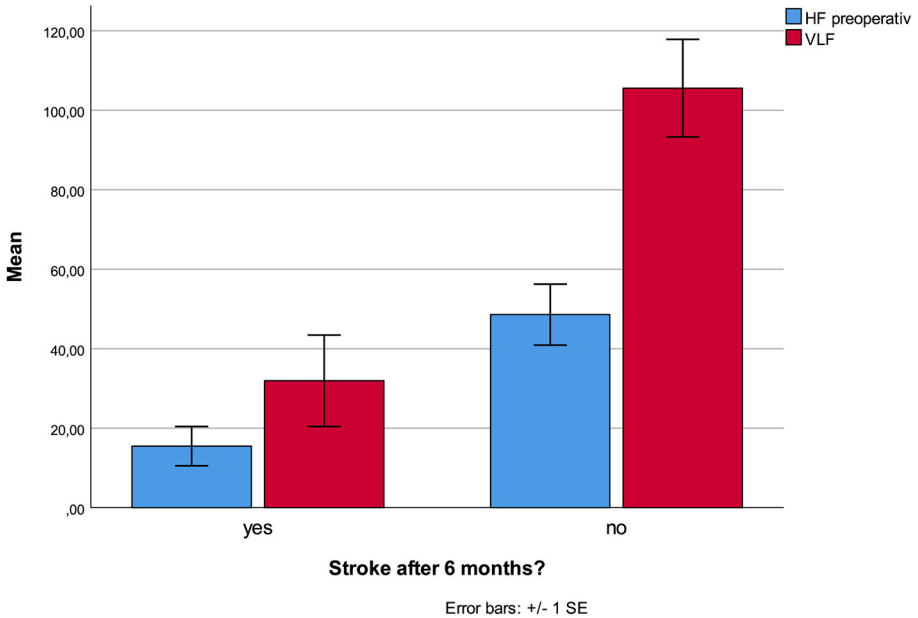

FIGURE 1 | Lower HF and VLF at admission was associated with increased stroke incidence within the first six postoperative months.

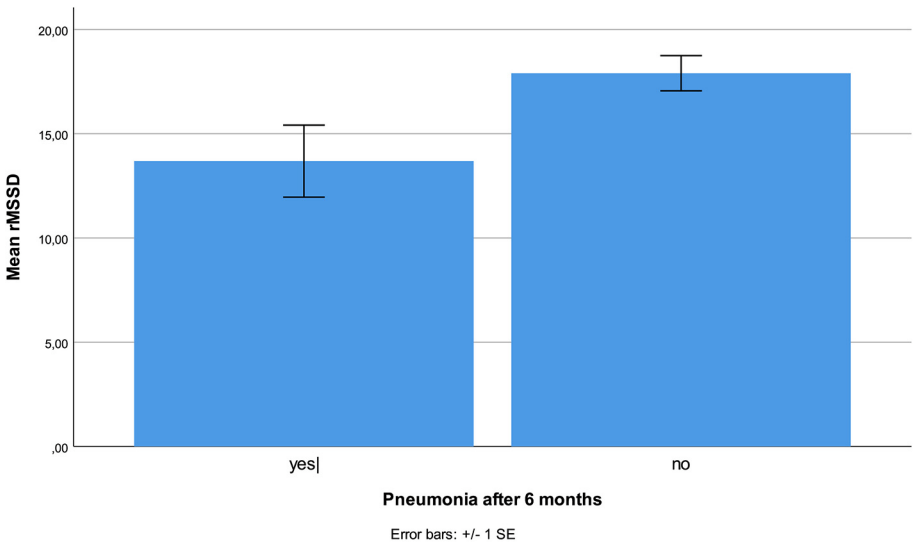

FIGURE 2 | Reduced RMSSD was associated with higher incidence of pneumonia during the 6-month follow-up period.

Our study has some limitations. Due to practical circumstances, we recorded ECG for 10 min in Kongsberg, and 5 min in Oslo. Previous studies reported no relevant differences in HRV assessments of 5 or 10 min (26). Mortality was analyzed by retrieving data from the central address register and can be considered as very reliable. Our follow up percent was high (95%). Since the diagnosis of stroke, myocardial infarction and pneumonia was made at different places, different criteria might have been used. On the other hand, especially diagnosis of stroke and myocardial infarction are highly standardized, and most patients were treated for these illnesses in the same two hospitals. The diagnosis of pneumonia, however, was established in hospitals, nursing homes, or by general physicians. It is possible that we were not able to identify all patients with milder forms of pneumonia, either because they were not recognized as pneumonia or because some patients did

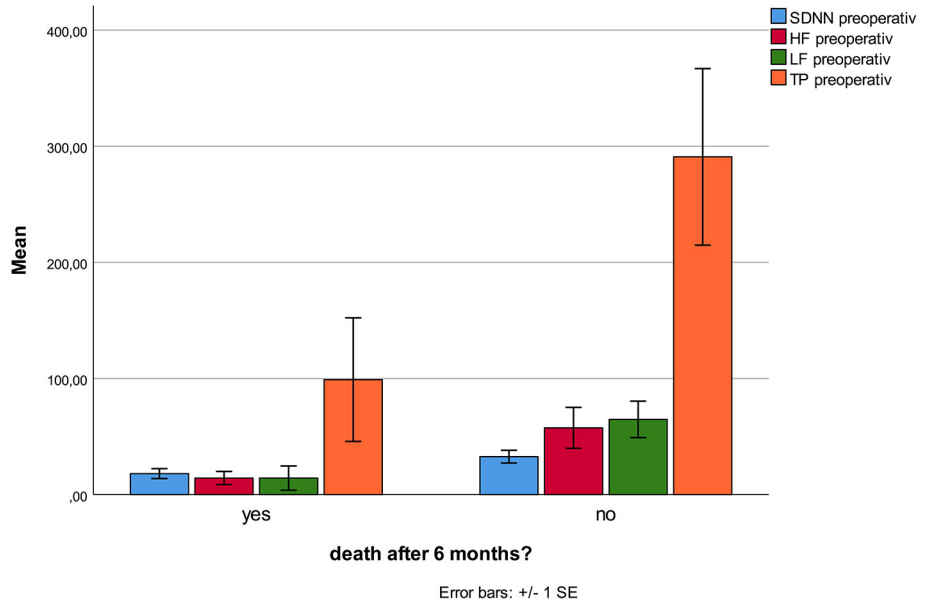

FIGURE 3 | Mortality in the subgroup of patients with coronary heart disease is associated with lower TP, HF, LF, and SDNN.

TABLE 3 | HRV results in hip fracture patients without and with complications during the first 6 months postoperatively.

Mean (S.E.M.)	No complications ($n = 131$)	Stroke ($n = 8$)	Pneumonia ($n = 21$)	Myocardial infarction ($n = 4$)
Heart rate	78.1 (± 1.0)	84.0 (± 3.3)	78.8 (±2.6)	81.1 (±7)
SDNN (ms)	35.9 (± 2.67)	54.8 (± 20.3)	49.1 (± 10.4)	28.8(± 11.8)
rMSSD (ms)	17.5 (± 0.86)	14.7 (± 1.5)	**13.7 (± 1.7)***	19.6 (± 6.4)
TP (ms^2)	1515.6 (± 331.4)	2436.9 (± 1718.8)	2486.1 (± 1260.8)	263.7 (± 195.1)
LF(ms^2)	164.1 (± 27.7)	101.3 (± 63.9)	131.6 (± 41.3)	56.3 (± 38.5)
HF(ms^2)	54.4 (± 7.8)	**14.6 (± 3.0)****	59.6 (± 21.8)	24.2 (± 15.7)
VLF (ms^2)	101.9 (± 11.6)	**31.9 (± 11.5)*****	105.9 (± 36.4)	56.3 (± 38.5)
LF/HF	1.88 (± 0.18)	2.61 (± 1.06)	1.82 (± 0.30)	1.50 (± 1.00)

*Higher numbers indicate higher variability. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, SDNN, standard deviation of all normal QRS-distances; rMSSD, square root of the mean squared differences of successive NN intervals; TP, Total Power; LF, Low Frequency Power; HF, High Frequency Power; VLF, Very Low Frequency Power.*

not contact a physician. The HRV measurements were carried out according to international standards (15) which secures a sufficient quality. This is an exploratory study where we tried to identify relevant HRV parameters as predictors of mortality and morbidity. Many associations were tested. Thus, we should consider some associations with caution. However, the *p*-values of the associations between stroke and HF and VLF are very low, indicating a high probability. The association between pneumonia and rMSSD was in accordance with previous findings seen during the hospital stay of the patients (23).

This is the first study showing an association between HRV parameters and stroke incidence during a 6-month postoperative period. Of particular importance is that most strokes occurred after discharge of the hospital. Only two studies have reported HRV as a predictor of stroke previously, none of them in a perioperative setting. The Copenhagen Holter study followed 678 healthy persons between age 65 and 75 and calculated night-time SDNN between 2.00 and 2.15 a.m. based on a 15 min measurement. In contrast to 24 h SDNN and Mean NN, night-time SDNN was significantly associated with stroke in the follow-up period (27). In the Atherosclerosis Risk in Communities (ARIC) study, lower HRV was associated with higher risk of stroke, but only in participants with prevalent diabetes mellitus (28). HRV has else only been used to characterize patients after stroke (29).

Reduced HF is associated with reduced parasympathetic activity. Reduced parasympathetic activity has been associated with hypercoagulation and increased blood viscosity, and is possibly associated with arrhythmias (30). The VLF-component is a major determinant of physical activity and reflects possibly efferent sympathetic activity (31), in other sources

modulated by the parasympathetic system (32), though its origin is controversial (33). Physiological studies indicate that VLF is mainly generated by stimulation of afferent sensory neurons in the heart provoking activation of feedback and feedforward loops (34, 35) and influenced by the renin-angiotensin system (32). Decreased VLF is often associated with increased inflammatory parameters like CRP, Il-6, and WBC (36). Increased inflammation and coagulation have often been associated with development of stroke (10, 37). Since both lower HF and VLF are also associated with these factors, they might reflect an increased tendency to inflammation and coagulation in patients predisposed to develop stroke. Most intervention studies have been conducted in cardiac surgery and showed that beta-blockers might prevent postoperative stroke in this patient group (38). Other predictive treatments, however, have shown conflicting results in studies (39). Our study is too small to establish HRV as a new risk assessment tool. A next step would be to include HRV-measurement together with other predictive parameters in a prediction model. This model and its possible guiding for interventions had to be tested in clinical investigations.

Increased incidence of pneumonia during the first 6 months of operation was associated with decreased rMSSD. A decreased rMSSD may indicate a lower parasympathetic activity and has been associated with immunologic changes in patients with hypertension (40), and in an experimental model where healthy human participants were treated with low dose endotoxin infusions (41). Decreased rMSSD has been suggested as an early marker of multiple organ dysfunction (42). The observed lower rMSSD in our patients with pneumonia might indicate an increased vulnerability to develop infections.

In patients with known coronary disease there was an association between mortality and lower SDNN, TP LF, and HF in our study. This is not surprising. SDNN, LF, and HF are all associated with increased risk for sudden cardiac death (43), although SDNN has been much more frequently measured in 24-h Holter monitoring. In risk populations with coronary heart disease these HRV parameters are correlated with long term mortality (44). Filipovic et al. followed patients scheduled for major elective non-cardiac surgery (e.g., vascular procedures of the abdominal aorta or lower limb, open intraperitoneal or intrathoracic procedures, major orthopedic procedures of the hip or spinal column, or major procedures on the neck). They observed an association between LF/HF < 2 and mortality, but did not measure SDNN or TP (19). In our study, we did not find a significant lower LF/HF ratio, probably because LF and HF were reduced similarly. Our results confirm these earlier results.

We did not find associations between HRV parameters and general mortality. This is in contrast with other earlier studies. A recent met analysis including at all 21,988 participants without cardiac disease at baseline and followed up in cohort studies, demonstrated a robust association between decreased HRV and later cardiovascular events. Individuals with low HRV have about 40% increased risk of fatal or non-fatal CVD compared to individuals with high HRV. Recent studies had a follow-up of 9 and 15 years (36, 45, 46), as opposed to our study of only 6 months follow-up. However, we did find associations between HRV parameters, stroke, and pneumonia. Presence of stroke and recurrent pneumonia are associated with increased mortality (47, 48). Thus, we could expect a further increase in mortality in our study group after 6 months, secondary to events like stroke and pneumonia.

CONCLUSIONS

We present for the first time a significant association between preoperative low HF, VLF, and stroke during a 6-month period after hip fracture. Pneumonia in this period was associated with low preoperative rMSSD. Mortality in cardiac patients was associated with low SDNN, TP, HF, and LF preoperatively. If these results can be confirmed, HRV might be a simple and effective tool to identify patients at risk. A better and more targeted follow-up of these patients might decrease morbidity and mortality.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committee for Medical and Health Research Ethics of Southern Norway (11.1.2008, S-07307b) and the Data Protection Officer of Oslo University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GE and MR developed the study protocol. GE, LW, and FF collected data. GE, LW, AD, and MR conducted the data analysis. All authors read and approved the final manuscript.

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REFERENCES

1. Konnopka A, Jerusel N, Konig HH. The health and economic consequences of osteopenia- and osteoporosis-attributable hip fractures in Germany: estimation for 2002 and projection until 2050. *Osteoporosis Int*. (2009) 20:1117–29. doi: 10.1055/s-0029-1239263
2. Siu CW, Sun NC, Lau TW, Yiu KH, Leung F, Tse HF. Preoperative cardiac risk assessment in geriatric patients with hip fractures: an orthopedic surgeons' perspective. *Osteoporosis Int*. (2010) 21(Suppl.4):S587–91. doi: 10.1007/s00198-010-1393-0
3. Dyer SM, Crotty M, Fairhall N, Magaziner J, Beaupre LA, Cameron ID, et al. A critical review of the long-term disability outcomes following hip fracture. *BMC Geriatr*. (2016) 16:158. doi: 10.1186/s12877-016-0332-0
4. Bertram M, Norman R, Kemp L, Vos T. Review of the long-term disability associated with hip fractures. *Injury Prev*. (2011) 17:365–70. doi: 10.1136/ip.2010.029579
5. Farahmand BY, Michaelsson K, Ahlbom A, Ljunghall S, Baron JA. Survival after hip fracture. *Osteoporosis Int*. (2005) 16:1583–90. doi: 10.1007/s00198-005-2024-z
6. Langslet E, Frihagen F, Opland V, Madsen JE, Nordsletten L, Figved W. Cemented versus uncemented hemiarthroplasty for displaced femoral neck fractures: 5-year followup of a randomized trial. *Clin Orthopaed Related Res*. (2014) 472:1291–9. doi: 10.1007/s11999-013-3308-9
7. Stoen RO, Lofthus CM, Nordsletten L, Madsen JE, Frihagen F. Randomized trial of hemiarthroplasty versus internal fixation for femoral neck fractures: no differences at 6 years. *Clin Orthopaed Related Res*. (2014) 472:360–7. doi: 10.1007/s11999-013-3245-7
8. Gjertsen JE, Dybvik E, Furnes O, Fevang JM, Havelin LI, Matre K, et al. Improved outcome after hip fracture surgery in Norway. *Acta Orthopaedica*. (2017) 88:505–11. doi: 10.1080/17453674.2017.1344456
9. Laitio T, Jalonen J, Kuusela T, Scheinin H. The role of heart rate variability in risk stratification for adverse postoperative cardiac events. *Anesth Analg*. (2007) 105:1548–60. doi: 10.1213/01.ane.0000287654.49358.3a
10. Gelb AW, Cowie DA. Perioperative stroke prevention. *Anesthesia Analgesia*. (2001) 2001:46–53. doi: 10.1097/00000539-200103001-00008
11. Wong GY, Warner DO, Schroeder DR, Offord KP, Warner MA, Maxson PM, et al. Risk of surgery and anesthesia for ischemic stroke. *Anesthesiology*. (2000) 92:425. doi: 10.1097/00000542-200002000-00024
12. Marufu TC, Mannings A, Moppett IK. Risk scoring models for predicting peri-operative morbidity and mortality in people with fragility hip fractures: qualitative systematic review. *Injury*. (2015) 46:2325–34. doi: 10.1016/j.injury.2015.10.025
13. de Jong Y, Ramspek CL, van der Endt VHW, Rookmaaker MB, Blankestijn PJ, Vernooij RWM, et al. A systematic review and external validation of stroke prediction models demonstrates poor performance in dialysis patients. *J Clin Epidemiol*. (2020) 123:69–79. doi: 10.1016/j.jclinepi.2020.03.015
14. Seely AJ, Macklem PT. Complex systems and the technology of variability analysis. *Crit Care*. (2004) 8:R367–84. doi: 10.1186/cc2948
15. Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, et al. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J*. (1996) 17:354–81. doi: 10.1093/oxfordjournals.eurheartj.a014868
16. Ernst G. *Heart Rate Variability*. London: Springer (2014). doi: 10.1007/978-1-4471-4309-3
17. Scheffler P, Muccio S, Egiziano G, Doonan RJ, Yu A, Carli F, et al. Heart rate variability exhibits complication-dependent changes postsurgery. *Angiology*. (2013) 64:597–603. doi: 10.1177/0003319712461932
18. Ushiyama T, Nakatsu T, Yamane S, Tokutake H, Wakabayashi H, Ishimura K, et al. Heart rate variability for evaluating surgical stress and development of postoperative complications. *Clin Exp Hypertension*. (2008) 30:45–55. doi: 10.1080/10641960701813908
19. Filipovic M, Jeger RV, Girard T, Probst C, Pfisterer M, Gurke L, et al. Predictors of long-term mortality and cardiac events in patients with known or suspected coronary artery disease who survive major non-cardiac surgery. *Anaesthesia*. (2005) 60:5–11. doi: 10.1111/j.1365-2044.2004.03996.x
20. Pousset F, Copie X, Lechat P, Jaillon P, Boissel JP, Hetzel M, et al. Effects of bisoprolol on heart rate variability in heart failure. *Am J Cardiol*. (1996) 77:612–7. doi: 10.1016/S0002-9149(97)89316-2
21. Lin JL, Chan HL, Du CC, Lin IN, Lai CW, Lin KT, et al. Long-term beta-blocker therapy improves autonomic nervous regulation in advanced congestive heart failure: a longitudinal heart rate variability study. *Am Heart J*. (1999) 137:658–65. doi: 10.1016/S0002-8703(99)70219-X
22. Watne LO, Torbergsen AC, Conroy S, Engedal K, Frihagen F, Hjorthaug GA, et al. The effect of a pre- and postoperative orthogeriatric service on cognitive function in patients with hip fracture: randomized controlled trial (Oslo Orthogeriatric Trial). *BMC Med*. (2014) 12:63. doi: 10.1186/1741-7015-12-63
23. Ernst G, Watne LO, Frihagen F, Wyller TB, Dominik A, Rostrup M. Decreases in heart rate variability are associated with postoperative complications in hip fracture patients. *PLoS ONE*. (2017) 12:e0180423. doi: 10.1371/journal.pone.0180423
24. Sztajzel J. Heart rate variability: a non-invasive electrocardiographic method to measure the autonomic nervous system. *Swiss Medical Weekly*. (2004) 134:514–22.
25. Dahl OE, Caprini JA, Colwell CW Jr, Frostick SP, Haas S, Hull RD, et al. Fatal vascular outcomes following major orthopedic surgery. *Thrombosis Haemostasis*. (2005) 93:860–6. doi: 10.1160/TH04-11-0769
26. Grant CC, van Rensburg DC, Strydom N, Viljoen M. Importance of tachogram length and period of recording during non-invasive investigation of the autonomic nervous system. *Ann Non-invasive Electrocardiol*. (2011) 16:131–9. doi: 10.1111/j.1542-474X.2011.00422.x
27. Binici Z, Mouridsen MR, Kober L, Sajadieh A. Decreased nighttime heart rate variability is associated with increased stroke risk. *Stroke*. (2011) 42:3196–201. doi: 10.1161/STROKEAHA.110.607697
28. Fyfe-Johnson AL, Muller CJ, Alonso A, Folsom AR, Gottesman RF, Rosamond WD, et al. Heart rate variability and incident stroke: the atherosclerosis risk in communities study. *Stroke*. (2016) 47:1452–8. doi: 10.1161/STROKEAHA.116.012662
29. Yperzeele L, van Hooff RJ, Nagels G, De Smedt A, De Keyser J, Brouns R. Heart rate variability and baroreceptor sensitivity in acute stroke: a systematic review. *Int J Stroke*. (2015) 10:796–800. doi: 10.1111/ijs.12573
30. Palatini P, Julius S. The role of cardiac autonomic function in hypertension and cardiovascular disease. *Curr Hypertension Rep*. (2009) 11:199–205. doi: 10.1007/s11906-009-0035-4
31. McCraty R, Shaffer F. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Glob Adv Health Med*. (2015) 4:46–61. doi: 10.7453/gahmj.2014.073
32. Stein PK, Pu Y. Heart rate variability, sleep and sleep disorders. *Sleep Med Rev*. (2011) 16:47–66. doi: 10.1016/j.smrv.2011.02.005
33. Frenneaux MP. Autonomic changes in patients with heart failure and in post-myocardial infarction patients. *Heart*. (2004) 90:1248–55. doi: 10.1136/hrt.2003.026146
34. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front Psychol*. (2014) 5:1040. doi: 10.3389/fpsyg.2014.01040
35. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public Health*. (2017) 5:258. doi: 10.3389/fpubh.2017.00258
36. Kop WJ, Stein PK, Tracy RP, Barzilay JI, Schulz R, Gottdiener JS. Autonomic nervous system dysfunction and inflammation contribute to the increased cardiovascular mortality risk associated with depression. *Psychosom Med*. (2010) 72:626–35. doi: 10.1097/PSY.0b013e3181eadd2b
37. Schuett KA, Lehrke M, Marx N, Burgmaier M. High-risk cardiovascular patients: clinical features, comorbidities, and interconnecting mechanisms. *Front Immunol*. (2015) 6:591. doi: 10.3389/fimmu.2015.00591
38. Amory DW, Grigore A, Amory JK, Gerhardt MA, White WD, Smith PK, et al. Neuroprotection is associated with beta-adrenergic receptor antagonists during cardiac surgery: evidence from 2,575 patients. *J Cardiothoracic Vascular Anesthesia*. (2002) 16:270–7. doi: 10.1053/jcan.2002.124132
39. Jovin DG, Katlaps KG, Ellis BK, Dharmaraj B. Neuroprotection against stroke and encephalopathy after cardiac surgery. *Int Med Appl Sci*. (2019) 11:27–37. doi: 10.1556/1646.11.2019.01
40. Celik A, Koc F, Kadi H, Ceyhan K, Erkorkmaz U. Inflammation is related to unbalanced cardiac autonomic functions in hypertension: an observational study. *Anatolian J Cardiol*. (2012) 12:233–40. doi: 10.5152/akd.2012.067

41. Ziegler D, Strom A, Strassburger K, Nowotny B, Zahiragic L, Nowotny PJ, et al. Differential patterns and determinants of cardiac autonomic nerve dysfunction during endotoxemia and oral fat load in humans. *PLoS ONE*. (2015) 10:e0124242. doi: 10.1371/journal.pone.0124242
42. Pontet J, Contreras P, Curbelo A, Medina J, Noveri S, Bentancourt S, et al. Heart rate variability as early marker of multiple organ dysfunction syndrome in septic patients. *J Crit Care*. (2003) 18:156–63. doi: 10.1016/j.jcrc.2003.08.005
43. Maheshwari A, Norby FL, Soliman EZ, Adabag S, Whitsel EA, Alonso A, et al. Low heart rate variability in a 2-minute electrocardiogram recording is associated with an increased risk of sudden cardiac death in the general population: the atherosclerosis risk in communities study. *PLoS ONE*. (2016) 11:e0161648. doi: 10.1371/journal.pone.0161648
44. Janszky I, Ericson M, Mittleman MA, Wamala S, Al-Khalili F, Schenck-Gustafsson K, et al. Heart rate variability in long-term risk assessment in middle-aged women with coronary heart disease: the Stockholm Female Coronary Risk Study. *J Intern Med*. (2004) 255:13–21. doi: 10.1046/j.0954-6820.2003.01250.x
45. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, et al. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care*. (2001) 24:1793–8. doi: 10.2337/diacare.24.10.1793
46. Kikuya M, Ohkubo T, Metoki H, Asayama K, Hara A, Obara T, et al. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis: the Ohasama study. *Hypertension*. (2008) 52:1045–50. doi: 10.1161/HYPERTENSIONAHA.107.104620
47. Hankey GJ. Stroke. *Lancet*. (2017) 389:641–54. doi: 10.1016/S0140-6736(16)30962-X
48. Phua J, Dean NC, Guo Q, Kuan WS, Lim HF, Lim TK. Severe community-acquired pneumonia: timely management measures in the first 24 h. *Crit Care*. (2016) 20:237. doi: 10.1186/s13054-016-1414-2

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.

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Gender- and Age-Specific Associations of Visit-to-Visit Blood Pressure Variability With Anxiety

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Background: There is a bidirectional relationship between blood pressure variability (BPV) and anxiety, but few studies have examined the gender- and age-specific effects of visit-to-visit BPV on incident anxiety. We examined the predictive value of BPV for the incidence of anxiety in a family clinic cohort.

Methods: Consecutive patients with a first attendance to family medicine clinics in Hong Kong between January 1, 2000, and December 31, 2002, with at least three blood pressure measurements available thereafter were included. The primary endpoint was incident anxiety as identified by ICD-9 coding.

Results: This study included 48,023 (50% males) patients with a median follow-up of 224 [interquartile range (IQR): 217–229] months. Females were more likely to develop incident anxiety compared to males (incidence rate: 7 vs. 2%), as were patients of older age. Significant univariate predictors were female gender, older age, preexisting cardiovascular diseases, respiratory diseases, diabetes mellitus, hypertension, and gastrointestinal diseases, various laboratory examinations, and the number of blood pressure measurements. Higher baseline, maximum, minimum, standard deviation (SD), coefficient of variation (CV), and variability score of diastolic blood pressure significantly predicted incident anxiety, as did all systolic blood pressure measures [baseline, latest, maximum, minimum, mean, median, variance, SD, root mean square (RMS), CV, and variability score].

Conclusions: The relationships between longer-term visit-to-visit BPV and incident anxiety were identified. Female and older patients with higher blood pressure and higher BPV were at the highest risks of incident anxiety.

Keywords: blood pressure variability, generalized anxiety disorder, risk prediction, visit-to-visit blood pressure variability, anxiety

INTRODUCTION

Anxiety is a common symptom, and anxiety disorder includes a group of conditions characterized by excessive worry associated with fatigue, restlessness, muscle tension, irritability, sleeping difficulty, and concentration problems. It is a major public health problem in many countries, damaging not only psychological health but also physical health and quality of life. There is a bidirectional relationship between blood pressure variability (BPV) and incident anxiety. The presence of anxiety can exert effects on BPV. Patients with depressive symptoms presented a significantly lower nighttime systolic blood pressure (BP) fall compared with non-depressed patients after controlling for age, sex, and traditional cardiovascular risk factors (1). The control of negative emotions has been shown to influence BP control and BPV (2). Conversely, increased beat-to-beat BPV has been associated with incident anxiety (3). Longer-term visit-to-visit BPV has also been reported as an independent predictor of cognitive impairment in several cohort studies (4–6). With the widespread measurement of BP measurements at home, fluctuations in BP, as well as very high or low BP readings at home, can cause anxiety in patients. However, few previous studies have examined the longitudinal relationship between BPV and anxiety disorders in older cohorts. In this study, we investigated the gender- and age-specific associations of longer-period visit-to-visit BPV with the incidence of anxiety.

METHODS

Research Design and Data Sources

The study was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee and Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. This was a retrospective cohort study of patients who attended family medicine clinics between January 1, 2000, and March 31, 2002, in the Hong Kong public sector. Patients with at least three BP measurements before being diagnosed with anxiety were included to calculate the variability measures. There were no exclusion criteria. The patients were identified from the Clinical Data Analysis and Reporting System (CDARS), a territory-wide database that centralizes patient information from individual local hospitals to establish comprehensive medical data, including clinical characteristics, disease diagnosis, laboratory results, and medication prescription details. The system has been previously used by both our team and other teams in Hong Kong to conduct studies on comparative drug action (7), specific diseases (8–10), model development (11), or visit-to-visit variability in metabolic parameters (12, 13). Data were obtained regarding consecutive patients diagnosed with anxiety, excluding those who died or discharged within 24 h after the first diastolic/systolic BP measurement and those with fewer than three diastolic/systolic BP measurements (study baseline). Mortality data were obtained from the Hong Kong Death Registry, a population-based official government registry with the registered death records of all Hong Kong citizens. Data on the clinical characteristics, disease diagnosis, laboratory results (including complete blood count, renal and liver function tests, glycemic and lipid profiles, and diastolic/systolic BP), and medication prescription details were extracted. Patients with anxiety were identified with the diagnosis codes 311, 296.3, 296.2, 308, 300.4, 292.84, 298, 300.02, 291.89, 293.84, 292.89, 294.9, 300.2, 309.24, 300.01, 309.21. The ICD-9 codes for past comorbidities and historical medication prescriptions are detailed in the **Supplementary Material**.

Primary Outcome and Statistical Analysis

The primary outcome was incident cases of anxiety from the study baseline in a time-to-event analysis. Follow-up was carried out until December 31, 2019. We extracted the baseline/latest/maximum/minimum values of diastolic and systolic BPs and calculated the temporal variability measures of diastolic and systolic BPs: (1) mean, (2) median, (3) standard deviation (SD), (4) root mean square (RMS) by first squaring all BP values and then calculating the square root of the mean of the squares, (5) coefficient of variation (CV) by dividing the BP SD by the mean BP and then multiplying by 100, and (6) a variability score [from 0 (low) to 100 (high)] defined as the number of changes in BP of 5 mmHg or more, i.e., 100 × (number of absolute BP change of each two successive measurements > 5)/number of measurements.

Clinical characteristics were summarized using statistical descriptive statistics. Continuous variables were presented as median [95% confidence interval (CI) or interquartile range (IQR)], and categorical variables were presented count (%). The Mann–Whitney *U*-test was used to compare continuous variables. The χ^2-test with Yates' correction was used for 2×2 contingency data, and Pearson's χ^2-test was used for contingency data for variables with more than two categories. Univariate Cox regression models were conducted based on cohorts of males and females, respectively, to identify the significant predictors of anxiety. Significant univariate predictors of demographics, prior comorbidities, clinical and biochemical tests, medication prescriptions, and BPV were used as input of a multivariate Cox analysis model, adjusted for demographics and comorbidities. Hazard ratios (HRs) with corresponding 95% CIs and *p*-values were reported. All significance tests were two-tailed and considered significant if *p*-values < 0.001. Data analysis was performed using the RStudio software (version: 1.1.456) and Python (version: 3.6).

RESULTS

Baseline Clinical Characteristics and Anxiety Incidence

This study included a total of 48,023 (50% males) patients with a median follow-up of 224 (IQR: 217–229) months (**Supplementary Figure 1**). Among the 23,964 male patients, 495 (incidence rate: 2.1%, median age: 70 [IQR: 57–79] years old) developed anxiety. By contrast, females had a higher incidence rate, with 1,687 of 24,059 (incidence rate: 7.0%, median age: 68 [IQR: 56–78] years old) developing anxiety.

The clinical characteristics of the included patients are provided in **Table 1**. Compared with female patients, male

TABLE 1 | Clinical characteristics of patients included in this cohort.

Characteristics	Males (*N* = 23,964; event: 495, incidence rate: 2.07%; mortality: 195, 39.4%) Median (IQR); Max; N or Count (%)	Females (*N* = 24,059; event: 1,687, incidence rate: 7.01%; mortality: 431, 25.68%) Median (IQR); Max; N or Count (%)	*p*
Demographics			
Age of first BP test, years	61.4 (50.8–69.8); *n* = 495	59.3 (49.3–69.3); *n* = 1,678	0.1453
Past comorbidity			
Cardiovascular	347 (70.10%)	1,209 (72.05%)	0.2253
Respiratory	315 (63.63%)	889 (52.97%)	<0.0001***
Kidney	148 (29.89%)	287 (17.10%)	<0.0001***
Endocrine	25 (5.05%)	83 (4.94%)	0.2588
Diabetes mellitus	79 (15.95%)	287 (17.10%)	0.1086
Hypertension	336 (67.87%)	1,131 (67.40%)	0.3494
Gastrointestinal	275 (55.55%)	942 (56.13%)	0.2378
Obesity	3 (0.60%)	6 (0.35%)	0.8561
Stroke	190 (38.38%)	475 (28.30%)	<0.0001***
Medications			
ACEI	90 (18.18%)	188 (11.20%)	0.0025**
ARB	2 (0.40%)	9 (0.53%)	0.8262
Calcium channel blockers	140 (28.28%)	379 (22.58%)	0.1477
Beta blockers	156 (31.51%)	537 (32.00%)	0.5235
Diuretics for heart failure	14 (2.82%)	51 (3.03%)	0.5597
Diuretics for hypertension	59 (11.91%)	197 (11.74%)	0.2936
Nitrates	74 (14.94%)	203 (12.09%)	0.8393
Antihypertensive drugs	114 (23.03%)	81 (4.82%)	<0.0001***
Antidiabetic drugs	54 (10.90%)	175 (10.42%)	0.5855
Statins and fibrates	75 (15.15%)	258 (15.37%)	0.6512
Complete blood count			
Mean corpuscular volume, fl	91.3 (88.3–94.0); *n* = 242	89.4 (86.2–92.5); *n* = 889	0.1994
Basophil, $\times 10^9$/L	0.04 (0.02–0.046); *n* = 113	0.03 (0.01–0.03); *n* = 390	0.35
Eosinophil, $\times 10^9$/L	0.1 (0.1–0.2); *n* = 132	0.1 (0.1–0.2); *n* = 485	0.3711
Lymphocyte, $\times 10^9$/L	1.78 (1.3–2.2); *n* = 133	1.8 (1.4–2.4); *n* = 489	0.1135
Monocyte, $\times 10^9$/L	0.5 (0.4–0.7); *n* = 132	0.4 (0.3–0.6); *n* = 486	0.6279
Neutrophil, $\times 10^9$/L	4.3 (3.5–6.55); *n* = 132	4.1 (3.2–5.53); *n* = 485	0.6455
White cell count, $\times 10^9$/L	7.16 (6.0–8.95); *n* = 243	6.8 (5.6–8.3); *n* = 892	0.787
Mean corpuscular hemoglobin, pg	31.1 (29.85–32.1); *n* = 242	30.5 (29.1–31.5); *n* = 889	0.8937
Platelet, $\times 10^9$/L	229.0 (191.0–265.0); *n* = 243	244.0 (209.0–293.0); *n* = 891	0.431
Reticulocyte, $\times 10^9$/L	31.9 (28.8–60.9); *n* = 3	60.9 (44.0–91.77); *n* = 17	<0.0001***
Red cell count, $\times 10^{12}$/L	4.65 (4.34–5.01); *n* = 241	4.31 (4.04–4.6); *n* = 889	0.3762
Hematocrit, L/L	0.43 (0.4–0.45); *n* = 226	0.38 (0.4–0.4); *n* = 836	0.9949
Renal and liver function tests			
Potassium, mmol/L	4.15 (3.89–4.5); *n* = 337	4.2 (3.9–4.47); *n* = 1,041	0.297
Urate, mmol/L	0.398 (0.35–0.475); *n* = 86	0.32 (0.26–0.39); *n* = 253	0.1253
Albumin, g/L	42.25 (40.0–44.368); *n* = 290	42.0 (39.8–44.0); *n* = 941	0.9327
Sodium, mmol/L	141.0 (139.0–142.0); *n* = 337	141.0 (139.0–142.0); *n* = 1,041	0.5236
Urea, mmol/L	5.7 (4.835–6.8); *n* = 336	5.3 (4.3–6.3); *n* = 1,039	0.9642
Protein, g/L	74.0 (70.69–77.35); *n* = 287	74.0 (71.0–78.0); *n* = 939	0.6126
Creatinine, μmol/L	98.0 (88.0–111.0); *n* = 337	75.0 (67.0–85.0); *n* = 1,041	<0.0001***
Alkaline phosphatase, U/L	75.0 (60.84–92.0); *n* = 248	77.0 (60.0–93.5); *n* = 815	0.7759
Aspartate transaminase, U/L	23.0 (17.0–27.0); *n* = 68	21.0 (18.0–26.0); *n* = 214	0.544
Alanine transaminase, U/L	23.0 (17.0–35.0); *n* = 225	19.0 (14.0–27.0); *n* = 740	0.0023**
Bilirubin, μmol/L	10.0 (7.5–13.0); *n* = 250	9.0 (6.3874–11.3); *n* = 824	0.3542

(Continued)

TABLE 1 | Continued

Characteristics	Males ($N = 23,964$; event: 495, incidence rate: 2.07%; mortality: 195, 39.4%) Median (IQR); Max; N or Count (%)	Females ($N = 24,059$; event: 1,687, incidence rate: 7.01%; mortality: 431, 25.68%) Median (IQR); Max; N or Count (%)	*p*
Glycemic and lipid profiles			
Triglyceride, mmol/mol	1.6 (1.1–2.3); $n = 156$	1.4 (0.97–2.0); $n = 546$	0.6491
LDL, mmol/mol	3.3 (2.8–3.7); $n = 116$	3.2 (2.6–3.9); $n = 373$	0.3887
HDL, mmol/mol	1.2 (1.02–1.4); $n = 118$	1.4 (1.195–1.66); $n = 390$	0.3572
HbA1c, g/dl	13.9 (12.9–15.1); $n = 215$	12.8 (11.7–13.6); $n = 788$	0.0031**
Cholesterol, mmol/L	5.2 (4.6–5.7); $n = 156$	5.4 (4.8–6.1); $n = 548$	0.128
Fasting glucose, mmol/L	5.8 (5.2–7.5); $n = 240$	5.6 (5.1–7.0); $n = 787$	0.9374
Diastolic blood pressure measurements			
Number of measurements	7 (5–10); $n = 495$	7 (6–10); $n = 1,678$	0.1225
Baseline, mmHg	77.0 (68.0–84.5); $n = 495$	72.0 (65.0–80.0); $n = 1,678$	0.041*
Latest, mmHg	74.0 (66.0–81.0); $n = 495$	70.0 (63.0–78.0); $n = 1,678$	0.6643
Maximum, mmHg	89.0 (81.5–97.0); $n = 495$	86.0 (79.0–93.0); $n = 1,678$	0.8432
Minimal, mmHg	63.0 (56.0–70.0); $n = 495$	58.0 (52.0–66.0); $n = 1,678$	0.0323*
Mean, mmHg	75.4 (69.8–81.6); $n = 495$	71.9 (66.2–77.2); $n = 1,678$	0.0065**
Median, mmHg	75.5(69.5–81.0); $n = 495$	71.5 (66.0–77.0); $n = 1,678$	0.0234*
Variance	54.3 (31.4–88.9); $n = 495$	56.1 (33.4–84.3); $n = 1,678$	0.2326
SD	7.4 (5.6–9.4); $n = 495$	7.5 (5.8–9.2); $n = 1,678$	0.5344
RMS	75.9 (70.3–81.9); $n = 495$	72.3 (66.6–77.6); $n = 1,678$	0.0422*
CV	0.09 (0.07–0.12); $n = 495$	0.1001 (0.073–0.1); $n = 1,678$	<0.0001***
Variability score	57.1 (46.2–66.7); $n = 495$	55.8 (47.4–66.2); $n = 1,678$	0.5416
Systolic blood pressure measurements			
Number of measurements			
Baseline, mmHg	135.0 (120.0–150.0); $n = 495$	132.0 (117.0–147.0); $n = 1,678$	0.1594
Latest, mmHg	132.0 (121.0–143.0); $n = 495$	131.0 (119.0–142.0); $n = 1,678$	0.7247
Maximum, mmHg	156.0 (144.0–169.0); $n = 495$	157.0 (140.0–173.0); $n = 1,678$	0.7289
Minimal, mmHg	111.0 (102.0–121.0); $n = 495$	109.0 (101.0–120.0); $n = 1,678$	0.1322
Mean, mmHg	134.0 (125.4–142.4); $n = 495$	132.5 (123.3–141.0); $n = 1,678$	0.3252
Median, mmHg	133.0 (125.0–142.0); $n = 495$	132.0 (122.5–141.0); $n = 1,678$	0.2253
Variance	165.7 (96.9–255.0); $n = 495$	161.8 (91.7–256.7); $n = 1,678$	0.1806
SD	12.9 (9.8–15.9); $n = 495$	12.7 (9.6–16.0); $n = 1,678$	0.2178
RMS	134.7 (126.2–143.0); $n = 495$	133.4 (124.0–141.9); $n = 1,678$	0.2509
CV	0.09 (0.07–0.11); $n = 495$	0.09 (0.07–0.12); $n = 1,678$	0.2264
Variability score	70.0 (60.0–77.0); $n = 495$	70.6 (57.1–78.6); $n = 1,678$	0.7872

ACEI, angiotensinogen-converting enzyme inhibitor; ARB, angiotensin receptor blocker; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; SD, standard deviation; RMS, root mean square; CV, coefficient of variation.
*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.*

patients were more likely to suffer from cardiovascular diseases (63.63 vs. 52.97%, $p < 0.0001$), kidney disease (29.89 vs. 17.10%, $p < 0.0001$), and stroke (38.38 vs. 28.30%, $p < 0.0001$). They were more likely to be prescribed angiotensinogen-converting enzyme inhibitors (ACEI) (18.18 vs. 11.20%) and other antihypertensive drugs (23.03 vs. 4.82%) than female patients.

Nevertheless, female patients had a higher reticulocyte level (median: 60.9, IQR: 44.0–91.77 vs. median: 31.9, IQR: 28.8–60.9, $p < 0.0001$), lower creatinine level (median: 75.0, IQR: 67.0–85.0 vs. median: 98.0, IQR: 88.0–111.0, $p < 0.0001$), lower alanine transaminase amount (median: 19.0, IQR: 14.0–27.0 vs. median: 23.0, IQR: 17.0–35.0, $p = 0.0023$), and lower HbA1c level (median: 12.8, IQR: 11.7–13.6 vs. median: 13.9, IQR: 12.9–15.1, $p = 0.0031$). Regarding diastolic BP measurements, female patients had a lower mean (median: 58.0, IQR: 52.0–66.0 vs. median: 63.0, IQR: 56.0–70.0, $p = 0.0323$), median (median: 71.9, IQR: 66.2–77.2 vs. median: 75.4, IQR: 69.8–81.6, $p = 0.0065$), RMS (median: 72.3, IQR: 66.6–77.6 vs. median: 75.9, IQR: 70.3–81.9, $p = 0.0422$), and CV (median: 0.1001, IQR: 0.073–0.1 vs. median: 0.09, IQR: 0.07–0.12, $p < 0.0001$).

Incidence of Anxiety on Follow-Up and Significant Predictors

The age-specific incidences of anxiety among male and female subgroups are shown in **Figure 1**. The number of female patients developing anxiety was more than double that of male patients

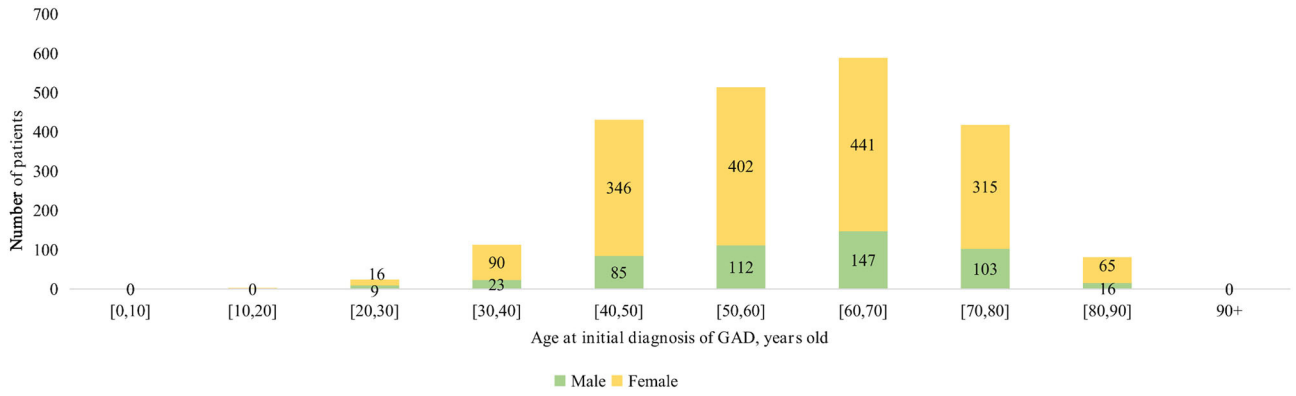

FIGURE 1 | Age-specific incidence of anxiety among male and female subgroups.

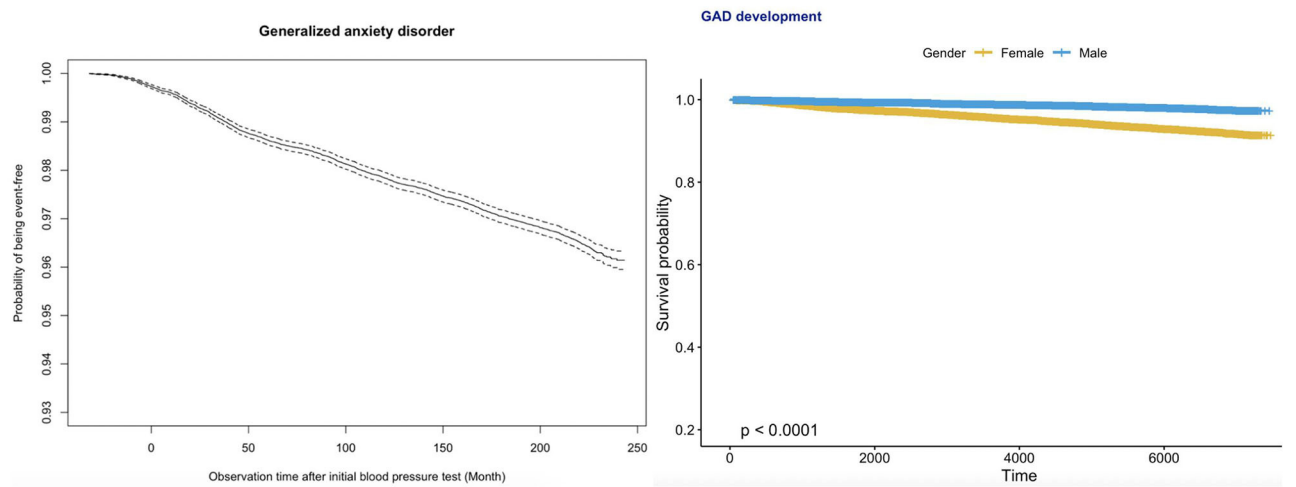

FIGURE 2 | Kaplan–Meier survival curves of incident anxiety development for the whole cohort (top) and stratified by gender (bottom).

among those over 30 years of age. Kaplan–Meier survival curves in **Figure 2** show that females had a higher risk of developing anxiety than males.

Univariate Cox regression demonstrated the following significant predictors for incident anxiety: demographics, namely, gender [female as comparison: HR for male: 0.30, 95% CI: [0.27, 0.33], $p < 0.0001^{***}$] and older age [HR: 1.23, 95% CI: [1.19, 2.03], $p < 0.0001$]. The specific risks differed between age groups: [40, 50] years old [HR: 1.42, 95% CI: [1.27, 1.57], $p < 0.0001$], [50, 60] years old [HR: 1.30, 95% CI: [1.18, 1.43], $p < 0.0001$], [60, 70] years old [HR: 1.11, 95% CI: [1.01, 1.22], $p = 0.0008$], [70, 80] years old [HR: 1.71, 95% CI: [1.64, 1.79], $p < 0.0001$], [80, 90] years old [HR: 1.46, 95% CI: [1.37, 1.57], $p < 0.0001$]; past history of cardiovascular diseases [HR: 4.00, 95% CI: [3.64, 4.39], $p < 0.0001$], respiratory diseases [HR: 1.33, 95% CI: [1.22, 1.45], $p < 0.0001$], diabetes mellitus [HR: 1.17, 95% CI: [1.05, 1.31], $p = 0.0062$], hypertension [HR: 1.17, 95% CI: [1.07, 1.28], $p = 0.0008$], and gastrointestinal disorders [HR: 1.90, 95% CI: [1.74, 2.07], $p < 0.0001$]; laboratory parameters, namely, lower neutrophil [HR: 0.35, 95% CI: [0.24, 0.50], $p < 0.0001$], less white cell count [HR: 0.92, 95% CI: [0.89, 0.95], $p < 0.0001$], lower mean corpuscular hemoglobin level [HR: 0.94, 95% CI: [0.92, 0.96], $p < 0.0001$], higher red cell count [HR: 1.001, 95% CI: [1.001, 1.002], $p = 0.0002$], lower urate level [HR: 0.03, 95% CI: [0.01, 0.07], $p < 0.0001$], higher albumin level [HR: 1.04, 95% CI: [1.03, 1.06], $p < 0.0001$], lower urea level [HR: 0.87, 95% CI: [0.85, 0.90], $p < 0.0001$], lower creatinine level [HR: 0.98, 95% CI: [0.979, 0.982], $p < 0.0001$], lower alkaline phosphatase level [HR: 0.995, 95% CI: [0.993, 0.997], $p < 0.0001$], lower bilirubin level [HR: 0.97, 95% CI: [0.95, 0.98], $p < 0.0001$], higher high-density lipoprotein (HDL) level [HR: 1.54, 95% CI: [1.25, 1.91], $p = 0.0001$], and lower fasting glucose level [HR: 0.96, 95% CI: [0.93, 0.98], $p = 0.0007$]; diastolic BP measures, namely, higher baseline value [HR: 1.49, 95% CI: [1.08, 2.45], $p < 0.0001$], higher maximum value [HR: 1.19, 95% CI: [1.06, 1.54], $p < 0.0001$], higher minimum value [HR: 1.23, 95% CI: [1.08, 2.06], $p < 0.0001$], larger SD [HR: 1.18, 95% CI: [1.03, 1.95], $p = 0.0008$], larger CV [HR: 1.13, 95% CI: [1.05, 1.38],

TABLE 2 | Univariate Cox analysis to predict incident anxiety and mortality.

Characteristics	Anxiety (*N* = 2,173) HR [95% CI]	*P*	Mortality (*N* = 626) HR [95% CI]	*p*
Demographics				
Male gender	0.30 [0.27, 0.33]	<0.0001***	1.74 [1.69, 1.79]	<0.0001***
Age at first blood pressure test, years	1.23 [1.19, 2.03]	<0.0001***	1.10 [1.10, 1.11]	<0.0001***
[0, 10]	-	-	-	-
[10, 20]	0.31 [0.10, 0.97]	0.0446*	0.01 [0.00, 0.07]	<0.0001***
[20, 30]	0.54 [0.36, 0.79]	0.0019**	0.13 [0.10, 0.17]	<0.0001***
[30, 40]	1.10 [0.91, 1.33]	0.3140	0.06 [0.05, 0.08]	<0.0001***
[40, 50]	1.42 [1.27, 1.57]	<0.0001***	0.11 [0.10, 0.12]	<0.0001***
[50, 60]	1.30 [1.18, 1.43]	<0.0001***	0.25 [0.24, 0.27]	<0.0001***
[60, 70]	1.11 [1.01, 1.22]	0.0008**	0.97 [0.94, 1.00]	0.0749
[70, 80]	1.71 [1.64, 1.79]	<0.0001***	3.51 [3.41, 3.61]	<0.0001***
[80, 90]	1.46 [1.37, 1.57]	<0.0001***	6.00 [5.78, 6.23]	<0.0001***
90+	-	0.9640	8.76 [7.75, 9.89]	<0.0001***
Past comorbidity				
Cardiovascular	4.00 [3.64, 4.39]	<0.0001***	2.18 [2.12, 2.24]	<0.0001***
Respiratory	1.33 [1.22, 1.45]	<0.0001***	3.90 [3.77, 4.02]	<0.0001***
Kidney	0.87 [0.78, 0.96]	0.0076**	2.15 [2.09, 2.22]	<0.0001***
Endocrine	1.12 [0.92, 1.36]	0.2500	2.08 [1.97, 2.20]	<0.0001***
Diabetes mellitus	1.17 [1.05, 1.31]	0.0062**	0.96 [0.92, 1.00]	0.0385*
Hypertension	1.17 [1.07, 1.28]	0.0008***	0.87 [0.84, 0.90]	<0.0001***
Gastrointestinal	1.90 [1.74, 2.07]	<0.0001***	1.20 [1.17, 1.23]	<0.0001***
Obesity	2.07 [1.08, 3.98]	0.0296*	0.39 [0.25, 0.62]	<0.0001***
Stroke	1.07 [0.98, 1.17]	0.1410	1.94 [1.89, 2.00]	<0.0001***
Medications				
ACEI	0.66 [0.59, 0.75]	<0.0001***	1.80 [1.74, 1.86]	<0.0001***
ARB	0.80 [0.44, 1.45]	0.4600	1.50 [1.29, 1.75]	<0.0001***
Calcium channel blockers	0.70 [0.64, 0.78]	<0.0001***	1.85 [1.80, 1.91]	<0.0001***
Beta blockers	1.20 [1.10, 1.32]	0.0001***	1.06 [1.03, 1.10]	0.0001***
Diuretics for heart failure	0.67 [0.52, 0.85]	0.0013**	3.98 [3.79, 4.18]	<0.0001***
Diuretics for hypertension	0.84 [0.73, 0.95]	0.0073**	1.40 [1.35, 1.45]	<0.0001***
Nitrates	1.05 [0.93, 1.20]	0.4130	1.91 [1.84, 1.98]	<0.0001***
Antihypertensive drugs	0.72 [0.62, 0.84]	<0.0001***	2.23 [2.15, 2.31]	<0.0001***
Antidiabetic drugs	0.59 [0.51, 0.67]	<0.0001***	1.40 [1.35, 1.45]	<0.0001***
Statins and fibrates	1.11 [0.98, 1.24]	0.0908	1.06 [1.02, 1.10]	0.0068**
Complete blood count				
Mean corpuscular volume, fl	0.995 [0.988, 1.002]	0.1930	1.021 [1.018, 1.024]	<0.0001***
Basophil, $\times 10^9$/L	0.20 [0.02, 1.68]	0.1370	2.00 [1.05, 3.79]	0.0339*
Eosinophil, $\times 10^9$/L	0.81 [0.54, 1.22]	0.3160	1.11 [1.02, 1.21]	0.0178*
Lymphocyte, $\times 10^9$/L	1.03 [1.01, 1.06]	0.0161*	0.66 [0.63, 0.68]	<0.0001***
Monocyte, $\times 10^9$/L	1.88 [0.33, 10.83]	0.4800	1.04 [0.65, 1.66]	0.871
Neutrophil, $\times 10^9$/L	0.35 [0.24, 0.50]	<0.0001***	2.10 [1.96, 2.26]	<0.0001***
White cell count, $\times 10^9$/L	0.92 [0.89, 0.95]	<0.0001***	1.08 [1.08, 1.09]	<0.0001***
Mean corpuscular hemoglobin, pg	0.94 [0.92, 0.96]	<0.0001***	1.06 [1.06, 1.06]	<0.0001***
Platelet, $\times 10^9$/L	1.00 [0.98, 1.01]	0.6170	1.03 [1.03, 1.04]	<0.0001***
Reticulocyte, $\times 10^9$/L	11.70 [0.38, 357.50]	0.1590	1.78 [0.89, 3.56]	0.105
Red cell count, $\times 10^{12}$/L	1.001 [1.001, 1.002]	0.0002***	0.998 [0.998, 0.998]	<0.0001***
Hematocrit, L/L	1.003 [0.993, 1.013]	0.5270	0.999 [0.996, 1.001]	0.312
Basophil, $\times 10^9$/L	0.97 [0.88, 1.06]	0.4620	0.57 [0.55, 0.59]	<0.0001***
Eosinophil, $\times 10^9$/L	0.63 [0.19, 2.07]	0.4490	0.50 [0.12, 0.81]	<0.0001***

(Continued)

TABLE 2 | Continued

Characteristics	Anxiety (*N* = 2,173) HR [95% CI]	*P*	Mortality (*N* = 626) HR [95% CI]	*p*
Renal and liver function tests				
Potassium, mmol/L	0.88 [0.79, 0.97]	0.0102*	0.95 [0.92, 0.98]	0.0026 **
Urate, mmol/L	0.03 [0.01, 0.07]	<0.0001***	10.65 [8.23, 13.77]	<0.0001***
Albumin, g/L	1.04 [1.03, 1.06]	<0.0001***	0.88 [0.87, 0.88]	<0.0001***
Sodium, mmol/L	1.02 [1.00, 1.04]	0.0196*	0.948 [0.943, 0.952]	<0.0001***
Urea, mmol/L	0.87 [0.85, 0.90]	<0.0001***	1.111 [1.108, 1.114]	<0.0001***
Protein, g/L	1.01 [1.00, 1.02]	0.0528	0.95 [0.95, 0.95]	<0.0001***
Creatinine, μmol/L	0.98 [0.979, 0.982]	<0.0001***	1.003 [1.003, 1.003]	<0.0001***
Alkaline phosphatase, U/L	0.995 [0.993, 0.997]	<0.0001***	1.002 [1.002, 1.002]	<0.0001***
Aspartate transaminase, U/L	1.000 [0.998, 1.001]	0.6580	1.001 [1.000, 1.001]	<0.0001***
Alanine transaminase, U/L	0.999 [0.997, 1.001]	0.4250	0.998 [0.997, 0.999]	<0.0001***
Bilirubin, μmol/L	0.97 [0.95, 0.98]	<0.0001***	1.007 [1.005, 1.009]	<0.0001***
Glycemic and lipid profiles				
Triglyceride, mmol/mol	0.98 [0.92, 1.04]	0.4300	0.97 [0.95, 0.99]	0.0024 **
LDL, mmol/mol	1.03 [0.93, 1.13]	0.6050	0.91 [0.88, 0.95]	<0.0001***
HDL, mmol/mol	1.54 [1.25, 1.91]	0.0001***	0.82 [0.75, 0.90]	<0.0001***
HbA1c, g/dl	1.01 [1.00, 1.03]	0.0613	0.98 [0.98, 0.98]	<0.0001***
Cholesterol, mmol/L	1.04 [0.98, 1.11]	0.2230	0.92 [0.90, 0.94]	<0.0001***
Fasting glucose, mmol/L	0.96 [0.93, 0.98]	0.0007***	1.05 [1.05, 1.06]	<0.0001***
Diastolic blood pressure measurements				
Number of measurements	0.89 [0.18, 1.23]	0.1352	0.53 [0.42, 1.71]	0.2342
Baseline, mmHg	1.49 [1.08, 2.45]	<0.0001***	1.03 [1.01, 1.24]	<0.0001***
Latest, mmHg	1.001 [0.997, 1.004]	0.6870	1.07 [1.02, 1.28]	<0.0001***
Maximum, mmHg	1.19 [1.06, 1.54]	<0.0001***	1.04 [1.02, 1.08]	<0.0001***
Minimal, mmHg	1.23 [1.08, 2.06]	<0.0001***	1.09 [1.01, 1.2]	0.0442*
Mean, mmHg	1.03 [1.01, 1.39]	0.0011**	1.1 [1.03, 1.7]	<0.0001***
Median, mmHg	1.04 [1.01, 1.53]	0.0026**	1.28 [1.16, 1.98]	<0.0001***
Variance	1.002 [1.001, 1.003]	0.0491*	1.003 [1.003, 1.003]	<0.0001***
SD	1.18 [1.03, 1.95]	0.0008***	1.06 [1.05, 1.06]	<0.0001***
RMS	1.23 [1.09, 1.6]	0.0078**	1.18 [1.02, 1.93]	<0.0001***
CV	1.13 [1.05, 1.38]	0.0002***	40.89 [28.15, 59.38]	<0.0001***
Variability score	1.16 [1.04, 1.85]	0.0003***	1.001 [1.000, 1.002]	0.0082**
Systolic blood pressure measurements				
Number of measurements				
Baseline, mmHg	1.25 [1.04, 1.93]	<0.0001***	1.015 [1.014, 1.015]	<0.0001***
Latest, mmHg	1.34 [1.12, 1.88]	<0.0001***	1.009 [1.008, 1.010]	<0.0001***
Maximum, mmHg	1.15 [1.04, 1.33]	<0.0001***	1.009 [1.009, 1.010]	<0.0001***
Minimal, mmHg	1.04 [1.01, 1.07]	<0.0001***	1.022 [1.021, 1.023]	<0.0001***
Mean, mmHg	1.26 [1.03, 1.56]	<0.0001***	1.031 [1.030, 1.032]	<0.0001***
Median, mmHg	1.16 [1.04, 1.29]	<0.0001***	1.030 [1.029, 1.031]	<0.0001***
Variance	1.04 [1.01, 1.1]	<0.0001***	1.001 [1.001, 1.001]	<0.0001***
SD	1.17 [1.07, 1.85]	<0.0001***	1.061 [1.059, 1.063]	<0.0001***
RMS	1.59 [1.18, 1.99]	<0.0001***	1.031 [1.030, 1.032]	<0.0001***
CV	1.07 [1.02, 1.23]	<0.0001***	629.90 [424.60, 934.40]	<0.0001***
Variability score	1.26 [1.04, 1.82]	0.0008 ***	1.000 [0.999, 1.001]	0.8170

ACEI, angiotensinogen-converting enzyme inhibitor; ARB, angiotensin receptor blocker; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; SD, standard deviation; RMS, root mean square; CV, coefficient of variation.
$^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

TABLE 3 | Multivariate Cox regression analysis to predict incident anxiety.

	HR [95% CI]	*Z*-value	*p*
Demographics			
Male gender	0.23 [0.11, 0.48]	−3.93	0.0001***
Age at first blood pressure test, years	1.04 [1.02, 1.07]	0.15	0.0029**
[20, 30]	1.24 [1.26, 5.98]	0.27	0.0019**
[30, 40]	1.07 [1.02, 2.14]	0.56	0.0034**
[40, 50]	1.98 [1.38, 10.24]	0.82	<0.0001***
[50, 60]	1.47 [1.22, 9.92]	0.39	0.0056**
[60, 70]	1.48 [1.05, 4.88]	0.63	<0.0001***
[70, 80]	1.06 [1.01, 16.38]	0.004	0.0069**
Past comorbidities			
Cardiovascular	3.69 [1.99, 6.84]	4.16	<0.0001***
Respiratory	1.68 [0.96, 2.94]	1.83	0.0670
Kidney	1.72 [0.98, 3.04]	1.88	0.0603
Diabetes mellitus	2.04 [1.14, 3.68]	2.39	0.0169*
Hypertension	1.42 [0.69, 2.88]	0.96	0.3390
Gastrointestinal	1.24 [0.74, 2.07]	0.82	0.4101
Medications			
ACEI	0.95 [0.54, 1.68]	−0.18	0.8545
Calcium channel blockers	0.62 [0.36, 1.09]	−1.67	0.0947
Beta blockers	1.54 [1.12, 2.59]	1.65	0.0016**
Diuretics for heart failure	1.68 [0.77, 3.67]	1.31	0.1915
Diuretics for hypertension	0.92 [0.51, 1.67]	−0.27	0.7879
Antihypertensive drugs	0.77 [0.36, 1.68]	−0.66	0.5125
Antidiabetic drugs	0.39 [0.20, 1.79]	−2.61	0.242
Complete blood count			
Neutrophil, $\times 10^9$/L	0.98 [0.85, 1.14]	−0.21	0.8336
White cell count, $\times 10^9$/L	1.00 [0.87, 1.14]	−0.03	0.9728
Mean corpuscular hemoglobin, pg	1.07 [0.95, 1.21]	1.09	0.2749
Red blood count, $\times 10^{12}$/L	1.08 [0.58, 1.99]	0.23	0.8179
Renal and liver function tests			
Urate, mmol/L	0.21 [0.02, 2.51]	−1.24	0.2161
Albumin, g/L	1.00 [0.93, 1.07]	−0.14	0.8891
Urea, mmol/L	0.97 [0.83, 1.13]	−0.42	0.6770
Creatinine, μmol/L	1.00 [0.98, 1.01]	−0.65	0.5184
Alkaline phosphatase, U/L	0.99 [0.98, 1.00]	−1.44	0.1498
Bilirubin, μmol/L	1.02 [1.00, 1.04]	2.22	0.0264*
Glycemic and lipid profiles			
HDL, mmol/mol	1.00 [0.50, 1.98]	−0.01	0.9921
Fasting glucose, mmol/L	1.04 [0.98, 1.11]	1.32	0.1885
Diastolic blood pressure measurements			
Baseline, mmHg	1.03 [1.01, 1.07]	1.69	<0.0001***
Maximum, mmHg	0.98 [0.91, 1.06]	−0.48	0.6316
Minimal, mmHg	1.04 [1.01, 1.14]	0.90	0.0071**
Mean, mmHg	1.10 [1.02, 1.46]	0.61	0.0011**
Median, mmHg	0.91 [0.74, 1.12]	−0.9	0.3679
SD	0.70 [0.40, 1.24]	−1.23	0.2204
CV	1.05 [1.01, 1.12]	1.05	0.0659*
Variability score	1.02 [1.00, 1.04]	2.01	0.0047**
Systolic blood pressure measurements			
Baseline, mmHg	1.15 [1.08, 1.33]	0.40	<0.0001***
Latest, mmHg	1.01 [0.99, 1.03]	1.18	0.2389
Maximum, mmHg	1.00 [0.96, 1.04]	0.09	0.9302
Minimal, mmHg	1.04 [1.01, 1.19]	2.27	0.003**
Mean, mmHg	0.49 [0.02, 11.33]	−0.44	0.6579
Median, mmHg	1.04 [0.93, 1.16]	0.68	0.4949
Variance	1.00 [0.99, 1.01]	0.38	0.7017
SD	1.08 [0.70, 1.67]	0.36	0.7162
RMS	1.96 [0.09, 44.35]	0.42	0.6719
CV	1.04 [1.01, 1.09]	1.14	0.2540
Variability score	1.03 [1.01, 1.05]	1.27	0.7872

*ACEI, angiotensinogen-converting enzyme inhibitor; HDL, high-density lipoprotein cholesterol; SD, standard deviation; RMS, root mean square; CV, coefficient of variation. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.*

$p = 0.0002$], and larger variability score [HR: 1.16, 95% CI: [1.04, 1.85], $p = 0.0003$]; and larger values of all systolic BP measures (baseline, latest, maximum, minimum, mean, median, variance, SD, RMS, CV, and variability score) (HR: >1, $p < 0.001$).

In addition, the significant univariate predictors of all-cause mortality after anxiety presentation were also identified (**Table 2**). Significant univariate predictors ($p < 0.05$) were entered into a multivariate Cox regression model, with most of the above univariate predictors remaining significant (**Table 3**). Next, we further analyzed different BP values in patients who developed anxiety with age stratification (**Figures 3**, **4** and **Supplementary Table 2**). There is an age-related increase in mean, median, and measures of variability for both diastolic and systolic BPs.

Comparisons of BP Measurements Before and After Anxiety Development

Gender-specific BP measure changes before and after initial anxiety presentation were identified as shown in **Table 4**. Regarding systolic BP, males had larger variance (median: 172.4, IQR: 99.5–262.7 vs. median: 165.7, IQR: 96.9–255.0, $p < 0.0001$), larger RMS (median: 137.1, IQR: 125.5–144.0 vs. median: 134.7, IQR: 126.2–143.0, $p = 0.0489$), and larger variability score (median: 73.6, IQR: 60.5–80.3 vs. median: 70.0, IQR: 60.0–77.0, $p = 0.0332$) after initial presentation of anxiety diseases; and females had larger variance (median: 174.2, IQR: 88.8–259.2 vs. median 161.8, IQR: 91.7–256.7, $p < 0.0001$) and larger variability score (median: 74.4, IQR: 60.5–79.3 vs. median: 70.6, IQR: 57.1–78.6, $p = 0.0136$) after initial presentation of anxiety diseases. In diastolic BP measurements, males had larger variance (median: 65.5, IQR: 30.3–81.0 vs. median: 54.3, IQR: 31.4–88.9, $p < 0.0001$) and larger variability score (median: 63.3, IQR: 40.2–71.2 vs. median: 57.1, IQR: 46.2–66.7, $p < 0.0001$) after initial presentation of anxiety; females had larger minimal test (median: 63.0, IQR: 54.0–67.0 vs. median: 58.0, IQR: 52.0–66.0, $p = 0.0003$), larger variance (median: 61.8, IQR: 31.4–81.4 vs. median: 56.1, IQR: 33.4–84.3, $p < 0.0001$), and larger variability score (median: 62.3, IQR: 43.2–67.54 vs. median: 55.8, IQR: 47.4–66.2, $p < 0.0001$).

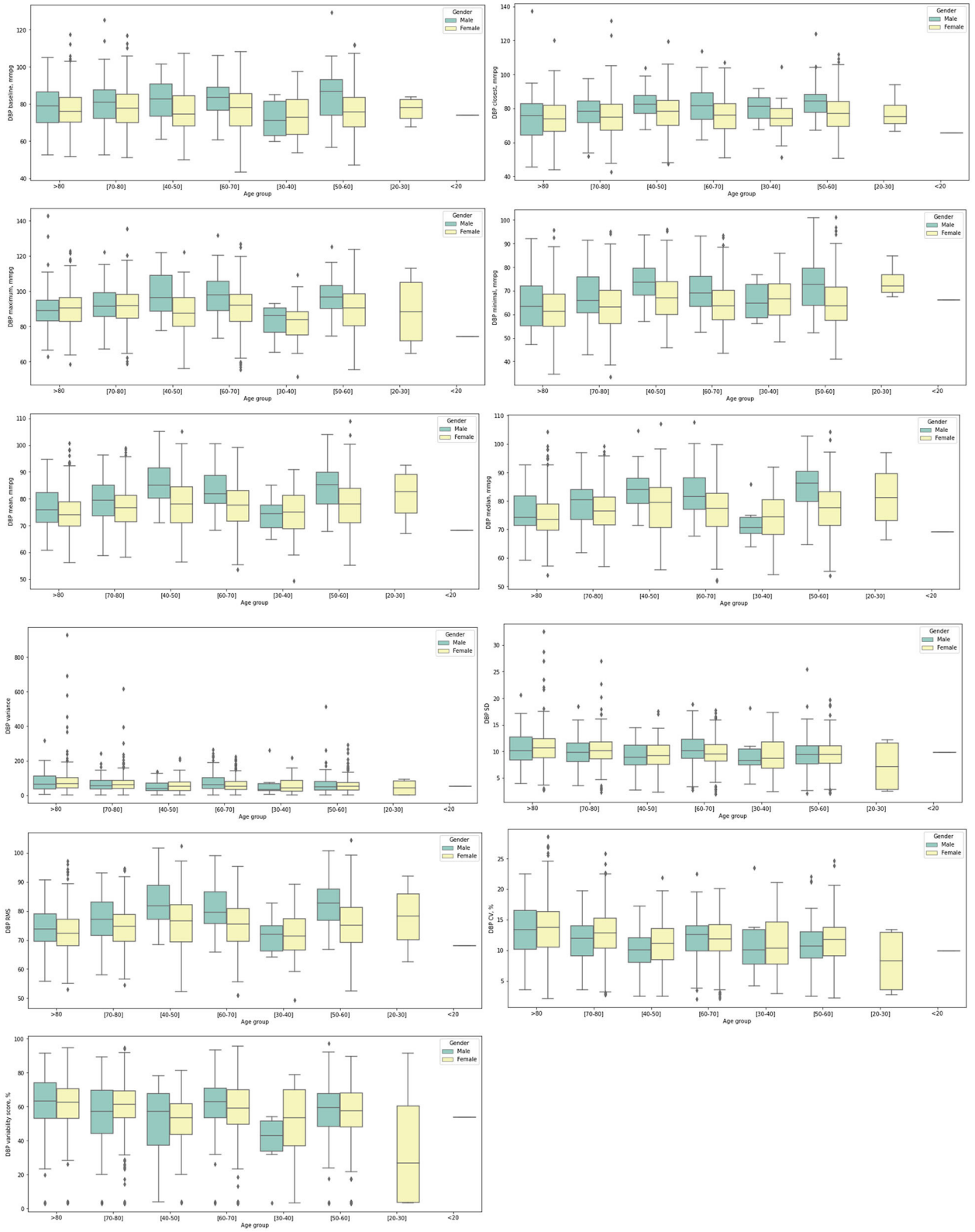

FIGURE 3 | Box plots showing diastolic blood pressure (DBP) measurements stratified by gender and age at anxiety presentation.

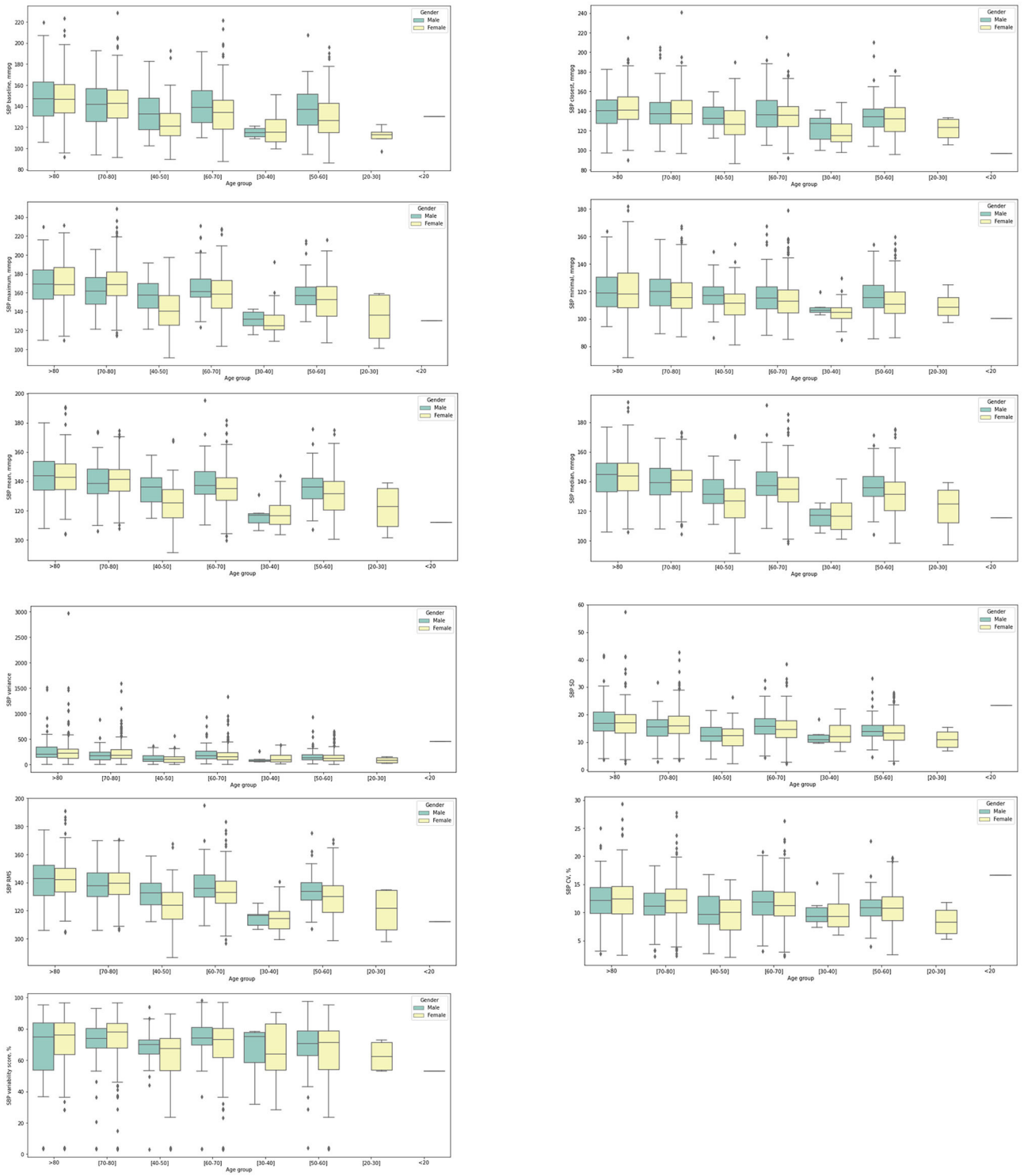

FIGURE 4 | Box plots showing systolic blood pressure (SBP) measurements stratified by gender and age at anxiety presentation.

DISCUSSION

The main findings of this study are that (1) higher baseline, maximum, minimum, SD, CV, and variability score of diastolic BP significantly predicted anxiety, as did all systolic BP measures (baseline, latest, maximum, minimum, mean, median, variance, SD, RMS, CV, and variability score) and (2) female and older patients with

TABLE 4 | Gender-specific blood pressure measurement changes before and after anxiety development.

	Males			Females		
	Before anxiety	**After anxiety**	***P***	**Before anxiety**	**After anxiety**	***p***
Systolic blood pressure measurements						
Closest	133.0 (121.5–144.5); 211.0	136.0 (120.0–150.0); 217.0	0.1024	132.0 (119.0–144.0); 222.0	134.0 (120.0–148.0); 225.0	0.2226
Maximum	156.0 (144.0–169.0); 233.0	156.0 (143.0–170.0); 233.0	0.8723	157.0 (140.0–173.0); 246.0	156.0 (140.0–171.0); 246.0	0.2414
Minimal	111.0 (102.0–121.0); 164.0	112.0 (103.0–122.5); 173.0	0.7634	109.0 (101.0–120.0); 183.0	110.0 (102.0–121.0); 196.0	0.6821
Mean	134.0 (125.4–142.4); 191.6	135.5 (124.9–143.45); 187.0	0.2356	132.5 (123.3–141.0); 193.3	135.1 (122.9–143.0); 214.7	0.1386
Median	133.0 (125.0–142.0); 187.0	134.0 (123.25–143.0); 184.0	0.7631	132.0 (122.5–141.0); 195.0	135.8 (121.0–143.0); 223.0	0.0986
Variance	165.7 (96.9–255.0); 1,512.5	172.4 (99.5–262.7); 930.5	<0.0001***	161.8 (91.7–256.7); 2,964.5	174.2 (88.8–259.2); 2,056.2	<0.0001***
SD	12.9 (9.8–15.9); 38.9	13.1 (10.0–16.2); 30.5	0.4429	12.7 (9.6–16.0); 54.4	12.4 (9.4–16.1); 45.4	0.6199
RMS	134.7 (126.2–143.0); 192.4	137.1 (125.5–144.0); 187.4	0.0489*	133.4 (124.0–141.9); 193.6	135.7 (123.6–143.7); 215.1	0.1332
CV	0.09 (0.07–0.11); 0.2	0.09 (0.07–0.1); 0.2	0.9125	0.09 (0.07–0.12); 0.27	0.09 (0.07–0.11); 0.28	0.2305
Variability score	70.0 (60.0–77.0); 94.4	73.6 (60.5–80.3); 90.4	0.0332*	70.6 (57.1–78.6); 93.8	74.4 (60.5–79.3); 90.5	0.0136*
Diastolic blood pressure measurements						
Closest	75.0 (67.0–82.0); 135.0	77.0 (68.0–84.0); 122.0	0.1853	71.0 (63.0–79.0); 115.0	72.0 (65.0–80.0); 110.0	0.0461
Maximum	89.0 (81.5–97.0); 135.0	88.0 (80.0–96.0); 135.0	0.2296	86.0 (79.0–93.0); 128.0	85.0 (78.0–91.0); 122.0	0.7141
Minimal	63.0 (56.0–70.0); 100.0	65.0 (57.0–70.0); 100.0	0.1777	58.0 (52.0–66.0); 98.0	63.0 (54.0–67.0); 90.0	0.0003***
Mean	75.4 (69.8–81.6); 106.4286	75.5 (69.5–81.3); 106.3	0.5467	71.9 (66.2–77.2); 102.0	72.3 (66.3–77.4); 98.8	0.7072
Median	75.5 (69.5–81.0); 110.0	75.0 (69.0–81.0); 109.0	0.6943	71.5 (66.0–77.0); 102.0	72.0 (66.0–77.8); 97.5	0.0968
Variance	54.3 (31.4–88.9); 512.0	65.5 (30.3–81.0); 273.3	<0.0001***	56.1 (33.4–84.3); 924.5	61.8 (31.4–81.4); 690.3	<0.0001***
SD	7.4 (5.6–9.4); 22.6	7.0 (5.5–9.0); 16.5	0.4272	7.5 (5.8–9.2); 30.4	7.7 (5.6–9.0); 26.3	0.3177
RMS	75.9 (70.3–81.9); 106.6	75.7 (69.9–81.7); 106.43	0.7934	72.3 (66.6–77.6); 102.1	73.7 (66.8–77.7); 99.6	0.7846
CV	0.09 (0.07–0.12); 0.25	0.09 (0.07–0.13); 0.2	0.3691	0.1001 (0.073–0.1); 0.31	0.095 (0.07–0.12); 0.3	0.1045
Variability score	57.1 (46.2–66.7); 93.8	63.3 (40.2–71.2); 93.2	<0.0001***	55.8 (47.4–66.2); 92.3	62.3 (43.2–67.54); 90.5	<0.0001***

SD, standard deviation; RMS, root mean square; CV, coefficient of variation.
$^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

higher BP and higher BPV were at the greatest risks of anxiety.

The effects of anxiety on BP and as a potential risk factor have been extensively examined in previous studies (14). However, whether BP influences the risk of incident anxiety has not been investigated in detail, and mixed results were seen in observational studies. Individuals with hypertension may be more likely to develop anxiety (14, 15), but this association is seen only when hypertension coexists with another chronic condition (16) or when the patients are aware of their hypertension diagnosis (17). Previously, higher beat-to-beat BPV has been associated with incident anxiety (3). Longer-term visit-to-visit BPV has also been reported as an independent predictor of neurological conditions such as cognitive impairment in cohort studies (4–6), but whether it can do so for incident anxiety has never been explored. In this population-based study of patients attending family medicine clinics in the public sector of Hong Kong, we established for the first time the predictive value of different metrics of BP and BPV on incident anxiety.

While the physiological mechanisms underlying the bidirectional relationship between hypertension and incident anxiety remain unclear, the phenomenon was reported in recent studies. Population-based studies demonstrated that patients with baseline anxiety had an increased risk of essential hypertension (18–20). By contrast, a territory-wide study of over two million patients in Sweden demonstrated that hypertensive patients were more likely to suffer from anxiety (21). The presence of anxiety increases the risk of poor drug compliance among hypertensive patients and thus worsens their BP control (22).

Various hypotheses have been proposed to explain the association between anxiety and hypertension. First of all, chronic stress, which induces a persistent maladaptive stress response that develops into anxiety, results in long-term cortisol elevation (23). Consequently, the hypothalamic–pituitary–adrenal axis becomes dysregulated and leads to hypertension (24). Furthermore, it is postulated that exaggerated neurobiological sensitivity toward threat results in prolonged activation of the hypothalamic–pituitary–adrenal axis, which results in both the autonomic dysregulation underlying hypertension and the biological change under anxiety (25). Other mechanisms including increased oxidative stress, physical inactivity, and hypercapnia were reported to be common in both the pathogenesis of hypertension and anxiety and thus may contribute to the association between the two diseases (26–28).

Similarly, hypotheses have been proposed to explain the predictive value of BPV for incident anxiety. Increased BPV has been shown to be due to reduced baroreflex sensitivity, which

may reflect sympathovagal imbalance likely due to sympathetic hyperactivity, which is observed in anxiety patients (3, 29, 30). The BP instability may reflect compensatory hemodynamic changes under reduced arterial compliance and increased aortic stiffness under systemic inflammatory response, which is both a cause of hypertension and a consequence of anxiety (31, 32). Furthermore, the pathological worrying in anxiety may be associated with increased compliance toward antihypertensives, which are known to increase BPV (33). Moreover, the use of medications such as beta blockers also predicted incident anxiety. It may be that anxious patients are more likely to receive such medications to reduce the symptoms of anxiety (34).

Limitations

Several limitations should be noted for the present study. Given its observational nature, there is information bias with regard to issues of under-coding, missing data, and documentation errors. Moreover, data on lifestyle risk factors of hypertension, such as smoking and alcoholism, were unavailable; thus, their potential influence on the relationship between BP and anxiety cannot be assessed. Furthermore, the clinical circumstances of BP measurement during hospital visits were susceptible to the effects of circumstantial factors, which may introduce additional variables that affect patients' BP and BPV. Circadian changes in BP may be a good predictor of the adverse outcomes. However, our BP values were measured within the clinical setting. It was therefore not possible to obtain BP values at nighttime. Heart rate variability is also an important predictor, and this should be evaluated for its predictive value and incorporated into predictive risk models in subsequent studies. Finally, the diagnosis of anxiety was reliant on ICD-9 coding, and therefore, not all diagnoses were made by a specialist in psychiatry. However, results of psychological tools such as Generalized Anxiety Disorder 7 and Beck Anxiety Inventory are not routinely coded, and it was therefore not possible to precisely define the presence of anxiety disorder that fulfills the specialist definition of this disease.

CONCLUSIONS

The relationships between longer-term visit-to-visit BPV and incident anxiety were identified. Female and older patients with higher BP and higher BPV were at the highest risks of anxiety. Future studies should examine the interacting effects between BPV and medication use to influence incident anxiety and anxiety-related outcomes.

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 The Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee and Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JZ and SL: data analysis, data interpretation, statistical analysis, manuscript drafting, and critical revision of the manuscript. WW, KL, RN, PL, and TL: project planning, data acquisition, data interpretation, and critical revision of manuscript. BC: study supervision, data interpretation, statistical analysis, and critical revision of manuscript. QZ and GT: study conception, study supervision, project planning, data interpretation, statistical analysis, manuscript drafting, and critical revision of 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.2021.650852/full#supplementary-material

REFERENCES

1. Scuteri A, Spalletta G, Cangelosi M, Gianni W, Assisi A, Brancati AM, et al. Decreased nocturnal systolic blood pressure fall in older subjects with depression. *Aging Clin Exp Res.* (2009) 21:292–7. doi: 10.1007/BF03324918
2. Symonides B, Holas P, Schram M, Sleszycka J, Bogaczewicz A, Gaciong Z. Does the control of negative emotions influence blood pressure control and its variability? *Blood Press.* (2014) 23:323–9. doi: 10.3109/08037051.2014.901006
3. Virtanen R, Jula A, Salminen JK, Voipio-Pulkki LM, Helenius H, Kuusela T, et al. Anxiety and hostility are associated with reduced baroreflex sensitivity and increased beat-to-beat blood pressure variability. *Psychosom Med.* (2003) 65:751–6. doi: 10.1097/01.PSY.0000088760.65046.CF

4. Nagai M, Hoshide S, Ishikawa J, Shimada K, Kario K. Visit-to-visit blood pressure variations: new independent determinants for cognitive function in the elderly at high risk of cardiovascular disease. *J Hypertens.* (2012) 30:1556–63. doi: 10.1097/HJH.0b013e3283552735
5. Sabayan B, Wijsman LW, Foster-Dingley JC, Stott DJ, Ford I, Buckley BM, et al. Association of visit-to-visit variability in blood pressure with cognitive function in old age: prospective cohort study. *BMJ*. (2013) 347:f4600. doi: 10.1136/bmj.f4600
6. Yano Y, Ning H, Allen N, Reis JP, Launer LJ, Liu K, et al. Long-term blood pressure variability throughout young adulthood and cognitive function in midlife: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Hypertension.* (2014) 64:983–8. doi: 10.1161/HYPERTENSIONAHA.114.03978
7. Ju C, Lai RWC, Li KHC, Hung JKF, Lai JCL, Ho J, et al. Comparative cardiovascular risk in users vs. non-users of xanthine oxidase inhibitors and febuxostat vs. allopurinol users. *Rheumatology*. (2020) 59:2340–9. doi: 10.1093/rheumatology/kez576
8. Zhou J, Wang X, Lee S, Wu WKK, Cheung BMY, Zhang Q, et al. Proton pump inhibitor or famotidine use and severe COVID-19 disease: a propensity score-matched territory-wide study. *Gut.* (2020). doi: 10.1136/gutjnl-2020-323668. [Epub ahead of print].
9. Zhou J, Lee S, Guo CL, Chang C, Liu T, Leung KSK, et al. Anticoagulant or antiplatelet use and severe COVID-19 disease: a propensity score-matched territory-wide study. *Pharmacol Res*. (2021) 165:105473. doi: 10.1016/j.phrs.2021.105473
10. Zhou J, Tse G, Lee S, Liu T, Cao Z, Zeng DD, et al. Interaction effects between angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and steroid or anti-viral therapies in COVID-19: a population-based study. *J Med Virol.* (2021) 93:2635–41. doi: 10.1002/jmv.26904
11. Lee S, Zhou J, Guo CL, Wong WT, Liu T, Wong ICK, et al. Predictive scores for identifying patients with type 2 diabetes mellitus at risk of acute myocardial infarction and sudden cardiac death. *Endocrinol Diabet Metabol.* (2021) e00240. doi: 10.1002/edm2.240. [Epub ahead of print].
12. Lee S, Liu T, Zhou J, Zhang Q, Wong WT, Tse G. Predictions of diabetes complications and mortality using hba1c variability: a 10-year observational cohort study. *Acta Diabetol.* (2020) 58:171–80. doi: 10.1007/s00592-020-01605-6
13. Lee S, Zhou J, Wong WT, Liu T, Wu WKK, Wong ICK, et al. Glycemic and lipid variability for predicting complications and mortality in diabetes mellitus using machine learning. *BMC Endocr Disord*. (2021).
14. Pan Y, Cai W, Cheng Q, Dong W, An T, Yan J. Association between anxiety and hypertension: a systematic review and meta-analysis of epidemiological studies. *Neuropsychiatr Dis Treat.* (2015) 11:1121–30. doi: 10.2147/NDT.S77710
15. Player MS, Peterson LE. Anxiety disorders, hypertension, and cardiovascular risk: a review. *Int J Psychiatry Med.* (2011) 41:365–77. doi: 10.2190/PM.41.4.f
16. Grimsrud A, Stein DJ, Seedat S, Williams D, Myer L. The association between hypertension and depression and anxiety disorders: results from a nationally-representative sample of South African adults. *PLoS ONE*. (2009) 4:e5552. doi: 10.1371/journal.pone.0005552
17. Hamer M, Batty GD, Stamatakis E, Kivimaki M. Hypertension awareness and psychological distress. *Hypertension.* (2010) 56:547–50. doi: 10.1161/HYPERTENSIONAHA.110.153775
18. Bacon SL, Campbell TS, Arsenault A, Lavoie KL. The impact of mood and anxiety disorders on incident hypertension at 1 year. *Int J Hypertens*. (2014) 2014:953094. doi: 10.1155/2014/953094
19. Stein DJ, Aguilar-Gaxiola S, Alonso J, Bruffaerts R, De Jonge P, Liu Z, et al. Associations between mental disorders and subsequent onset of hypertension. *Gen Hosp Psychiatry.* (2014) 36:142–9. doi: 10.1016/j.genhosppsych.2013.11.002
20. Perez-Pinar M, Mathur R, Foguet Q, Ayis S, Robson J, Ayerbe L. Cardiovascular risk factors among patients with schizophrenia, bipolar, depressive, anxiety, and personality disorders. *Eur Psychiatry*. (2016) 35:8–15. doi: 10.1016/j.eurpsy.2016.02.004
21. Sandstrom YK, Ljunggren G, Wandell P, Wahlstrom L, Carlsson AC. Psychiatric comorbidities in patients with hypertension–a study of registered diagnoses 2009-2013 in the total population in Stockholm County, Sweden. *J Hypertens.* (2016) 34:414–20. doi: 10.1097/HJH.0000000000000824
22. Bautista LE, Vera-Cala LM, Colombo C, Smith P. Symptoms of depression and anxiety and adherence to antihypertensive medication. *Am J Hypertens.* (2012) 25:505–11. doi: 10.1038/ajh.2011.256
23. Patriquin MA, Mathew SJ. The neurobiological mechanisms of generalized anxiety disorder and chronic stress. *Chronic Stress.* (2017) 1:2470547017703993. doi: 10.1177/2470547017703993
24. Gold SM, Dziobek I, Rogers K, Bayoumy A, Mchugh PF, Convit A. Hypertension and hypothalamo-pituitary-adrenal axis hyperactivity affect frontal lobe integrity. *J Clin Endocrinol Metab*. (2005) 90:3262–7. doi: 10.1210/jc.2004-2181
25. O'donovan A, Slavich GM, Epel ES, Neylan TC. Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. *Neurosci Biobehav Rev.* (2013) 37:96–108. doi: 10.1016/j.neubiorev.2012.10.013
26. Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K. Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. *Brain Res.* (2010) 1359:178–85. doi: 10.1016/j.brainres.2010.08.093
27. Johnson PL, Samuels BC, Fitz SD, Lightman SL, Lowry CA, Shekhar A. Activation of the orexin 1 receptor is a critical component of CO_2-mediated anxiety and hypertension but not bradycardia. *Neuropsychopharmacology*. (2012) 37:1911–22. doi: 10.1038/npp.2012.38
28. Ushakov AV, Ivanchenko VS, Gagarina AA. Psychological stress in pathogenesis of essential hypertension. *Curr Hypertens Rev.* (2016) 12:203–14. doi: 10.2174/1573402112666161230121622
29. Januzzi JLJr, Stern TA, Pasternak RC, Desanctis RW. The influence of anxiety and depression on outcomes of patients with coronary artery disease. *Arch Intern Med.* (2000) 160:1913–21. doi: 10.1001/archinte.160.13.1913
30. Tully PJ, Tzourio C. Psychiatric correlates of blood pressure variability in the elderly: the Three City cohort study. *Physiol Behav.* (2017) 168:91–7. doi: 10.1016/j.physbeh.2016.10.024
31. Mitchell GF. Arterial stiffness and wave reflection: biomarkers of cardiovascular risk. *Artery Res.* (2009) 3:56–64. doi: 10.1016/j.artres.2009.02.002
32. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet.* (2010) 375:938–48. doi: 10.1016/S0140-6736(10)60309-1
33. Parker G, Hyett M, Hadzi-Pavlovic D, Brotchie H, Walsh W. GAD is good? Generalized anxiety disorder predicts a superior five-year outcome following an acute coronary syndrome. *Psychiatry Res.* (2011) 188:383–9. doi: 10.1016/j.psychres.2011.05.018
34. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and management of blood-pressure variability. *Nat Rev Cardiol.* (2013) 10:143–55. doi: 10.1038/nrcardio.2013.1

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Presence of Extra-Criteria Antiphospholipid Antibodies Is an Independent Risk Factor for Ischemic Stroke

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Background: Ischemic stroke is the most common and severe arterial thrombotic event in Antiphospholipid syndrome (APS). APS is an autoimmune disease characterized by the presence of thrombosis and antiphospholipid antibodies (aPL), which provide a pro-coagulant state. The aPL included in the classification criteria are lupus anticoagulant, anti-cardiolipin (aCL) and anti-β2-glycoprotein-I antibodies (aB2GPI) of IgG and IgM isotypes. Extra-criteria aPL, especially IgA aB2GPI and IgG/IgM anti-phosphatidylserine/prothrombin antibodies (aPS/PT), have been strongly associated with thrombosis. However, their role in the general population suffering from stroke is unknown. We aim (1) to evaluate the aPL prevalence in ischemic stroke patients, (2) to determine the role of aPL as a risk factor for stroke, and (3) to create an easy-to-use tool to stratify the risk of ischemic stroke occurrence considering the presence of aPL and other risk factors.

Materials and Methods: A cohort of 245 consecutive ischemic stroke patients was evaluated in the first 24 h after the acute event for the presence of classic aPL, extra-criteria aPL (IgA aB2GPI, IgG, and IgM aPS/PT) and conventional cardiovascular risk factors. These patients were followed-up for 2-years. A group of 121 healthy volunteers of the same age range and representative of the general population was used as reference population. The study was approved by the Ethics Committee for Clinical Research (Reference numbers CEIC-14/354 and CEIC-18/182).

Results: The overall aPL prevalence in stroke patients was 28% and IgA aB2GPI were the most prevalent (20%). In the multivariant analysis, the presence of IgA aB2GPI (OR 2.40, 95% CI: 1.03–5.53), dyslipidemia (OR 1.70, 95% CI: 1.01–2.84), arterial hypertension (OR 1.82, 95% CI: 1.03–3.22), atrial fibrillation (OR 4.31, 95% CI: 1.90–9.78), and active smoking (OR 3.47, 95% CI: 1.72–6.99) were identified as independent

risk factors for ischemic stroke. A risk stratification tool for stroke was created based on these factors (AUC: 0.75).

Conclusions: IgA aB2GPI are an important independent risk factor for ischemic stroke. Evaluation of aPL (including extra-criteria) in cardiovascular risk factor assessment for stroke can potentially increase the identification of patients at risk of thrombotic event, facilitating a decision on preventive treatments.

Keywords: antiphospholipid syndrome, ischemic stroke, antiphospholipid antibodies, IgA anti-b2-glycoprotein-I antibodies, thrombosis

INTRODUCTION

Stroke is a very serious life-threatening medical condition. It is the second leading cause of death and one of the most important causes of disability worldwide (50% of survivors will be permanently disabled) (1, 2). Strokes can be classified into two main types: ischemic strokes (about 90%) caused by an obstruction of blood supply, and haemorrhagic strokes due to the rupture of blood vessels (3). Ischemic stroke can be due to intracranial thrombosis or embolism, either from atherosclerotic plaque in the aortic arch, cervical arteries or the heart (4). When a cerebral artery is occluded by a clot and blood flow decreases, the oxygen and nutrients supply is interrupted and neuronal electrical function ceases. If circulation isn't restored quickly, diverse mechanisms of ischemic damage are involved in neuronal death and irreversible tissue injury causing long-term disability or even death (5).

Stroke is a complex disease with numerous aetiological factors and has been associated with numerous risk factors such as age, hypertension, diabetes mellitus, dyslipidemia, diet, smoking, or alcohol consumption (6). Furthermore, the presence of other previous diseases such as carotid artery disease, atrial fibrillation, mitral stenosis, coronary artery disease, and antiphospholipid syndrome (APS) are considered as facilitators of the appearance of arterial occlusions by thrombotic or cardioembolic mechanisms (7, 8). APS is a systemic autoimmune disease characterized by venous or arterial thrombotic events and/or gestational morbidity in patients positive for antiphospholipid antibodies (aPL). The presence of aPL contributes to the activation of immune cells, platelets, trophoblast, and endothelial cells (9–13) through the interaction of B2GPI-antibody complexes with several membrane receptors such as toll-like receptors (TLR2, TLR4), annexin 2 or LDL receptor-related protein 8 (LRP8) (14). Inflammatory mechanisms mediated by these complexes may be due to the release of neutrophil extracellular traps (NETs) and the complement activation. Activation of endothelial cells causes the loss of its anticoagulant properties and the acquisition of a pro-inflammatory and pro-coagulant phenotype (15). B2GPI-antibody complexes also reduce the tissue factor pathway inhibitor and activated protein C activity (16). However, although aPL are present, thrombotic events only occur occasionally, suggesting that aPL-associated clotting must be triggered by an additional "second hit" that involves a strong activation of the innate immunity, such as surgery, trauma, or infection (17). This "second hit" may induce the exposition of cryptic epitopes not previously accessible.

To date, there are no diagnostic criteria for APS. Classification criteria (clinical and laboratory criteria) were updated at a workshop in Sydney (Australia, 2004) in order to identify patients with APS for research purposes (18). The classification of a patient as APS requires the combination of one clinical and one laboratory criterion. Clinical criteria include arterial, venous or small vessel thrombosis in any tissue or organ, and/or pregnancy morbidity (19). The laboratory criteria are comprised by lupus anticoagulant (LA), anti-cardiolipin antibodies (aCL), and anti-β2-glycoprotein-I antibodies (aB2GPI), both of IgG or IgM isotypes. The importance of ischemic stroke in APS was already specified in the initial description of the syndrome in 1983 (20, 21) and the presence of aPL has been reported as an independent risk factor for the etiology of ischemic stroke (22–29).

There are patients who present APS clinical features where the evaluation of aPL recognized in the classification criteria is systematically negative. To describe these types of patients, Hughes and Khamashta defined the concept of "seronegative APS" and a growing interest in the development of new biomarkers that improve the diagnostic accuracy of APS has emerged (30). New aPL associated with APS clinical manifestations but not included in the current classification criteria have been described and are called extra-criteria aPL (31). Among them, aB2GPI of IgA isotype, IgG and IgM anti-phosphatidylserine/prothrombin (aPS/PT) antibodies, and IgG anti-domain I of B2GPI antibodies stand out for their strong association with APS clinical characteristics and for the existence of well-standardized diagnostic tests (32–34). Although IgA aB2GPI were not included in the APS classification criteria, their clinical importance has increased over the last 16 years (35–39). Since 2010, the task force of the 13th International Congress on Antiphospholipid Antibodies recommended testing for IgA aB2GPI in negative cases for IgG and IgM isotypes where APS is still suspected (40). The presence of IgA aB2GPI was demonstrated as a risk factor for the appearance of thrombosis and stroke. Asymptomatic IgA aB2GP1 carriers had a higher incidence of strokes than negative aPL people (OR 5.17, 95% CI: 1.13–23.59) within a 5-year follow-up study (37).

In this work, we analyse the prevalence of aPL (classic and extra-criteria) and well-known cardiovascular risk factors in a consecutive series of 245 patients with acute ischemic stroke and in a control group representing the general population. In addition, we propose a scoring model to stratify the risk of

ischemic stroke considering the risk factors associated with this disorder (including aPL) that can be helpful to evaluate patients for preventive treatments.

MATERIALS AND METHODS

Study Design

We performed a prospective and observational study that included 245 patients who suffered from acute ischemic stroke who were followed-up for 2 years. The presence of aPL was determined in the first 24 h after the acute event.

In this work, we aim (1) to analyse the prevalence of aPL (classic and extra-criteria) in ischemic stroke patients compared to a control population, and to assess the clinical evolution of aPL-positive patients in relation to those negative in a 2-year follow-up; (2) to quantify the role of aPL as a risk factor for ischemic stroke; and (3) to propose a risk stratification model based on the independent risk factors associated with stroke.

Ischemic Stroke Patients

Two hundred and sixty-eight consecutive subjects with suspected acute stroke were attended at the Stroke Unit of the Hospital 12 de Octubre (Madrid, Spain) from November 1st 2017 to April 6th 2018. These 268 patients constitute the total number of patients who attended the Emergency Room for suspected stroke in this period. All patients were assessed by the on-duty neurologists at admission.

Patients underwent an emergency study including blood analysis (routine laboratory test, complete blood count, and coagulation test), electrocardiography study, cerebral imaging by computer tomography (CT), and CT-angiography. Complementary tests were performed depending on the patient's clinical condition and aetiological suspicion, including duplex ultrasound of intracranial, and supra-aortic vessels, transthoracic magnetic resonance imaging, transoesophageal echocardiography, or Holter ECG monitoring. Detailed information on vascular risk factors including hypertension, cardiac disease, hiperlipemia, diabetes mellitus, cigarette smoking, or alcohol intake was collected (defined below).

Twenty-three subjects were excluded due to a non-vascular origin of the event or a stroke not confirmed by brain imaging study. Therefore, 245 patients with a confirmed diagnosis were included (**Supplementary Figure 1**) and followed-up for 2 years. Stroke severity was assessed according to the National Institute of Health Stroke Scale (NIHSS) and the level of functional independence by the modified Rankin Score (mRS).

Patients whose symptoms were compatible with ischaemic stroke were treated with systemic intravenous thrombolysis using intravenous tissue plasminogen activator (IV-tPA) or by mechanical thrombectomy according to the Stroke Care Plan.

Control Group and Reference Population

In order to compare the aPL prevalence between ischemic stroke patients and the healthy population, aPL were evaluated in a group of 501 anonymous blood donors (age, sex, and clinical data unknown). The blood donor group has the disadvantage that it does not include individuals over 65 years old, and those aged above 50 are underrepresented (age bias).

A second control group of 121 healthy individuals (reference population) representative of the general adult population of our area was constituted by 33 volunteers in the age range of 18–50 recruited consecutively at the blood bank's donor room and 88 volunteers aged over 50. Older participants were recruited from people who underwent a preoperative study for cataract surgery or other minor conditions (not related to any vascular pathology) and had no symptoms at the time of the medical examination except for minor age-related symptoms. This reference population and stroke patients were similar in both ethnicity (96% Caucasians) and area of residence. Individuals with antecedents of cardiovascular disease, neoplasia, or serious disease were excluded. Clinical and laboratory data of the 121 members of the reference population were stored in an anonymized database.

Definitions

Ischemic stroke: was defined as rapidly developing signs of focal or global disturbance of cerebral functions, lasting more than 24 h or leading to death, with no apparent cause other than that of vascular origin. Stroke was confirmed by brain imaging study.

Arterial hypertension: was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg recorded on different days during evaluations prior to the stroke (with evidence of at least 2 readings) or use of medication.

Diabetes mellitus: was defined as hyperglycemia resulting from defects of insulin secretion, insulin action, or both. It was diagnosed by medical evaluation.

Dyslipidemia: was defined as serum cholesterol concentrations >220 mg/dL, LDL >130mg/dL, triglycerides >150 mg/dL or use of medication in patients with dyslipidemia antecedents.

Smoking: was considered in active or former smokers.

Atrial fibrillation: was confirmed by electrocardiogram performed during evaluations prior to the stroke or at time of admission.

Alcohol consumption: was defined as >60 g/day in active or former drinkers.

Obesity: was considered when the body mass index (BMI) was >30, or in accordance with the current and past medical history.

Ischaemic heart disease: was considered when a history of angina or myocardial infarction was present.

Peripheral artery disease: was defined as medical diagnosis of intermittent claudication during exercise.

Classic aPL: any of the aPL included in the updated APS classification criteria excluding lupus anticoagulant.

Extra-criteria aPL: aPL not included in the updated APS classification criteria (IgA aβ2GPI and aPS/PT antibodies of IgG and IgM isotypes).

Autoantibodies Determination

Both classic aPL and extra-criteria aPL levels were quantified in the first 24 h after the acute event at the Autoimmunity

Laboratory of the Immunology Department. Classic aPL (aCL and aB2GPI of IgG and IgM isotypes) were quantified using BioPLex 2200 multiplex immunoassay system APLS (Bio-Rad, Hercules CA, USA). Antibody levels higher than 18 U/ml were considered positive for classic aPL (99th percentile of a healthy population, $N = 270$).

For extra-criteria aPL, IgA aB2GPI were quantified by enzyme-linked immunosorbent assay (ELISA) using the QUANTA Lite B2 GPI IgA (INOVA Diagnostics Inc., San Diego, CA, USA), and aPS/PT of IgG and IgM isotypes were evaluated using QUANTA Lite aPS/PT (INOVA DIAGNOSTICS, San Diego, CA, USA). The cut-off values considered positive were >20 U/ml for IgA anti-B2GPI, >30 U/ml for IgG aPS/PT, and >40 U/ml for IgM aPS/PT (41), based on the 99th percentile of a healthy population ($N = 718$). The donor population and the stroke cohort were similar in both ethnicity and area of residence.

Ethical Issues

Individuals were included in the study in accordance with the Declaration of Helsinki. To assure the data anonymity, including both sera (blood drawn) and clinical data, a blind code was assigned to each patient. Approval was obtained from the Ethics Committee for Clinical Research of Hospital 12 de Octubre and a favorable report was received (Reference numbers CEIC-14/354 and CEIC-18/182). Written informed consents were obtained from all patients and the reference population.

Statistical Methods

Demographic, clinical, and pathological characteristics of patients were described by the median with interquartile range (IQR) or relative frequencies. Comparisons of non-normally distributed scaled variables were performed using the Wilcoxon-Mann-Whitney test. The Kolmogorov-Smirnov test was used to evalaute the normality of a distribution.

Comparisons between qualitative variables were determined with Pearson's Chi-square test or Fisher's exact test, where appropriate. The relative measure of an effect was expressed as odds ratio (OR), indicating a 95% confidence interval (95% CI).

Logistic regression analysis was used to estimate the association between risk factors and the outcome (stroke) (42). The model discrimination was quantified using an area under a receiver operating characteristic (ROC) curve and the agreement between predicted and observed probabilities using the Hosmer-Lemeshow test. The intercept used to estimate the ischemic stroke risk was adjusted for the prevalence of ischemic stroke in the general population (43). A scoring system was created based on the regression coefficient. Different score values were evaluated in terms of sensitivity, specificity, likelihood of positive and negative ratio with their corresponding confidence intervals (44, 45).

Probabilities <0.05 were considered significant. Statistical analysis of data were performed using *MedCalc* Statistical Software version 19.5 (MedCalc Software, Ostend, Belgium).

RESULTS

Patient's Characteristics and Conventional Cardiovascular Risk Factors

The stroke cohort had a similar proportion of men (131, 53.5%) and women (114, 46.5%). Men were significantly younger than women ($p < 0.001$), with a median age of 67 (IQR: 57–77) vs. 79 (IQR: 63–85), respectively. In the reference population, no significant age differences between men (median: 64 years, IQR: 50–75) and women (median: 70 years, IQR: 50–75) were detected ($p = 0.963$) (**Table 1**). Comparing both cohorts, women were significantly older in the stroke cohort ($p < 0.001$) but no significant differences in the male age were observed ($p = 0.091$).

Furthermore, significant differences were found in the main cardiovascular risk factors between the reference population and stroke patients (**Table 1**): dyslipidemia (35.5 vs. 55.5%, $p < 0.001$), diabetes mellitus (14.0 vs. 30.2%, $p < 0.001$), arterial hypertension (46.3 vs. 71.0%, $p < 0.001$), and atrial fibrillation (6.6 vs. 29.0%, $p < 0.001$), respectively. No differences in sex (50.4 vs. 53.5%, $p = 0.582$) or presence of autoimmune/inflammatory diseases (5.0 vs. 9.4%, $p = 0.141$) were found between both populations. Other analytical parameters of stroke patients at admission are described in **Supplementary Table 1**.

Prevalence of Antiphospholipid Antibodies

The prevalence of classic aPL in ischemic stroke patients was 6.1%. The prevalence of each classic aPL individually was 2.0% for IgG aB2GPI, 4.1% for IgM aB2GPI, 1.6% for IgG aCL, and 4.1% for IgM aCL antibodies. The prevalence of extra-criteria aPL was 7.8% for IgG/IgM aPS/PT, and 20% for IgA aB2GPI ($n = 49$) (**Table 1**). Eighty-two percent of IgA aB2GPI-positive patients were isolated positive (negative for other aPL).

If we consider any aPL positivity (antibody levels above the cut-off for any classic or extra-criteria aPL), a total of 70 stroke patients (28.6%) were positive for any aPL and 40 of these were isolated positive for IgA aB2PI. The number and type of aPL positivity in stroke patients is described in Venn's diagrams in **Figure 1**.

Comparing the aPL prevalence between a control population of 501 blood donors and stroke patients, we found statistically significant differences in the prevalence of IgG aB2GPI (0.2 vs. 2.0%, $p = 0.008$), IgM aB2GPI (0.2 vs. 4.1%, $p < 0.001$), IgA aB2GPI (1.0 vs. 20.0%, $p < 0.001$), IgM aCL (0.0 vs. 4.1%, $p < 0.001$), and IgG/IgM aPS/PT (1.2 vs. 7.8%, $p < 0.001$), respectively (**Supplementary Table 2**). The absolute levels of criteria and extra-criteria aPL in both cohorts are represented in **Supplementary Figure 2**.

However, using as control the reference population with a similar age range to stroke patients, no differences in the prevalence of most of these antibodies were found between both populations except for IgA aB2GPI, that was significantly higher in stroke patients (20%, $n = 49$) than in the reference population (6.6%, $n = 8$) (OR: 3.53, 95% CI: 1.61–7.72, $p < 0.001$). If we consider any aPL positivity, a total of 70 stroke patients (28.6%) vs. 16 individuals in the reference population

TABLE 1 | Clinical characteristics, conventional cardiovascular risk factors, and antiphospholipid antibodies in the ischemic stroke cohort and the reference population.

Variables	Reference population (*N* = 121)		Stroke patients (*N* = 245)						
	N/median	%/IQR	N/median	%/IQR	*p*-value	OR	95% CI	AUC	95% CI
Age (years)	66	49.8–75.0	72	60.0–82.3	<0.001	1.03	1.02–1.05	0.63	0.58–0.68
Sex (men)	61	50.4	131	53.5	0.582				
Dyslipidemia	43	35.5	136	55.5	<0.001	2.26	1.44–3.55	0.6	0.55–0.65
Diabetes mellitus	17	14.0	74	30.2	<0.001	2.65	1.48–4.73	0.58	0.53–0.63
Former smoker	20	16.5	46	18.8	0.587				
Active smoker	14	11.6	48	19.6	0.054	1.86	0.98–3.53	0.54	0.49–0.59
Arterial hypertension	56	46.3	174	71.0	<0.001	2.84	1.81–4.47	0.62	0.57–0.67
Atrial fibrillation	8	6.6	71	29.0	<0.001	5.76	2.67–12.43	0.61	0.56–0.66
Obesity	24	19.8	37	15.1	0.254				
IgG aB2GPI	0	0.0	5	2.0	0.114				
IgM aB2GPI	3	2.5	10	4.1	0.436				
IgA aB2GPI	8	6.6	49	20.0	<0.001	3.53	1.61–7.72	0.57	0.51–0.62
IgG aCL	1	0.8	4	1.6	0.536				
IgM aCL	3	2.5	10	4.1	0.436				
IgG/IgM aPS-PT	5	4.1	19	7.8	0.188				
Classic aPL	3	2.5	15	6.1	0.130				
Any aPL	16	13.2	70	28.6	0.001	2.63	1.45–4.76	0.57	0.51–0.62

aB2GPI, anti-β2-glycoprotein-I antibodies; aCL, anti-cardiolipin antibodies; aPL, antiphospholipid antibodies; aPS/PT, anti-phosphatidylserine/prothrombin antibodies; AUC, area under the ROC curve.

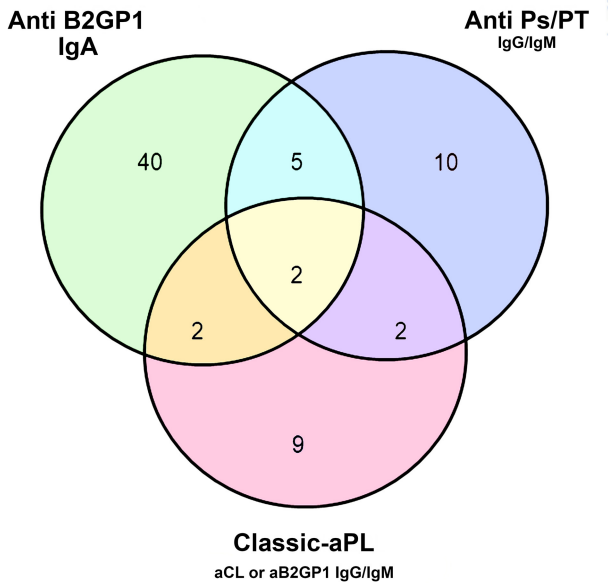


FIGURE 1 | Number and type of aPL positivity in the ischemic stroke cohort. aB2GPI, anti-β2-glycoprotein-I antibodies; aCL, anti-cardiolipin antibodies; aPS/PT, anti-phosphatidylserine/prothrombin antibodies.

(13.2%) were positive for any aPL (OR 2.63, 95% CI: 1.45–4.76, $p = 0.001$) (**Table 1**). Extra-criteria aPL levels in the reference population, blood donors, and stroke patients are represented in **Figure 2**. Mean and median levels of the different aPL are shown in **Supplementary Table 3**.

Multivariate Analysis

The association of aPL and stroke-related conventional cardiovascular risk factors was examined by univariate analysis comparing stroke patients with the reference population. All factors with a *p*-value <0.10 in the univariate evaluation were included in a multivariate analysis model. The presence of any aPL (OR 2.28, 95% CI: 1.09–4.78, $p = 0.028$), dyslipidemia (OR 1.69, 95% CI: 1.01–2.83, $p = 0.046$), arterial hypertension (OR 1.82, 95% CI: 1.03–3.19, $p = 0.038$), atrial fibrillation (OR 4.40, 95% CI: 1.95–9.90, $p < 0.001$), and active smoker (OR 3.45, 95% CI: 1.71–6.93, $p < 0.001$) were independent and significant risk factors for ischemic stroke (**Table 2A**). A second multivariate model where the presence of any aPL was separated into classic aPL, IgG/IgM aPS/PT and IgA aB2GPI positivity was performed (**Table 2B**). This second model had a similar area under the ROC curve (0.756 in the first model vs. 0.758 in the second model) and the presence of IgA aB2GP1 was an independent risk factor for stroke, ranking in order of importance below atrial fibrillation, and smoking habit.

Predictive Model Based on Independent Risk Factors

The exact probability of stroke can be determined using the logistic regression formula: probability = 1/[1 + exp –(intercept value + 0.8141*Arterial hypertension + 0.6392*Dyslipidemia + 1.6108*Atrial fibrillation + 1.1722*Active smoker + 0.9541*IgA aB2GPI Positive)]. The intercept value (−4.81) was adjusted for the prevalence of ischemic stroke in the general population by subtracting −4.18 (odds(3%)/odds(66.93%)) from the previous intercept (−0.62).

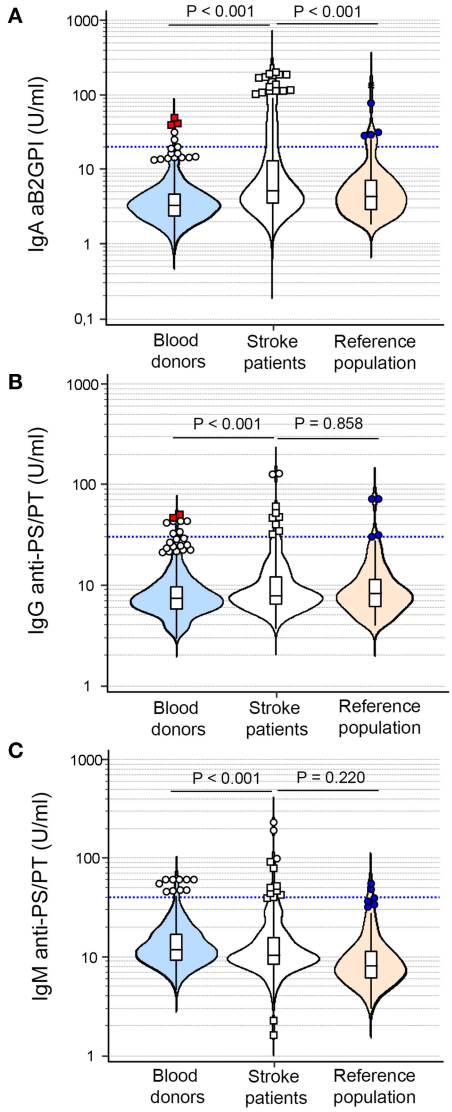


FIGURE 2 | Distribution of extra-criteria aPL levels in blood donors (blue), stroke patients (white), and reference population (orange). **(A)** IgA anti-b2-glycoprotein-I antibodies. **(B)** IgG anti-phosphatidylserine/prothrombin antibodies. **(C)** IgM anti-phosphatidylserine/prothrombin antibodies. The cut-off of each antibody is represented by the blue lines.

An easy risk-scoring system for stroke was constructed based on the independent variables identified in the multivariate model 2 (**Table 2C**). The points associated with each variable of the risk model and the probability of stroke according to the total score are described in **Table 3**. The total score can be calculated using this formula:

$$Score = (Dyslipidemia \times 1) + (Hypertension \times 1) + (IgA\ aB2GPI \times 2) + (Atrial\ fibrillation \times 3) + (Active\ smoking \times 2)$$

For each patient, the value for the presence of atrial fibrillation, active smoking, IgA aB2GP1, dyslipidemia, and hypertension is 1 in this formula. The value for the absence of these conditions is 0. The ROC analysis of the risk predictive model presents an area under the curve of 0.75 (95% CI: 0.70–0.79). Positive and negative predictive values, diagnostic odds, and Youden index are described in **Supplementary Table 4**.

Using the specificity score value, 3-risk categories were created (**Figure 3**): (1) moderate, specificity <80–90% (score = 3, yellow); (2) high, specificity of 90–95% (score = 4, blue), and (3) very high, specificity >95% (score ≥ 5, red). For example, if we had a patient with hypertension, positive for IgA aB2GPI, and smoker, the calculated score would be the following: Score = 0 + 1 + 2 + 0 + 2 = 5. The stroke probability associated with this score value would be 16.7% (**Table 3**).

Clinical Evolution After a 2-Year Follow-Up

Differences in the level of functional independence using mRS between patients with any aPL and without aPL were not detected (OR 1.01, 95% CI: 0.87–1.15, $p = 0.972$). Besides, no significant differences between both populations were observed in the appearance of new strokes (OR 1.43, 95% CI: 0.48–4.32, $p = 0.517$), ischaemic heart disease (OR 0.81, 95% CI: 0.33–1.97, $p = 0.638$), peripheral artery disease (OR 1.39, 95% CI: 0.41–4.71, $p = 0.590$) or death (OR 0.99, 95% CI: 0.44–2.24, $p = 0.980$). Similarly, the presence of IgA aB2GP1 was not associated with the clinical prognosis, recurrence of vascular events or mortality.

DISCUSSION

The role of IgA aB2GPI in stroke etiology has been largely unstudied and its involvement in this pathology is unknown. In this work we demonstrate that IgA aB2GPI are the most prevalent autoantibodies in ischemic stroke patients and their presence constitutes an important independent risk factor associated with the occurrence of sporadic ischemic strokes, regardless of whether or not patients have a previous history of systemic autoimmune disease. The importance of IgA aB2GPI as a risk factor for stroke is higher than some conventional risk factors such as dyslipidemia or hypertension. Only smoking habit and presence of atrial fibrillation constitute risk factors of greater intensity than IgA aB2GPI.

Although aB2GPI of IgA isotype were not included in the APS classification criteria, their clinical importance has increased over the last 16 years (35–39) and in the 13th International Congress on Antiphospholipid Antibodies the task force recommended testing for IgA anti-B2GPI when other aPL are negative and APS is still suspected (40).

In clinical studies with APS and other autoimmune disease patients, control populations are usually blood donors, a very homogeneous group of people with good health, and an age range of 18–65 (mainly between 30 and 50 years). However, it has been known for a long time that stroke has a higher incidence in the elderly and the presence of autoantibodies (anti-nuclear, aPL, and anti-thyroid antibodies) is also more frequent in older populations than in young individuals (46,

TABLE 2 | (A) Multivariate analysis including variables with a *p*-value <0.10 identified in the univariate analysis, **(B)** Second multivariate analysis in which the presence of any positive aPL was separated into classic aPL, IgG/IgM aPS/PT, and IgA aB2GPI positivity, **(C)** Multivariate analysis including only the significant variables described in the second model.

A) 1st Multivariate analysis

Variable	Univariate		Multivariate		
	Odds ratio	95% CI	Odds ratio	95% CI	*p*-value
Age (years)	1.03	1.02–1.05	1.01	0.99–1.03	0.213
Any positive aPL	2.63	1.45–4.76	2.28	1.09–4.78	**0.028**
Dyslipidemia	2.26	1.44–3.55	1.69	1.01–2.83	**0.046**
Diabetes mellitus	2.65	1.48–4.73	1.60	0.84–3.06	0.151
Arterial hypertension	2.84	1.81–4.47	1.82	1.03–3.19	**0.038**
Atrial fibrillation	5.76	2.67–12.43	4.40	1.95–9.90	**<0.001**
Active smoker	1.86	0.98–3.53	3.45	1.71–6.93	**0.001**
			AUC 0.756	**0.71–0.80**	

B) 2nd Multivariate analysis

Variable	Univariate		Multivariate		
	Odds ratio	95% CI	Odds ratio	95% CI	*p*-value
Age (years)	1.03	1.02–1.05	1.01	0.99–1.03	0.218
Positive classic aPL	2.56	0.72–9.03	2.03	0.53–7.85	0.304
Positive IgA B2GPI	3.53	1.61–7.72	2.40	1.03–5.53	**0.042**
Positive IgG/IgM aPSPT	1.95	0.71–5.36	1.14	0.34–3.80	0.835
Dyslipidemia	2.26	1.44–3.55	1.70	1.01–2.84	**0.045**
Diabetes mellitus	2.65	1.48–4.73	1.59	0.83–3.05	0.161
Arterial hypertension	2.84	1.81–4.47	1.82	1.03–3.22	**0.041**
Atrial fibrillation	5.76	2.67–12.43	4.31	1.90–9.78	**<0.001**
Active smoker	1.86	0.98–3.53	3.47	1.72–6.99	**<0.001**
			AUC 0.758	**0.71–0.80**	

C) Analysis of significant variables in second model

Variable	Odds ratio	95% CI	Coefficient	Std. Error	Wald
Positive IgA aB2GPI	2,60	1,13 to 5,94	0,954	0,422	5,106
Dyslipidemia	1,90	1,15 to 3,11	0,639	0,253	6,380
Arterial hypertension	2,26	1,36 to 3,75	0,814	0,259	9,877
Atrial fibrillation	5,01	2,26 to 11,10	1,611	0,406	15,724
Active smoker	3,23	1,63 to 6,41	1,172	0,35	11,207
Raw constant			−0,625	0,222	7,905
Adjusted* constant			−4.810	0,222	7,905

aB2GPI, anti-β2-glycoprotein-I antibodies; aCL, anti-cardiolipin antibodies; aPL, antiphospholipid antibodies; aPS/PT, anti-phosphatidylserine/prothrombin antibodies; AUC, area under the ROC curve.

**Adjusted to prevalence in general population. Significant values are written in bold.*

47). The autoantibodies in the elderly can be pathology-related or age-related. Most of the age-related autoantibodies are scavenger antibodies (not associated with an autoimmune response) produced in response to an increase of apoptotic cells caused by tissue damage in the context of the senescence process (48). In order to minimize the influence of age as a confounding variable and to properly assess whether the presence of aPL is associated with a higher incidence of stroke, we used the reference population as control group of the healthy population with an age range similar to that of stroke patients.

Neurological involvement in APS is relatively frequent (49). It has long been known that ischemic stroke is the most common and severe arterial thrombotic event in APS, with significant morbidity (50, 51). There are several studies that reported an increased frequency of stroke in APS patients (52–54), with a prevalence of 19.8% for stroke in the Euro-Phospholipid Project (55). When this cohort was followed-up for 10 years, stroke

TABLE 3 | Stroke risk scoring system.

A) Score points associated with each variable

Variable	Condition	Score
Arterial hypertension	Absence	0 points
	presence	1 points
Dyslipidemia	Absence	0 points
	Presence	1 points
Atrial fibrillation	Absence	0 points
	Presence	3 points
Active smoker	Absence	0 points
	Presence	2 points
IgA anti-B2GPI Positive	Absence	0 points
	Presence	2 points

B) Total score and stroke probability

Total score	Stroke probability
0	0.008
1	0.015
2	0.029
3	0.053
4	0.095
5	0.167
6	0.275
7	0.418
8	0.576
9	0.720

(A) *Points associated with each variable of the risk model.* ***(B)*** *Probability of the stroke occurrence according to the total score. The score can be calculated using the formula: Score = (Dyslipidemia*1) + (Hypertension*1) + (IgA aB2GPI*2) + (Atrial fibrillation*3) + (Active smoking*2). The value for the presence and absence of the variables is 1 and 0, respectively.*

was the most common thrombotic event that appeared in that period. Similarly, a well-known association of aPL and ischemic stroke has been described (56–61), highlighting a meta-analysis by Chighizola et al. (58) that describes an aPL prevalence of 10% in stroke patients and a study by Gaspersic et al. (29) that shows an aPL prevalence of 22% in stroke patients (including extra-criteria aPL), concluding that aPL represent an independent risk factor for cerebrovascular events. The lower prevalence of classic aPL in our group (6.1%) may be due to differences in the cut-off points and because we did not evaluate the lupus anticoagulant. Furthermore, an overall aPL frequency of 17.2% was reported in young patients (<50 years) with a stroke, supporting that aPL could be considered a leading cause of strokes below the age of 50 (62).

Despite the widespread association of criteria aPL with stroke, the role of extra-criteria aPL in ischemic stroke is not sufficiently known. In a previous study, our group observed a high incidence of thrombotic events (especially ischemic strokes) in asymptomatic carriers of isolated IgA aB2GPI, proposing a score to calculate the risk of thrombotic events (37). Besides, other published studies have also suggested a relevant role for this antibody in the stroke etiology (63, 64). In addition, the presence of criteria aPL has been reported as an independent risk factor for strokes (22–29).

The findings observed at initial evaluation of stroke patients were not related with the clinical evolution in the 2-year follow-up in terms of level of functional independence, recurrence of vascular events or mortality, suggesting that aPL have their role at the time of the event, and there are no differences from strokes caused by other reasons. Some previous studies have not found (60, 65) or have found a weak correlation (66) between serum aPL levels and the stroke severity or outcome.

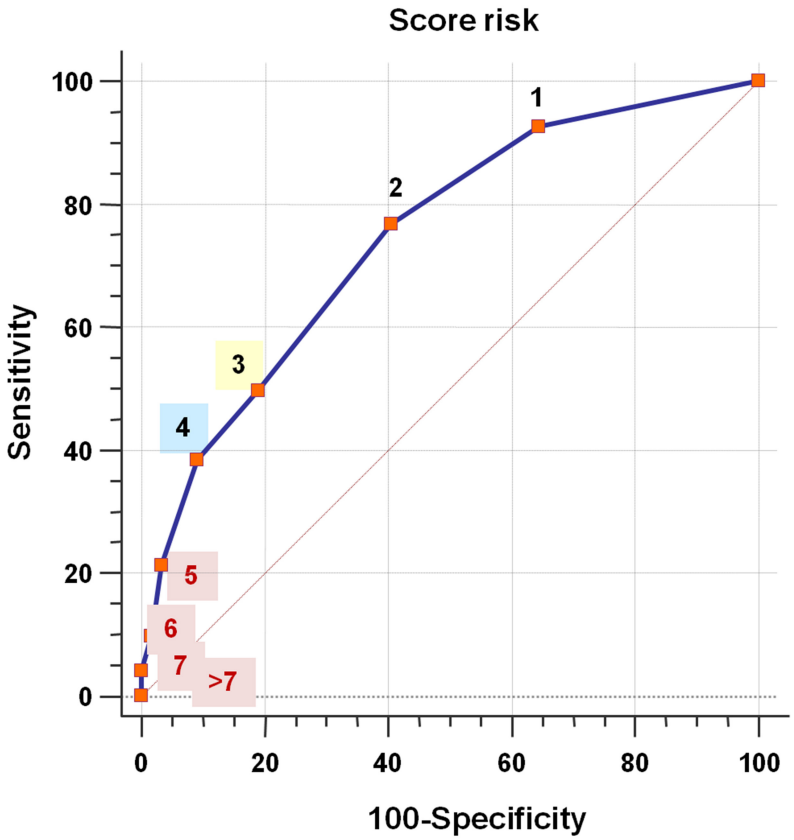
Atrial fibrillation is a leading cause of stroke in the elderly and its prevalence increases with age (67). About a quarter of acute strokes in older patients are caused by atrial fibrillation, however, anticoagulation reduces the stroke risk by 70% in atrial fibrillation (68, 69). In this cohort, we found a prevalence of 29.0% for atrial fibrillation, which is in accordance with the prevalence described in other large cohorts of stroke patients [23.3% for patients aged between 64 and 74, 34.3% aged between 75 and 84 in the Barcelona Stroke Registry (7) or 20.1% in the stroke cohort of the Framingham Study (70)].

Up to 85% of all strokes could be prevented by acting on risk factors with effective medical interventions and modifying the lifestyle of patients (69). In patients with high thromboembolic risk, such as those with atrial fibrillation, the accumulated evidence advises the establishment of antithrombotic prophylaxis (71, 72). An effective thromboprophylaxis requires a balance between efficacy and safety: the benefits of prevention must outweigh the risk of bleeding, so tools which help decide when to start antithrombotic prophylaxis are very useful in clinical practice. The CHA_2DS_2-VASc score allows the estimation of stroke risk in the case of patients with atrial fibrillation based on the presence of different factors and assessing which should have anticoagulant treatment (73).

The inclusion of aPL testing in the profile of tests used in Vascular/Heart health screening could identify people at risk of thrombotic event which could be controlled with preventive treatments (74–76). However, when a patient presents IgA aB2GPI it is not clear whether the beneficial effects of an antithrombotic prophylaxis would justify assuming the inherent risks of the treatment. In this work, we propose a risk stratification model that allows the calculation of the risk of stroke taking into account the simultaneous presence of several risk factors, so it can be useful in helping decide on the convenience of establishing prophylactic treatments. In our model, we have observed that the presence of IgA aB2GPI as the only risk factor means a lower risk than atrial fibrillation. However, IgA aB2GPI-carriers with additional risk factors may have a higher risk than those with only atrial fibrillation. In this sense, the cumulative risk due to the presence of hypertension and dyslipidemia in an IgA aB2GPI-carrier would be greater than the risk of a patient with atrial fibrillation. The value of our prediction model should be verified by independent studies on other cohorts of patients.

Limitations

This work has several limitations. Although, the sample size is large, it is a single center study and the findings described



Score	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	P
0	100	98.5-100	0	0.0-3.0	1	1.0-1.0			0.008
1	92.7	88.6-95.6	35.5	27.0-44.8	1.44	1.3-1.6	0.21	0.1-0.3	0.015
2	76.7	70.9-81.9	59.5	50.2-68.3	1.89	1.5-2.4	0.39	0.3-0.5	0.029
3	49.8	43.4-56.2	81.0	72.9-87.6	2.62	1.8-3.9	0.62	0.5-0.7	0.053
4	38.4	32.2-44.8	90.9	84.3-95.4	4.22	2.4-7.6	0.68	0.6-0.8	0.095
5	21.2	16.3-26.9	96.7	91.8-99.1	6.42	2.4-17.3	0.81	0.8-0.9	0.167
6	9.8	6.4-14.2	98.4	94.2-99.8	5.93	1.4-24.7	0.92	0.9-1.0	0.275
>=7	4.08	2.0-7.4	100	97.0-100			0.96	0.9-1.0	0.418

FIGURE 3 | Risk stratification model, predicted probability of stroke, and representation of the score values.

here should be evaluated in future multicentre studies. Another limitation is that we have no data about lupus anticoagulant in most patients of the study, since it is not routinely evaluated in patients with a first thrombotic event in our hospital (only in patients with recurrent thrombosis). However, this is partially compensated by the fact that patients were tested for anti-PS/PT. A study of Meroni's group showed that the good correlation between the levels of aPS/PT and LA supports the use of these antibodies as a surrogate test for LA (77). Subsequent studies also support that aPS/PT represent an additional diagnostic tool when LA test is not available or the results are uncertain (78, 79). The lack of a second aPL evaluation is another drawback of this study,

but the systematic evaluation of IgA B2GPI in patients with APS has demonstrated that IgA aB2GPI positivity is extremely stable and persists for years. Six months after the first diagnosis, 96% of positive patients continue to be positive (80). The remaining cases were patients with antibody levels very close to the cut-off (gray zone) who became intermittently positive in successive samples. A similar situation occurs with aPL of IgG isotype. Besides, we cannot rule out the possible contribution of other unmeasured factors.

Future Directions

Cerebrovascular events are one of the main causes of mortality with important social consequences but limited data exist about the frequency of non-conventional risk factors in this pathology. The potential role of IgA aB2GPI in stroke should be confirmed by further studies using immunoassays with appropriate and proven sensitivities.

In conclusion, this study demonstrates that IgA aB2GPI are an important independent risk factor for ischemic stroke. The incorporation of aPL evaluation (including extra-criteria aPL) in the assessment of cardiovascular risk factors for ischemic stroke during health examinations could increase the identification of patients at risk of thrombotic events, allowing evaluation to establish preventive treatments.

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 for Clinical Research of Hospital 12 de Octubre (Reference numbers CEIC-14/354 and CEIC-18/182). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM-S and AS conceived and designed the study. FO, AM-S, JH-G, RD-S, and MC participated in the recruitment and screening of patients and in clinical data acquisition. LN, ÓC-M, DP, and FG-E performed laboratory tests and contributed to the collection of samples. LN, AS, and AM-S incorporated clinical and analytical information to the database, performed the first data analysis, and wrote the first draft of the article. AS and DL made the statistical analysis. LN and AS wrote the final draft of the article and made all the changes suggested by the co-authors. 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.2021.665741/full#supplementary-material

REFERENCES

1. Donkor ES. Stroke in the 21(st) century: a snapshot of the burden, epidemiology, and quality of life. *Stroke Res Treat.* (2018) 2018:3238165. doi: 10.1155/2018/3238165
2. Katan M, Luft A. Global burden of stroke. *Semin Neurol.* (2018) 38:208–11. doi: 10.1055/s-0038-1649503
3. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2013) 44:2064–89. doi: 10.1161/STR.0b013e318296aeca
4. Campbell BCV, Khatri P. Stroke. *Lancet.* (2020) 396:129–42. doi: 10.1016/S0140-6736(20)31179-X
5. Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. *Neuropharmacology.* (2008) 55:310–8. doi: 10.1016/j.neuropharm.2008.01.005
6. Boehme AK, Esenwa C, Elkind MS. Stroke risk factors, genetics, and prevention. *Circ Res.* (2017) 120:472–95. doi: 10.1161/CIRCRESAHA.116.308398
7. Arboix A. Cardiovascular risk factors for acute stroke: risk profiles in the different subtypes of ischemic stroke. *World J Clin Cases.* (2015) 3:418–29. doi: 10.12998/wjcc.v3.i5.418
8. Andreoli L, Chighizola CB, Banzato A, Pons-Estel GJ, Ramire de Jesus G, Erkan D. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Arthritis Care Res.* (2013) 65:1869–73. doi: 10.1002/acr.22066
9. Lim W. Antiphospholipid antibody syndrome. *Hematology Am Soc Hematol Educ Program.* (2009) 2009:233–9. doi: 10.1182/asheducation-2009.1.233
10. Corban MT, Duarte Garcia A, McBane RD, Matteson EL, Lerman LO, Lerman A. Antiphospholipid syndrome: role of vascular endothelial cells and implications for risk stratification and targeted therapeutics. *J Am Coll Cardiol.* (2017) 69:2317–30. doi: 10.1016/j.jacc.2017.02.058
11. Pierangeli SS, Chen PP, Raschi E, Scurati S, Grossi C, Borghi MO, et al. Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms. *Semin Thromb Hemost.* (2008) 34:236–50. doi: 10.1055/s-0028-1082267
12. Campbell AL, Pierangeli SS, Wellhausen S, Harris EN. Comparison of the effects of anticardiolipin antibodies from patients with the antiphospholipid syndrome and with syphilis on platelet activation and aggregation. *Thromb Haemost.* (1995) 73:529–34. doi: 10.1055/s-0038-1653808
13. Simantov R, LaSala JM, Lo SK, Gharavi AE, Sammaritano LR, Salmon JE, et al. Activation of cultured vascular endothelial cells by antiphospholipid antibodies. *J Clin Invest.* (1995) 96:2211–9. doi: 10.1172/JCI118276

14. Lambrianides A, Carroll CJ, Pierangeli SS, Pericleous C, Branch W, Rice J, et al. Effects of polyclonal IgG derived from patients with different clinical types of the antiphospholipid syndrome on monocyte signaling pathways. *J Immunol.* (2010) 184:6622–8. doi: 10.4049/jimmunol.0902765
15. Schreiber K, Sciascia S, de Groot PG, Devreese K, Jacobsen S, Ruiz-Irastorza G, et al. Antiphospholipid syndrome. *Nat Rev Dis Primers.* (2018) 4:17103. doi: 10.1038/nrdp.2017.103
16. Garcia D, Erkan D. Diagnosis and management of the antiphospholipid syndrome. *N Engl J Med.* (2018) 378:2010–21. doi: 10.1056/NEJMra1705454
17. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat Rev Rheumatol.* (2011) 7:330–9. doi: 10.1038/nrrheum.2011.52
18. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost.* (2006) 4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x
19. Sciascia S, Amigo MC, Roccatello D, Khamashta M. Diagnosing antiphospholipid syndrome: 'extra-criteria' manifestations and technical advances. *Nat Rev Rheumatol.* (2017) 13:548–60. doi: 10.1038/nrrheum.2017.124
20. Hughes GR. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Br Med J Clin Res Ed.* (1983) 287:1088–9. doi: 10.1136/bmj.287.6399.1088
21. Asherson RA, Mercey D, Phillips G, Sheehan N, Gharavi AE, Harris EN, et al. Recurrent stroke and multi-infarct dementia in systemic lupus erythematosus: association with antiphospholipid antibodies. *Ann Rheum Dis.* (1987) 46:605–11. doi: 10.1136/ard.46.8.605
22. Kenet G, Sadetzki S, Murad H, Martinowitz U, Rosenberg N, Gitel S, et al. Factor V Leiden and antiphospholipid antibodies are significant risk factors for ischemic stroke in children. *Stroke.* (2000) 31:1283–8. doi: 10.1161/01.STR.31.6.1283
23. Saidi S, Mahjoub T, Almawi WY. Lupus anticoagulants and anti-phospholipid antibodies as risk factors for a first episode of ischemic stroke. *J Thromb Haemost.* (2009) 7:1075–80. doi: 10.1111/j.1538-7836.2009.03446.x
24. Lien LM, Chen WH, Chiu HC, Pan WH, Chen JR, Bai CH. High titer of anticardiolipin antibody is associated with first-ever ischemic stroke in Taiwan. *Cerebrovasc Dis.* (2006) 22:225–30. doi: 10.1159/000094008
25. Tuhrim S, Rand JH, Wu XX, Weinberger J, Horowitz DR, Goldman ME, et al. Elevated anticardiolipin antibody titer is a stroke risk factor in a multiethnic population independent of isotype or degree of positivity. *Stroke.* (1999) 30:1561–5. doi: 10.1161/01.STR.30.8.1561
26. Janardhan V, Wolf PA, Kase CS, Massaro JM, D'Agostino RB, Franzblau C, et al. Anticardiolipin antibodies and risk of ischemic stroke and transient ischemic attack: the Framingham cohort and offspring study. *Stroke.* (2004) 35:736–41. doi: 10.1161/01.STR.0000117575.48205.2D
27. Brey RL, Abbott RD, Curb JD, Sharp DS, Ross GW, Stallworth CL, et al. Beta(2)-Glycoprotein 1-dependent anticardiolipin antibodies and risk of ischemic stroke and myocardial infarction: the honolulu heart program. *Stroke.* (2001) 32:1701–6. doi: 10.1161/01.STR.32.8.1701
28. Dragoni F, Chiarotti F, Rosano G, Simioni P, Tormene D, Mazzucconi MG, et al. Thrombophilic screening in young patients (< 40 years) with idiopathic ischemic stroke: a controlled study. *Thromb Res.* (2011) 127:85–90. doi: 10.1016/j.thromres.2010.11.013
29. Gaspersic N, Zaletel M, Kobal J, Zigon P, Cucnik S, Semrl SS, et al. Stroke and antiphospholipid syndrome-antiphospholipid antibodies are a risk factor for an ischemic cerebrovascular event. *Clin Rheumatol.* (2019) 38:379–84. doi: 10.1007/s10067-018-4247-3
30. Pires da Rosa G, Bettencourt P, Rodriguez-Pinto I, Cervera R, Espinosa G. "Non-criteria" antiphospholipid syndrome: a nomenclature proposal. *Autoimmun Rev.* (2020) 19:102689. doi: 10.1016/j.autrev.2020.102689
31. Salle V. Seronegative antiphospholipid syndrome: Myth or reality?. *Rev Med Interne.* (2020) 41:265–74. doi: 10.1016/j.revmed.2020.02.005
32. Cabrera-Marante O, Rodriguez de Frias E, Serrano M, Lozano Morillo F, Naranjo L, Gil-Etayo FJ, et al. The weight of IgA anti-beta2glycoprotein I in the antiphospholipid syndrome pathogenesis: closing the gap of seronegative antiphospholipid syndrome. *Int J Mol Sci.* (2020) 21:8972. doi: 10.3390/ijms21238972
33. Misasi R, Longo A, Recalchi S, Caissutti D, Riitano G, Manganelli V, et al. Molecular mechanisms of "antiphospholipid antibodies" and their paradoxical role in the pathogenesis of "seronegative APS". *Int J Mol Sci.* (2020) 21:8411. doi: 10.3390/ijms21218411
34. de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE, et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost.* (2009) 7:1767–73. doi: 10.1111/j.1538-7836.2009.03588.x
35. Ruiz-Garcia R, Serrano M, Angel Martinez-Flores J, Mora S, Morillas L, Martin-Mola MA, et al. Isolated IgA anti- beta 2 glycoprotein I antibodies in patients with clinical criteria for antiphospholipid syndrome. *J Immunol Res.* (2014) 2014:704395. doi: 10.1155/2014/704395
36. Murthy V, Willis R, Romay-Penabad Z, Ruiz-Limon P, Martinez-Martinez LA, Jatwani S, et al. Value of isolated IgA anti-beta2 -glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. *Arthritis Rheum.* (2013) 65:3186–93. doi: 10.1002/art.38131
37. Tortosa C, Cabrera-Marante O, Serrano M, Martinez-Flores JA, Perez D, Lora D, et al. Incidence of thromboembolic events in asymptomatic carriers of IgA anti ss2 glycoprotein-I antibodies. *PLoS ONE.* (2017) 12:e0178889. doi: 10.1371/journal.pone.0178889
38. Perez D, Tincani A, Serrano M, Shoenfeld Y, Serrano A. Antiphospholipid syndrome and IgA anti-beta2-glycoprotein I antibodies: when Cinderella becomes a princess. *Lupus.* (2018) 27:177–8. doi: 10.1177/0961203317738227
39. Mehrani T, Petri M. Association of IgA Anti-beta2 glycoprotein I with clinical and laboratory manifestations of systemic lupus erythematosus. *J Rheumatol.* (2011) 38:64–8. doi: 10.3899/jrheum.100568
40. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC, et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum.* (2012) 64:1–10. doi: 10.1002/art.33349
41. Serrano M, Espinosa G, Lalueza A, Bravo-Gallego LY, Diaz-Simon R, Garcinuno S, et al. Beta-2-glycoprotein-i deficiency could precipitate an antiphospholipid syndrome-like prothrombotic situation in patients with coronavirus disease 2019. *ACR Open Rheumatol.* (2021). doi: 10.1002/acr2.11245. [Epub ahead of print].
42. Annesi I, Moreau T, Lellouch J. Efficiency of the logistic regression and Cox proportional hazards models in longitudinal studies. *Stat Med.* (1989) 8:1515–21. doi: 10.1002/sim.4780081211
43. Steyerberg E. Clinical prediction models. In: Gail M, editor. *A Practical Approach to Development, Validation, and Updating.* New York, NY: Springer Science+Business Media (2009). p. 508.
44. Moons KG, Harrell FE, Steyerberg EW. Should scoring rules be based on odds ratios or regression coefficients? *J Clin Epidemiol.* (2002) 55:1054–5. doi: 10.1016/S0895-4356(02)00453-5
45. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMJ.* (2015) 350:g7594. doi: 10.1136/bmj.g7594
46. Fields RA, Toubbeh H, Searles RP, Bankhurst AD. The prevalence of anticardiolipin antibodies in a healthy elderly population and its association with antinuclear antibodies. *J Rheumatol.* (1989) 16:623–5.
47. Manoussakis MN, Tzioufas AG, Silis MP, Pange PJ, Goudevenos J, Moutsopoulos HM. High prevalence of anti-cardiolipin and other autoantibodies in a healthy elderly population. *Clin Exp Immunol.* (1987) 69:557–65.
48. Vadasz Z, Haj T, Kessel A, Toubi E. Age-related autoimmunity. *BMC Med.* (2013) 11:94. doi: 10.1186/1741-7015-11-94
49. Fleetwood T, Cantello R, Comi C. Antiphospholipid syndrome and the neurologist: from pathogenesis to therapy. *Front Neurol.* (2018) 9:1001. doi: 10.3389/fneur.2018.01001
50. Sanna G, D'Cruz D, Cuadrado MJ. Cerebral manifestations in the antiphospholipid (Hughes) syndrome. *Rheum Dis Clin North Am.* (2006) 32:465–90. doi: 10.1016/j.rdc.2006.05.010
51. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramon E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis.* (2015) 74:1011–8. doi: 10.1136/annrheumdis-2013-204838

52. Miesbach W, Gilzinger A, Gokpinar B, Claus D, Scharrer I. Prevalence of antiphospholipid antibodies in patients with neurological symptoms. *Clin Neurol Neurosurg.* (2006) 108:135–42. doi: 10.1016/j.clineuro.2005.03.005
53. Hughes GR. Migraine, memory loss, and "multiple sclerosis". Neurological features of the antiphospholipid (Hughes') syndrome. *Postgrad Med J.* (2003) 79:81–3. doi: 10.1136/pmj.79.928.81
54. Ali HY, Abdullah ZA. Anti-beta(2)-glycoprotein I autoantibody expression as a potential biomarker for strokes in patients with anti-phospholipid syndrome. *J Immunotoxicol.* (2008) 5:173–7. doi: 10.1080/15476910802129638
55. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* (2002) 46:1019–27. doi: 10.1002/art.10187
56. Nencini P, Baruffi MC, Abbate R, Massai G, Amaducci L, Inzitari D. Lupus anticoagulant and anticardiolipin antibodies in young adults with cerebral ischemia. *Stroke.* (1992) 23:189–93. doi: 10.1161/01.STR.23.2.189
57. Fiallo P, Tomasina C, Clapasson A, Cardo PP. Antibodies to beta(2)-glycoprotein I in ischemic stroke. *Cerebrovasc Dis.* (2000) 10:293–7. doi: 10.1159/000016073
58. Chighizola CB, Andreoli L, de Jesus GR, Banzato A, Pons-Estel GJ, Erkan D, et al. The association between antiphospholipid antibodies and pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Lupus.* (2015) 24:980–4. doi: 10.1177/0961203315572714
59. Brey RL, Chapman J, Levine SR, Ruiz-Irastorza G, Derksen RH, Khamashta M, et al. Stroke and the antiphospholipid syndrome: consensus meeting Taormina 2002. *Lupus.* (2003) 12:508–13. doi: 10.1191/0961203303lu390oa
60. Verro P, Levine SR, Tietjen GE. Cerebrovascular ischemic events with high positive anticardiolipin antibodies. *Stroke.* (1998) 29:2245–53. doi: 10.1161/01.STR.29.11.2245
61. Nojima J, Kuratsune H, Suehisa E, Kitani T, Iwatani Y, Kanakura Y. Strong correlation between the prevalence of cerebral infarction and the presence of anti-cardiolipin/beta2-glycoprotein I and anti-phosphatidylserine/prothrombin antibodies–Co-existence of these antibodies enhances ADP-induced platelet activation in vitro. *Thromb Haemost.* (2004) 91:967–76. doi: 10.1160/TH03-10-0608
62. Sciascia S, Sanna G, Khamashta MA, Cuadrado MJ, Erkan D, Andreoli L, et al. The estimated frequency of antiphospholipid antibodies in young adults with cerebrovascular events: a systematic review. *Ann Rheum Dis.* (2015) 74:2028–33. doi: 10.1136/annrheumdis-2014-205663
63. Kahles T, Humpich M, Steinmetz H, Sitzer M, Lindhoff-Last E. Phosphatidylserine IgG and beta-2-glycoprotein I IgA antibodies may be a risk factor for ischaemic stroke. *Rheumatology (Oxford).* (2005) 44:1161–5. doi: 10.1093/rheumatology/keh698
64. Staub HL, Norman GL, Crowther T, da Cunha VR, Polanczyk A, Bohn JM, et al. Antibodies to the atherosclerotic plaque components beta2-glycoprotein I and heat-shock proteins as risk factors for acute cerebral ischemia. *Arq Neuropsiquiatr.* (2003) 61:757–63. doi: 10.1590/S0004-282X2003000500010
65. Heinzlef O, Abuaf N, Cohen A, Amarenco P, French Study of Aortic Plaques in Stroke G. Recurrent stroke and vascular events in elderly patients with anticardiolipin antibodies: a prospective study. *J Neurol.* (2001) 248:373–9. doi: 10.1007/s004150170176
66. Rodriguez-Sanz A, Martinez-Sanchez P, Prefasi D, Fuentes B, Pascual-Salcedo D, Blanco-Banares MJ, et al. Antiphospholipid antibodies correlate with stroke severity and outcome in patients with antiphospholipid syndrome. *Autoimmunity.* (2015) 48:275–81. doi: 10.3109/08916934.2014.988329
67. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation: a major contributor to stroke in the elderly. The Framingham Study. *Arch Intern Med.* (1987) 147:1561–4. doi: 10.1001/archinte.147.9.1561
68. Arboix A, Garcia-Eroles L, Massons J, Oliveres M, Targa C. Lacunar infarcts in patients aged 85 years and older. *Acta Neurol Scand.* (2000) 101:25–9. doi: 10.1034/j.1600-0404.2000.00005.x
69. Sarikaya H, Ferro J, Arnold M. Stroke prevention–medical and lifestyle measures. *Eur Neurol.* (2015) 73:150–7. doi: 10.1159/000367652
70. Lin HJ, Wolf PA, Kelly-Hayes M, Beiser AS, Kase CS, Benjamin EJ, et al. Stroke severity in atrial fibrillation. The Framingham Study. *Stroke.* (1996) 27:1760–4. doi: 10.1161/01.STR.27.10.1760
71. Deedwania PC, Huang GW. Role of emerging antithrombotic therapy in the prevention of cardioembolic complications in patients with atrial fibrillation. *Am J Cardiovasc Drugs.* (2011) 11:265–75. doi: 10.2165/11592410-000000000-00000
72. Lip GYH, Banerjee A, Boriani G, Chiang CE, Fargo R, Freedman B, et al. Antithrombotic therapy for atrial fibrillation: CHEST guideline and expert panel report. *Chest.* (2018) 154:1121–201. doi: 10.1016/j.chest.2018.07.040
73. Lip GY. Stroke and bleeding risk assessment in atrial fibrillation: when, how, and why? *Eur Heart J.* (2013) 34:1041–9. doi: 10.1093/eurheartj/ehs435
74. Roldan JF, Brey RL. Neurologic manifestations of the antiphospholipid syndrome. *Curr Rheumatol Rep.* (2007) 9:109–15. doi: 10.1007/s11926-007-0004-3
75. Sanna G, Bertolaccini ML, Hughes GR. Hughes syndrome, the antiphospholipid syndrome: a new chapter in neurology. *Ann N Y Acad Sci.* (2005) 1051:465–86. doi: 10.1196/annals.1361.088
76. Rodrigues CE, Carvalho JF, Shoenfeld Y. Neurological manifestations of antiphospholipid syndrome. *Eur J Clin Invest.* (2010) 40:350–9. doi: 10.1111/j.1365-2362.2010.02263.x
77. Pregnolato F, Chighizola CB, Encabo S, Shums Z, Norman GL, Tripodi A, et al. Anti-phosphatidylserine/prothrombin antibodies: an additional diagnostic marker for APS? *Immunol Res.* (2013) 56:432–8. doi: 10.1007/s12026-013-8421-z
78. Sciascia S, Radin M, Cecchi I, Rubini E, Scotta A, Rolla R, et al. Reliability of lupus anticoagulant and anti-phosphatidylserine/prothrombin autoantibodies in antiphospholipid syndrome: a multicenter study. *Front Immunol.* (2019) 10:376. doi: 10.3389/fimmu.2019.00376
79. Shi H, Zheng H, Yin YF, Hu QY, Teng JL, Sun Y, et al. Antiphosphatidylserine/prothrombin antibodies (aPS/PT) as potential diagnostic markers and risk predictors of venous thrombosis and obstetric complications in antiphospholipid syndrome. *Clin Chem Lab Med.* (2018) 56:614–24. doi: 10.1515/cclm-2017-0502
80. Serrano A, Garcia F, Serrano M, Ramirez E, Alfaro FJ, Lora D, et al. IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. *Kidney Int.* (2012) 81:1239–44. doi: 10.1038/ki.2011.477

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CASE REPORT
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The Brain–Heart Link: A Case Report of a Critically Located Multiple Sclerosis Lesion in the Brainstem Leading to Recurrent Takotsubo Syndrome

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Various central nervous system (CNS) diseases, including neurovascular and neuroinflammatory diseases, can lead to stress cardiomyopathy, also known as Takotsubo syndrome (TTS). We present a case of a 69-year-old woman with cardiovascular comorbidities, suffering from repeated episodes of TTS and respiratory failure due to a critical lesion in the brainstem, leading to a diagnosis of multiple sclerosis (MS). Despite aggressive treatment, intractable and recurrent symptoms in our patient occurred. Repeated bouts of autonomic dysfunction and respiratory failure ultimately led to installment of palliative care and the patient passing away. TTS should raise suspicion for underlying neurological diseases. Thorough questioning of previous neurological symptoms and extensive neurological workup is warranted. MS should be considered as a trigger of TTS also in elderly patients with cardiovascular risk factors.

Keywords: multiple sclerosis, brainstem, autonomic dysfunction, case report, Takotsubo syndrome, stress cardiomyopathy

INTRODUCTION

Stress cardiomyopathy, also known as Takotsubo syndrome (TTS) or broken-heart syndrome (1, 2), can be caused by acute or chronic central nervous system (CNS) diseases, including subarachnoid or intracerebral hemorrhage, epilepsy, ischemic stroke, migraine, encephalitis, traumatic brain injury, posterior reversible encephalopathy syndrome (PRES), and amyotrophic lateral sclerosis (ALS) (3–5). Both classic TTS and inverted TTS have also been described in patients known to suffer from multiple sclerosis (MS) (3, 4, 6–19). We report a case of recurrent classic TTS in combination with episodes of respiratory failure and other signs of autonomic dysfunction, which led to a diagnosis of MS in an elderly woman with cardiovascular risk factors.

CASE DESCRIPTION

A 69-year-old woman with a history of cigarette smoking, arterial hypertension, type 2 diabetes mellitus, hypercholesterolemia, right subcapital femur fracture, and possible stroke was transferred to our institution for coronary angiography (CAG) because of an episode of thoracic discomfort and persistent dyspnea. The working diagnosis was inferolateral ST-segment elevation myocardial infarction (STEMI). The provided print of 6-lead electrocardiogram (ECG) monitor strip showed inferolateral STEMI (**Figure 1A**). On admission, she had a blood pressure of 100/70 mmHg, heart rate of 110 beats per minute, and peripheral oxygen saturation of 100% with 2 L of oxygen. Tachypnea, hyperhidrosis, bilateral rhonchi, and right-sided facial palsy were present on physical examination. Biochemical findings included elevated cardiac troponin I (peak level: 5.68 μg/L, normal value <0.045 μg/L; day 1, 5.21 μg/ml; day 2 morning, 4.74 μg/ml; day 2 evening 5.68 μg/ml) and creatine kinase-MB (CK-MB) (peak level: 32.6 μg/L, normal value <3.6 μg/L). Natriuretic peptide levels were not assessed at any timepoint. Chest radiograph was normal. CAG showed mild (50%) stenosis of the mid left anterior descending artery and a significant (70%) stenosis of the right coronary artery (RCA) (**Figures 1B,C**). A 12-lead ECG showed slight residual ST elevation in the infero-anterolateral leads with Q wave formation in the same region (**Supplementary File 12-lead ECG 1**). Ventriculography revealed apical ballooning (**Figures 2A,B**) with a decreased left ventricular ejection fraction (LVEF) of 33%. She was diagnosed with TTS, although typical (emotional) triggers were absent.

Besides thoracic complaints, the patient mentioned blurred vision, double vision, and dysphagia, which had been present for more than 2 weeks prior to admission. She had experienced an episode of gait imbalance and right-sided facial palsy attributed to a possible stroke 4 months earlier. Her current neurological symptoms were attributed to a new stroke after excluding hemorrhage on a brain computed tomography (CT) scan, which was completely normal, without signs of recent or old brain infarction. Eight days later, she underwent uncomplicated percutaneous coronary intervention (PCI) with drug-eluting stent (DRES) implantation of the coronary artery stenosis of the RCA.

Seven days after PCI, she was readmitted to the intensive care unit (ICU) due to cardiogenic shock and respiratory failure. Supportive treatment with IV dobutamine, switched to IV noradrenaline after 2 days, and mechanical ventilation was started. ECG revealed non-STEMI with non-significant ST elevation and biphasic T waves in anterior leads (**Supplementary File 12-lead ECG 2**). Serum troponin I levels remained low (0.118 μg/ml for 2 days in a row). CAG did not demonstrate restenosis of the proximal RCA at the level of the DRES. Ventriculography revealed apical ballooning of the left ventricle with hyperdynamic midsegments, compatible with TTS with a different morphology than at first presentation (**Figures 2C,D**). This was confirmed by transesophageal echocardiography (TEE). Intracardiac thrombi were absent. TTS resolved in the next days, and cardiac function improved with residual mild hypokinesia of the left ventricle and an LVEF of 53% with evolving deep negative T waves on ECG (**Supplementary File 12-lead ECG 3**).

Several episodes of acute arterial hypertension (maximum 220 mmHg systolic) occurred, accompanied by respiratory failure with hypoventilation and hypercapnia necessitating invasive ventilation and treatment for hypertension with IV nicardipine. Episodes of brady- and tachycardia occurred, and profound

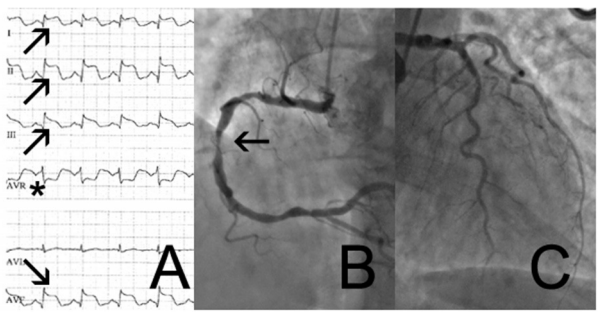

FIGURE 1 | 6-lead monitor ECG **(A)** with ST-elevation in the inferolateral leads I,II,III,aVF (arrows) and ST depression in aVR (asterix). Coronary angiogram showing right coronary artery **(B)** with significant stenosis of the mid section of the right coronary artery (arrow) and coronary atheromatosis with not significant coronary artery disease of the left circumflex- and left anterior artery **(C)**.

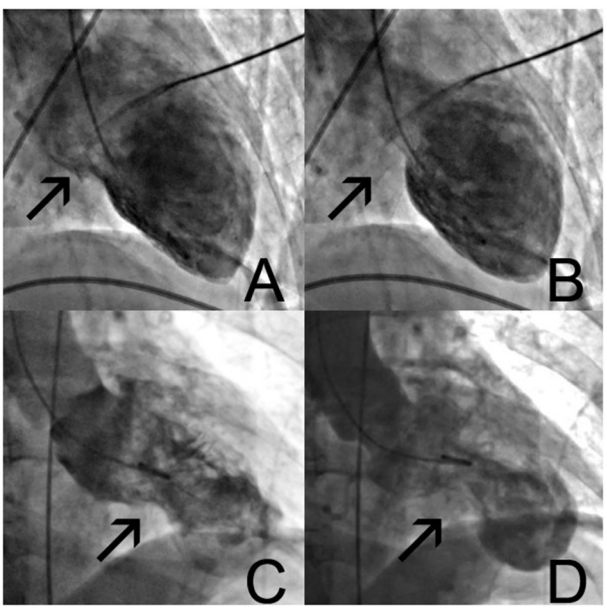

FIGURE 2 | Left ventricular ventriculography **(A–D)**. In normal diastole **(A)**, with typical apical ballooning and contraction of only the basal parts of the ventricle **(B)**. Control angiogram after readmission 7 days later **(C)**, showing different morphologies with mid-section hypercontraction and apical sparing **(D)**. See also **Supplementary Data Clips 1**, **2**.

sweating despite hypothermia (lowest temperature 33.5°C) was present. Two days after cessation of IV noradrenaline, plasma catecholamines were measured: metanephrine level was normal (87 pg/ml, reference value 90 pg/ml), but normetanephrine was increased (949 pg/ml, reference value 200 pg/ml). The 24-h urinary excretion of adrenaline, dopamine, and metanephrine was normal. Excreted levels of norepinephrine (416 μg/24 h, reference values 14–50) and normetanephrine (1,709 μg/24 h, reference value upper limit 769) were increased in urine. CT of thorax and abdomen did not show lesions suggestive of paraganglioma nor pheochromocytoma. Iodine-123-labeled MIBG scintigraphy was normal.

Neurological evaluation showed diplopia, right-sided hemiparesis, a bilateral pyramidal syndrome, and Cheyne–Stokes breathing pattern. An underlying CNS disease triggering TTS and autonomic dysregulation was suspected. Electroencephalography showed no epileptic activity. Brain magnetic resonance imaging (MRI) showed a well-demarcated, oval-shaped T2 hyperintense lesion with diffusion restriction in the left cerebellar peduncle extending to the posterolateral part of the medulla oblongata (**Figures 3A,B**). Other T2 hyperintense lesions were found in the pons, in the left temporal and right frontal periventricular white matter, and in the cervical spinal cord at the level of C4 (**Figures 3C,D**). One juxtacortical T2 hyperintense lesion was found in the left parietal lobe. In the differential diagnosis, systemic autoimmune diseases and neuroinflammatory diseases such as MS and neuromyelitis optica were considered. Infectious and autoimmune diseases other than MS were excluded via laboratory tests. Bedside fundoscopy was normal without signs of vasculitis. Due to the need for dual antiaggregant therapy after recent PCI of RCA, lumbar puncture was not performed.

In retrospect, 33 years prior, two episodes of spontaneously remitting neurological symptoms occurred with an interval of 2 months. MRI of the cervical spine, performed around that time, demonstrated a small hyperintensity in the spinal cord at the level of the craniocervical junction and a hyperintense signal at the levels of C4–C5 and C5–C6, with discus hernia. Furthermore, the patient had experienced another neurological episode of discrete right-sided hemiparesis of unknown etiology 14 years earlier. Investigation with MRI of the cervical spine at that time showed a myelopathy at C4 and cervical stenosis at the level below. MS had been considered in the differential diagnosis but was not confirmed at that point in time, as investigation of the cerebrospinal fluid did not demonstrate oligoclonal bands. The previously mentioned "stroke" in our patient's medical history had been an interpretation of an episode of right facial palsy, loss of strength in the right arm, diplopia, and tendency to fall to the right side. However, a brain MRI was not performed to confirm the clinically suspected diagnosis of stroke. We believe the previous episodes of neurological dysfunction, including the "possible stroke," were in fact MS relapses. Indeed, brain MRI showed evidence of inflammatory T2 hyperintense lesions and no lesions compatible with previous stroke(s).

Based on the MRI findings and combined with previous and current clinical neurological symptomatology, the diagnosis of relapsing–remitting MS (RRMS) was made. The patient fulfilled the 2010 McDonald criteria for MS, with clinical episodes providing evidence for dissemination in time and space (20). Brain and cervical spinal cord MRIs were compatible with dissemination in space according to the 2010 McDonald criteria (20).

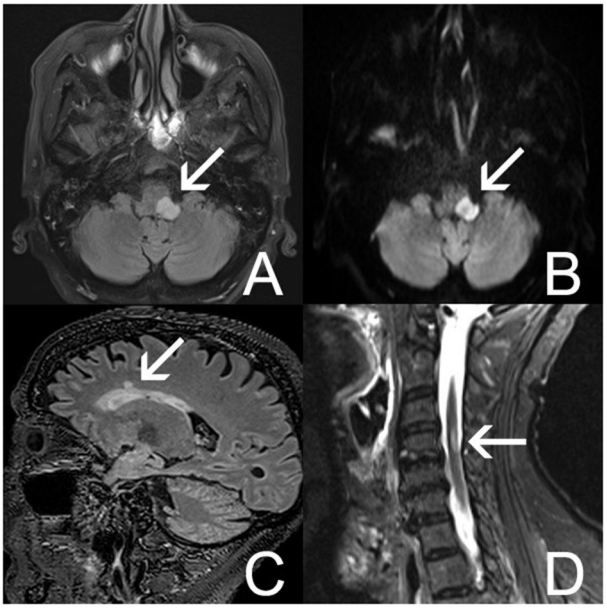

FIGURE 3 | Brain MRI **(A–D)**. **(A)** Axial fluid-attenuated inversion recovery (FLAIR) MRI image shows a well-demarcated, hyperintense lesion in the left posterolateral part of the medulla oblongata (arrow). **(B)** Axial diffusion-weighted imaging (DWI) shows restriction of diffusion in this lesion (arrow). **(C)** Sagittal FLAIR MRI image shows a nodular hyperintensity in the right frontal lobe (arrow). **(D)** Sagittal short tau inversion recovery (STIR) image shows a hyperintense lesion in the cervical medulla at C4 (arrow).

Transthoracic echocardiogram (TTE) showed almost complete recovery of the systolic left ventricular function. ECG showed normalization of previous changes, and only non-specific ST-T changes were present (**Supplementary File 12-lead ECG 4**). During her stay at the ICU, there were recurrent episodes of uncontrolled hypertension, perspiration without fever or distress, and repetitive hypercapnic respiratory failure based on central apnea, necessitating intubation with ventilatory support for four times. She was treated with pulsed high-dose intravenous methylprednisolone, plasma exchange, and intravenous immunoglobulins (400 mg/kg) for 5 days. This was followed by treatment with high-dose cyclophosphamide due to lack of clinical response to previous treatments and severity of clinical symptoms.

Three months after admission to the ICU, she was discharged to the neurology ward but readmitted 2 weeks later with recurrence of central hypercapnic respiratory failure due to autonomic dysfunction, probably caused by the brainstem lesion. Again, the episode was accompanied by hypertension (216/110 mmHg) and sinus tachycardia (120 bpm). TTE did not show recurrence of TTS at that time. Because of relapsing episodes of untreatable autonomic dysfunction and

the poor prognosis for further recovery, the patient decided to stop supportive treatment, and she died 4 months after initial presentation.

DISCUSSION

While MS exacerbations and lesions in the medulla oblongata with cardiopulmonary presentations have been described before (21), we present the first case of recurrent TTS leading to a diagnosis of MS in an elderly patient. In older patients, a diagnosis of MS may be challenging because of the atypical age and medical history with cardiovascular risk factors. However, when reassessing the patient history, we identified several episodes compatible with MS relapses over the course of more than 30 years. The age of our patient, 69 years, is comparable with the mean age to develop TTS (1), but older than has been published in the literature (MS patients with TTS had an age range of 14–55 years) (21). Our patient recovered from two episodes of TTS but eventually died after stopping the supportive treatment in the ICU. Several negative prognostic factors were present in our patient. Dyspnea on admission is known to correlate with worse outcome, including in-hospital complications and mortality (22). Neurological disorders, low LVEF on admission, and cardiogenic shock, all present in our case, have been associated with worse outcome (2, 23). The InterTAK registry has shown that physical triggers carry a higher mortality risk than emotional ones (24). Moreover, patients with TTS secondary to neurological diseases had the worst short-term (30 days) prognosis and neurological diseases, as a cause of TTS remained a negative prognostic factor in the long term (5 years) (24).

The cardiovascular comorbidities and the presence of coronary artery disease (CAD) at presentation complicated the diagnosis in this case. However, two points need to be made here. First, TTS can be triggered by acute coronary syndrome (25). Hence, significant CAD does not exclude the diagnosis of TTS, which is also reflected in the International Expert Consensus Document on TTS (26). Our case fulfills the suggested diagnostic criteria for TTS (26). Second, the regional wall abnormalities did not match the location of the CAD in our patient and were consistent with TTS. The recovery of the ventricular function without intervention, after the first episode, supports the diagnosis of TTS in our patient. The normal MIBG scan does not exclude a diagnosis of TTS in our patient, as this scan was performed ∼1 week after a TTE demonstrated almost full recovery of TTS, showing only a mild global reduced contractility without regional wall motion disturbances.

While the pathogenesis of TTS remains incompletely understood, the association with emotional or psychological stress factors and neurological diseases preceding the onset of TTS emphasizes the existence of a link between the heart and the brain (26). Indeed, TTS has been associated with a variety of neurological causes, among which subarachnoid hemorrhage, status epilepticus, and seizures have been reported as the strongest associated acute neurological diseases (5, 26–28). Data analysis from the InterTAK registry demonstrated that 4.7% of patients ($N = 66/1,402$) had TTS recurrence with intervals ranging from 30 days to 9.9 years (29). Both neurological and psychiatric disorders were found to be independent predictors of recurrence (29). In 34.8% ($N = 23/66$) patients, the ballooning pattern differed between the first and subsequent presentations (29). All patients with multiple recurrent events had psychiatric or neurological comorbidities (29). Recent research has linked a decreased functioning of brain regions, which are associated with autonomic functions and the occurrence of TTS (1, 26, 30). A recent review of published cases of MS and TTS found that in the majority, a demyelinating lesion located in the medulla oblongata was present (21).

One hypothesis of the pathophysiology of TTS is based on an increase of catecholamines. This can lead, via multiple mechanisms, to direct catecholamine toxicity and adrenoceptor-mediated damage resulting in epicardial and microvascular coronary vasoconstriction and/or spasm and increased cardiac workload. This induces myocardial damage, which has a functional counterpart of transient apical left ventricular ballooning (31, 32). In our patient, increased plasma and 24-h urinary excretion levels of norepinephrine and normetanephrine were detected during the second episode of TTS. Since levels at first presentation were not measured and only measured once during the disease course, it is difficult to draw firm conclusions on the relevance of the catecholamine levels in the development of TTS in our case. A recently published meta-analysis of catecholamine plasma levels in TTS demonstrated that levels of norepinephrine, epinephrine, and dopamine are elevated in TTS, while marked elevation is rare (32). A link to prognosis of TTS remains to be proven.

We hypothesize that in our case, direct suppression of vagal-mediated cardio-inhibition played a role in the development of TTS. Indeed, our patient had a critically located MS lesion in the dorsolateral medulla oblongata involving the nucleus ambiguus, which contains cardio-inhibitory cholinergic preganglionic parasympathetic neurons (33). The role of impairment of vagal nerve fibers in development of TTS and blood pressure dysregulation has been reviewed extensively by Norcliffe-Kaufmann (34).

LIMITATIONS

Several limitations of our case study need to be addressed. Autopsy was not performed, and therefore, the diagnosis of MS was not confirmed postmortem. However, our patient fulfilled McDonald 2010 diagnostic criteria for MS, and other potential causes for the brain lesions were excluded (20). Also, pathological examination of the heart is missing, which could have confirmed the clinical findings of limited or no cardiac ischemia, supporting the diagnosis of TTS. Despite the lack of autopsy material, additional investigations were compatible with a diagnosis of TTS. Moreover, the presence of CAD does not exclude a diagnosis of TTS (26). Finally, while no formal autonomic function tests were performed in our patient due to her clinical

condition, the clinical symptoms with profound blood pressure fluctuations, hypothermia, and hypoventilation are suggestive of autonomic dysregulation and compatible with symptoms occurring as a consequence of the lesion in the medulla oblongata.

FUTURE DIRECTIONS

This case supports the hypothesis of the link between neurological disease and TTS, especially with involvement of the medulla oblongata. Further research of the pathophysiology of brain stem disease and/or lesions, especially located in the medulla oblongata, and the occurrence of TTS is needed and may provide new insights in the pathophysiology and treatment of this syndrome.

CONCLUSIONS

This case illustrates a link between a single critical demyelinating lesion in the dorsolateral medulla oblongata and TTS. Diagnosis of TTS in combination with signs of autonomic dysfunction warrants thorough investigation of possible underlying or concomitant neurological disease, including MS, even in older patients with cardiovascular risk factors.

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

Ethical review and approval was not required for this case study on human participants in accordance with the local legislation and institutional requirements. The patient's relative provided their written informed consent to participate in this study. Written informed consent was obtained from the patient's relative for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FR, PM, and BW conceived the idea for the manuscript. FR and PM drafted the first version. FR and BW drafted the revised manuscript. PM, IB, CF, and BW critically revised the paper for important intellectual content. SN selected the MRI images and provided the description. All authors approved the final version of the 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.2021.674118/full#supplementary-material

Supplementary Figure 1. 12-lead ECG 1 | 12-lead ECG on 25 mm/s paper speed and 10 mm/mV y-axis with regular sinus-rhythm at 111 beats per minute (tachycardia). There is slight residual 1 mm ST-elevation in lead II and V4-V6. There is Q-wave formation present on inferior (II, III, and aVF) and anterolateral leads (I, V2-V6) and a small R wave on anterior leads (V1-V6).

Supplementary Figure 2. 12-lead ECG 2 | 12-lead ECG on 25 mm/s paper speed and 10 mm/mV y-axis with regular sinus-rhythm at 86 beats per minute. There is convex ST elevation, most appreciated in two of the inferior leads (II, aVF) and anterolateral (I, V2-V6). Biphasic T wave formation anterior (V2-V4) and negative T waves in aVL. Q-waves are present inferior (II, III, and aVF), lateral (I, aVL, V5-V6) and anterior (V3-V4).

Supplementary Figure 3. 12-lead ECG 3 | 12-lead ECG on 25 mm/s paper speed and 10 mm/mV y-axis with regular sinus-rhythm at 78 beats per minute. Negative T waves are present inferior (II, III, and aVF), lateral (I, V5-V6) and anterior (V2-V4). ST-segment in aVL is flattened. There is only a small Q wave remaining in the lateral (I, aVL, V5, V6) leads.

Supplementary Figure 4. 12-lead ECG 4 | 12-lead ECG on 25 mm/s paper speed and 10 mm/mV y-axis with regular sinus-rhythm at 124 beats per minute (tachycardia). There is recuperation of the earlier mentioned ECG-changes with no more pathological ST-segments, T-waves, or Q-waves present. There is a diffuse flattened ST-segment.

REFERENCES

1. Rawish E, Stiermaier T, Santoro F, Brunetti ND, Eitel I. Current knowledge and future challenges in takotsubo syndrome: part 1-pathophysiology and diagnosis. *J Clin Med.* (2021) 10:30479. doi: 10.3390/jcm10030479
2. Santoro F, Mallardi A, Leopizzi A, Vitale E, Rawish E, Stiermaier T, et al. Current knowledge and future challenges in takotsubo syndrome: part 2-treatment and prognosis. *J Clin Med.* (2021) 10:30468. doi: 10.3390/jcm10030468
3. Finsterer J, Wahbi K. CNS disease triggering Takotsubo stress cardiomyopathy. *Int J Cardiol.* (2014) 177:322–9. doi: 10.1016/j.ijcard.2014.08.101
4. Ennezat PV, Pesenti-Rossi D, Aubert JM, Rachenne V, Bauchart JJ, Auffray JL, et al. Transient left ventricular basal dysfunction without coronary stenosis in acute cerebral disorders: a novel heart syndrome (inverted Takotsubo). *Echocardiography.* (2005) 22:599–602. doi: 10.1111/j.1540-8175.2005.40046.x
5. Buchmann SJ, Lehmann D, Stevens CE. Takotsubo cardiomyopathy-acute cardiac dysfunction associated with neurological and psychiatric disorders. *Front Neurol.* (2019) 10:917. doi: 10.3389/fneur.2019.00917
6. Biesbroek PS, Nijveldt R, de Jong BA, van Rossum AC, van Loon RB. Reversed takotsubo cardiomyopathy in a patient with new-onset multiple sclerosis: Cause, link, or just coincidence? *Int J Cardiol.* (2016) 207:217–9. doi: 10.1016/j.ijcard.2016.01.174
7. Peller M, Balsam P, Budnik M, Marchel M, Opolski G. Reverse Takotsubo syndrome in a patient with diagnosed multiple sclerosis. *Kardiol Pol.* (2016) 74:1029. doi: 10.5603/KP.2016.0125
8. Yalta K, Taylan G, Yalta T, Yetkin E. Takotsubo cardiomyopathy in the setting of multiple sclerosis: a multifaceted phenomenon with important implications. *Monaldi Arch Chest Dis.* (2020) 90:1420. doi: 10.4081/monaldi.2020.1420
9. Rapp D, Keßler M, Pinkhardt E, Otto M, Tumani H, Senel M. Stress cardiomyopathy associated with the first manifestation of multiple sclerosis:

a case report. *BMC Neurol.* (2020) 20:227. doi: 10.1186/s12883-020-01757-6
10. Dell'Aquila A, Sciatti E, Vizzardi E, Metra M. The brain-heart connection: a multiple sclerosis relapse presenting as Takotsubo Syndrome. A case report and literature review. *Monaldi Arch Chest Dis.* (2020) 90:1153. doi: 10.4081/monaldi.2020.1153
11. Madias JE. Takotsubo syndrome triggered in the setting of multiple sclerosis: The need to monitor blood catecholamines and the CNS sympathetic input to the heart. *Multiple Sclerosis Relat Disord.* (2019) 27:391. doi: 10.1016/j.msard.2018.11.028
12. Prestipino E, Squitieri M, Razzolini L, Pastò L, Forleo P, Amato MP. A case of Takotsubo syndrome during a multiple sclerosis brainstem relapse. *Multiple Sclerosis Relat Disord.* (2018) 24:1–2. doi: 10.1016/j.msard.2018.05.005
13. Prestipino E, Squitieri M, Razzolini L, Pastò L, Forleo P, Amato MP. Corrigendum to: "A case of Takotsubo syndrome during a multiple sclerosis brainstem relapse." *Multiple Sclerosis Relat Disord.* (2019). 30:292. doi: 10.1016/j.msard.2019.03.010
14. London F, Gonzalez Rodriguez de Azero N, Philippart M, Higny J, Mulquin N. Reverse takotsubo cardiomyopathy triggered by a multiple sclerosis relapse. *Acta Neurol Belg.* (2019) 119:295–7. doi: 10.1007/s13760-018-1056-z
15. Bayer AD, Cahill JF, Rizvi SA. Multiple sclerosis relapse presenting as an acute cardiomyopathy. *Multiple Sclerosis Relat Disord.* (2019) 27:7–8. doi: 10.1016/j.msard.2018.09.021
16. Androdias G, Bernard E, Biotti D, Collongues N, Durand-Dubief F, Pique J, et al. Multiple sclerosis broke my heart. *Ann Neurol.* (2017) 81:754–8. doi: 10.1002/ana.24935
17. Midaglia L, Juega Mariño JM, Sastre-Garriga J, Rovira A, Vidal-Jordana A, López-Pérez MA, et al. An uncommon first manifestation of multiple sclerosis: Tako-Tsubo cardiomyopathy. *Multiple Sclerosis.* (2016) 22:842–6. doi: 10.1177/1352458516638557
18. Kozu K, Suzuki H, Nishiyama S, Yaoita N, Yamamoto S, Tatebe S, et al. Multiple sclerosis lesion in the medulla oblongata in a patient with takotsubo cardiomyopathy. *Int J Cardiol.* (2016) 222:980–1. doi: 10.1016/j.ijcard.2016.08.128
19. Hilz MJ. Cardiac stunning as first manifestation of multiple sclerosis: A case report reminding us not to overlook cardiovascular autonomic dysfunction in multiple sclerosis. *Multiple Sclerosis.* (2016) 22:847–8. doi: 10.1177/1352458516638559
20. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* (2011) 69:292–302. doi: 10.1002/ana.22366
21. Valencia-Sanchez C, Goodman BP, Carter JL, Wingerchuk DM. The spectrum of acute cardiopulmonary events associated with multiple sclerosis exacerbations. *Mult Scler.* (2019) 25:758–65. doi: 10.1177/1352458518823482
22. Arcari L, Musumeci MB, Stiermaier T, El-Battrawy I, Möller C, Guerra F, et al. Incidence, determinants and prognostic relevance of dyspnea at admission in patients with Takotsubo syndrome: results from the international multicenter GEIST registry. *Sci Rep.* (2020) 10:13603. doi: 10.1038/s41598-020-70445-9
23. Ghadri JR, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International expert consensus document on Takotsubo syndrome (Part II): diagnostic workup, outcome, and management. *Eur Heart J.* (2018) 39:2047–62. doi: 10.1093/eurheartj/ehy077
24. Ghadri JR, Kato K, Cammann VL, Gili S, Jurisic S, Di Vece D, et al. Long-term prognosis of patients with Takotsubo syndrome. *J Am College Cardiol.* (2018) 72:874–82. doi: 10.1016/j.jacc.2018.06.016
25. S YH. Takotsubo syndrome triggered by acute coronary syndrome in a cohort of 20 patients: an often missed diagnosis. *Int J Cardiol Res.* (2015) 2:28–33. doi: 10.19070/2470-4563-150007
26. Ghadri JR, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International expert consensus document on Takotsubo syndrome (Part I): clinical characteristics, diagnostic criteria, and pathophysiology. *Eur Heart J.* (2018) 39:2032–46. doi: 10.1093/eurheartj/ehy076
27. Morris NA, Chatterjee A, Adejumo OL, Chen M, Merkler AE, Murthy SB, et al. The risk of Takotsubo cardiomyopathy in acute neurological disease. *Neurocrit Care.* (2019) 30:171–6. doi: 10.1007/s12028-018-0591-z
28. Templin C, Ghadri JR, Diekmann J, Napp LC, Bataiosu DR, Jaguszewski M, et al. Clinical features and outcomes of Takotsubo (stress) cardiomyopathy. *N Engl J Med.* (2015) 373:929–38. doi: 10.1056/NEJMoa1406761
29. Kato K, Di Vece D, Cammann VL, Micek J, Szawan KA, Bacchi B, et al. Takotsubo recurrence: morphological types and triggers and identification of risk factors. *J Am College Cardiol.* (2019) 73:982–4. doi: 10.1016/j.jacc.2018.12.033
30. Templin C, Hänggi J, Klein C, Topka MS, Hiestand T, Levinson RA, et al. Altered limbic and autonomic processing supports brain-heart axis in Takotsubo syndrome. *Eur Heart J.* (2019) 40:1183–7. doi: 10.1093/eurheartj/ehz068
31. Pelliccia F, Kaski JC, Crea F, Camici PG. Pathophysiology of Takotsubo syndrome. *Circulation.* (2017) 135:2426–41. doi: 10.1161/CIRCULATIONAHA.116.027121
32. Madias JE. Blood norepinephrine/epinephrine/dopamine measurements in 108 patients with takotsubo syndrome from the world literature: pathophysiological implications. *Acta Cardiol.* (2020) 2020:1–9. doi: 10.1080/00015385.2020.1826703
33. Machado BH, Brody MJ. Role of the nucleus ambiguus in the regulation of heart rate and arterial pressure. *Hypertension.* (1988) 11:602–7. doi: 10.1161/01.HYP.11.6.602
34. Norcliffe-Kaufmann L. The vagus and glossopharyngeal nerves in two autonomic disorders. *J Clin Neurophysiol.* (2019) 36:443–51. doi: 10.1097/WNP.0000000000000604

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The Role of Transthoracic Echocardiography in the Evaluation of Patients With Ischemic Stroke

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Background: Ischemic stroke can be classified into five etiological types, according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, and its adequate investigation and characterization can aid in its clinical management and in preventing new events. Transthoracic echocardiography (TTE) plays a key role in investigating its etiology; approximately one-third of the patients remain without an adequate definition of the etiology or are classified as the undetermined TOAST type.

Objectives: To evaluate if the percentage of patients with indeterminate etiology according to the TOAST classification decreased after transthoracic echocardiography, to determine whether or not the prognosis after ischemic stroke is worse among patients classified as the undetermined TOAST type, and to verify the predictive capacity of echocardiography on the prognosis after ischemic stroke.

Methods: In this retrospective cohort study, clinical, neurological, and echocardiographic examinations were conducted when the patient was hospitalized for stroke. In-hospital mortality and functional capacity were evaluated at hospital discharge and 90 days thereafter. Multiple linear regression and multiple logistic regression models were adjusted for confounding factors. The level of significance was 5%.

Results: A total of 1,100 patients (men = 606; 55.09%), with a mean age of 68.1 ± 13.3 years, were included in this study. Using TTE, 977 patients (88.82%) were evaluated and 448 patients (40.7%) were classified as the undetermined TOAST type. The patients who underwent TTE were 3.1 times less likely to classified as the undetermined TOAST type (OR = 0.32; $p < 0.001$). Echocardiography during hospitalization was a protective factor against poor prognosis, and reduced the odds of in-hospital death by 11.1 times (OR: 0.090; $p < 0.001$). However, the presence of the undetermined TOAST classification elevated the chance of mortality during hospitalization by 2.0 times (OR: 2.00; $p = 0.013$).

Conclusions: Echocardiography during hospitalization for ischemic stroke reduces the chances of an undetermined TOAST classification and the risk of in-hospital

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mortality. However, being classified as the undetermined TOAST type increases the chance of mortality during hospitalization, suggesting that evaluating patients using echocardiography during hospitalization for acute ischemic stroke is important.

Keywords: stroke, ischemic stroke, echocardiography, cardioembolism, stroke etiology

INTRODUCTION

A stroke is characterized by an acute neurological deficit attributed to a focal lesion of vascular origin in the central nervous system (CNS), which may be secondary to an ischemic infarction, or a parenchymal or subarachnoid hemorrhage (1). A CNS infarction is defined as the death of the brain, retinal, or spinal cord cells due to ischemia, as confirmed by pathological evidence on imaging examination. A CNS infarction may also be defined by other evidences of injury to the vascular territory or by the persistence of symptoms for more than 24 h after excluding other causes (1).

It is estimated that during their lifetime, one in six men and one in five women present with stroke (2), which is the second leading cause of death and is responsible for approximately one in eight deaths worldwide (3). In Brazil, stroke is the second leading cause of death and the leading cause of disability (4, 5).

Stroke can be classified according to the pathology, etiology, and clinical presentation (6). According to the pathological classification, a stroke may be hemorrhagic or ischemic, with the latter corresponding to 80% of the total stroke cases. Etiologically, ischemic stroke can be categorized into five types, according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification: (1) large-artery atherosclerosis, (2) cardioembolism, (3) small-vessel occlusion, (4) stroke of other determined etiology, and (5) stroke of undetermined etiology (7). The proportion of patients in each group differed among the studied populations. The definition of the etiological mechanism is important for evaluating severity, progression, and prognosis. The cardioembolic type is responsible for 14–30% of ischemic conditions and has higher mortality, greater severity, and worse functional outcome compared to the other etiologies (8, 9); the affected patients are predisposed to early recurrence. Atrial fibrillation (AF) is the main finding associated with this type of stroke.

Rücker et al. studied 3,346 ischemic stroke patients to determine the long-term survival and recurrence after ischemic stroke according to the etiological subtype (the TOAST classification) in a population-based stroke registry in Germany. Their study showed that the 5 year survival rate was higher in patients with stroke, due to the occlusion of the small arteries, and lower in patients with cardioembolic stroke. Furthermore, the 5 year recurrence rates were lower in women with stroke, due to small artery occlusion, and in men with large artery atherosclerosis. The highest recurrence rates, in both women and men, were seen in indeterminate stroke (10). Existing literature still reports a certain degree of conflict in the clinical prognosis, mortality, and recurrence rate in the undetermined TOAST type, and this can be attributed to the heterogeneity of this etiological subtype, which comprises different pathophysiological mechanisms.

The cardiovascular risk profile and echocardiographic findings in patients with AF detected after a stroke are comparable to those of patients previously diagnosed with AF, but differ from those of patients without AF. Preexisting heart disease is the major cause of AF and is first diagnosed after a stroke (11).

Some disorders are considered to be high-risk sources for the cardioembolic type, such as mitral stenosis, heart valve prosthesis, myocardial infarction in the previous 4 weeks, mural thrombus in the left cavities, left ventricular aneurysm, any documented history of permanent or transient fibrillation or atrial flutter with or without spontaneous contrast echocardiogram or left atrial thrombus, sinus node disease, dilated cardiomyopathy, ejection fraction <35%, endocarditis, intracardiac mass, patent foramen ovale with *in situ* thrombosis, and patent foramen ovale associated with pulmonary thromboembolism or peripheral venous thrombosis prior to the ischemic stroke (12).

Furthermore, with regard to structural heart diseases, four studies considered left ventricular dysfunction defined as recent heart failure, a 25% reduction in left ventricular ejection fraction, and an ejection fraction inferior to 50% as independent risk factors for stroke, despite a population overlap in three of the four studies. Two of the studies also considered ventricular hypertrophy and a left ventricular mass >110 g/m^2 in women and 134 g/m^2 in men as independent risk factors for stroke (13).

Left atrial enlargement is an independent factor for stroke and is associated with a 20% chance of thromboembolism per year in the presence of a left atrium >2.5 cm/m^2 with moderate to severe left ventricular contractility changes (14).

Left ventricular dysfunction and left atrial size were the strongest independent predictors of late thromboembolism. Patients without these two predictors on echocardiography, or without the three identified clinical predictors of thromboembolism (history of hypertension, recent heart failure, and previous thromboembolism) had a low risk of thromboembolism (1% per year). However, patients

Abbreviations: TOAST, Trial of Org 10172 in Acute Stroke Treatment; TTE, transthoracic echocardiography; mRS, Modified Rankin scale; OR, odds ratio; 95% CI, 95% confidence interval; LA, left atrium diameter; LVM, left ventricular mass; LVEF, left ventricular ejection fraction by the Teichholz method; ASC, alteration of segmental contractility; LVH, left ventricular hypertrophy; LVR, left ventricle remodeling; SDD, severe diastolic dysfunction; MoDD, moderate diastolic dysfunction; MiDD, mild diastolic dysfunction; S AoV Insuf, severe aortic valve insufficiency; Mo AoV Insuf, moderate aortic valve insufficiency; Mi AoV Insuf, mild aortic valve insufficiency; NIHSS, National Institute of Health Stroke Scale; LACS, lacunar syndromes; PACS, partial anterior circulation syndromes; POCS, posterior circulation syndromes; TACS, total anterior circulation syndromes; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

with no thromboembolism predictors but with one or both echocardiographic predictors had a 6% risk of stroke per year, showing that in addition to clinical assessment, echocardiography can stratify patients with AF and guide their therapy (15).

Despite all investigations, about a third of ischemic stroke patients cannot be categorized etiologically and are classified as the undetermined TOAST type, which can comprise potential cardiac sources of embolism, atherothrombotic causes, and cerebral embolism from indeterminate sources (16). The American Heart Association and American Stroke Association (AHA/ASA) guidelines recommend echocardiography for evaluating a patient with ischemic stroke only in selected cases (class IIa/class IIb). A recent study published by Harris et al. aimed to investigate the utility of transthoracic echocardiography (TTE) as a part of an acute ischemic stroke workup and revealed that the overall yield of TTE in acute ischemic stroke was low (17). Conversely, TTE has been performed as part of the assessment of stroke patients in recent years. More recently, point-of-care ultrasound (POCUS) has increased its field of application and TTE has been used as a screening method in the stroke unit (18). Robust registries, such as The Cornell Acute Stroke Academic Registry (CAESAR), routinely perform echocardiography as a strategy for evaluating patients with ischemic stroke (19).

Although current literature has not been able to clarify the role of echocardiography in the routine examination of patients with ischemic stroke, it is an important investigation in them. It is an easily available, non-invasive, relatively inexpensive method, which is easy to perform in centers that have integrated stroke and cardiology units, providing information that can change both the treatment and the understanding of the etiological mechanism of stroke.

Therefore, the objectives of this study were to assess the following: (1a) Whether or not the percentage of patients with ischemic stroke classified as the undetermined TOAST type decreased as a result of echocardiographic examination, (1b) Whether or not the prognosis after ischemic stroke is worse in patients with an undetermined TOAST classification, and (2) The predictive capacity of echocardiography in determining the prognosis of patients with ischemic stroke.

It was hypothesized that transthoracic echocardiography, in the routine investigation of patients with ischemic stroke, permits better etiological assessment, and consequently, improves the prognosis after the event.

MATERIALS AND METHODS

Study Design

This retrospective cohort study was performed at the Stroke Unit (SU) of the Clinical Hospital of the School of Medicine of Botucatu (HC-FMB-UNESP), and included 1,100 inpatients diagnosed with ischemic stroke. Data collection was conducted at two time points: at hospital admission and 90 days after hospital discharge.

The study was approved by the Research Ethics Committee (REC) of the School of Medicine of Botucatu under no. 2,698,569.

Study Population

The sample size was estimated based on simple random sampling, with a normal distribution for the numerical outcomes, type I error = 0.05, and, of all possible associations, the association between left ventricular remodeling (one of the echocardiographic examination variables) and an unfavorable modified Rankin scale (mRS) at 90 days to estimate the test power. Based on the descriptive findings obtained from this association, the test power was estimated to be above 80% for the analyzed association, indicating that the sample size to analyze objective 1a ($n = 1{,}100$), objective 1b ($n = 994$), and objective 2 ($n = 927$) was large enough to ensure test powers >80%.

The study included adults diagnosed with ischemic stroke after clinical evaluation and imaging, such as computed tomography (CT) at admission, and control evaluations, between October 2012 and February 2018.

Clinical Evaluation

The following data were collected from the electronic medical records of clinical evaluations performed by the assistant medical team during the hospitalization period: age, sex, race (white/non-white), the presence of comorbidities (systemic arterial hypertension, type 2 diabetes mellitus, dyslipidemia, smoking, alcohol use, illicit drug use, arrhythmias such as AF or atrial flutter, and a history of previous stroke), the continuous use of medications [acetylsalicylic acid, clopidogrel, anticoagulants, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB), and statins].

Neurological Evaluation

Data pertaining to neurological assessments conducted by the medical team during the hospitalization of the patients at the SU and at 90 days after hospital discharge were collected from the electronic medical records. This data included the score obtained using the National Institutes of Health Stroke Scale (NIHSS) (20) at admission, on hospital discharge, and at 90 days after discharge; the clinical condition classified by the Oxfordshire or Bamford scale (21); the TOAST classification (7); the modified Rankin scale (22) (previous, at hospital discharge, and 90 days after discharge); the recurrence of stroke; the presence of carotid and vertebrobasilar system stenosis and its quantification.

All the variables were obtained from the stroke data bank of Botucatu Medical School. The database is audited monthly by the stroke unit coordinator.

Echocardiographic Evaluation

The patients underwent TTE during hospitalization at the SU. Transesophageal echocardiography was performed when a right-left intracardiac shunt was suspected on TTE, or in case of other findings that required better diagnostic interpretation.

The following parameters were verified with these examinations: left atrial diameter (LA) (mm), left ventricular mass (LVM) (g), left ventricular ejection fraction by the Teichholz method (LVEF) (%), the presence of alterations in segmental contractility (ASC), the presence of left ventricular hypertrophy (LVH), left ventricle remodeling (LVR), severe diastolic dysfunction (SDD), moderate diastolic dysfunction

(MoDD), mild diastolic dysfunction (MiDD), severe aortic valve insufficiency (S AoV Insuf), moderate aortic valve insufficiency (Mo AoV Insuf), mild aortic valve insufficiency (Mi AoV Insuf), severe mitral valve insufficiency (S MiV Insuf), moderate mitral valve insufficiency (Mo MiV Insuf), and mild mitral valve insufficiency (Mi MiV Insuf).

The Etiological Investigation Protocol in Patients With Ischemic Stroke

The investigation protocol at the institution was based on the TOAST classification. All the patients underwent a brain CT at admission, while some underwent an additional scan after 24 h. Depending on the clinical progression, MRI was done for the patients with posterior circulation events or in those with a doubtful diagnosis.

CT angiography of the cerebral and cervical arteries was performed when the patient arrived within 8 h of the ictus, and duplex ultrasound of the cervical arteries and transcranial Doppler were performed 8 h after the ictus. The study was complemented by an anatomical examination (CT angiography or digital angiography) whenever required.

TTE was conducted to locate a cardioembolic source other than AF, while a transesophageal echocardiogram was requested to assess a right-left circulation shunt, left atrial appendage thrombus, and an atheroma in the thoracic aorta.

All patients underwent electrocardiography at admission followed by 24 h of cardiac monitoring. The 24 h Holter test was performed for patients older than 55 years with suspected arrhythmias, and for cryptogenic strokes.

The patient underwent laboratory investigations for syphilis, Chagas disease, glycated hemoglobin, thyroid stimulating hormone (TSH), total cholesterol and fractions, and triglycerides. An autoimmune panel was also performed for patients aged <55 years.

Statistical Analysis

Continuous variables were expressed as mean and standard deviation, while categorical variables were presented as absolute values and percentages. The statistical models were built to separately answer each objective defined in the study.

The potential confounders (variables identified in the maximal model that were clinically relevant, with $p < 0.20$) considered for all the objectives of this study were as follows: age; sex; race; systemic arterial hypertension; type 2 diabetes mellitus; dyslipidemia; smoking; alcoholism; the use of illicit drugs; AF; previous stroke; the continuous use of acetylsalicylic acid, clopidogrel, anticoagulant, ACEI, ARB, and statins; NIHSS at admission; TOAST classification at admission; mRS at admission.

The association between the echocardiographic examination and being classified as the undetermined TOAST type was analyzed using the multiple logistic regression model, including the potential pre-established confounders. The variables included were those presenting statistical significance in the univariate analysis (Objective 1a).

To verify the association between the classification as the undetermined TOAST type and the NIHSS scale score at discharge and 90 days after hospital discharge, the multiple linear regression model was used independently after adjusting for potential confounders. The multiple logistic regression model, adjusted for potential pre-established confounders, was also used to verify the association between the classification as the undetermined TOAST type and unfavorable mRS (mRS > 3 at discharge and 90 days after hospital discharge), and in-hospital mortality. The variables included were those presenting statistical significance in the univariate analysis (Objective 1b).

The association between the echocardiographic variables previously described and the NIHSS scale score at discharge and 90 days after hospital discharge was analyzed using the multiple linear regression model independently and adjusted for potential confounders. The multiple logistic regression model was used to

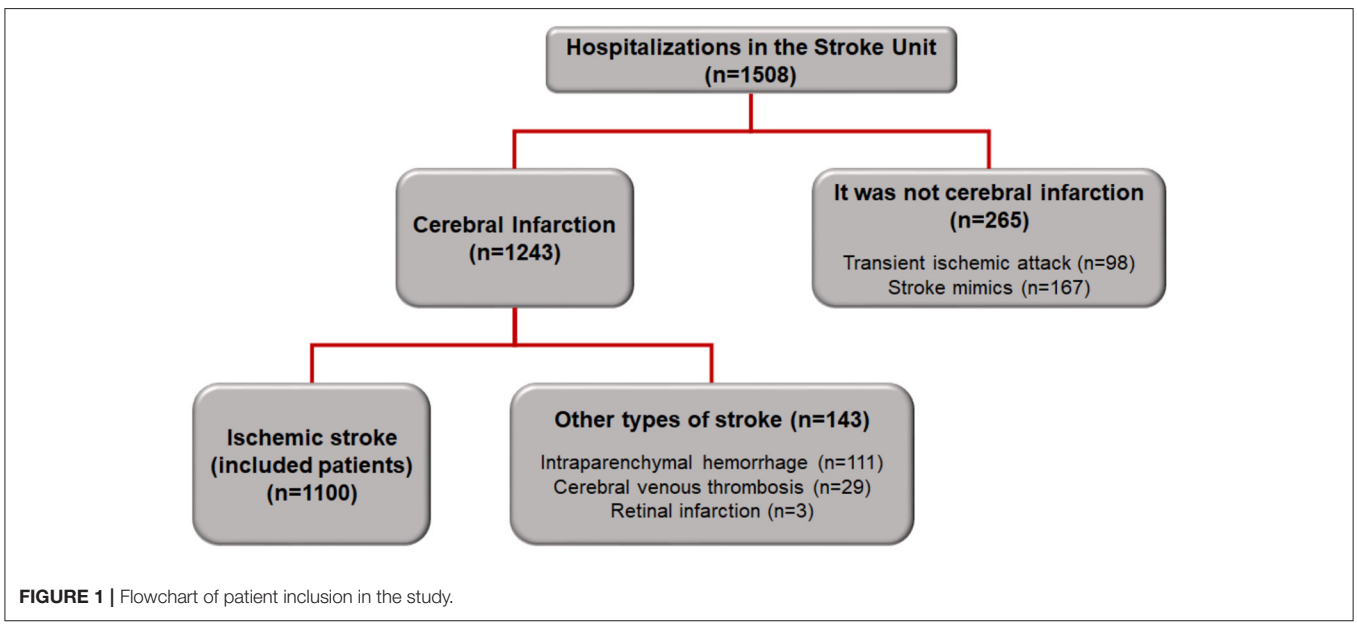

FIGURE 1 | Flowchart of patient inclusion in the study.

verify the association between the echocardiographic variables and unfavorable mRS scores (mRS > 3) at discharge and 90 days after hospital discharge, and in-hospital mortality. The models were adjusted for potential pre-established confounders. The variables included were those presenting statistical significance in the univariate analysis (Objective 2).

A comparison between the TOAST types and the echocardiographic variables was performed using the Kruskal-Wallis non-parametric test, followed by the Dunn test for multiple comparisons. Statistical significance was set at $p < 0.05$. The analysis was performed using the SPSS version 21 software.

RESULTS

Patient Inclusion in the Study

A total of 1,508 patients were admitted to the SU between October 2012 and February 2018. Of these, 1,243 patients had a confirmed diagnosis of cerebral infarction, and the 1,100 patients diagnosed with ischemic stroke were included in this study (**Figure 1**).

Demographic and Clinical Characteristics of Patients Admitted With Ischemic Stroke

Table 1 shows the demographic characteristics of the patients admitted with ischemic stroke, as well as the neurological assessments regarding the TOAST classification, Bamford clinical classification, and the degree of disability using the modified Rankin scale. Echocardiography was performed in 977 patients (88.82%).

Table 2 shows the risk factors for cardiovascular diseases and the medications used being at the time of hospitalization.

Association Between Echocardiography and Classification as the Undetermined TOAST Type (Objective 1a)

Table 3 shows that patients undergoing TTE were 3.1 times less likely to be classified as the undetermined TOAST type (OR = 0.32; 95% CI: 0.21-0.51; $p < 0.001$).

The number needed to treat was calculated to be 3.4, implying that for every 3.4 TTEs performed, one patient would be prevented from being classified as the undetermined TOAST type.

Association Between Being Classified as the Undetermined TOAST Type and the Outcomes at Discharge and at 90 Days After Hospital Discharge (Objective 1b)

There was no association between being classified as the undetermined TOAST type and the outcomes at hospital discharge, as can be seen from the following: NIHSS score (β: −0.040; $p = 0.871$) and mRS score >3 (OR: 0.901; $p = 0.544$) using the multiple linear regression model corrected for confounding variables (alcoholism, history of previous stroke, TACS ischemic stroke, POCS ischemic stroke, PACS ischemic stroke, echocardiogram, age, and NIHSS at admission), and by the multiple logistic regression model corrected for confounding variables (age, male, non-white race, diabetes mellitus, alcoholism, use of oral anticoagulants, NIHSS at

TABLE 1 | Demographic and clinical characteristics of patients admitted with ischemic stroke ($n = 1,100$).

Variables	*n* (%)
Male	606 (55.09)
Age (years)	68.1 ± 13.3
Race (non-white)	107 (9.73)
History of previous stroke	103 (9.36)
NIHSS at admission	8.37 ± 7.44
Echocardiogram	977 (88.82)
Neurological evaluation	
Undetermined TOAST	448 (40.7)
Cardioembolic TOAST	255 (23.2)
Large vessel TOAST	164 (14.9)
Small vessel TOAST	181 (16.5)
TOAST due to other causes	52 (4.7)
LACS ischemic stroke	416 (37.8)
PACS ischemic stroke	346 (31.5)
POCS ischemic stroke	141 (12.8)
TACS ischemic stroke	197 (17.9)
Previous mRS 0	816 (74.2)
Previous mRS 1	159 (14.5)
Previous mRS 2	51 (4.6)
Previous mRS 3	53 (4.8)
Previous mRS 4	19 (1.7)
Previous mRS 5	2 (0.2)

Values are expressed as numbers and percentages or as mean and standard deviation. NIHSS, National Institute of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LACS, lacunar syndromes; PACS, partial anterior circulation syndromes; POCS, posterior circulation syndromes; TACS, total anterior circulation syndromes; mRS, Modified Rankin scale.

TABLE 2 | Risk factors for cardiovascular diseases and medications being used previously in patients admitted to the Stroke Unit ($n = 1,100$ patients).

Variables	*n* (%)
Risk factors for CVD	
Systemic arterial hypertension	838 (76.18)
Diabetes mellitus	383 (34.82)
Dyslipidemia	191 (17.36)
Atrial fibrillation	203 (18.45)
Smoking	479 (43.55)
Alcoholism	256 (23.27)
Use of illicit drugs	11 (1.00)
Previously used medications	
Acetylsalicylic acid	302 (27.45)
Clopidogrel	45 (4.09)
Oral anticoagulant	54 (4.91)
ACEI or ARB	470 (42.73)
Statins	300 (27.27)

Values are expressed as numbers and percentages. CVD, cardiovascular disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

TABLE 3 | Multiple logistic regression model adjusted to explain the chance of classification as the undetermined TOAST type on echocardiography, corrected for confounding variables ($n = 1{,}100$ patients).

Variables	OR	95% CI		p
Age (years)	1.014	1.003	1.025	0.010
Sex (male)	0.781	0.592	1.031	0.081
Race (non-white)	1.482	0.955	2.301	0.079
Dyslipidemia	0.689	0.483	0.985	0.041
Smoking	0.848	0.641	1.121	0.247
Atrial fibrillation	0.087	0.054	0.143	0.000
History of previous stroke	1.424	0.897	2.260	0.133
LACS ischemic stroke				0.000
PACS ischemic stroke	2.360	1.706	3.266	0.000
POCS ischemic stroke	1.928	1.255	2.964	0.003
TACS ischemic stroke	2.161	1.450	3.220	0.000
Previous mRS	1.197	1.028	1.395	0.021
Echocardiogram	**0.324**	**0.206**	**0.510**	**<0.001**

A parsimonious model, including only variables with $p < 0.20$, in the multiple logistic regression for the chance of being classified as the undetermined TOAST type that showed an association. TOAST, Trial of Org 10172 in Acute Stroke Treatment; OR, odds ratio; 95% CI, 95% confidence interval; LACS, lacunar syndromes; PACS, partial anterior circulation syndromes; POCS, posterior circulation syndromes; TACS, total anterior circulation syndromes; mRS, Modified Rankin scale.

TABLE 4 | Multiple logistic regression model to explain in-hospital mortality due to the undetermined TOAST type classification, corrected for confounding variables ($n = 1{,}100$ patients).

Variables	OR	95% CI	p
Age (years)	1.022	1.000–1.050	0.033
Male	1.870	1.070–3.260	0.028
SAH	1.880	0.930–3.800	0.078
Diabetes	1.540	0.860–2.750	0.143
Use of ASA	1.450	0.820–2.580	0.205
Use of oral anticoagulant	0.250	0.050–1.290	0.099
NIHSS at admission	1.110	1.070–1.150	<0.001
Bamford			<0.001
PACS ischemic stroke	14.560	1.860–114.110	0.011
TACS ischemic stroke	28.550	3.480–234.550	0.002
LACS ischemic stroke	40.530	5.050–325.310	<0.001
Echocardiogram	**0.090**	**0.050–0.170**	**<0.001**
Undetermined TOAST	**2.000**	**1.160–3.460**	**0.013**

TOAST, Trial of Org 10172 in Acute Stroke Treatment; OR, odds ratio; 95% CI, 95% confidence interval; SAH, systemic arterial hypertension; ASA, acetylsalicylic acid; NIHSS, National Institutes of Health Stroke Scale; PACS, partial anterior circulation syndrome; TACS, total anterior circulation syndrome; LACS, lacunar syndrome; POCS, posterior circulation syndrome.

admission, LACS ischemic stroke, PACS ischemic stroke, POCS ischemic stroke, and TACS ischemic stroke).

With regard to the prognosis 90 days after hospital discharge, no association was found between the classification as the undetermined TOAST type and NIHSS score outcomes (β: −0.160; $p = 0.560$) and mRS score >3 (OR: 0.812; $p = 0.261$) 90 days after hospital discharge, using the multiple linear regression model corrected for confounding variables (AF, history of previous stroke, use of clopidogrel, use of oral anticoagulants, TACS ischemic stroke, POCS ischemic stroke, PACS ischemic stroke, echocardiogram, age, NIHSS at admission, previous mRS), and the multiple logistic regression model corrected for confounding variables (age, male sex, diabetes mellitus, dyslipidemia, alcoholism, use of oral anticoagulants, NIHSS at admission, LACS ischemic stroke, PACS ischemic stroke, POCS ischemic stroke, TACS ischemic stroke, previous mRS, and echocardiogram).

Undergoing an echocardiogram was a protective factor against death during hospitalization, and reduced the possibility of in-hospital death by 11.1 times (OR: 0.090; $p < 0.001$). Conversely, being classified as the undetermined TOAST type increased the chances of mortality during hospitalization by 2.0 times (OR: 2.00; $p = 0.013$), as shown in **Table 4**.

Association Between Echocardiographic Variables and Outcomes at Discharge and at 90 Days After Hospital Discharge (Objective 2)

There was no association between the echocardiographic variables and NIHSS score outcomes (**Table 5**) and mRS > 3 (**Table 6**) at discharge.

TABLE 5 | Association between the echocardiographic variables of patients hospitalized due to ischemic stroke and NIHSS scores at hospital discharge ($n = 977$ patients).

Variables	β	95% CI	p
(Intercept)	−2.125	−5.019–0.770	0.150
LA (mm)	0.004	−0.042–0.050	0.861
LVM (g)	−0.002	−0.007–0.004	0.554
LVEF (%)	0.012	−0.012–0.037	0.328
ASC	0.108	−0.853–1.069	0.826
LVH	0.635	−0.031–1.301	0.062
LVR	0.158	−0.507–0.824	0.641
SDD	−1.238	−3.841–1.366	0.351
MoDD	−0.506	−1.741–0.728	0.421
MiDD	0.077	−0.455–0.610	0.776
S AoV Insuf	−0.179	−2.472–2.113	0.878
Mo AoV Insuf	−0.366	−1.773–1.041	0.610
Mi AoV Insuf	−0.051	−0.673–0.571	0.873
Thrombus in the LA	−1.703	−5.330–1.924	0.357

The parsimonious model included only variables with $p < 0.20$, in the multiple linear regression for the NIHSS score at discharge that showed an association. NIHSS, National Institutes of Health Stroke Scale; 95% CI, 95% confidence interval; LA, left atrium diameter; LVM, left ventricular mass; LVEF, left ventricular ejection fraction by the Teichholz method; ASC, alteration of segmental contractility; LVH, left ventricular hypertrophy; LVR, left ventricle remodeling; SDD, severe diastolic dysfunction; MoDD, moderate diastolic dysfunction; MiDD, mild diastolic dysfunction; S AoV Insuf, severe aortic valve insufficiency; Mo AoV Insuf, moderate aortic valve insufficiency; Mi AoV Insuf, mild aortic valve insufficiency.
Model adjusted for potential confounders: alcoholism, the use of anticoagulants, Bamford classification, age, and the NIHSS and mRS scores at admission.

At 90 days after hospital discharge, there was no association between the echocardiographic findings and NIHSS score outcomes. Furthermore, there was no association between the

TABLE 6 | Association between the echocardiographic variables of patients hospitalized due to ischemic stroke and mRS > 3 at hospital discharge ($n = 977$ patients).

Variables	OR	95% CI	*p*
LA (mm)	0.992	0.958–1.028	0.663
LVM (g)	1.001	0.997–1.005	0.600
LVEF (%)	1.000	0.982–1.018	0.986
ASC	1.386	0.678–2.834	0.371
LVR	1.516	0.907–2.532	0.112
LVH	1.344	0.803–2.248	0.260
SDD	1.172	0.779–1.765	0.446
MoDD	1.209	0.463–3.157	0.699
MiDD	0.473	0.053–4.209	0.502
S AoV Insuf	1.119	0.700–1.787	0.639
Mo AoV Insuf	0.846	0.285–2.509	0.763
Mi AoV Insuf	0.481	0.090–2.578	0.393
Thrombus in the LA	0.520	0.035–7.710	0.634

A parsimonious model, including only variables with $p < 0.20$, in the multiple logistic regression for the chance of mRS > 3 at discharge showing an association. mRS, Modified Rankin scale; OR, odds ratio; 95% CI, 95% confidence interval; LA, left atrium diameter; LVM, left ventricular mass; LVEF, left ventricular ejection fraction by the Teichholz method; ASC, alteration of segmental contractility; LVH, left ventricular hypertrophy; LVR, left ventricle remodeling; SDD, severe diastolic dysfunction; MoDD, moderate diastolic dysfunction; MiDD, mild diastolic dysfunction; S AoV Insuf, severe aortic valve insufficiency; Mo AoV Insuf, moderate aortic valve insufficiency; Mi AoV Insuf, mild aortic valve insufficiency.

Model adjusted for potential confounders: age, sex, alcoholism, previous stroke, use of anticoagulant, NIHSS and mRS at admission, and Bamford classification (PACS, POCS, TACS).

echocardiographic findings and mRS>3 score outcomes, except for left ventricular remodeling (OR = 1.78; 95% CI: 1.06–2.98; $p = 0.028$).

An evaluation of the echocardiographic variables and their correlation with the TOAST classification type showed significantly greater values for left atrial diameter and LVM and significantly lower left ventricular ejection fraction values in patients with stroke classified as the cardioembolic TOAST type, as shown in **Table 7**.

DISCUSSION

In the present study, echocardiography during hospitalization due to ischemic stroke reduced the possibility of being classified as the undetermined TOAST type and was associated with lower in-hospital mortality.

The distribution of the different TOAST classifications was similar to that reported previously in literature; >30% (40.7%) of the patients were classified as the undetermined type, despite the investigation protocol (23). The incidence of small vessel TOAST classification was 16.45%, that of large vessels was 14.81%, and that of other causes was 4.72%. Of the 23.14% patients classified as the cardioembolic type, the main associated factor was the presence of AF (in 18% of all ischemic strokes), with a higher incidence than seen in previous studies on Brazilian patients (24).

In this study, echocardiography decreased the number of patients classified without a defined etiology, and this relationship confirmed the study hypothesis. TTE is a non-invasive and low-cost examination, and the association described above proves the importance of including it in an investigation protocol for patients hospitalized with ischemic stroke.

Although echocardiography did not correlate with a better patient prognosis, as measured by the NIHSS and Rankin scale scores, both at discharge and after 90 days, it increased the chance of identifying specific TOAST classifications, thereby decreasing the chance of inappropriate patient treatment. Although the highest NIHSS was found among patients who did not undergo echocardiography, this variable was taken into account and adjusted in the multiple regression for the mortality outcome. The correlation between echocardiography and the lower frequency of death at admission reinforces the importance of this examination for proper patient management.

The 5 year survival probability was higher in patients with small artery occlusion stroke (73.8 [95% CI, 70.4–77.3]) and lower in patients with cardioembolic stroke (40.9 [95% CI, 37.2–45.0]) and in indeterminate stroke patients (50.3 [95% CI, 47.2–53.7]) (10).

There was no association between the variables assessed on the echocardiogram and the NIHSS and mRS scores at 90 days. These data do not corroborate with those of studies that evaluated systolic function through ejection fraction and diastolic dysfunction and reported a worse outcome in stroke (25, 26). Ventricular mass was reported to be a risk factor for non-fatal ischemic stroke (27), as well as for recurrence and death in cases of severe LVH (28).

A possible reason for this difference in the correlation between the echocardiogram measurements and prognosis in previous literature is the evaluation of these variables without considering the TOAST etiological classification. Patients with a cardioembolic TOAST classification generally present with more changes in the echocardiographic measurements. In this study, for example, patients with a cardioembolic TOAST classification had a higher ventricular mass, larger atrial diameter, and a lower EF. Thus, such echocardiogram findings are possibly collinear with a cardioembolic TOAST classification and are not independent prognostic factors.

A large number of patients with ischemic stroke were on antiplatelet therapy (31.54%). A comprehensive investigation of these patients can help in identifying conditions in which anticoagulation would be the appropriate prophylaxis; echocardiography may have an important role here. Thus, a thorough investigation of the patient is important for the proper characterization and management of ischemic stroke. Emerging evidence suggests that atrial enlargement may be a biomarker of AF-independent underlying thrombogenic atrial heart disease with an independent risk of indeterminate or recurrent cardioembolic stroke (17).

Despite its findings, this study has its limitations. It is a retrospective study involving a single center only,

TABLE 7 | Comparison between TOAST types and echocardiographic variables ($n = 977$ patients).

	Other causes	Undetermined classification	TOAST Large vessels	Small vessels	Cardioembolic	$p^{(1)}$
LA	37.0 (30.0–52.0)	40.0 (27.0–63.0)	39.0 (29.0–55.0)	39.0 (28.0–58.0)	46.0 (30.0–77.0)	**<0.001**
LVM	141.5 (85.4–364.8)	167.6 (88.8–635.8)	169.3 (85.2–345.5)	173.0 (96.8–401.1)	199.6 (89.7–470.7)	**<0.001**
EF	69.5 (26.0–78.0)	70.0 (24.0–86.0)	70.0 (28.0–86.0)	70.0 (31.0–89.5)	62.0 (20.0–88.0)	**<0.001**

Values expressed as median (minimum-maximum). (1) Kruskal-Wallis for independent samples. Statistically significant contrasts ($p < 0.05$; Dunn's test for independent samples): LA, Other causes, Undetermined, Large vessels, Small vessels <Cardioembolic; LVM, Other causes, Undetermined, Large vessels, Small vessels <Cardioembolic, Other causes <Small, Large vessels; EF, Other causes, Undetermined, Large vessels, Small vessels <Cardioembolic. TOAST, Trial of Org 10172 in Acute Stroke Treatment; Med, median; Min, minimum value; Max, maximum value; LA, left atrium diameter (mm); LVM, left ventricular mass (g); LVEF, left ventricular ejection fraction by the Teichholz method (%).

making it impossible to obtain the measurement of the left atrial volume for analysis. Left atrial volume has been shown to be a powerful prognostic variable in heart disease.

We also understand as a limitation that the design of this study did not allow the identification of mechanisms by which the echocardiogram correlated with a reduction in mortality, which opens up frontiers for future studies in this area.

Based on the results of the present study, we can conclude that echocardiography during hospitalization for ischemic stroke may be associated with a decreased chance of an undetermined TOAST classification, and also with lower mortality during hospitalization. On the other hand, an undetermined TOAST classification may correlate with higher mortality during hospitalization, suggesting the importance of including echocardiography in the hospital investigation protocol for patients with ischemic stroke.

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 Research Ethics Committee (REC) of the School of Medicine of Botucatu under no. 2,698,569. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RT contributed to the literature search, study design, data collection, data analysis and interpretation, and writing of the manuscript. GS, GM, and ST participated in the literature search, study design, data analysis and interpretation, and in the writing of the manuscript. JS, GL, LM, and RB conducted the literature search, data analysis and interpretation, and wrote the manuscript. HN and SZ participated in the literature search, study design, data analysis and interpretation, and wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.710334/full#supplementary-material

REFERENCES

1. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJB, Culebras A, et al. An updated definition of stroke for the 21st century. *Stroke.* (2013) 44:2064–89. doi: 10.1161/STR.0b013e318296aeca
2. Seshadri S, Wolf PA. Lifetime risk of stroke and dementia: current concepts, and estimates from the Framingham Study. *Lancet Neurol.* (2007) 6:1106–14. doi: 10.1016/S1474-4422(07)70291-0
3. Adamson J, Beswick A, Ebrahim S. Is stroke the most common cause of disability? *J Stroke Cerebrovasc Dis.* (2004) 13:171–7. doi: 10.1016/j.jstrokecerebrovasdis.2004.06.003
4. de Santana NM, dos Santos Figueiredo FW, de Melo Lucena DM, Soares FM, Adami F, de Carvalho Pádua Cardoso L, et al. The burden of stroke in Brazil in 2016: an analysis of the Global Burden of Disease study findings. *BMC Res Notes.* (2018) 11:735. doi: 10.1186/s13104-018-3842-3
5. de Campos LM, Martins BM, Cabral NL, Franco SC, Pontes-Neto OM, Mazin SC, et al. How many patients become functionally dependent after a stroke? A 3-year population-based study in Joinville, Brazil. *PLoS ONE.* (2017) 12:e0170204. doi: 10.1371/journal.pone.0170204
6. Hankey GJ. Stroke. *Lancet.* (2016) 389:641–54. doi: 10.1016/S0140-6736(16)30962-X
7. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter classification of subtype of acute ischemic stroke definitions for use in a multicenter clinical trial. *Stroke.* (1993) 24:35–41. doi: 10.1161/01.str.24.1.35
8. Arboix A, Alio J. Cardioembolic stroke: clinical features, specific cardiac disorders and prognosis. *Curr Cardiol Rev.* (2010) 6:150–61. doi: 10.2174/157340310791658730
9. Lin HJ, Wolf PA, Kelly-Hayes M, Beiser AS, Kase CS, Benjamin EJ, et al. Stroke severity in atrial fibrillation: the Framingham Study. *Stroke.* (1996) 27:1760–4. doi: 10.1161/01.str.27.10.1760
10. Rücker V, Heuschmann PU, O'Flaherty M, Weingärtner M, Hess M, Sedlak C, et al. Twenty-year time trends in long-term case-fatality and recurrence

rates after ischemic stroke stratified by etiology. *Stroke*. (2020) 51:2778–85. doi: 10.1161/STROKEAHA.120.029972
11. Rizos T, Horstmann S, Dittgen F, Täger T, Jenetzky E, Heuschmann P, et al. Preexisting heart disease underlies newly diagnosed atrial fibrillation after acute ischemic stroke. *Stroke*. (2016) 47:336–41. doi: 10.1161/STROKEAHA.115.011465
12. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. New approach to stroke subtyping: the A-S-C-O (phenotypic) classification of stroke. *Cerebrovasc Dis*. (2009) 27:502–8. doi: 10.1159/000210433
13. Pisters R, Lane DA, Marin F, Camm AJ, Lip GYH. Stroke and thromboembolism in atrial fibrillation. *Circ J*. (2012) 76:2289–304. doi: 10.1253/circj.cj-12-1036
14. The Stroke Prevention in Atrial Fibrillation Investigators. Predictors of thromboembolism in atrial fibrillation: II. Echocardiographic features of patients at risk. The Stroke Prevention in Atrial Fibrillation Investigators. *Ann Intern Med*. (1992) 116:6–12. doi: 10.7326/0003-4819-116-1-6
15. Lerakis S, Nicholson WJ. Part I: use of echocardiography in the evaluation of patients with suspected cardioembolic stroke. *Am J Med Sci*. (2005) 329:310–6. doi: 10.1097/00000441-200506000-00011
16. Hart RG, Diener H, Coutts SB, Easton JD, Granger CB, O'Donnell MJ, et al. Embolic strokes of undetermined source: the case for a new clinical construct. *Lancet Neurol*. (2014) 13:429–38. doi: 10.1016/S1474-4422(13)70310-7
17. Harris J, Yoon J, Salem M, Selim M, Kumar S, Lioutas VA. Utility of transthoracic echocardiography in diagnostic evaluation of ischemic stroke. *Front Neurol*. (2020) 11:103. doi: 10.3389/fneur.2020.00103
18. Pagola J, Pagola C, Juega J, González-Alujas T, Alvarez-Sabin J, Molina CA. The role of echocardiography screening at the stroke unit. *Front Neurol*. (2020) 11:1003. doi: 10.3389/fneur.2020.01003
19. Kamel H, Okin PM, Merkler AE, Navi BB, Campion TR, Devereux RB, et al. Relationship between left atrial volume and ischemic stroke subtype. *Ann Clin Transl Neurol*. (2019) 6:1480–6. doi: 10.1002/acn3.50841
20. Appelros P, Terént A. Characteristics of the National Institute of Health stroke scale: results from a population-based stroke cohort at baseline and after one year. *Cerebrovasc Dis*. (2004) 17:21–7. doi: 10.1159/000073894
21. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. (1991) 337:1521–6. doi: 10.1016/0140-6736(91)93206-o
22. Kasner SE. Clinical interpretation and use of stroke scales. *Lancet Neurol*. (2006) 5:603–12. doi: 10.1016/S1474-4422(06)70495-1
23. Hart RG, Catanese L, Perera KS, Ntaios G, Connolly SJ. Embolic stroke of undetermined source: a systematic review and clinical update. *Stroke*. (2017) 48:867–72. doi: 10.1161/STROKEAHA.116.016414
24. Carod-Artal FJ, JOC Lanchipa, LMC Ramírez, Pérez NS, FMS Aguayo, Moreno IG, et al. Stroke subtypes and comorbidity among ischemic stroke patients in brasilia and cuenca: a brazilian-spanish cross-cultural study. *J Stroke Cerebrovasc Dis*. (2014) 23:140–7. doi: 10.1016/j.jstrokecerebrovasdis.2012.11.012
25. Ryu S, Park J, Ko S, Hwang S, Kim Y, Kim D, et al. Diastolic dysfunction and outcome in acute ischemic stroke. *Cerebrovasc Dis*. (2016) 41:148–55. doi: 10.1159/000442006
26. Devulapalli S, Dunlap S, Wilson N, Cockburn S, Kurukumbi M, Mehrotra P, et al. Prevalence and clinical correlation of left ventricular systolic dysfunction in African Americans with ischemic stroke. *J Stroke Cerebrovasc Dis*. (2014) 23:1965–8. doi: 10.1016/j.jstrokecerebrovasdis.2014.01.026
27. Bouzas-Mosquera A, Broullón FJ, Álvarez-García N, Peteiro J, Mosquera VX, Castro-Beiras A. Association of left ventricular mass with all-cause mortality, myocardial infarction and stroke. *PLoS ONE*. (2012) 7:e45570. doi: 10.1371/journal.pone.0045570
28. Miles JA, Garber L, Ghosh S, Spevack DM. Association of transthoracic echocardiography findings and long-term outcomes in patients undergoing workup of stroke. *J Stroke Cerebrovasc Dis*. (2018) 27:2943–50. doi: 10.1016/j.jstrokecerebrovasdis.2018.06.023

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Early Coagulation Disorder Is Associated With an Increased Risk of Atrial Fibrillation in Septic Patients

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Background: Atrial fibrillation (AF) and coagulation disorder, two common complications of sepsis, are associated with the mortality. However, the relationship between early coagulation disorder and AF in sepsis remains elusive. This study aimed to evaluate the interaction between AF and early coagulation disorder on mortality.

Methods: In this retrospective study, all data were extracted from the Medical Information Mart for Intensive Care III (MIMIC-III) database. Septic patients with coagulation tests during the first 24 h after admission to intensive care units (ICUs) meeting study criteria were included in the analysis. Early coagulation disorder is defined by abnormalities in platelet count (PLT), international normalized ratio (INR) and activated partial thromboplastin time (APTT) within the first 24 h after admission, whose score was defined with reference to sepsis-induced coagulopathy (SIC) and coagulopathy. Patients meeting study criteria were divided into AF and non-AF groups.

Results: In total, 7,528 septic patients were enrolled, including 1,243 (16.51%) with AF and 5,112 (67.91%) with early coagulation disorder. Compared with patients in the non-AF group, patients in the AF group had higher levels of INR and APTT ($P < 0.001$). Multivariable logistic regression analyses showed that stroke, early coagulation disorder, age, gender, congestive heart failure (CHF), chronic pulmonary disease, renal failure, and chronic liver disease were independent risk factors for AF. In addition, AF was related to in-hospital mortality and 90-day mortality. In the subgroup analysis stratified by the scores of early coagulation disorder, AF was associated with an increased risk of 90-day mortality when the scores of early coagulation disorder were 1 or 2 and 3 or 4.

Conclusion: In sepsis, coagulation disorder within the first 24 h after admission to the ICUs is an independent risk factor for AF. The effect of AF on 90-day mortality varies with the severity of early coagulation disorder.

Keywords: neurocardiovascular, atrial fibrillation, coagulation disorder, ischemic stroke, sepsis

INTRODUCTION

In sepsis, AF is the most common arrhythmia, with an incidence of 20–30% (1). High levels of circulating stress hormones and inflammatory cytokines, autonomic nervous system dysfunction, blood volume change, and cardiovascular injury promote atrial structure and electrical remodeling to act as substrates of AF (2). Therefore, patients accompanied with AF have more thrombosis, longer hospital stay, as well as a higher risk of death, especially in severe cases (3–6).

Coagulation dysfunction, including SIC and DIC, are common complications in sepsis (7). After being triggered by an acute systemic inflammatory response of sepsis, coagulation initiates and induces extensive crosstalk with inflammation and immunity to promote SIC and DIC (8, 9). This process results in massive consumption of coagulation substances, then pathological coagulation occurs to further damage the vascular endothelial barrier and contribute to micro thrombosis and micro bleeding (10). These pathophysiologic mechanisms contributing to multiple system organ ischemia and ischemia-reperfusion injury (11) are, in part, related to a high risk of cardiovascular events. Therefore, SIC is proposed to identify the earlier stage of coagulation abnormalities in sepsis.

Based on preclinical findings, hyper coagulant-transgenic animals were more likely to have AF than wild-type animals, suggesting that hypercoagulation may cause or promote AF (12). However, it remains elusive whether abnormal coagulation is a risk factor for AF in the real world. Therefore, the present work aimed to evaluate the effects between early coagulation disorder and AF on the mortality in sepsis.

MATERIALS AND METHODS

Study Design

The retrospective study was performed with data from the Medical Information Mart for Intensive Care-III (MIMIC-III) database, consisting of data on 53,423 different hospital admissions for adult patients presenting to the ICUs between 2001 and 2012. MIMIC-III is a large, freely available database comprising de-identified health-related data associated with patients admitted to the critical care units of Beth Israel Deaconess Medical Center. As a result, the informed consent and approval of the Institutional Review Board were waived. Data were not eligible for access until approved by MIT's (Massachusetts Institute of Technology) institutional review board. All data were extracted by an author (Record ID: 28572693) who completed the CITI "Data or Specimens Only Research" course and passed the exam.

Patients

All septic adult patients with coagulation function tests within 24 h after admission to the ICUs were evaluated for enrollment in this study. The diagnostic criteria for sepsis were consistent with sepsis 3.0, which is defined after meeting the following conditions: patients with documented or suspected infection; an acute increase of Sequential Organ Failure Assessment (SOFA) score of $\geq$2. Suspected infection was identified as prescriptions of antibiotics and sampling of bodily fluids for microbiological culture (13). The exclusion criteria were age>80 years, pregnancy, congenital heart diseases, valvular heart diseases, congenital coagulation disorders (e.g., hemophilia, vitamin K deficiency), coronary artery stenosis, implanted cardiac devices, and admission to the cardiac care unit (CCU) as well as cardiac surgery intensive care unit (CSICU). If there were multiple ICU admissions in a patient, the first information was only included for analysis. Eventually, patients were divided into two groups based on the status of AF, which was recognized from the database by ICD-9 codes.

Variables

Baseline characteristics of the patients included the following: age, gender, ethnicity, height, weight, insurance type, marital status, ICU type, mean arterial pressure (MAP), heart rate (HR), temperature (T), and respiratory rate (RR), complications including hypertension, diabetes, CHF, peripheral vascular disease, renal failure, chronic liver disease, chronic pulmonary disease, stroke, DIC, critical illness scores including Elixhauser comorbidity index (ECI), SOFA score, Acute Physiological Score III (APS III), Systemic Inflammatory Response Syndrome (SIRS) score, Simplified Acute Physiology Score II (SAPS II), Overall Anxiety Severity and Impairment Scale (OASIS) score, Glasgow Coma Score (GCS), PLT, INR, APTT, and AF. ECI, a comprehensive comorbidity scoring system with 30 comorbidities, is appropriate for studies of databases. INR reflects the standardized prothrombin time, which is a ratio of prothrombin time between patients and normality after being adjusted with the international sensitivity index (ISI). If there were several reports on a variable within the first 24 h, the worst value was chosen for analysis.

Outcomes

The primary outcome was 90-day mortality. Secondary outcomes were the morbidity of AF, in-hospital, ICU, and 28-day mortality.

Statistical Analysis

Variables are divided into continuous and categorical variables. Continuous variables were described as the medians with interquartile range (IQR). The Kolmogorov-Smirnov test was used to evaluate the normality of variables. Student's *t*-test and Wilcoxon Mann–Whitney test were applied to compare continuous variables between AF and non-AF groups. Categorical variables were shown as frequencies with percentages and were compared with the chi-square test or Fisher's exact test.

Additive PLT, INR, and APTT scores were used to define early coagulation disorder with reference to SIC and coagulopathy (**Table 2**) (12, 14). Propensity score matching (PSM) minimized the imbalance of AF and non-AF groups with age, gender, ICU type, ethnicity, and marital status. Univariate and multivariate logistic regression analyses were used to evaluate the association of risk factors and outcomes. Baseline variables that were considered clinically relevant or that showed a univariate relationship with the outcome were included in a multivariate logistic regression model as covariates. The interaction between

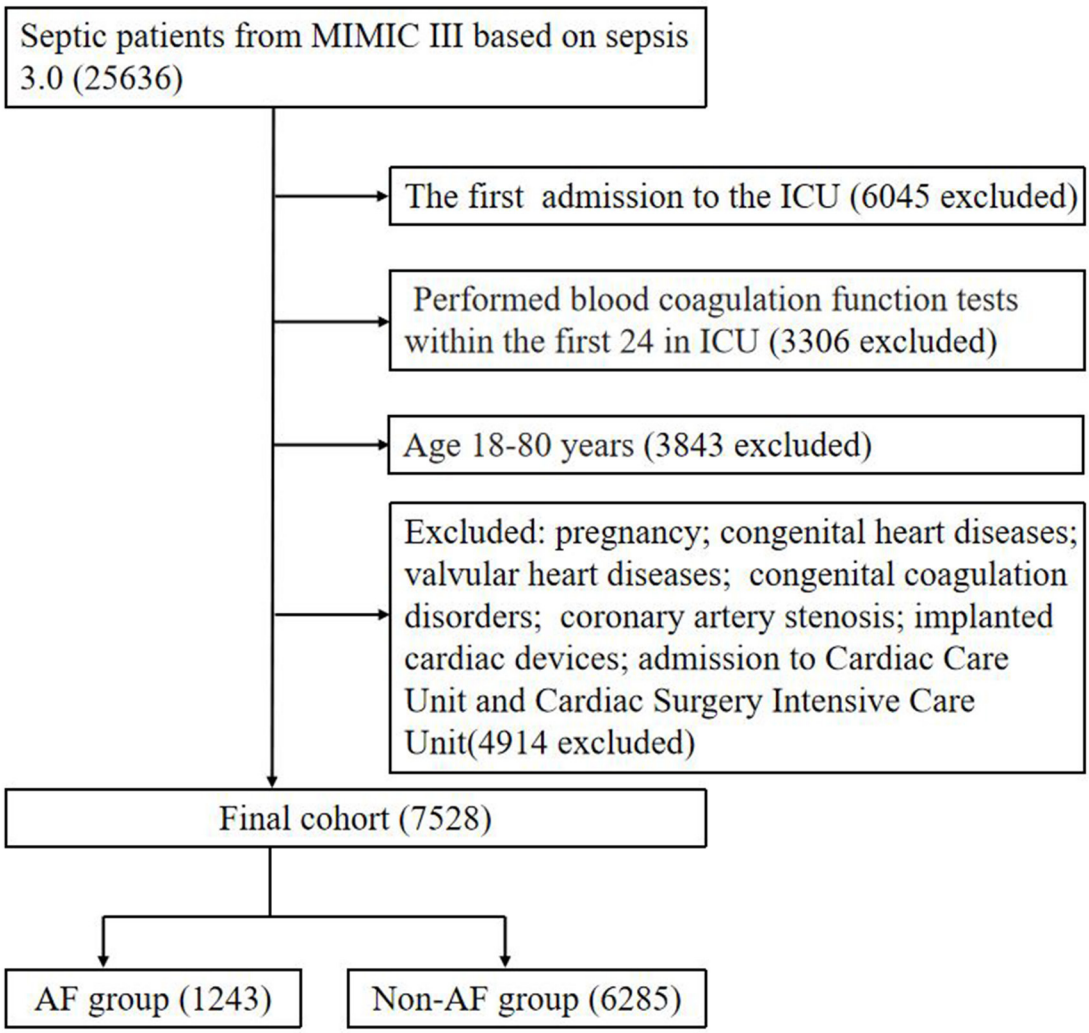

FIGURE 1 | The flowchart of study patients.

early coagulation disorder and AF on mortality was assessed by multivariate logistic regression analyses.

Statistical analyses were performed using SPSS 26.0. $P < 0.05$ was statistically significant.

RESULTS

Demographic and Baseline Characteristics

Overall, 7,528 septic patients were enrolled in this study, of whom 1,243 (16.51%) had AF, and 5,112 (67.91%) had coagulation disorder (**Figure 1**). The median age was 61, with a range from 49 to 70 years old. Female patients comprised 43.65% of the total. The demographic data between the AF group and non-AF group are described (**Table 1**). The patients accompanied with AF were older than that of patients without AF (70, 63–76 vs. 58, 48–69; $P < 0.001$). Furthermore, stroke (0.76 vs. 3.30%; $P < 0.001$), CHF (16.23 vs. 41.43%; $P < 0.001$), hypertension (43.90 vs. 56.48%; $P < 0.001$), diabetes (24.47 vs. 35.32%; $P < 0.001$), ECI (11, 5–17 vs. 17.12–22; $P < 0.001$), APS III (49, 37–63 vs. 53, 42–67; $P < 0.001$), INR (1.3, 1.2–1.7 vs. 1.5, 1.2–2.2; $P < 0.001$), and APTT (32.7, 27.7–43.9, vs. 34.6, 29–46.1; $P < 0.001$) were significantly different in patients between the AF and non-AF groups. Among septic patients, the AF group had higher in-hospital mortality and 90-day mortality (19.71 vs. 13.79%, $P < 0.001$; 59.21 vs. 56.04%, $P = 0.001$). After 1:1 PSM, consisting of 1039 septic patients with AF and 1039 matched individuals without AF as controls, differences in INR, APTT and 90-day mortality between two groups still remained ($P < 0.05$; **Supplementary Table 1**).

Associations Between Early Coagulation Disorder and Atrial Fibrillation

There were more abnormal values of coagulation variables in the AF group ($P < 0.05$). Therefore, early coagulation disorder was classified by adding the PLT, INR, and APTT scores (**Table 2**).

Multivariate logistic regression analyses (**Table 3**) showed that the incidence of AF was associated with the following:

TABLE 1 | Demographic characteristics between AF and Non-AF groups within the first 24 h after admission to ICUs before PSM.

Variables	Total *n* = 7,528	Non-AF *n* = 6,285	AF *n* = 1,243	*P*-value
Age (years)	61 (49–70)	58 (48–69)	70 (63–76)	<0.001
Female, (*n*, %)	3,286 (43.65)	2,775 (42.41)	511 (41.11)	0.048
Ethnicity, *n* (%)				0.001
Asian	225 (2.99)	192 (3.05)	33 (2.65)	
Black	773 (10.27)	667 (10.61)	773 (8.53)	
Hispanic	316 (4.20)	281 (4.47)	316 (2.82)	
White	5,333 (70.84)	4,391 (69.86)	5,333 (75.78)	
Other	881 (11.70)	754 (12.00)	881 (10.22)	
BMI (kg/m^2)	27.7 (23.9–33.0)	27.6 (23.7–32.8)	28.7 (24.7–34.1)	<0.001
Insurance type, *n* (%)				<0.001
Government	269 (3.57)	256 (4.07)	13 (1.05)	
Medicaid	967 (12.85)	913 (14.53)	54 (4.34)	
Medicare	3,479 (46.21)	2,590 (41.20)	889 (71.52)	
Private	2,707 (35.96)	2,428 (38.63)	279 (22.44)	
Self-pay	106 (1.41)	98 (1.56)	8 (0.64)	
Marital status, *n* (%)				<0.001
Single	2,394 (36.92)	2,118 (38.91)	276 (26.51)	
Married	3,442 (53.08)	2,797 (51.38)	645 (61.96)	
Divorced	552 (8.51)	447 (8.21)	105 (10.09)	
Other	97 (1.50)	82 (1.51)	15 (1.44)	
ICU type, *n* (%)				<0.001
SICU	1,683 (22.36)	1,408 (22.40)	275 (22.12)	
TSICU	1,242 (16.50)	1,089 (17.33)	153 (12.31)	
MICU	4,603 (61.15)	3,788 (60.27)	815 (65.57)	
Vital signs				
HR (bpm)	90 (78–102)	91 (79–102)	89 (77–102)	0.056
RR (bpm)	19 (17–22)	19 (17–22)	20 (17–23)	<0.001
T (°C)	36.9 (36.5–37.4)	37.0 (36.5–37.5)	36.9 (36.4–37.3)	<0.001
MAP (mmHg)	77 (70–85)	77 (70–85)	75 (68–82)	<0.001
Complications, *n* (%)				
Hypertension	3,463 (45.98)	2,759 (43.90)	702 (56.48)	<0.001
Diabetes	1,977 (26.26)	1,538 (24.47)	439 (35.32)	<0.001
CHF	1,534 (20.39)	1,019 (16.23)	515 (41.43)	<0.001
Peripheral vascular disease	411 (5.46)	300 (4.78)	111 (8.93)	<0.001
Renal failure	1,127 (14.98)	818 (13.03)	309 (24.86)	<0.001
Chronic liver disease	1,506 (20.02)	1,340 (21.34)	166 (13.35)	<0.001
Chronic pulmonary disease	1,569 (20.86)	1,196 (19.05)	373 (30.00)	<0.001
Stroke	89 (1.18)	48 (0.76)	41 (3.30)	<0.001
DIC	179 (2.38)	151 (2.40)	28 (2.25)	0.206
Critical illness score				
ECI	11 (5-17)	11 (5-17)	17 (12–22)	<0.001
SOFA	5 (3-7)	5 (3-7)	5 (3-7)	<0.001
APSIII	47 (35–62)	49 (37–63)	53 (42–67)	<0.001
SAPSII	36 (28-46)	37 (29–46)	41 (34–51)	<0.001
OASIS	33 (27–39)	33 (27–39)	35 (29–42)	<0.001
GCS	15 (13–15)	15 (14–15)	15 (14–15)	0.547
Coagulation function				
PLT(10^9/L)	179 (109–256)	168 (97–251)	179 (107–256)	0.102
INR	1.3 (1.1–1.7)	1.3 (1.2–1.7)	1.5 (1.2–2.2)	<0.001
APTT (s)	31.5 (27–40.5)	32.7 (27.7–43.9)	34.6 (29–46.1)	<0.001

(Continued)

TABLE 1 | Continued

Variables	Total *n* = 7,528	Non-AF *n* = 6,285	AF *n* = 1,243	*P*-value
Outcomes				
ICU morality, *n* (%)	785 (10.43)	626 (9.96)	159 (12.79)	0.003
In-hospital mortality, *n* (%)	1,112 (14.77)	867 (13.79)	245 (19.71)	<0.001
28-day mortality, *n* (%)	1,236 (16.41)	968 (15.40)	268 (21.56)	<0.001
90-day mortality, *n* (%)	4,528 (56.56)	3,522 (56.04)	736 (59.21)	0.001

AF, atrial fibrillation; BMI, body mass index; SICU, surgical intensive care unit; TSICU, trauma surgical intensive care unit; MICU, medical intensive care unit; HR, heart rate; RR, respiratory rate; T, temperature; MAP, mean arterial pressure; CHF, congestive heart failure; DIC, disseminated intravascular coagulation; ECI, Elixhauser comorbidity index; SOFA, sequential organ failure assessment; APSIII, acute physiological score III; SAPSII, simplified acute physiology score II; OASIS, overall anxiety severity and impairment scale; GCS, Glasgow coma score; PLT, platelet count; INR, international normalized ratio; APTT, activated partial thromboplastin time.

TABLE 2 | Comparisons of early coagulation disorder between AF group and Non-AF group before PSM.

Coagulation	Total	Non-AF	AF	*P*-value
PLT score				0.002
0 (≥150 × 10^9/L)	4,508 (59.88)	3,733 (59.40)	775 (62.35)	
1 (100–150 × 10^9/L)	1,440 (19.13)	1,186 (18.87)	254 (20.43)	
2 (<100 × 10^9/L)	1,580 (20.99)	1,366 (21.73)	214 (17.22)	
INR score				<0.001
0 (≤1.2)	4,198 (55.77)	3,664 (58.30)	534 (42.96)	
1 (1.2-1.4)	1,587 (21.08)	1,325 (21.08)	262 (21.08)	
2 (>1.4)	1,743 (23.15)	1,296 (20.62)	447 (35.96)	
APTT score				<0.001
0 (≤37 s)	5,153 (68.45)	4,387 (69.80)	766 (61.62)	
1 (37–39 s)	290 (3.85)	227 (3.61)	63 (5.07)	
2 (>39 s)	2,085 (27.70)	1,671 (26.59)	414 (33.30)	
Total score		1 (0–3)	2 (1–4)	<0.001
Coagulation disorder type				<0.001
Coagulation disorder score = 0	2,416 (32.09)	2,132 (33.92)	284 (22.85)	
Coagulation disorder score = 1 or 2	2,708 (35.97)	2,231 (35.50)	477 (38.37)	
Coagulation disorder score = 3 or 4	1,591 (21.13)	1,242 (19.76)	349 (28.08)	
Coagulation disorder score = 5 or 6	813 (10.80)	680 (10.82)	133 (10.70)	

AF, atrial fibrillation; PLT, platelet count; INR, international normalized ratio; APTT, activated partial thromboplastin time.

stroke (adjusted OR = 3.756; 95% CI: 2.269–6.215; $P < 0.001$), coagulation disorder types (early coagulation disorder type = 1; as reference; $P < 0.001$), age (adjusted OR = 1.072; 95% CI: 1.064–1.080; $P < 0.001$), gender (adjusted OR = 1.276; 95% CI: 1.096–1.487; $P = 0.002$), CHF (adjusted OR = 2.378; 95% CI: 2.022–2.797; $P < 0.001$), chronic pulmonary disease (adjusted OR = 1.350; 95% CI: 1.139–1.600; $P = 0.001$), renal failure (adjusted OR = 1.459; 95% CI: 1.203–1.769; $P < 0.001$) and chronic liver disease (adjusted OR = 0.664; 95% CI: 0.531–0.829; $P < 0.001$). After 1:1 PSM, early coagulation disorder was still an independent risk factor for AF (reference as early coagulation disorder type = 1; $P < 0.001$).

The Interaction of AF and Early Coagulation Disorder on Mortality

With the population before PSM, univariate regression analyses showed that AF was relevant to short-term outcomes, including in-hospital mortality (OR = 1.534; 95% CI: 1.311–1.795; $P < 0.001$), ICU mortality (OR = 1.326; 95% CI: 1.101–1.597; $P = 0.003$), 28-day mortality (OR = 1.510; 95% CI: 1.298–1.757; $P < 0.001$) and 90-day mortality (OR = 1.139; 95% CI: 1.006–1.289; $P = 0.039$). On multivariate logistic regression analyses, AF was an independent risk factor for in-hospital mortality (adjusted OR = 1.323; 95% CI: 1.078–1.624; $P = 0.008$) and 90-day mortality (adjusted OR = 1.243; 95% CI: 1.073–1.441; $P = 0.004$). In septic patients with different coagulation disorder scores, AF was significantly associated with an increased risk of 90-day mortality when coagulation disorder scores were 1 or 2 and 3 or 4 (adjusted OR = 1.291; 95% CI: 1.017–1.639; $p = 0.036$; adjusted OR = 1.355; 95% CI: 1.004–1.828; $p = 0.047$). However, there were no differences in patients with all coagulation scores for in-hospital mortality. Among patients with coagulation scores of 0 and 5 or 6, AF was not associated with 90-day mortality. (**Tables 4**, **5**).

TABLE 3 | Association between early coagulation disorder and AF in multivariate logistic regression before and after PSM.

Variables	Before PSM		After PSM	
	OR; 95% CI	*P*-value	OR; 95% CI	*P*-value
Age (years)	1.072; 1.064–1.080	<0.001	0.994; 0.985–1.003	0.196
Gender		0.002		0.959
Female	1			
Male	1.276; 1.096–1.487		0.995; 0.824–1.202	
Ethnicity		0.458		0.109
Asian	1			
Black	0.789; 0.485–1.283	0.339	0.577; 0.313–1.063	0.078
Hispanic	0.810; 0.450–1.457	0.481	0.945; 0.446–2.003	0.882
White	0.991; 0.649–1.513	0.967	0.868; 0.510–1.478	0.602
Marital status		0.479		0.365
Divorced	1		1	
Married	0.835; 0.648–1.076;	0.163	0.897; 0.654–1.230	0.501
Single	0.900; 0.686–1.180	0.445	1.094; 0.777–1.541	0.608
Other	1.027; 0.541–1.952	0.934	0.891; 0.403–1.970	0.775
ICU type		0.310		0.157
SICU	1			
TSICU	0.875; 0.677–1.130	0.306	0.951; 0.689–1.311	0.758
MICU	0.871; 0.726–1.044	0.135	0.814; 0.649–1.019	0.073
Complications, *n* (%)				
CHF	2.378; 2.022–2.797	<0.001	1.549; 1.274–1.884	<0.001
Peripheral vascular disease	1.051; 0.795–1.389	0.726	1.167; 0.823–1.653	0.386
Chronic pulmonary disease	1.350; 1.139–1.600	0.001	1.531; 1.237–1.897	<0.001
Hypertension	0.955; 0.816–1.117	0.563	1.183; 0.977–1.433	0.086
Diabetes	1.111; 0.944–1.308	0.204	1.194; 0.977–1.460	0.082
Renal failure	1.459;1.203–1.769	<0.001	1.599; 1.254–2.040	<0.001
Chronic liver disease	0.664; 0.531–0.829	<0.001	0.707; 0.539–0.926	0.012
Stroke	3.756; 2.269–6.215	<0.001	5.047; 2.300–11.073	<0.001
SOFA	1.051; 0.795–1.389	0.267	0.979; 0.949–1.011	0.194
Coagulation disorder		<0.001		<0.001
Coagulation disorder score = 0	1		1	
Coagulation disorder score = 1 or 2	1.566; 1.295–1.894	<0.001	1.592; 1.261–2.010	<0.001
Coagulation disorder score = 3 or 4	2.022; 1.628–2.510	<0.001	1.780; 1.372–2.309	<0.001
Coagulation disorder score = 5 or 6	2.126; 1.559–2.898	<0.001	1.763; 1.218–2.551	0.003

AF, atrial fibrillation; CHF, congestive heart failure; SOFA, sequential organ failure assessment; PSM, propensity score matching, with the covariates of age, gender, ICU type, ethnicity and marital status. Multivariate logistic regression analysis includes the factors of age, gender, ethnicity, marital status, ICU type, CHF, peripheral vascular disease, chronic pulmonary disease, hypertension, diabetes, renal failure, chronic liver disease, stroke, SOFA score, and coagulation disorder type.

DISCUSSION

This study suggested an interaction between AF and early coagulation disorder on in-hospital mortality as well as 90-day mortality. In the subgroup analysis stratified by early coagulation disorder scores, AF was associated with an increased risk of 90-day mortality when the scores of early coagulation disorder were 1 or 2 and 3 or 4. The risk of AF was also related to stroke, age, gender, CHF, chronic pulmonary disease, renal failure, and chronic liver disease.

In this study, AF was significantly correlated with higher in-hospital mortality and 90-day mortality than non-AF in sepsis, which is consistent with previous studies (15, 16). Desai et al. (16) reported that although in-hospital mortality and hospital stay decreased between 2010 and 2014 in the sepsis-AF cohort, septic patients with AF had increased all-cause mortality during hospitalization, a longer hospital stay, and higher hospitalization expenses than non-AF individuals. The review also assessed the morbidity of AF and the relationship between AF and adverse events in sepsis. The morbidity of new-onset AF in sepsis was 20.6%. New-onset AF was correlated with excess mortality during hospitalization and 28 days, 1 year as well as 5 years after discharge (15). Therefore, AF is regarded as a marker of disease severity and risk factor of death. The pathogenesis of AF in sepsis can be divided into two steps: the changes of the atrial matrix and trigger events (17, 18). Before the onset of sepsis, patient characteristics, such as advanced age, diabetes, acute renal impairment, and CHF, lead to atrial structure and electrical

remodeling through the production of TGF-β, angiotensin, ROS, inflammatory factors, and changes in intracellular ion channels. During sepsis, infection, overloading volume, and thyroid crisis can further trigger AF. In addition, AF is connected with decreased cardiac output and increased cardiac afterload. AF can also aggravate atrial cardiomyopathy, further increasing the risk of thromboembolism. Thus, AF and its adverse consequences dramatically increase short-term and long-term mortality.

AF is correlated with an increased risk of thrombosis (19), but it is unknown whether early abnormal coagulation function is a risk factor for AF. The present studies indicate that sepsis is associated with early coagulation disorder, including a fall in platelet count and lengthening of clotting time. SIC is used to identify the earlier stage of DIC in sepsis, which was proposed by the Scientific and Standardization Committee on DIC (20). The main mechanisms contributing to SIC and DIC consist of coagulation initiation, activation of platelets, increased inflammatory cells, and damage to vascular endothelium (8, 10, 21) due to damage-associated molecular patterns (DAMPs) (22, 23), neutrophil extracellular traps (NETs) (24), extracellular vehicles (EVs) (25) and endothelial glycocalyx damage (26). It is worth noting that vascular endothelial dysfunction and anticoagulant/fibrinolytic disorder are major markers of SIC. Vascular endothelium connects with membrane-binding proteoglycans and glycosaminoglycan side chains which can bind to antithrombin so that thrombosis is negatively affected in normal circumstances. However, the inhibition weakens or disappears when the vascular endothelium is damaged. Endothelial cells also have a vital influence on fibrinolysis through tissue-type plasminogen activators and their inhibitors, while the balance is disrupted and tends to inhibit fibrinolysis during sepsis (13). Thus, derangement of the coagulation/fibrinolytic system is a hallmark of SIC. It is speculated that abnormal coagulation function caused by sepsis is associated with a disturbance in hemodynamics (23), which can contribute to the development of AF.

In sepsis, there are a large proportion of patients exhibiting DIC, which is diagnosed by coagulation indicators of prothrombin time, platelet count, fibrinogen, D-dimer and fibrin degradation products. However, the DIC scoring system is only suitable for the evaluation of severe coagulation dysfunction (8). To provide an early and valuable prediction of coagulation disorder, this work defines early coagulation disorder with reference to SIC and coagulopathy. This is because SIC provides an earlier diagnose and includes most cases of DIC. Septic patients with SIC can also benefit from anticoagulant therapy even though they do not meet the criteria for DIC (23). In addition, sepsis-associated DIC is characterized by excessive inhibition of fibrinolysis due to overproduction of plasminogen activator inhibitors. Therefore, hypofibrinogenemia is uncommon and fibrin-related markers are not correlated with the severity of illness. In contrast, platelet count and PT play a vital role in the mortality of septic patients. Therefore, PLT, INR, and APTT were selected as common and major coagulation indicators to evaluate early coagulation dysfunction (27).

Currently, there are few models to predict the risk of AF. Based on the findings of our work, early coagulation status should be considered, in part, as a clue of AF risk in septic patients and may be regarded as an indicator of the AF prediction model accompanied with other indicators (27). Because of the poor prognosis in septic patients with AF, this model is vital for the risk stratification to promote the early management of these patients. However, a retrospective study in these patients makes robust conclusions difficult. Therefore, more prospective, multicenter trials to evaluate the relationship between early coagulation and AF are necessary.

TABLE 4 | Univariate and multivariate logistic regression analysis of AF and mortality.

	Univariate		Multivariate	
	OR; 95% CI	***P*-value**	**OR; 95% CI**	***P*-value**
In-hospital mortality	1.534; 1.311–1,795	<0.001	1.323; 1.078–1.624	0.008
ICU mortality	1.326; 1.101–1.597	0.003	1.195; 0.937–1.525	0.150
28-day mortality	1.510; 1.298–1.757	<0.001	1.195; 0.983–1.453	0.074
90-day mortality	1.139; 1.006–1.289	0.039	1.243; 1.073–1.441	0.004

Multivariate logistic regression analysis includes the factors of age, gender, ethnicity, marital status, ICU type, CHF, peripheral vascular disease, chronic pulmonary disease, hypertension, diabetes, renal failure, chronic liver disease, stroke, SOFA score, and coagulation disorder type.

Limitations

Moreover, we acknowledge certain limitations in this work. First, it was a retrospective study covering hospital admissions

TABLE 5 | The interaction between AF and early coagulation disorder on morality.

	In-hospital mortality		90-day mortality	
	OR; 95% CI	***p*-value**	**OR; 95% CI**	***p*-value**
Coagulation disorder score = 0	1.417; 0.875–2.296	0.157	1.139; 0.850–1.526	0.383
Coagulation disorder score = 1 or 2	1.340; 0.960–1.869	0.085	1.291; 1.017–1.639	0.036
Coagulation disorder score = 3 or 4	1.242; 0.835–1.849	0.285	1.355; 1.004–1.828	0.047
Coagulation disorder score = 5 or 6	1.187; 0.690–2.045	0.535	1.356; 0.812–2.264	0.245

Multivariate logistic regression analysis includes the factors of age, gender, ethnicity, marital status, ICU type, CHF, peripheral vascular disease, chronic pulmonary disease, hypertension, diabetes, renal failure, chronic liver disease, stroke, and SOFA score.

of more than 10 years. During this period, the decision-making process of AF and sepsis management developed dramatically, which is a confounding factor affecting mortality (17, 28). Second, information on medication in septic patients before the onset of AF, such as glucocorticoids and vasopressin, was not available (29). Owing to the influence of these medications on AF morbidity, the relationship between coagulation disorders and AF can interfere. Third, we have only taken coagulation analysis with PLT, INR, and APTT. Given the retrospective nature of our study, clinical information such as other coagulation factors and fibrinogen were not available, so further analysis about the association between coagulation and AF cannot be fully explored. Finally, given the retrospective nature of the study, clinical information on several aspects of AF and sepsis in the cohort, such as AF loading assessment, AF classification and treatment, were not be fully available.

Future Directions

The present results highlight the influence of early coagulation dysfunction on septic patients with AF. However, larger, prospective trials are necessary to determine the relationship between coagulation disorder and AF. This study may provide the basis for future research on the relationship between early coagulation and AF.

CONCLUSION

Coagulation disorder within the first 24 h after admission to the ICUs is related to a higher risk of AF in individuals with sepsis. AF is an independent risk factor of in-hospital mortality and 90-day mortality in sepsis, and the effect of AF on 90-day mortality varies with the severity of early coagulation disorder. It is of great importance for clinicians to make personalized management for septic patients with AF based on their early coagulation status.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YL, YT, RF, and JD: methodology, writing, and revision. YL, RM, XC, JW, and JS: data curation and investigation. GL and CL: supervision, reviewing, and editing the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.724942/full#supplementary-material

REFERENCES

1. Wetterslev M, Moller MH, Granholm A, Haase N, Hassager C, Lange T, et al. New-onset atrial fibrillation in the intensive care unit: protocol for an international inception cohort study (AFIB-ICU). *Acta Anaesthesiol Scand.* (2021) 65:846–51. doi: 10.1111/aas.13827
2. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med.* (2017) 43:304–77. doi: 10.1007/s00134-017-4683-6
3. Bedford J, Harford M, Petrinic T, Young JD, Watkinson PJ. Risk factors for new-onset atrial fibrillation on the general adult ICU: protocol for a systematic review. *BMJ Open.* (2018) 8:e024640. doi: 10.1136/bmjopen-2018-024640
4. Ou SM, Chu H, Chao PW, Lee YJ, Kuo SC, Chen TJ, et al. Long-term mortality and major adverse cardiovascular events in sepsis survivors. A Nationwide Population-based Study. *Am J Respir Crit Care Med.* (2016) 194:209–17. doi: 10.1164/rccm.201510-2023OC
5. Walkey AJ, Hammill BG, Curtis LH, Benjamin EJ. Long-term outcomes following development of new-onset atrial fibrillation during sepsis. *Chest.* (2014) 146:1187–95. doi: 10.1378/chest.14-0003
6. Klein Klouwenberg PM, Frencken JF, Kuipers S, Ong DS, Peelen LM, van Vught LA, et al. Incidence, predictors, and outcomes of new-onset atrial fibrillation in critically ill patients with sepsis. A Cohort Study. *Am J Respir Crit Care Med.* (2017) 195:205–11. doi: 10.1164/rccm.201603-0618OC
7. Giustozzi M, Ehrlinder H, Bongiovanni D, Borovac JA, Guerreiro RA, Gasecka A, et al. Coagulopathy and sepsis: pathophysiology, clinical manifestations and treatment. *Blood Rev.* (2021). doi: 10.1016/j.blre.2021.100864. [Epub ahead of print].
8. Iba T, Levy JH. Sepsis-induced coagulopathy and disseminated intravascular coagulation. *Anesthesiology.* (2020) 132:1238–45. doi: 10.1097/ALN.0000000000003122
9. Wu CQ, Lu W, Zhang Y, Zhang GY, Shi XY, Hisada Y, et al. Inflammasome activation triggers blood clotting and host death through pyroptosis. *Immunity.* (2019) 50:1401. doi: 10.1016/j.immuni.2019.04.003
10. Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. *Nat Rev Dis Primers.* (2016) 2:16037. doi: 10.1038/nrdp.2016.37
11. Iba T, Di Nisio M, Thachil J, Wada H, Asakura H, Sato K, et al. Revision of the Japanese Association for Acute Medicine (JAAM) disseminated intravascular coagulation (DIC) diagnostic criteria using antithrombin activity. *Crit Care.* (2016) 20:287. doi: 10.1186/s13054-016-1468-1
12. Spronk HM, De Jong AM, Verheule S, De Boer HC, Maass AH, Lau DH, et al. Hypercoagulability causes atrial fibrosis and promotes atrial fibrillation. *Eur Heart J.* (2017) 38:38–50. doi: 10.1093/eurheartj/ehw119
13. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* (2016) 315:801–10. doi: 10.1001/jama.2016.0287
14. Bodilsen J, Mariager T, Vestergaard HH, Christiansen MH, Kunwald M, Luttichau HR, et al. Association of lumbar puncture with spinal hematoma in patients with and without coagulopathy. *JAMA.* (2020) 324:1419–28. doi: 10.1001/jama.2020.14895
15. Aibar J, Schulman S. New-onset atrial fibrillation in sepsis: a narrative review. *Semin Thromb Hemost.* (2021) 47:18–25. doi: 10.1055/s-0040-1714400
16. Desai R, Hanna B, Singh S, Omar A, Deshmukh A, Kumar G, et al. Trends and outcomes in sepsis hospitalizations with and without atrial

fibrillation: a nationwide inpatient analysis. *Crit Care Med.* (2019) 47:e630–e8. doi: 10.1097/CCM.0000000000003806
17. Lévy S, Santini L, Cappato R, Steinbeck G, Capucci A, Saksena S. Clinical classification and the subclinical atrial fibrillation challenge: a position paper of the European Cardiac Arrhythmia SocietyJ Interv Card Electrophysiol. *J Interv Card Electrophysiol.* (2020) 59:495–507. doi: 10.1007/s10840-020-00859-y
18. Levi M, Schultz MJ. What do sepsis-induced coagulation test result abnormalities mean to intensivists? *Intens Care Med.* (2017) 43:581–3. doi: 10.1007/s00134-017-4725-0
19. Kamel H, Okin PM, Elkind MS, Iadecola C. Atrial fibrillation and mechanisms of stroke: time for a new model. *Stroke.* (2016) 47:895–900. doi: 10.1161/STROKEAHA.115.012004
20. Iba T, Arakawa M, Levy JH, Yamakawa K, Koami H, Hifumi T, et al. Sepsis-Induced Coagulopathy and Japanese Association for Acute Medicine DIC in coagulopathic patients with decreased antithrombin and treated by antithrombin. *Clin Appl Thromb Hemost.* (2018) 24:1020–6. doi: 10.1177/1076029618770273
21. Boral BM, Williams DJ, Boral LI. Disseminated intravascular coagulation. *Am J Clin Pathol.* (2016) 146:670–80. doi: 10.1093/ajcp/aqw195
22. Liaw PC, Ito T, Iba T, Thachil J, Zeerleder S. DAMP and DIC: the role of extracellular DNA and DNA-binding proteins in the pathogenesis of DIC. *Blood Rev.* (2016) 30:257–61. doi: 10.1016/j.blre.2015.12.004
23. Lupu F, Kinasewitz G, Dormer K. The role of endothelial shear stress on haemodynamics, inflammation, coagulation and glycocalyx during sepsis. *J Cell Mol Med.* (2020) 24:12258–71. doi: 10.1111/jcmm.15895
24. McDonald B, Davis RP, Kim SJ, Tse M, Esmon CT, Kolaczkowska E, et al. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood.* (2017) 129:1357–67. doi: 10.1182/blood-2016-09-741298
25. Zhang Y, Meng H, Ma R, He Z, Wu X, Cao M, et al. Circulating microparticles, blood cells, and endothelium induce procoagulant activity in sepsis through phosphatidylserine exposure. *Shock.* (2016) 45:299–307. doi: 10.1097/SHK.0000000000000509
26. Dogne S, Flamion B. Endothelial glycocalyx impairment in disease: focus on hyaluronan shedding. *Am J Pathol.* (2020) 190:768–80. doi: 10.1016/j.ajpath.2019.11.016
27. Iba T, Umemura Y, Watanabe E, Wada T, Hayashida K, Kushimoto S, et al. Diagnosis of sepsis-induced disseminated intravascular coagulation and coagulopathy. *Acute Med Surg.* (2019) 6:223–32. doi: 10.1002/ams2.411
28. Andrade JG, Verma A, Mitchell LB, Parkash R, Leblanc K, Atzema C, et al. 2018 focused update of the canadian cardiovascular society guidelines for the management of atrial fibrillation. *Can J Cardiol.* (2018) 34:1371–92. doi: 10.1016/j.cjca.2018.08.026
29. Bedford JP, Harford M, Petrinic T, Young JD, Watkinson PJ. Risk factors for new-onset atrial fibrillation on the general adult ICU: a systematic review. *J Crit Care.* (2019) 53:169–75. doi: 10.1016/j.jcrc.2019.06.015

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Antithrombotic Therapy With Ticagrelor in Atrial Fibrillation Subjects After Percutaneous Coronary Intervention

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Background: Warfarin, along with aspirin and clopidogrel, has long been recommended for patients with atrial fibrillation (AF) who are undergoing percutaneous coronary intervention with a drug-eluting stent (PCI-DES). However, this triple therapy has been known to increase the risk of bleeding complications. Meanwhile, there is no evidence from prospective trials on the use of ticagrelor in a dual therapy. We here aimed to compare the antiplatelet drug ticagrelor as a dual antithrombotic agent to aspirin and clopidogrel in bleeding events.

Methods: In this multicenter, active-controlled, open-label, randomized trial, patients with AF taking warfarin who had undergone PCI-DES were randomly assigned to the ticagrelor therapy group (Dual group) or the clopidogrel plus aspirin therapy group (Triple group). The primary and secondary endpoints were overall bleeding events and major bleeding events, respectively, according to the Thrombolysis in Myocardial Infarction (TIMI) criteria at 6 months. Cardiovascular events [re-PCI, surgical bypass, myocardial infarction (MI), heart failure, rehospitalization due to angina pectoris, stent thrombosis and death due to cardiovascular causes] at 6 months were also recorded.

Results: A total of 296 patients from 12 medical centers in China were randomized after PCI-DES to either the Dual therapy group ($n = 148$) or the Triple group ($n = 146$) for 6 months. The overall incidence of bleeding events at 6 months was 36.49% in the Dual therapy group and 35.62% in the Triple group [hazard ratio, 0.930; 95% confidence interval (CI), 0.635 to 1.361; $P = 0.7088$]. The incidence of the secondary endpoint over 6 months was 4.73% in the Dual therapy group and 1.37% in the Triple group (hazard ratio, 0.273; 95% CI, 0.057 to 1.315; $P = 0.1056$). Cardiovascular event occurrence was

also comparable in both groups at 6 months (18.24 vs. 16.44%; hazard ratio, 0.845; 95% CI, 0.488 to 1.465; $P = 0.5484$).

Conclusions: The incidence of total bleeding events in AF patients treated with ticagrelor was comparable to that in patients treated with clopidogrel plus aspirin at 6 month; Meanwhile, the incidence of cardiovascular events were also comparable between the groups.

Clinical Trial Registration: MANJUSRI, ClinicalTrials.gov# NCT02206815, 2014, August 1st

Keywords: antithrombic therapy, atrial fibrillation, drug eluting stent, percutaneous coronary intervention, coronary artery disease

INTRODUCTION

Chronic treatment with oral anticoagulants (e.g., warfarin) is essential for most atrial fibrillation (AF) patients with CHA_2DS_2VASc scores ≥ 2. Treatment of patients with AF who have undergone percutaneous coronary intervention with a drug-eluting stent (PCI-DES) further requires follow-up antiplatelet therapy to minimize thrombotic events. Triple therapy, such as warfarin plus dual antiplatelet agents (DAPT), aspirin and clopidogrel, has been used for many years in patients with AF who have undergone PCI-DES (1).

The European Society of Cardiology recommended a short period of therapy with VKA, aspirin, and clopidogrel for such patients (2). The 2019 AHA/ACC/HRS guidelines for the management of patients with AF suggested that "Triple therapy should be administered to these patients with bare-metal stents for 1–3 months and much longer in patients with a drug-eluting stent (3–6 months) followed by one anticoagulant plus clopidogrel 75 mg/day or aspirin 100 mg/day" (3).

RCT studies as well as retrospective analyses all suggested that the combination of oral anticoagulation with a P2Y12 inhibitor and aspirin in patients with AF undergoing PCI-DES is associated with a high bleeding risk (4, 5). Triple antithrombotic therapy, particularly if consisting of a OAC, aspirin and a P2Y12 inhibitor, is associated with a increasing of bleeding, including major and intracranial hemorrhages (6). However, pooled data from meta-analysis (7) have shown a possible increase of ischemic events in the dual therapy with clopidogrel, for example Galli et al. confirmed that dual therapy with clopidogrel with a significant increase of stent thrombosis risk in the overall population and a significant 43% increase of MI in the ACS/PCI subgroup (8) sparkling the interest for the use of alternative antithrombotic agents. In addition to this, clopidogrel, but not ticagrelor, is characterized by an interindividual variability in the pharmacodynamic profile, leading to insufficient platelet inhibition and increased ischemic events in up to 40% of treated patients and thus Most recent investigations support the clinical benefit of a genetic guided selection of antiplatelet therapy in patients undergo PCI (i.e., switching to prasugrel or ticagrelor) (9, 10).

Recently, randomized clinical trials and recent evidence have supported the ESC recommendation for dual antithrombotic therapy in patients with AF who have undergone PCI-DES (6, 11). As is well-known, the recent guidelines (2018–2020 ECS Guidelines) supported the current trend of antithrombotic therapy using a combination of one antiplatelet drug and one anticoagulant (12–14), for example,2020 ESC Guidelines presented that In AF patients with ACS undergoing an uncomplicated PCI, early cessation (<1 week) of aspirin and continuation of dual therapy with an OAC and a P2Y12 inhibitor for up to 12 months is recommended if the risk of stent thrombosis is low or if concerns about bleeding risk prevail over concerns about risk of stent thrombosis, irrespective of the type of stent used. However,1 week triple therapy with clopidogrel can be associated to increased ischemic events.

The TWILIGHT trial proved that ticagrelor monotherapy was associated with a lower incidence of clinically relevant bleeding than ticagrelor plus aspirin, without higher risks of death, myocardial infarction, or stroke, again suggesting that ticagrelor is a more potent antiplatelet drug (15, 16).

The recent AUGUSTUS study was designed for AF patients who had ACS or had undergone PCI and were taking a P_2Y_{12} inhibitor to receive apixaban or warfarin and aspirin or a matching placebo for 6 months (17, 18). However, more than 90% of the individuals in this trial were treated with clopidogrel instead of more potent agents, such as ticagrelor. Among published clinical studies such as PIONEER trial, ENTRUST trial and Re-Dual trial, the Re-Dual study included the most patients received ticagrelor were only 12% (19–21). The use of ticagrelor can potentially overcome the increased rate of ischemic events as the rate of non-responders to this drug is very low.

On the other hand, triple therapy with ticagrelor in these previous trials was somewhat unfounded and in particular lacked the support of ESC guidelines. Thus, the rationale for our study is that ticagrelor were used only in a minority of the 4 RCTs on the topic (PIONEER, RE-DUAL, AUGUSTUS and ENTRUST-AF), which implied that there are not many data from prospective studies on a dual therapy including this potent P2Y12 inhibitors.

Therefore, we performed the present study to compare the combination of ticagrelor and warfarin with traditional triple therapy, with the aim to evaluate the safety and efficacy of ticagrelor as one of dual antithrombins.

METHODS

Study Design

The trial rationale and design were published previously (22). In brief, the MANJUSRI trial was a prospective, multicenter, open-label, randomized clinical trial that compared the potent antiplatelet drug ticagrelor with the traditional antithrombotic drugs clopidogrel plus aspirin in patients with AF taking warfarin and receiving PCI-DES (all of the patients in the trial received drug-eluting stents). This clinical trial was registered with ClinicalTrials.gov (NCT02206815). The study was performed in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The institutional ethics committee associated with the participating centers approved the trial protocol. All of the patients provided written informed consent.

Study Population

Eligible patients were recruited from 12 participating centers in China from September 2014 to February 2019. The inclusion criteria were men or nonpregnant women $\geq$ 18 and $\leq$ 75 years of age with a severe coronary lesion ($\geq$ 75% angiographically or fractional flow reserve, FFR $<$ 0.8) with an indication for coronary stent implantation and patients with a CHA_2DS_2-VASc score $\geq$ 2. The exclusion criteria are listed in **Supplementary Table 1.** CHA_2DS_2-VASc scores, which reflect the risk of stroke, and HAS-BLED scores, which reflect the risk of bleeding, were assessed in all of the patients.

Randomization and Trial Regimen

Patients who met all of the inclusion criteria and none of the exclusion criteria were randomly assigned at a 1:1 ratio to receive ticagrelor and warfarin (Dual therapy group) or clopidogrel + aspirin + warfarin (Triple group) just after PCI-DES. The randomization sequence and allocation were accomplished using sealed envelopes containing a computer-generated sequence (generated at ZhongDa Hospital Affiliated with Southeast University). The randomized enrollment progress and patient distribution are shown in **Supplementary Figure 1**. All of the patients in the trial took warfarin, and the doses were adjusted to reach a target international normalized ratio (INR) within the range of 2.0 to 2.5. Before PCI-DES, a loading dose of 180 mg of ticagrelor or 300 mg of clopidogrel and 300 mg of aspirin were administered. After PCI-DES, patients on Triple group received clopidogrel 75 mg/day + aspirin 100 mg/day, whereas patients in the Dual therapy group received ticagrelor 90 mg/bid. The use of proton pump inhibitors to reduce the risk of bleeding was recommended but not mandatory. Follow-ups were performed during routinely scheduled outpatient visits at 1, 3, and 6 months and via telephone calls at 15 days and 7 months after randomization. After 6 months, the patients were transitioned from their 2-trial interventions to receive antiplatelet and anticoagulant therapy at the discretion of the attending physician according to the local standard of care.

Outcomes and Endpoints

The primary endpoint of the study was the occurrence of overall bleeding events (minimal, minor, and major) during the first 6 months of follow-up. The secondary endpoint was the occurrence of major bleeding events during the 6-month follow-up. Bleeding events in the trial were assessed according to the Thrombolysis in Myocardial Infarction (TIMI) bleeding criteria (23–26): (1) Major: any intracranial bleeding; clinically overt signs of hemorrhage associated with a drop in hemoglobin of $\geq$5-g/dL or a $\geq$15% absolute decrease in hematocrit; and fatal bleeding; (2) Minor: clinically overt signs (including imaging), resulting in a hemoglobin drop of 3- to $<$5-g/dL or a $\geq$10% decrease in hematocrit; no observed blood loss: $\geq$4 g/dL decrease in the hemoglobin concentration or $\geq$12% decrease in hematocrit; requiring medical attention: any overt sign of hemorrhage that met one of the following criteria and did not meet the criteria for a major or minor bleeding event, as defined above; requiring intervention: medical treatment to stop or treat bleeding, including changing the dose of the study drug; leading to or prolonging hospitalization; and prompting evaluation (unscheduled visit to a healthcare professional and diagnostic testing); (3) Minimal: any overt bleeding event not meeting the criteria above; any clinically overt sign of hemorrhage (including imaging) associated with a $<$3-g/dL decrease in hemoglobin concentration or $<$9% decrease in hematocrit.

Assessment of Cardiovascular Events

Cardiovascular events reported were re-PCI (percutaneous coronary intervention) or surgical bypass, MI (myocardial infarction), heart failure and rehospitalization due to angina pectoris, stent thrombosis and death due to cardiovascular causes, all of which were in accordance with the Academic Research Consortium criteria. Standardized questions were used to assess cardiovascular events, and the use of medications. A committee of clinical events that was unaware of treatment allocations adjudicated and verified all events that required medical attention from the medical records of the referring doctors and hospitals.

Statistical Analysis

Data management and statistical analyses were performed using SAS software, version 9.4 (SAS Institute, USA).

Based on previous research mainly retrospective analysis at the time, we anticipated a bleeding complication rate of 18% in the dual ticagrelor therapy group and 30% in the clopidogrel plus aspirin group at 6 months. The proportion of patients dropping out of the treatment and the control groups was assumed to be 5%. The sample size was estimated at 296 subjects.

The primary and secondary endpoints were the first occurrences of events. The primary analysis compared the time from randomization to the first occurrence of any event in the composite endpoint using the log-rank test.

The null and alternative hypotheses were the following.

H0: The distribution of the first occurrence of bleeding in the 2 groups is the same.

H1: The distribution of the first occurrence of bleeding in the 2 groups is not the same.

The hypotheses were tested at an overall significance level of 5% (2 sided). Patients who failed to record any event at the primary

composite efficacy endpoint were censored at the close of the study (i.e., date of the end-of-treatment visit) or at the time of the last available information, if not earlier. Kaplan-Meier and Cox proportional hazard regression estimates of the cumulative risk for the first occurrence of any event were calculated in the 2 groups. The hazard ratio and 95% confidence intervals (CIs) are reported.

Role of the Funding Source

The sponsors of the study played no role in the entire process, including the study design, data collection, and data analysis. The corresponding author is fully responsible for the trial and manuscript publishing.

RESULTS

Baseline Characteristics of Patients With AF

From September 2014 to February 2019, a total of 296 patients from 12 sites in China were enrolled. Totals of 148 and 146 AF patients were assigned to the ticagrelor therapy group (Dual group) and clopidogrel plus aspirin (Triple group) therapy group, respectively (**Figure 1**). After a follow-up period of 6 months for both groups, the final collection of follow-up data occurred on February 20, 2019. A total of 3 patients died during the study period. The direct cause of death of a patient in the ticagrelor therapy group was heart failure; One patient died of trauma following a fall in Triple therapy group, and the other also in the Triple group died of acute exacerbation of renal insufficiency.

The mean age of the patients in the trial was 69.39 years old. In all, 65.54% of patients in the Dual therapy group and 62.33% in the Triple therapy group were male. Clinical comorbidities, including hypertension, diabetes, and history of stroke/TIA, and the use of medication were comparable between the 2 groups (**Table 1**). The average CHA_2DS_2-VASc score was 3.41 in the ticagrelor therapy group and 3.22 in the Triple therapy group. The mean HAS-BLED scores were 2.01 in the ticagrelor therapy group and 1.97 in the triple therapy group. The mean INR at randomization was 2.08 in the ticagrelor therapy group and 2.13 in the Triple therapy group, respectively. Baseline procedural characteristics were similar between the two groups, including PCI-related vessels and periprocedural treatments, are shown in **Table 2**.

Bleeding Events

After a 6-month follow-up, 54 patients (36.49%) in the ticagrelor therapy group and 52 patients (35.62%) in the Triple group experienced bleeding events. The time-to-first bleeding event analysis revealed comparable bleeding rates between the 2 groups [hazard ratio (HR), 0.930; 95% CI, 0.635 to 1.361; $P = 0.7088$] (**Figure 2A**). Similarly, 7 (4.73%) patients in the ticagrelor therapy group and 2 (1.37%) patients in the Triple group experienced major bleeding events (HR, 0.273; 95% CI, 0.057 to 1.315; $P = 0.1056$) according to the TIMI bleeding classifications (**Figure 2B**).

The majority of bleeding events in the trial were minor bleeding events mainly concentrated in the nose (27.78 vs. 21.15%) and mouth (29.63 vs. 32.69%) in the ticagrelor therapy and triple therapy groups (**Table 3**). We observed comparable distributions and proportions of bleeding events between the 2 groups. The occurrence of multiple bleeding events especially twice bleeding seems more frequently in the Triple therapy group (36.54%) and it was 20.37% in the ticagrelor therapy group, but showed no significant difference between the groups.

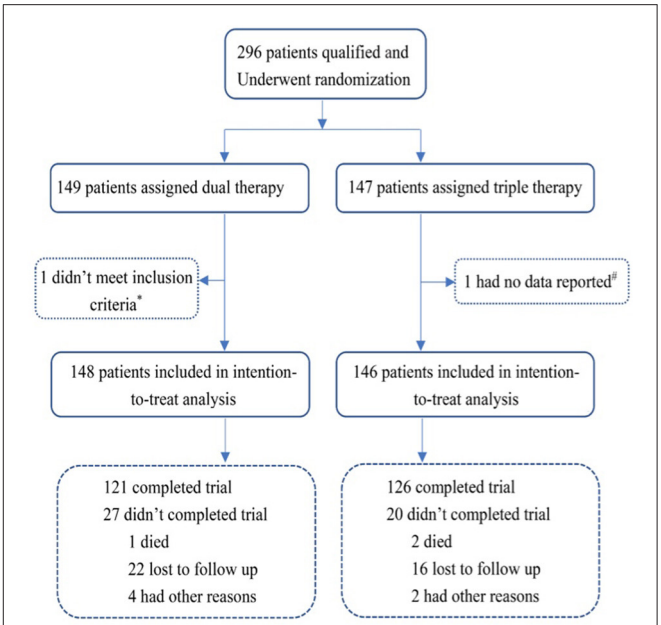

FIGURE 1 | Randomization and follow-up. *One patient in the ticagrelor therapy group did not meet the inclusion criteria immediately after randomization; #one patient in the Triple therapy group had no data reported. Dual therapy: ticagrelor therapy group; Triple therapy: clopidogrel plus aspirin group.

According to the data analysis and patients lost to follow-up or other reasons, we obtained 247 patients for the per protocol set (PPS). In the set, 48 of the 121 patients (39.67%) in the ticagrelor therapy group experienced total bleeding events compared with 46 of the 126 (36.51%) patients in the Triple group, representing comparable bleeding rates between the 2 groups (HR, 0.889; 95% CI, 0.593 to 1.332; $P = 0.5684$) (**Figure 3A**; **Supplementary Table 2**). Considering the secondary endpoints in the PPS, 6 (4.96%) patients in the ticagrelor therapy group experienced major bleeding events, whereas 2 (1.59%) patients in the Triple therapy group experienced major bleeding events. Time-to-event analysis showed no significant difference (HR, 0.313; 95% CI, 0.063 to 1.552; $P = 0.1550$) (**Figure 3A**; **Supplementary Table 2**) between the 2 groups.

Ischemic Events

Cardiovascular events, including re-PCI, surgical bypass, MI, heart failure, rehospitalization due to angina pectoris, stent thrombosis and death due to cardiovascular causes, at the end of the 6-month follow-up are shown in **Figure 4**; **Table 4**. We reported cardiovascular events in 27 (18.24%) patients in the

TABLE 1 | Characteristics of the patients at baseline.

Characteristics	Ticagrelor/dual group ($n = 148$)	Triple group ($n = 146$)	*P*-value
Age (years, mean ± SD)	69.33 ± 8.25	69.45 ± 7.65	0.8909
Sex (male), N	97 (65.54%)	91 (62.33%)	0.6274
BMI (kg/m^2, mean ± SD)	25.53±2.99	25.85±4.33	0.4608
Smoke, *N*	41 (27.70%)	27 (18.49%)	0.0611
Comorbidities			
Diabetes	52 (35.14%)	41 (28.08%)	0.1935
Hypertension	111 (75.00%)	105 (71.92%)	0.5981
Hypercholesterolemia	4 (2.70%)	5 (3.42%)	0.7488
History of stroke/TIA	40 (27.03%)	30 (20.55%)	0.1922
NYHA(III-IV)	76 (51.35%)	68 (46.58%)	0.4127
Peripheral vascular diseases	4 (2.70%)	7 (4.79%)	0.3447
Medication			
β-blocker	122 (82.43%)	113 (77.40%)	0.3099
Statin	132 (89.19%)	138 (94.52%)	0.1348
ACEI/ARB	93 (62.84%)	88 (60.27%)	0.7193
PPI	58 (39.19%)	61 (41.78%)	0.7216
Nitrates	70 (47.30%)	65 (44.52%)	0.6417
INR at randomization	2.08 ± 0.84	2.13 ± 0.60	0.5698
CHA_2DS_2-VASc score	3.41 ± 1.56	3.22 ± 1.48	0.4364
HASBLED score	2.01 ± 1.09	1.97 ± 1.14	0.7104

Data are expressed as the mean ± standard deviation and n (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; INR, International Normalized Ratio; NYHA, New York Heart Association classification grading of cardiac function; PPI, proton pump inhibitor; TIA, transient ischemic attack.

ticagrelor therapy group, whereas 24 (16.44%) patients in the Triple group experienced these events (HR, 0.845; 95% CI, 0.488 to 1.465; $P = 0.5484$).

Furthermore, at the end of the 6-month follow-up in the PPS, 22 (18.18%) patients in the ticagrelor therapy group experienced cardiovascular events compared to 20 events (15.87%) in the Triple group. Time-to-event analysis showed no significant difference (HR, 0.844; 95% CI, 0.461 to 1.547; $P = 0.5836$) (**Figure 5**; **Supplementary Table 3**) between the 2 groups.

DISCUSSION

There are many issues surrounding antithrombotic treatment of patients with AF undergoing PCI that remain unresolved. Whether ticagrelor as a part of dual therapy would exhibit favorable safety and efficacy is unknown. Some meta-analysis suggested that the use of ticagrelor as part of dual or triple antithrombotic therapy is associated with significantly higher rates of clinically relevant hemorrhagic complications compared with clopidogrel (27).

What's different is that our trial primarily confirmed the safety of ticagrelor as a dual therapy regimen didn't increasing bleeding rates, especially total bleeding rates. We further found that most of the total TIMI bleeding events were minimal and minor events. This result indicates that ticagrelor, even with its potent antithrombotic effect, did not increase the risk of fatal bleeding after 6 months as dual therapy when combined with warfarin and administered to AF patients after they underwent PCI-DES.

2019 AHA/ACC/HRS guidelines suggested that in patients with AF at increased risk of stroke (based on CHA2 DS2 -VASc risk score of 2 or greater) who have undergone PCI with stenting for ACS, double therapy with a P2Y12 inhibitor (clopidogrel or ticagrelor) and dose-adjusted vitamin K antagonist is reasonable to reduce the risk of bleeding as compared with triple therapy (3). Our results provide further evidence support and supplement for the clinical application of dual therapy with different P2Y12 inhibitor. On the other hand our results are consistent with and support the trends of using dual drug regimen in patients with atrial fibrillation and PCI-DES, as recommended by recent guidelines (12, 28, 29). In the "North American Perspective 2016–2021 Update" regarding antithrombotic treatment for these patients, triple therapy was only recommended during the peri-PCI period and dual therapy as soon as possible after hospital discharge (30–32). In other words, our study suggests a possibility that a slightly stronger dual antithrombotic with ticagrelor therapy might be used immediately after PCI in AF patients, instead of 1 week to 1 month of triple antithrombotic therapy.

The MANJUSRI trial showed that, among AF patients who had undergone PCI-DES, the anticoagulation regimen warfarin plus ticagrelor resulted in comparable total bleeding events compared with Triple therapy. The difference in risk between the ticagrelor therapy group and the Triple therapy group was 6.8% (0.87 percentage points) over ∼6 months of treatment. Compared to previously published trials, bleeding rates (33, 34) in our trial appeared to be amplified. This might be attributed to several factors, firstly, patients in triple group received a

TABLE 2 | Baseline Procedural Characteristics.

Characteristics	Dual therapy group ($n = 148$)	Triple group ($n = 146$)
ACS patients	36 (24.33%)	28 (19.18%)
Radial artery	145 (97.97%)	142 (97.26%)
Number of drug-eluting stents		
1	106 (71.62%)	98 (67.12%)
2	34 (22.97%)	37 (25.34%)
≥3	8 (5.41%)	11 (7.53%)
PCI vessel		
LM	1 (0.61%)	9 (5.33%)
LAD	95 (57.93%)	90 (53.25%)
LCX	34 (20.73%)	34 (20.12%)
RCA	34 (20.73%)	36 (21.30%)
Periprocedural treatment		
Continuation of OAC	112 (29.79%)	111 (31.09%)
Unfractionated heparin	140 (37.23%)	129 (36.13%)
Bridging with LMWH	72 (19.15%)	79 (22.12%)
GPIIbIIIa	52 (13.83%)	38 (10.64%)

Data are expressed as n (%). ACS, acute coronary syndrome; GPIIbIIIa, glycoprotein IIbIIIa receptor blocker; LAD, left anterior descending artery; LCX, left circumflex artery; LM, left main coronary artery; LMWH, low-molecular-weight heparin; OAC, oral anticoagulation; PCI, percutaneous coronary intervention; RCA, right coronary artery.

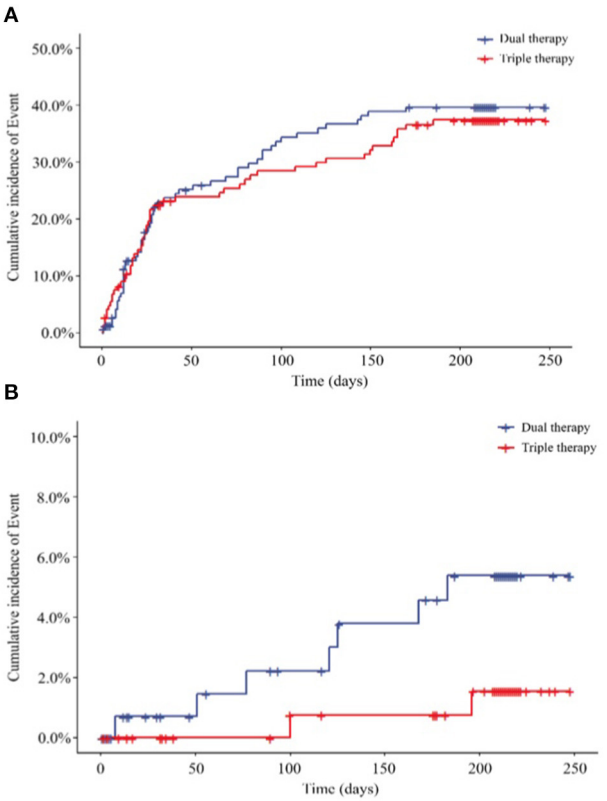

FIGURE 2 | Endpoints, **(A)** Primary outcome for the incidence of total bleeding (HR 0.930; 95% CI, 0.635 to 1.361; $P = 0.7088$); **(B)** secondary outcome for the incidence of major bleeding (HR, 0.273; 95% CI, 0.057 to 1.315; $P = 0.1056$) HR, hazard ratio. Dual therapy: ticagrelor therapy group; triple therapy: clopidogrel plus aspirin group.

relatively longer time of Antithrombotic therapy, then nearly one-third of the patients in our study used glycoprotein receptor antagonists during the perioperative period and Glycoprotein IIbIIIa receptor antagonists seemed to be used more frequently in the trial of ticagrelor group.

The rates of major TIMI bleeding were higher in the ticagrelor therapy group than in the Triple group, but the difference was not statistically significant. The difference in risk between the groups was 27.5% (3.36 percentage points), however in our trial, we must consider the particularity of certain cases themselves. In the ticagrelor therapy group, only 1 of 7 major bleeding patients suffered from intracranial bleeding, and the others all experienced gastrointestinal bleeding. However, it is important to note that 2 of the patients who experienced major bleeding were at high risk at the time of enrollment. One patient had a HASBLED score ≥3, and the other patient had an INR of 4.43. Another 3 patients' specific situations were similar to those of the 2 patients in the Triple group. In conclusion, these findings suggest a relatively balanced risk of bleeding in the prevention of thromboembolism, at least to some extent, providing clinicians with one more regimen option when considering a AF patient's risk of bleeding and the risk of thromboembolic events after PCI-DES.

The strategies for dual therapy with ticagrelor that we tested incorporated two changes relative to the previous trials of antithrombotic therapy. The first change is the incorporation of ticagrelor as part of the dual therapy regimen for all patients with AF just after PCI-DES. The proportion of patients taking ticagrelor in our trial (every patient in the dual therapy group took oral ticagrelor, so the usage rate was 100%) was greater to that in the few other trials that have investigated ticagrelor as part of the regimens in patients with atrial fibrillation and PCI-DES (3–5.5% in the PIONEER trial; 7–8% in ENTRUST trial) (15, 16). The second change refers to the use of warfarin as the basic anticoagulant for the two groups of patients, instead of NOVC. This change was mainly based on economic reasons given that most Chinese patients prefer warfarin due to its relatively lower price, and at the time of enrollment, more than 90% of the patients were taking warfarin once daily as antithrombotic therapy for AF (35, 36); Secondly, access to NOACs in China was possible only after 2016.

There several limitations in the trial though we are the first trial that systematically evaluated the safety of ticagrelor as part of a dual antithrombotic regimen. First, the primary analyses showed that the safety of ticagrelor was similar to that of Triple therapy, and the result just reached a point of non-inferiority, rather than superiority, as anticipated. This outcome might be related to the potent antiplatelet effect of ticagrelor as discussed (37), and longer duration of antithrombotic administration as well as more additional antithrombotic administration during perioperative period. Secondly, We did not compare the risk

TABLE 3 | Bleeding Times and Specific Location of Bleeding in 2 Groups of Patients; "Others" were cases in which the bleeding site could not be identified, but were identified as bleeding by the research team due to Hb decrease.

Bleeding specific	Dual therapy group (n = 148) primary events (n = 54)	Triple therapy group (n = 146) Primary events (n = 52)
Bleeding times		
1 time	37 (68.52%)	30 (57.69%)
2 times	11 (20.37%)	19 (36.54%)
≥3 times	6 (11.11%)	3 (5.77%)
Bleeding location		
Intracranial	1 (1.85%)	0 (0%)
Eye	1 (1.85%)	2 (3.85%)
Mouth	16 (29.63%)	17 (32.69%)
Gastrointestinal	4 (7.41%)	2 (3.85%)
Nose	15 (27.78%)	11 (21.15%)
Respiratory tract	3 (5.56%)	2 (3.85%)
Urinary system	0 (0%)	2 (3.85%)
Skin hematoma	9 (16.67%)	9 (15.38%)
Other	5 (9.26%)	8 (15.38%)

Data are expressed as n (%).

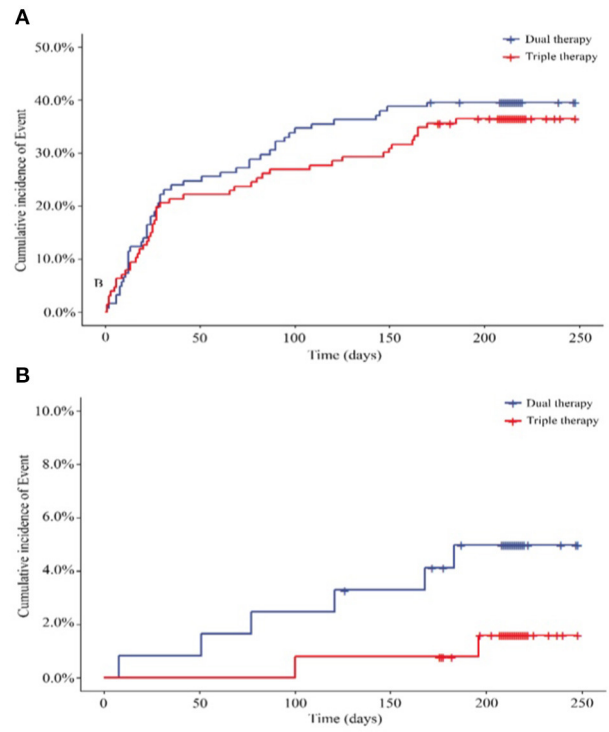

FIGURE 3 | (A) Primary outcome for the incidence of total bleeding in PPS (N = 247) (HR, 0.889; 95% CI, 0.593 to 1.332; P = 0.5684); **(B)** Secondary outcome for the incidence of major bleeding (HR, 0.313; 95% CI, 0.063 to 1.552; P = 0.1550). HR, hazard ratio. Dual therapy: ticagrelor therapy group; triple therapy: clopidogrel plus aspirin group.

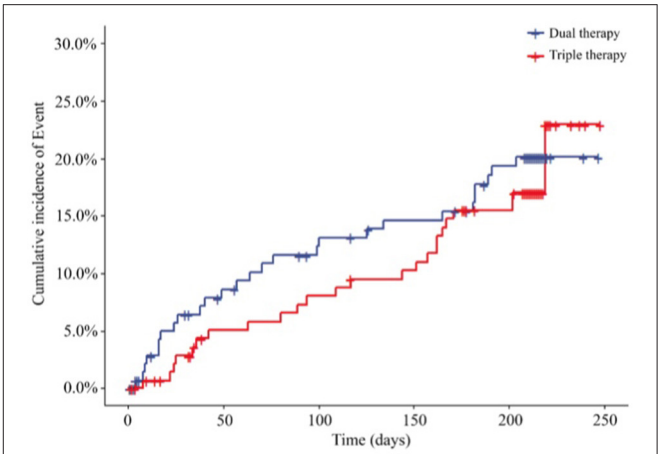

FIGURE 4 | Incidence of cardiovascular event outcomes of the 2 groups (HR, 0.845; 95% CI, 0.488 to 1.465; P = 0.5484). HR, hazard ratio. Dual therapy: ticagrelor therapy group; triple therapy: clopidogrel plus aspirin group.

of bleeding between the two groups at 1 week and 1 month, because current guidelines only recommend the shorter-term triple antithrombotic therapy (6, 28, 29, 38). Thirdly, the rates of primary endpoints of bleeding in this study were higher than those in the previous trials on this topic; in addition to over verified minor bleeding as discussed above, we suspected that to some extent this outcome might be related to increased variability in INR during the follow-up process and Asians being prone to bleeding when treated with warfarin (39, 40). In the end, the number of patients in our trial was relatively small for various reasons, including research funding, thus restricting the power of the study to detect worthwhile differences between the two groups. In addition, the progress of the trial was relatively slow due to physicians' and patients' concerns about various issues, such as safety in subcenters during the first year. However, though the trial did take us almost 5 years to complete, the patients were not highly selected, all of the data in the trial met the random requirements and the inclusion and exclusion criteria, and the two groups of patients were relatively homogenous.

CONCLUSION

Patients with AF undergoing PCI-DES treated with ticagrelor and warfarin in this trial experienced an incidence of total bleeding events that was comparable to those receiving a traditional triple antithrombotic regimen consisting of clopidogrel, aspirin, and warfarin. In this study we found that a dual antithrombotic therapy with ticagrelor is associated with similar incidence of bleeding and ischemic events as compared to a triple therapy with clopidogrel among AF patients undergoing PCI, however, we did not demonstrated dual therapy using ticagrelor reduced bleeding events compared to triple therapy. Further studies are

TABLE 4 | Specific description of cardiovascular events in 2 groups of patients.

Cardiovascular events	Dual therapy group ($n = 148$) Cardiovascular events ($n = 27$)	Triple therapy group ($n = 146$) Cardiovascular events ($n = 24$)
Re-PCI	3 (11.11%)	2 (8.33%)
Surgical bypass	0 (0.0%)	0 (0.0%)
Myocardial infarction	3 (11.11%)	2 (8.33%)
Heart failure	3 (11.11%)	4 (16.67%)
Rehospitalization due to angina pectoris	15 (55.55%)	15 (62.55%)
Stent thrombosis	0 (0.0%)	0 (0.0%)
Death due to cardiovascular causes	1 (3.70%)	1 (4.67%)
Others	2 (7.40%)	2 (8.33%)

"Others" referred patients who were reported symptoms of chest tightness or chest pain or heart failure but refused to go to the hospital for further determination and were considered by the study team to be compatible with cardiovascular events. Data are expressed as n (%). PCI, percutaneous coronary intervention.

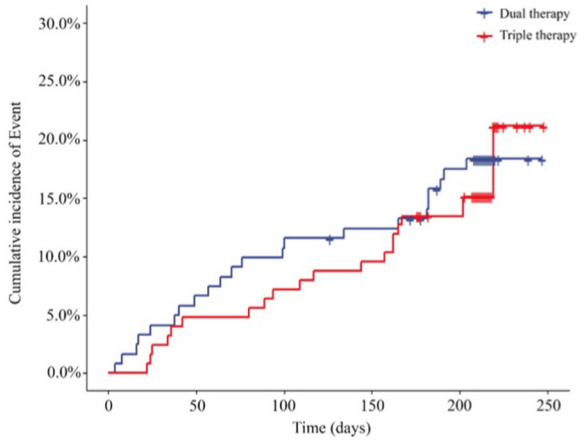

FIGURE 5 | Incidence of cardiovascular event outcomes of the two groups (PPS, $N = 247$) (HR, 0.844; 95% CI, 0.461 to 1.547; $P = 0.5836$). HR, hazard ratio. Dual therapy: ticagrelor therapy group; triple therapy: clopidogrel plus aspirin group.

warranted to shed light on the potential benefit of implementing a dual antithrombotic therapy with potent P2Y12 inhibitor in this setting.

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 ICE for Clinical research of Zhongda Hospital Affiliated to Southeast University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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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.2021.745549/full#supplementary-material

REFERENCES

1. Mishra A, Singh M, Acker WW, Kamboj S, Sporn D, Stapleton D, et al. Antithrombotic therapy in patients with atrial fibrillation and coronary artery disease undergoing percutaneous coronary intervention. *J Cardiovasc Pharmacol.* (2019) 74:82–90. doi: 10.1097/FJC.0000000000000697
2. European Heart Rhythm Association, European Association for Cardio-Thoracic Surgery, Camm AJ, Kirchhof P, Lip GY, Schotten U, et al. Guidelines for the management of atrial fibrillation: the task force for the management of atrial fibrillation of the European society of cardiology (ESC). *Eur Heart J.* (2010) 31:2369–429. doi: 10.1093/eurheartj/ehq278
3. January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JC Jr, et al. 2019 AHA/ACC/HRS focused update of the 2014 AHA/ACC/HRS

guideline for the management of patients with atrial fibrillation: a report of the American college of cardiology/American heart association task force on clinical practice guidelines and the heart rhythm society in collaboration with the society of thoracic surgeons. *Circulation.* (2019) 140:e125–51. doi: 10.1161/CIR.0000000000000665

4. Haller PM, Sulzgruber P, Kaufmann C, Geelhoed B, Tamargo J, Wassmann S, et al. Bleeding and ischaemic outcomes in patients treated with dual or triple antithrombotic therapy: systematic review and meta-analysis. *Eur Heart J Cardiovasc Pharmacother.* (2019) 5:226–36. doi: 10.1093/ehjcvp/pvz021
5. Gargiulo G, Goette A, Tijssen J, Eckardt L, Lewalter T, Vranckx P, et al. Safety and efficacy outcomes of double vs. Triple antithrombotic therapy in patients with atrial fibrillation following percutaneous coronary intervention: a systematic review and meta-analysis of non-vitamin K antagonist oral anticoagulant-based randomized clinical trials. *Eur Heart J.* (2019) 40:3757–67. doi: 10.1093/eurheartj/ehz732
6. Khan SU, Osman M, Khan MU, Khan MS, Zhao D, Mamas MA, et al. Dual versus triple therapy for atrial fibrillation after percutaneous coronary intervention: a systematic review and meta-analysis. *Ann Intern Med.* (2020) 172:474–83. doi: 10.7326/M19-3763
7. Galli M, Andreotti F, D'Amario D, Vergallo R, Montone RA, Porto I, et al. Dual therapy with direct oral anticoagulants significantly increases the risk of stent thrombosis compared to triple therapy. *Eur Heart J Cardiovasc Pharmacother.* (2020) 6:128–9. doi: 10.1093/ehjcvp/pvz030
8. Galli M, Andreotti F, Porto I, Crea F. Intracranial haemorrhages vs. Stent thromboses with direct oral anticoagulant plus single antiplatelet agent or triple antithrombotic therapy: a meta-analysis of randomized trials in atrial fibrillation and percutaneous coronary intervention/acute coronary syndrome patients. *Europace.* (2020) 22:538–46. doi: 10.1093/europace/euz345
9. Galli M, Benenati S, Capodanno D, Franchi F, Rollini F, D'Amario D, et al. Guided versus standard antiplatelet therapy in patients undergoing percutaneous coronary intervention: a systematic review and meta-analysis. *Lancet.* (2021) 397:1470–83. doi: 10.1016/S0140-6736(21)00533-X
10. Galli M, Franchi F, Rollini F, Cavallari LH, Capodanno D, Crea F, et al. Genetic testing in patients undergoing percutaneous coronary intervention: rationale, evidence and practical recommendations. *Expert Rev Clin Pharmacol.* (2021) 14:963–78. doi: 10.1080/17512433.2021.1927709
11. Eyileten C, Postula M, Jakubik D, Toma A, Mirowska-Guzel D, Patti G, et al. Non-Vitamin K Oral anticoagulants (NOAC) versus vitamin k antagonists (VKA) for atrial fibrillation with elective or urgent percutaneous coronary intervention: a meta-analysis with a particular focus on combination type. *J Clin Med.* (2020) 9:1120. doi: 10.3390/jcm9041120
12. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J.* (2021) 42:373–498. doi: 10.1093/eurheartj/ehaa612
13. Steffel J, Verhamme P, Potpara TS, Albaladejo P, Antz M, Desteghe L, et al. The 2018 European heart rhythm association practical guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. *Eur Heart J.* (2018) 39:1330–93. doi: 10.1093/eurheartj/ehy136
14. Lip GYH, Collet JP, Haude M, Byrne R, Chung EH, Fauchier L, et al. 2018 Joint European consensus document on the management of antithrombotic therapy in atrial fibrillation patients presenting with acute coronary syndrome and/or undergoing percutaneous cardiovascular interventions: a joint consensus document of the European Heart Rhythm Association (EHRA), European Society of Cardiology Working Group on Thrombosis, European Association of Percutaneous Cardiovascular Interventions (EAPCI), and European Association of Acute Cardiac Care (ACCA) endorsed by the Heart Rhythm Society (HRS), Asia-Pacific Heart Rhythm Society (APHRS), Latin America Heart Rhythm Society (LAHRS), and Cardiac Arrhythmia Society of Southern Africa (CASSA). *Europace.* (2019) 21:192–3. doi: 10.1093/europace/euy174
15. Mehran R, Baber U, Sharma SK, Cohen DJ, Angiolillo DJ, Briguori C, et al. Ticagrelor with or without Aspirin in high-risk patients after PCI. *N Engl J Med.* (2019) 381:2032–42. doi: 10.1056/NEJMoa1908419
16. Baber U, Zafar MU, Dangas G, Escolar G, Angiolillo DJ, Sharma SK, et al. Ticagrelor with or without aspirin after PCI: the TWILIGHT platelet substudy. *J Am Coll Cardiol.* (2020) 75:578–86. doi: 10.1016/j.jacc.2019.11.056
17. Lopes RD, Vora AN, Liaw D, Granger CB, Darius H, Goodman SG, et al. An open-Label, 2 × 2 factorial, randomized controlled trial to evaluate the safety of apixaban vs. Vitamin K antagonist and aspirin vs. placebo in patients with atrial fibrillation and acute coronary syndrome and/or percutaneous coronary intervention: Rationale and design of the AUGUSTUS trial. *Am Heart J.* (2018) 200:17–23. doi: 10.1016/j.ahj.2018.03.001
18. Jones DW, Minhas S, Fierro JJ, Ardeshna D, Rawal A, Cave B, et al. From WOEST to AUGUSTUS: a review of safety and efficacy of triple versus dual antithrombotic regimens in patients with atrial fibrillation requiring percutaneous coronary intervention for acute coronary syndrome. *Ann Transl Med.* (2019) 7:405. doi: 10.21037/atm.2019.08.21
19. Gibson CM, Mehran R, Bode C, Halperin J, Verheugt FW, Wildgoose P, et al. Prevention of bleeding in patients with atrial fibrillation undergoing PCI. *N Engl J Med.* (2016) 375:2423–34. doi: 10.1056/NEJMoa1611594
20. Vranckx P, Valgimigli M, Eckardt L, Tijssen J, Lewalter T, Gargiulo G, et al. Edoxaban-based versus vitamin K antagonist-based antithrombotic regimen after successful coronary stenting in patients with atrial fibrillation (ENTRUST-AF PCI): a randomised, open-label, phase 3b trial. *Lancet.* (2019) 394:1335–43. doi: 10.1016/S0140-6736(19)31872-0
21. Cannon CP, Bhatt DL, Oldgren J, Lip GYH, Ellis SG, Kimura T, et al. RE-DUAL PCI steering committee and investigators. Dual antithrombotic therapy with dabigatran after PCI in atrial fibrillation. *N Engl J Med.* (2017) 377:1513–24. doi: 10.1056/NEJMoa1708454
22. Lu W, Chen L, Wang Y, Yao Y, Fu C, Zuo P, et al. Rationale and design of MANJUSRI trial: a randomized, open-label, active-controlled multicenter study to evaluate the safety of combined therapy with ticagrelor and warfarin in AF subjects after PCI-DES. *Contemp Clin Trials.* (2015) 40:166–71. doi: 10.1016/j.cct.2014.12.002
23. Mehran R, Rao SV, Bhatt DL, Gibson CM, Caixeta A, Eikelboom J, et al. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding academic research consortium. *Circulation.* (2011) 123:2736–47. doi: 10.1161/CIRCULATIONAHA.110.009449
24. Rao SV, O'Grady K, Pieper KS, Granger CB, Newby LK, Van de Werf F, et al. Impact of bleeding severity on clinical outcomes among patients with acute coronary syndromes. *Am J Cardiol.* (2005) 96:1200–6. doi: 10.1016/j.amjcard.2005.06.056
25. Bovill EG, Terrin ML, Stump DC, Berke AD, Frederick M, Collen D, et al. Hemorrhagic events during therapy with recombinant tissue-type plasminogen activator, heparin, and aspirin for acute myocardial infarction. Results of the thrombolysis in myocardial infarction (TIMI), Phase II trial. *Ann Intern Med.* (1991) 115:256–65. doi: 10.7326/0003-4819-115-4-256
26. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al. TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* (2007) 357:2001–15. doi: 10.1056/NEJMoa0706482
27. Andreou I, Briasoulis A, Pappas C, Ikonomidis I, Alexopoulos D. Ticagrelor versus clopidogrel as part of dual or triple antithrombotic therapy: a systematic review and meta-analysis. *Cardiovasc Drugs Ther.* (2018) 32:287–94. doi: 10.1007/s10557-018-6795-9
28. Valgimigli M, Bueno H, Byrne RA, Collet JP, Costa F, Jeppsson A, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: the task force for dual antiplatelet therapy in coronary artery disease of the European society of cardiology (ESC) and of the European association for cardio-thoracic surgery (EACTS). *Eur Heart J.* (2018) 39:213–60. doi: 10.1093/eurheartj/ehx419
29. Núñez-Gil IJ, Riha H, Ramakrishna H. Review of the 2017 European society of cardiology's guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation and focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with the European association for cardio-thoracic surgery. *J Cardiothorac Vasc Anesth.* (2019) 33:2334–43. doi: 10.1053/j.jvca.2018.09.032
30. Angiolillo DJ, Bhatt DL, Cannon CP, Eikelboom JW, Gibson CM, Goodman SG, et al. Antithrombotic therapy in patients with atrial fibrillation treated with oral anticoagulation undergoing percutaneous coronary intervention:

a north American perspective: 2021 update. *Circulation.* (2021) 143:583–96. doi: 10.1161/CIRCULATIONAHA.120.050438
31. Angiolillo DJ, Goodman SG, Bhatt DL, Eikelboom JW, Price MJ, Moliterno DJ, et al. Antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention: a north american perspective-2016 update. *Circ Cardiovasc Interv.* (2016) 9:e004395. doi: 10.1161/CIRCINTERVENTIONS.116.004395
32. Angiolillo DJ, Goodman SG, Bhatt DL, Eikelboom JW, Price MJ, Moliterno DJ, et al. Antithrombotic therapy in patients with atrial fibrillation treated with oral anticoagulation undergoing percutaneous coronary intervention: a north American perspective-2018 update. *Circulation.* (2018) 138:527–36. doi: 10.1161/CIRCULATIONAHA.118.034722
33. Lopes RD, Heizer G, Aronson R, Vora AN, Massaro T, Mehran R, et al. AUGUSTUS investigators. Antithrombotic therapy after acute coronary syndrome or PCI in atrial fibrillation. *N Engl J Med.* (2019) 380:1509–24. doi: 10.1056/NEJMoa1817083
34. Moustafa A, Khan MS, Marei A, Alsamman MA, Baig M, Saad M. Safety and efficacy of dual versus triple antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention: a meta-analysis. *Avicenna J Med.* (2020) 10:232–40. doi: 10.4103/ajm.ajm_40_20
35. Wang Z, Tang Z, Zhu W, Ge L, Ge J. Efficacy and safety of traditional Chinese medicine on thromboembolic events in patients with atrial fibrillation: a systematic review and meta-analysis. *Complement Ther Med.* (2017) 32:1–10. doi: 10.1016/j.ctim.2017.03.006
36. Li J, Wang L, Hu J, Xu G. Warfarin use and the risks of stroke and bleeding in hemodialysis patients with atrial fibrillation: a systematic review and a meta-analysis. *Nutr Metab Cardiovasc Dis.* (2015) 25:706–13. doi: 10.1016/j.numecd.2015.03.013
37. Navarese EP, Khan SU, Kołodziejczak M, Kubica J, Buccheri S, Cannon CP, et al. Comparative efficacy and safety of oral $P2Y_{12}$ inhibitors in acute coronary syndrome: network meta-analysis of 52 816 patients from 12 randomized trials. *Circulation.* (2020) 142:150–60. doi: 10.1161/CIRCULATIONAHA.120.046786
38. Kuno T, Ueyama H, Takagi H, Ando T, Numasawa Y, Briasoulis A, et al. Meta-analysis of antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention. *Am J Cardiol.* (2020) 125:521–7. doi: 10.1016/j.amjcard.2019.11.022
39. Chiang CE, Wang KL, Lip GY. Stroke prevention in atrial fibrillation: an Asian perspective. *Thromb Haemost.* (2014) 111:789–97. doi: 10.1160/TH13-11-0948
40. Wei H, Cui C, Cui X, Liu Y, Li D. Cost-effectiveness analysis of dabigatran, rivaroxaban and warfarin in the prevention of stroke in patients with atrial fibrillation in China. *BMC Health Serv Res.* (2021) 21:96. doi: 10.1186/s12913-021-06084-1

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Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors vs. Dipeptidyl Peptidase-4 (DPP4) Inhibitors for New-Onset Dementia: A Propensity Score-Matched Population-Based Study With Competing Risk Analysis

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Introduction: The effects of sodium-glucose cotransporter 2 inhibitors (SGLT2I) and dipeptidyl peptidase-4 inhibitors (DPP4I) on new-onset cognitive dysfunction in type 2 diabetes mellitus remain unknown. This study aimed to evaluate the effects of the two novel antidiabetic agents on cognitive dysfunction by comparing the rates of dementia between SGLT2I and DPP4I users.

Methods: This was a population-based cohort study of type 2 diabetes mellitus patients treated with SGLT2I and DPP4I between January 1, 2015 and December 31, 2019 in Hong Kong. Exclusion criteria were < 1-month exposure or exposure to both medication classes, or prior diagnosis of dementia or major neurological/psychiatric diseases. Primary outcomes were new-onset dementia, Alzheimer's, and Parkinson's. Secondary outcomes were all-cause, cardiovascular, and cerebrovascular mortality.

Results: A total of 13,276 SGLT2I and 36,544 DPP4I users (total $n = 51,460$; median age: 66.3 years old [interquartile range (IQR): 58–76], 55.65% men) were studied (follow-up: 472 [120–792] days). After 1:2 matching (SGLT2I: $n = 13,283$; DPP4I: $n = 26,545$), SGLT2I users had lower incidences of dementia (0.19 vs. 0.78%, $p < 0.0001$), Alzheimer's (0.01 vs. 0.1%, $p = 0.0047$), Parkinson's disease (0.02 vs. 0.14%, $p = 0.0006$), all-cause (5.48 vs. 12.69%, $p < 0.0001$), cerebrovascular (0.88 vs. 3.88%, $p < 0.0001$), and cardiovascular mortality (0.49 vs. 3.75%, $p < 0.0001$). Cox regression showed that SGLT2I use was associated with lower risks of dementia (hazard ratio [HR]: 0.41, 95% confidence interval [CI]: [0.27–0.61], $P < 0.0001$), Parkinson's (HR:0.28, 95% CI: [0.09–0.91], $P = 0.0349$), all-cause (HR:0.84, 95% CI:

[0.77–0.91], $P < 0.0001$), cardiovascular (HR:0.64, 95% CI: [0.49–0.85], $P = 0.0017$), and cerebrovascular (HR:0.36, 95% CI: [0.3–0.43], $P < 0.0001$) mortality.

Conclusions: The use of SGLT2I is associated with lower risks of dementia, Parkinson's disease, and cerebrovascular mortality compared with DPP4I use after 1:2 ratio propensity score matching.

Keywords: SGLT2, SGLT2 (sodium-glucose cotransporter 2) inhibitor, DPP4, DPP4 inhibitor, dementia, cognitive dysfunction, Alzheimer's disease, Parkinson's disease

INTRODUCTION

Type-2 diabetes mellitus is a complex multi-systemic disorder with wide-ranging complications affecting the retinal, cardiovascular, renal, and peripheral nervous systems (1–5). Increasingly, cognitive dysfunction is being recognized as a clinically important complication of type-2 diabetes (6). Diabetic patients are associated with a 1.5-fold increased risk of cognitive dysfunction, 1.9-fold increased risk of dementia, and 2.2-fold increased risk of stroke (7–9). While the underlying pathophysiology is still unclear, several mechanisms have been proposed including insulin resistance, hypoglycemia, hyperglycemia-induced cerebral microvascular and macrovascular dysfunction, as well as amyloid deposition (10, 11). It is highly likely that the cognitive dysfunction is multifactorial and caused by a combination of these mechanisms specific to the demographic and comorbidities of the patient.

Several studies have suggested that improved glycemic control, reduced HbA1c levels, and use of anti-diabetic medication are associated with a reduced risk of cognitive dysfunction (12–15). This has consequently raised the prospect of anti-diabetic agents reducing cognitive dysfunction in type 2 diabetes patients. Of interest are novel second-line anti-diabetic agents including sodium-glucose cotransporter 2 inhibitors (SGLT2I) and dipeptidyl peptidase-4 inhibitors. Multiple preclinical studies have suggested that DPP4I and SGLT2I improve cognition in animal models *via* a variety of mechanisms (16–20). However, few clinical studies have explored SGLT2I and DPP4I in their effects on cognitive dysfunction in diabetic patients. A randomized controlled trial in 2018 found no cognitive decline in SGLT2I and DPP4I users within 12 months while a case-control study in 2019 found that DPP4I and SGLT2I use are associated with a lower risk of dementia compared with other anti-diabetic agents (21, 22). Until recent times, no study has directly compared the risk of cognitive dysfunction and major neurocognitive disorders among SGLT2I and DPP4I users.

Therefore, the present study aimed to compare the incidence of dementia in SGLT2 users against DPP4I users in a Chinese population to evaluate the effects of the two novel antidiabetic agents on cognitive dysfunction.

METHODS

Study Design and Population

This was a retrospective, territory-wide cohort study of type-2 diabetes mellitus patients with SGLT2I/DPP4I use between January 1, 2015, and December 31, 2019 in Hong Kong (**Figure 1**). Patients during the aforementioned period were enrolled and followed up until December 31, 2019, or until death. Patients with <1 month SGLT2I/DPP4I exposure ($N = 3,225$), with both SGLT2I and DPP4I therapy ($N = 15,276$), or with a prior diagnosis of all-cause dementia, Alzheimer's disease, dementia with Lewy bodies, vascular dementia, frontotemporal dementia, or other major neurological/psychiatric diseases ($N = 2,785$) were excluded.

The patients were identified from the Clinical Data Analysis and Reporting System (CDARS), a city-wide database that centralizes patient information from individual local hospitals to establish comprehensive medical data, including clinical characteristics, disease diagnosis, laboratory results, and drug treatment details. The system has been previously used by both our team and other teams in Hong Kong (23–25). Clinical and biochemical data were extracted for the present study. The demographics of the patients include gender and age of initial drug use (baseline). Prior comorbidities were extracted based on standard *International Classification of Diseases Ninth Edition* (ICD-9) codes (**Supplementary Table 1**). The Charlson comorbidity index and neutrophil-to-lymphocyte ratio (NLR) were calculated. Mortality was recorded using the *International Classification of Diseases Tenth Edition* (ICD-10) coding. ICD-10 codes I00-I09, I11, I13, I20-I51 were used to identify cardiovascular mortality outcomes. ICD-10 codes I60-I69 identified cerebrovascular mortality. Medication histories and baseline laboratory examinations were extracted. Mortality data were obtained from the Hong Kong Death Registry, a population-based official government registry with the registered death records of all Hong Kong citizens linked to CDARS.

Outcomes and Statistical Analysis

The primary outcomes were new-onset dementia, new-onset Alzheimer's disease, and new-onset Parkinson's disease. The secondary outcomes were all-cause mortality, cardiovascular mortality, and cerebrovascular mortality. Descriptive statistics were used to summarize baseline clinical and biochemical characteristics of patients with SGLT2I and DPP4I use. For baseline clinical characteristics, the continuous variables were presented as median (95% confidence interval [CI]/interquartile range [IQR]) and the categorical variables were presented as total number (percentage). Continuous variables were compared using the two-tailed Mann-Whitney U test, while the two-tailed Chi-square test with Yates' correction was used to test 2×2 contingency data. Propensity score matching

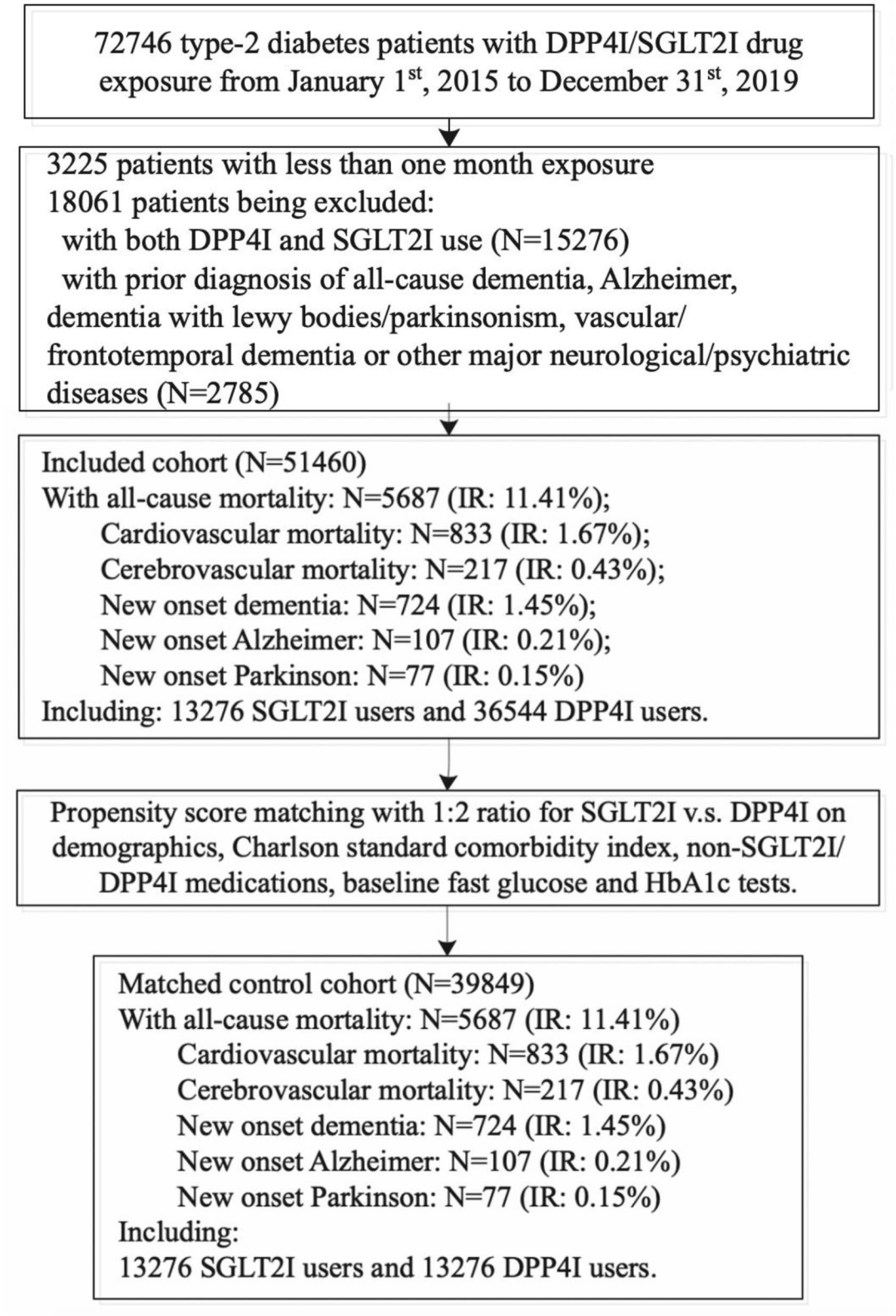

FIGURE 1 | Procedures of data processing for the study cohort.

TABLE 1 | Baseline and clinical characteristics of patients with DPP4I vs. SGLT2I uses before and after propensity score matching (1:2).

Characteristics	Before matching			*P*-value	After matching			*P*-value
	All (*N* = 39828) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13276) Median (IQR); N or Count(%)	DPP4I (users *N* = 36554) Median (IQR); N or Count(%)		All (*N* = 49830) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13283) Median (IQR); N or Count(%)	DPP4I users (*N* = 26545) Median (IQR); N or Count(%)	
Adverse events								
All-cause mortality	5,687 (11.41%)	695 (5.23%)	4,992 (13.65%)	<0.0001***	5,687 (11.41%)	729 (5.48%)	3,371 (12.69%)	<0.0001***
Cardiovascular mortality	833 (1.67%)	108 (0.81%)	725 (1.98%)	<0.0001***	833 (1.67%)	66 (0.49%)	998 (3.75%)	<0.0001***
Cerebrovascular mortality	217 (0.43%)	18 (0.13%)	199 (0.54%)	<0.0001***	217 (0.43%)	117 (0.88%)	1,030 (3.88%)	<0.0001***
New onset dementia	724 (1.45%)	72 (0.54%)	652 (1.78%)	<0.0001***	724 (1.45%)	26 (0.19%)	208 (0.78%)	<0.0001***
New onset Alzheimer's	107 (0.21%)	12 (0.09%)	95 (0.25%)	0.0005***	107 (0.21%)	2 (0.01%)	27 (0.10%)	0.0047**
New onset Parkinson's	77 (0.15%)	10 (0.07%)	67 (0.18%)	0.0099**	77 (0.15%)	3 (0.02%)	39 (0.14%)	0.0006***
Demographics								
Male gender	27,734 (55.65%)	8,229 (61.98%)	19,505 (53.35%)	<0.0001***	27,734 (55.65%)	8,194 (61.68%)	15,714 (59.19%)	0.0175*
Female gender	22,096 (44.34%)	5,047 (38.01%)	17,049 (46.64%)	<0.0001***	22,096 (44.34%)	5,089 (38.31%)	10,831 (40.80%)	0.0017**
Baseline age, year	66.27 (58.08–75.59); *n* = 49,830	61.17 (53.89–68.42); *n* = 13,276	68.38 (59.92–77.97); *n* = 36,554	<0.0001***	66.27 (58.08–75.59); *n* = 49,830	61.18 (53.9–68.22); *n* = 13,283	62.08 (54.14–69.68); *n* = 26,545	<0.0001***
<40	1,161 (2.32%)	658 (4.95%)	503 (1.37%)	<0.0001***	1,161 (2.32%)	658 (4.95%)	1,304 (4.91%)	0.8837
[40, 50]	3,480 (6.98%)	1,553 (11.69%)	1,927 (5.27%)	<0.0001***	3,480 (6.98%)	1,552 (11.68%)	3,069 (11.56%)	0.7611
[50–60]	10,637 (21.34%)	3,831 (28.85%)	6,806 (18.61%)	<0.0001***	10,637 (21.34%)	3,829 (28.82%)	6,963 (26.23%)	<0.0001***
[60–70]	15,373 (30.85%)	4,495 (33.85%)	10,878 (29.75%)	<0.0001***	15,373 (30.85%)	4,579 (34.47%)	8,787 (33.10%)	0.0559
[70–80]	10,969 (22.01%)	1,979 (14.90%)	8,990 (24.59%)	<0.0001***	10,969 (22.01%)	1,965 (14.79%)	4,984 (18.77%)	<0.0001***
≥80	8,210 (16.47%)	760 (5.72%)	7,450 (20.38%)	<0.0001***	8,210 (16.47%)	700 (5.26%)	1,438 (5.41%)	0.5759
Charlson score	2.0 (1.0–3.0); *n* = 49,830	2.0 (1.0–3.0); *n* = 13,276	3.0 (2.0–4.0); *n* = 36,554	<0.0001***	2. 0(1.0–3.0); *n* = 49,830	2.0 (1.0–3.0); *n* = 13,283	2.0 (1.0–3.0); *n* = 26,545	<0.0001***
NLR	2.39 (1.75–3.54); *n* = 19,776	2.17 (1.64–3.0); *n* = 5,560	2.5 (1.81–3.77); *n* = 14,216	<0.0001***	2.39 (1.75–3.54); *n* = 19,776	2.1 4(1.62–2.95); *n* = 5,597	2.13 (1.33–3.56); *n* = 10,176	0.9764
Past comorbidities								
Hypertension	11,993 (24.06%)	3,075 (23.16%)	8,918 (24.39%)	0.0262*	11,993 (24.06%)	3,036 (22.85%)	4,884 (18.39%)	<0.0001***
Heart failure	850 (1.70%)	208 (1.56%)	642 (1.75%)	0.167	850 (1.70%)	204 (1.53%)	253 (0.95%)	<0.0001***
Renal diseases	2,998 (6.01%)	193 (1.45%)	2,805 (7.67%)	<0.0001***	2,998 (6.01%)	178 (1.34%)	712 (2.68%)	<0.0001***
Liver diseases	351 (0.70%)	53 (0.39%)	298 (0.81%)	<0.0001***	351 (0.70%)	53 (0.39%)	114 (0.42%)	0.7193
Stroke/TIA	1,539 (3.08%)	390 (2.93%)	1,149 (3.14%)	0.2676	1,539 (3.08%)	385 (2.89%)	617 (2.32%)	0.0009***
Gastrointestinal bleeding	969 (1.94%)	204 (1.53%)	765 (2.09%)	0.0001***	969 (1.94%)	205 (1.54%)	313 (1.17%)	0.0033**
History of falls	3,405 (6.83%)	644 (4.85%)	2,761 (7.55%)	<0.0001***	3,405 (6.83%)	627 (4.72%)	1,134 (4.27%)	0.0529
Pneumonia and influenza	1,201 (2.41%)	156 (1.17%)	1,045 (2.85%)	<0.0001***	1,201 (2.41%)	143 (1.07%)	387 (1.45%)	0.0023**
Endocrine	1,047 (2.10%)	219 (1.64%)	828 (2.26%)	<0.0001***	1,047 (2.10%)	216 (1.62%)	416 (1.56%)	0.6931
Atrial fibrillation	2,139 (4.29%)	383 (2.88%)	1,756 (4.80%)	<0.0001***	2,139 (4.29%)	372 (2.80%)	1,323 (4.98%)	<0.0001***
Ischemic heart disease	5,355(10.74%)	1,811 (13.64%)	3,544 (9.69%)	<0.0001***	5,355(10.74%)	1,787 (13.45%)	2,339 (8.81%)	<0.0001***

(Continued)

TABLE 1 | Continued

Characteristics	Before matching			*P*-value	After matching			*P*-value
	All (*N* = 39828) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13276) Median (IQR); N or Count(%)	DPP4I (users *N* = 36554) Median (IQR); N or Count(%)		All (*N* = 49830) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13283) Median (IQR); N or Count(%)	DPP4I users (*N* = 26545) Median (IQR); N or Count(%)	
Peripheral vascular disease	556 (1.11%)	86 (0.64%)	470 (1.28%)	<0.0001***	556 (1.11%)	82 (0.61%)	232 (0.87%)	0.0080**
Malignancy	1,380 (2.76%)	241 (1.81%)	1,139 (3.11%)	<0.0001***	1,380 (2.76%)	238 (1.79%)	278 (1.04%)	<0.0001***
Metastatic solid tumor	399 (0.80%)	42 (0.31%)	357 (0.97%)	<0.0001***	399 (0.80%)	42 (0.31%)	73 (0.27%)	0.5345
Medications								
SGLT2I vs. DPP4I	13,276 (26.64%)	13,276 (100.00%)	0 (0.00%)	<0.0001***	13,276 (26.64%)	13,283 (100.00%)	0 (0.00%)	<0.0001***
Beta blockers	1,547 (3.10%)	1,544 (11.63%)	3 (0.00%)	<0.0001***	1,547 (3.10%)	1,633 (12.29%)	2,557 (9.63%)	<0.0001***
Diuretics	1,378 (2.76%)	1,373 (10.34%)	5 (0.01%)	<0.0001***	1,378 (2.76%)	1,372 (10.32%)	699 (2.63%)	<0.0001***
Anticoagulants	49,566 (99.47%)	13,271 (99.96%)	36,295 (99.29%)	0.6437	49,566 (99.47%)	13,278 (99.96%)	26,535 (99.96%)	0.994
Antiplatelets	3,331 (6.68%)	3,320 (25.00%)	11 (0.03%)	<0.0001***	3,331 (6.68%)	3,408 (25.65%)	1,650 (6.21%)	<0.0001***
Antihypertensive drugs	1,007 (2.02%)	1,005 (7.57%)	2 (0.00%)	<0.0001***	1,007 (2.02%)	1,005 (7.56%)	2 (0.00%)	<0.0001***
Lipid–lowering drugs	7,394 (14.83%)	7,379 (55.58%)	15 (0.04%)	<0.0001***	7,394(14.83%)	7,467 (56.21%)	2,568 (9.67%)	<0.0001***
Statins and fibrates	7,226 (14.50%)	2,954 (22.25%)	4,272 (11.68%)	<0.0001***	7,226(14.50%)	2,932 (22.07%)	4,816 (18.14%)	<0.0001***
Non–steroidal anti–inflammatory drugs	3,152 (6.32%)	3,141(23.65%)	11 (0.03%)	<0.0001***	3,152 (6.32%)	3,229 (24.30%)	1,650 (6.21%)	<0.0001***
Other antidiabetic drugs	45,436 (91.18%)	11,341 (85.42%)	34,095 (93.27%)	<0.0001***	45,436(91.18%)	11,350 (85.44%)	22,735 (85.64%)	0.8878
Complete blood counts								
Mean corpuscular volume, fL	88.5 (85.0–91.7); *n* = 24,270	88.3 (84.9–91.3); *n* = 6,939	88.7 (85.0–91.9); *n* = 17,331	<0.0001***	88.5 (85.0–91.7); *n* = 24,270	88.3 (84.9–91.3); *n* = 6,967	89.6 (85.8–91.3); *n* = 12,055	<0.0001***
Basophil, × 10^9/L	0.02 (0.0–0.05); *n* = 17,555	0.03 (0.0–0.06); *n* = 4,496	0.02 (0.0–0.05); *n* = 13,059	0.5161	0.02 (0.0–0.05); *n* = 17,555	0.02 (0.0–0.05); *n* = 4,538	0.03 (0.0–0.06); *n* = 9,599	<0.0001***
Eosinophil, × 10^9/L	0.19 (0.1–0.3); *n* = 19,755	0.2 (0.1–0.3); *n* = 5,558	0.18 (0.1–0.3); *n* = 14,197	0.0061**	0.1 9 (0.1–0.3); *n* = 19,755	0.2 (0.1–0.3); *n* = 5,595	0.2 (0.1–0.22); *n* = 10,166	0.23
Lymphocyte, × 10^9/L	1.9 (1.4–2.4); *n* = 19,776	2.06 (1.6–2.58); *n* = 5,560	1.81 (1.36–2.33); *n* = 14,216	<0.0001***	1.9 (1.4–2.4); *n* = 19,776	2.1 (1.63–2.56); *n* = 5,597	2.1 (1.46–2.6); *n* = 10,176	0.026*
Monocyte, × 10^9/L	0.5 (0.38–0.6); *n* = 19,776	0.5 (0.4–0.6); *n* = 5,560	0.5 (0.37–0.6); *n* = 14,216	0.001**	0.5 (0.38–0.6); *n* = 19,776	0.5 (0.4–0.6); *n* = 5,597	0.5 (0.4–0.62); *n* = 10,176	<0.0001***
Neutrophil, × 10^9/L	4.65 (3.67–6.08); *n* = 19,776	4.54 (3.61–5.86); *n* = 5,560	4.7 (3.69–6.18); *n* = 14,216	<0.0001***	4.65 (3.67–6.08); *n* = 19,776	4.5 (3.6–5.8); *n* = 5,597	4.4 (3.5–6.22); *n* = 10,176	0.307
White blood count, × 10^9/L	7.48 (6.2–9.0); *n* = 24,278	7.5 (6.3–9.0); *n* = 6,946	7.43 (6.2–9.0); *n* = 17,332	0.0491*	7.48 (6.2–9.0); *n* = 24,278	7.5 (6.3–9.0); *n* = 6,974	7.71 (6.58–9.2); *n* = 12,054	<0.0001***
Mean cell haemoglobin, pg	29.9 (28.5–31.0); *n* = 24,270	29.8 (28.5–30.9); *n* = 6,939	29.9 (28.5–31.1); *n* = 17,331	0.0003***	29.9 (28.5–31.0); *n* = 24,270	29.8 (28.5–30.9); *n* = 6,967	30.2 (28.9–31.1); *n* = 12,055	<0.0001***
Platelet, × 10^9/L	231.0 (190.0–277.0); *n* = 24,279	235.0 (197.0–280.0); *n* = 6,946	228.0 (188.0–276.0); *n* = 17,333	<0.0001***	231.0 (190.0–277.0); *n* = 24,279	236.0 (197.0–279.0); *n* = 6,974	238.0 (207.0–267.0); *n* = 12,054	0.0778
Red blood count, × 10^12/L	4.46 (4.03–4.88); *n* = 24,270	4.7 (4.36–5.07); *n* = 6,939	4.36 (3.9–4.78); *n* = 17,331	<0.0001***	4.46 (4.03–4.88); *n* = 24,270	4.7 (4.35–5.07); *n* = 6,967	4.35 (4.17–4.85); *n* = 12,055	<0.0001***

(Continued)

TABLE 1 | Continued

Characteristics	Before matching			*P*-value	After matching			*P*-value
	All (*N* = 39828) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13276) Median (IQR); N or Count(%)	DPP4I (users *N* = 36554) Median (IQR); N or Count(%)		All (*N* = 49830) Median (IQR); N or Count(%)	SGLT2I users (*N* = 13283) Median (IQR); N or Count(%)	DPP4I users (*N* = 26545) Median (IQR); N or Count(%)	
Liver and renal biochemical tests								
K/Potassium, mmol/L	4.3 (4.0–4.6); *n* = 40,605	4.28 (4.0–4.51); *n* = 10,416	4.31 (4.01–4.7); *n* = 30,189	<0.0001***	4.3 (4.0–4.6); *n* = 40,605	4.3 (4.0–4.55); *n* = 10,429	4.3 (4.0–4.7); *n* = 20,235	<0.0001***
Urate, mmol/L	0.4 (0.32–0.48); *n* = 6,169	0.37 (0.3–0.44); *n* = 1,953	0.41 (0.34–0.49); *n* = 4,216	<0.0001***	0.4 (0.32–0.48); *n* = 6,169	0.37 (0.3–0.44); *n* = 1,943	0.4 (0.33–0.49); *n* = 2,394	<0.0001***
Albumin, g/L	42.0(39.4–44.0); *n* = 30, 323	43.0 (41.0–45.0); *n* = 8,761	41.8 (39.0–44.0); *n* = 21,562	<0.0001***	42.0 (39.4–44.0); *n* = 30,323	43.0 (40.9–45.0); *n* = 8,786	41.26 (38.1–44.0); *n* = 14,994	<0.0001***
Na/Sodium, mmol/L	139.8(138.0–141.0); *n* = 40, 626	139.9 (138.0–141.0); *n* = 10,420	139.78 (138.0–141.0); *n* = 30,206	0.0007***	139.8 (138.0–141.0); *n* = 40,626	139.86 (138.0–141.0); *n* = 10,433	139.0 (137.8–141.0); *n* = 20,241	<0.0001***
Urea, mmol/L	5.9 (4.7–7.7); *n* = 40,610	5.4 (4.5–6.59); *n* = 10,411	6.16 (4.8–8.26); *n* = 30,199	<0.0001***	5.9 (4.7–7.7); *n* = 40,610	5.4 (4.44–6.51); *n* = 10,424	5.7 (4.43–7.2); *n* = 20,241	<0.0001***
Protein, g/L	74.0 (70.2–77.1); *n* = 28,453	74.7 (71.1–78.0); *n* = 8,313	73.7 (70.0–77.0); *n* = 20,140	<0.0001***	74.0 (70.2–77.1); *n* = 28,453	74.6 (71.0–78.0); *n* = 8,340	73.4 (69.0–77.9); *n* = 14,208	<0.0001***
Creatinine, umol/L	82.0 (67.0–108.0); *n* = 40,731	76.0 (64.0–90.0); *n* = 10,428	86.0 (68.5–117.4); *n* = 30,303	<0.0001***	82.0 (67.0–108.0); *n* = 40,731	75.0 (64.0–89.2); *n* = 10,441	78.0 (65.0–99.0); *n* = 20,308	<0.0001***
Alkaline phosphatase, U/L	72.0 (59.0–88.0); *n* = 30,432	70.0 (58.0–85.1); *n* = 8,761	73.0 (60.0–89.0); *n* = 21,671	<0.0001***	72.0 (59.0–88.0); *n* = 30,432	70.0 (58.0–86.0); *n* = 8,786	71.0 (59.0–91.0); *n* = 15,083	<0.0001***
Aspartate transaminase, U/L	21.0 (16.0–29.0); *n* = 8,137	22.0 (17.0–30.25); *n* = 2,326	21.0 (15.0–28.0); *n* = 5,811	<0.0001***	21.0 (16.0–29.0); *n* = 8,137	21.1 (16.0–30.0); *n* = 2,382	19.0 (14.0–30.0); *n* = 5,846	<0.0001***
Alanine transaminase, U/L	22.0 (15.0–33.0); *n* = 24,264	26.0 (18.0–39.0); *n* = 6,993	20.0 (14.0–30.0); *n* = 17,271	<0.0001***	22.0 (15.0–33.0); *n* = 24,264	26.0 (18.0–39.0); *n* = 7,030	23.0 (17.0–32.0); *n* = 11,850	<0.0001***
Bilirubin, umol/L	10.0 (7.4–13.5); *n* = 30,260	10.2 (7.8–13.7); *n* = 8,741	10.0 (7.2–13.4); *n* = 21,519	<0.0001***	10.0 (7.4–13.5); *n* = 30,260	10.3 (7.8–13.9); *n* = 8,766	10.7 (8.0–15.0); *n* = 14,969	<0.0001***
Glycemic and lipid profiles								
Triglyceride, mmol/L	1.38 (0.97–2.0); *n* = 38,215	1.42 (1.0–2.09); *n* = 9,949	1.35 (0.96–1.98); *n* = 28,266	<0.0001***	1.38 (0.97–2.0); *n* = 38,215	1.44 (1.01–2.1); *n* = 9,973	1.4 (1.0–2.19); *n* = 18,675	0.0222*
Total cholesterol, mmol/L	4.08 (3.45–4.73); *n* = 38,246	4.14 (3.53–4.84); *n* = 9,956	4.05 (3.41–4.7); *n* = 28,290	<0.0001***	4.08 (3.45–4.73); *n* = 38,246	4.16 (3.53–4.87); *n* = 9,980	4.14 (3.41–4.94); *n* = 18,691	<0.0001***
Low–density lipoprotein (LDL), mmol/L	2.27 (1.83–2.79); *n* = 34,071	2.27 (1.83–2.85); *n* = 9,174	2.27 (1.84–2.76); *n* = 24,897	0.0879	2.27 (1.83–2.79); *n* = 34,071	2.28 (1.83–2.86); *n* = 9,206	2.36 (1.86–2.86); *n* = 16,683	0.0004***
High–density lipoprotein (LDL), mmol/L	1.14 (0.97–1.36); *n* = 34,635	1.13 (0.97–1.34); *n* = 9,344	1.14 (0.97–1.37); *n* = 25,291	0.0006***	1.14 (0.97–1.36); *n* = 34,635	1.13 (0.97–1.33); *n* = 9,375	1.12 (0.94–1.3); *n* = 17,006	<0.0001***
Fast glucose, mmol/L	7.9 (6.5–9.79); *n* = 34,961	8.0 (6.6–10.16); *n* = 8,745	7.89 (6.5–9.66); *n* = 26,216	<0.0001***	7.9 (6.5–9.79); *n* = 34,961	8.01 (6.6–10.24); *n* = 8,769	8.3 (6.71–10.9); *n* = 17,192	<0.0001***
HbA1C, g/dL	12.6 (10.5–14.0); *n* = 24,738	13.5 (11.8–14.6); *n* = 7,032	12.3 (10.1–13.7); *n* = 17,706	<0.0001***	12.6 (10.5–14.0); *n* = 24,738	13.5 (11.9–14.6); *n* = 7,059	13.0 (11.4–13.8); *n* = 12,259	<0.0001***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; SGLT2I, Sodium–glucose cotransporter–2 inhibitors; DPP4I, Dipeptidyl peptidase–4 inhibitors; NLR, neutrophil–to–lymphocyte ratio; TIA, transient ischemic attack.*

TABLE 2 | Baseline and clinical characteristics of patients with new–onset dementia, Alzheimer's, and Parkinson's before and after propensity score matching (1:2).

Characteristics	Before matching			*P*-value	After matching			*P*-value
	New onset dementia (*N* = 724) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 107) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 77) Median (IQR); *N* or Count (%)		New onset dementia (*N* = 234) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 29) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 42) Median (IQR); *N* or Count (%)	
Demographics	131 (55.98%)	12 (41.37%)	30 (71.42%)	0.5571	298 (41.16%)	36 (33.64%)	45 (58.44%)	<0.0001***
Male gender								
Female gender	103 (44.01%)	17 (58.62%)	12 (28.57%)	0.4487	426 (58.83%)	71 (66.35%)	32 (41.55%)	<0.0001***
Baseline age, year	78.97 (68.6–84.2); *n* = 234	84.19 (77.67–87.79); *n* = 29	71.36 (63.64–77.66); *n* = 42	<0.0001***	81.72 (76.01–86.58); *n* = 724	83.68 (79.4–87.18); *n* = 107	77.29 (69.59–83.04); *n* = 77	<0.0001***
<40	0 (0.00%)	0 (0.00%)	0 (0.00%)	0.0012**	0 (0.00%)	0 (0.00%)	0 (0.00%)	<0.0001***
[40, 50]	3 (1.28%)	0 (0.00%)	0 (0.00%)	<0.0001***	1 (0.13%)	0 (0.00%)	0 (0.00%)	<0.0001***
[50–60]	11 (4.70%)	0 (0.00%)	7 (16.66%)	<0.0001***	13 (1.79%)	0 (0.00%)	5 (6.49%)	<0.0001***
[60–70]	55 (23.50%)	3 (10.34%)	13 (30.95%)	0.0199*	80 (11.04%)	4 (3.73%)	15 (19.48%)	<0.0001***
[70–80]	65 (27.77%)	8 (27.58%)	15 (35.71%)	0.0011**	202 (27.90%)	29 (27.10%)	27 (35.06%)	0.0030**
≥80	100 (42.73%)	18 (62.06%)	7 (16.66%)	<0.0001***	428 (59.11%)	74 (69.15%)	30 (38.96%)	<0.0001***
Charlson score	4.0 (3.0–4.0); *n* = 234	4.0 (3.0–4.0); *n* = 29	3.0 (2.0–3.0); *n* = 42	<0.0001***	4.0 (3.0–4.0); *n* = 724	4.0 (4.0–4.0); *n* = 107	3.0 (3.0–4.0); *n* = 77	<0.0001***
NLR	3.1 (1.92–4.97); *n* = 108	3.6 (1.87–7.99); *n* = 12	5.69 (2.18–14.2); *n* = 19	<0.0001***	3.08 (2.13–4.81); *n* = 362	3.11 (2.33–5.55); *n* = 44	3.23 (2.25–5.55); *n* = 33	<0.0001***
Past comorbidities								
Hypertension	76 (32.47%)	7 (24.13%)	13 (30.95%)	0.0002***	259 (35.77%)	26 (24.29%)	22 (28.57%)	<0.0001***
Heart failure	5 (2.13%)	1 (3.44%)	0 (0.00%)	0.2732	26 (3.59%)	3 (2.80%)	1 (1.29%)	0.0002***
Renal diseases	15 (6.41%)	2 (6.89%)	2 (4.76%)	<0.0001***	65 (8.97%)	7 (6.54%)	4 (5.19%)	0.0022**
Liver diseases	0 (0.00%)	0 (0.00%)	1 (2.38%)	0.6276	3 (0.41%)	0 (0.00%)	1 (1.29%)	0.4774
Stroke/TIA	11 (4.70%)	0 (0.00%)	0 (0.00%)	0.0631	39 (5.38%)	5 (4.67%)	1 (1.29%)	0.0008***
Gastrointestinal bleeding	9 (3.84%)	0 (0.00%)	2 (4.76%)	0.0021**	28 (3.86%)	4 (3.73%)	3 (3.89%)	0.0004***
History of falls	38 (16.23%)	4 (13.79%)	5 (11.90%)	<0.0001***	135 (18.64%)	20 (18.69%)	12 (15.58%)	<0.0001***
Pneumonia and influenza	16 (6.83%)	5 (17.24%)	2 (4.76%)	<0.0001***	51 (7.04%)	7 (6.54%)	4 (5.19%)	<0.0001***
Endocrine	6 (2.56%)	2 (6.89%)	3 (7.14%)	0.3606	22 (3.03%)	4 (3.73%)	1 (1.29%)	0.1102
Atrial fibrillation	12 (5.12%)	1 (3.44%)	0 (0.00%)	0.6375	48 (6.62%)	4 (3.73%)	0 (0.00%)	0.0041**
Ischemic heart disease	31 (13.24%)	4 (13.79%)	1 (2.38%)	0.2348	92 (12.70%)	13 (12.14%)	5 (6.49%)	0.1422
Peripheral vascular disease	1 (0.42%)	0 (0.00%)	0 (0.00%)	0.8017	12 (1.65%)	1 (0.93%)	2 (2.59%)	0.2298
Malignancy	8 (3.41%)	0 (0.00%)	0 (0.00%)	0.0115*	15 (2.07%)	1(0.93%)	1 (1.29%)	0.3124
Metastatic solid tumor	2 (0.85%)	0 (0.00%)	0 (0.00%)	0.3174	3 (0.41%)	0 (0.00%)	1 (1.29%)	0.3384
Medications								
SGLT2I vs. DPP4I	26 (11.11%)	2 (6.89%)	3 (7.14%)	<0.0001***	72 (9.94%)	12 (11.21%)	10 (12.98%)	<0.0001***
Beta blockers	1 (0.42%)	0 (0.00%)	0 (0.00%)	<0.0001***	1 (0.13%)	0 (0.00%)	0 (0.00%)	<0.0001***

(Continued)

TABLE 2 | Continued

Characteristics	Before matching			*P*-value	After matching			*P*-value
	New onset dementia (*N* = 724) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 107) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 77) Median (IQR); *N* or Count (%)		New onset dementia (*N* = 234) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 29) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 42) Median (IQR); *N* or Count (%)	
Diuretics	0 (0.00%)	0 (0.00%)	0 (0.00%)	0.0008***	0 (0.00%)	0 (0.00%)	0 (0.00%)	<0.0001***
Anticoagulants	234 (100.00%)	29 (100.00%)	42 (100.00%)	0.9663	717 (99.03%)	106 (99.06%)	76 (98.70%)	0.954
Antiplatelets	1 (0.42%)	0 (0.00%)	0 (0.00%)	<0.0001***	2 (0.27%)	0 (0.00%)	0 (0.00%)	<0.0001***
Antihypertensive drugs	2 (0.85%)	0 (0.00%)	0 (0.00%)	0.1623	2 (0.27%)	0 (0.00%)	0 (0.00%)	0.0014**
Lipid–lowering drugs	4 (1.70%)	1 (3.44%)	0 (0.00%)	<0.0001***	5 (0.69%)	1 (0.93%)	0 (0.00%)	<0.0001***
Statins and fibrates	26 (11.11%)	3 (10.34%)	10 (23.80%)	0.0076**	84 (11.60%)	10 (9.34%)	11 (14.28%)	0.0575
Non–steroidal anti–inflammatory drugs	1 (0.42%)	0 (0.00%)	0 (0.00%)	<0.0001***	2 (0.27%)	0 (0.00%)	0 (0.00%)	<0.0001***
Other antidiabetic drugs	210 (89.74%)	28 (96.55%)	37 (88.09%)	0.6502	685 (94.61%)	100 (93.45%)	72 (93.50%)	0.502
Complete blood counts								
Mean corpuscular volume, fL	90.0 (87.15–92.95); *n* = 127	88.7 (87.3–94.1); *n* = 15	89.3 (86.0–90.0); *n* = 25	0.0006***	89.0 (85.65–92.3); *n* = 427	88.4 (85.95–90.9); *n* = 55	88.75 (84.3–91.4); *n* = 44	0.023*
Basophil, × 10^9/L	0.02 (0.0–0.04); *n* = 96	0.04 (0.0–0.07); *n* = 12	0.02 (0.0–0.05); *n* = 16	0.0242*	0.02 (0.0–0.04); *n* = 322	0.03 (0.0–0.05); *n* = 41	0.03 (0.02–0.04); *n* = 28	0.013*
Eosinophil, × 10^9/L	0.15 (0.08–0.29); *n* = 108	0.19 (0.0–0.24); *n* = 12	0.1 (0.05–0.1); *n* = 19	0.0385*	0.17 (0.1–0.3); *n* = 361	0.2 (0.1–0.27); *n* = 44	0.13 (0.09–0.2); *n* = 33	0.0379*
Lymphocyte, × 10^9/L	1.53 (1.04–1.98); *n* = 108	1.28 (0.9–1.93); *n* = 12	1.37 (0.84–1.5); *n* = 19	<0.0001***	1.56 (1.1–2.1); *n* = 362	1.45 (1.06–1.98); *n* = 44	1.4 (0.99–1.9); *n* = 33	<0.0001***
Monocyte, × 10^9/L	0.47 (0.36–0.6); *n* = 108	0.5 (0.38–0.8); *n* = 12	0.5 (0.26–0.65); *n* = 19	0.1121	0.5 (0.36–0.6); *n* = 362	0.5 (0.37–0.6); *n* = 44	0.5 (0.33–0.7); *n* = 33	0.1745
Neutrophil, × 10^9/L	4.61 (3.6–6.6); *n* = 108	5.15 (3.4–7.75); *n* = 12	7.32 (3.4–11.79); *n* = 19	0.6069	4.8 (3.78–6.59); *n* = 362	5.11 (4.04–6.48); *n* = 44	5.2 (3.44–7.32); *n* = 33	0.0402*
White blood count, × 10^9/L	7.31 (5.85–9.5); *n* = 127	7.3 (5.38–10.51); *n* = 15	8.5 (5.1–9.42); *n* = 25	0.4192	7.6 (6.2–9.1); *n* = 427	7.49 (6.41–8.96); *n* = 55	7.28 (5.62–9.28); *n* = 44	0.5659
Mean cell haemoglobin, pg	30.2 (29.1–31.6); *n* = 127	29.5 (29.0–31.2); *n* = 15	29.9 (29.3–30.9); *n* = 25	0.0144*	30.0 (28.6–31.2); *n* = 427	30.0 (28.6–31.0); *n* = 55	29.85 (28.85–31.25); *n* = 44	0.1277
Platelet, × 10^9/L	220.0 (175.5–274.5); *n* = 127	190.0 (153.5–223.5); *n* = 15	239.0 (222.0–275.0); *n* = 25	0.0018**	223.0 (184.0–271.0); *n* = 427	217.0 (183.0–265.0); *n* = 55	234.0 (200.5–277.0); *n* = 44	0.0208*
Red blood count, × 10^12/L	4.07 (3.56–4.44); *n* = 127	4.16 (3.38–4.44); *n* = 15	4.29 (3.96–4.77); *n* = 25	<0.0001***	4.08 (3.63–4.51); *n* = 427	4.25 (3.73–4.52); *n* = 55	4.16 (3.82–4.5); *n* = 44	<0.0001***
Liver and renal biochemical tests								
K/Potassium, mmol/L	4.3 (4.0–4.7); *n* = 190	4.3 (3.9–4.5); *n* = 24	4.3 (4.01–4.7); *n* = 37	0.8572	4.32 (4.0–4.7); *n* = 621	4.2 (4.1–4.5); *n* = 87	4.4 (4.2–4.7); *n* = 65	0.3277

(Continued)

TABLE 2 | Continued

Characteristics	Before matching			*P*-value	After matching			*P*-value
	New onset dementia (*N* = 724) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 107) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 77) Median (IQR); *N* or Count (%)		New onset dementia (*N* = 234) Median (IQR); *N* or Count (%)	New onset Alzheimer's (*N* = 29) Median (IQR); *N* or Count (%)	New onset Parkinson's (*N* = 42) Median (IQR); *N* or Count (%)	
Urate, mmol/L	0.35 (0.29–0.48); *n* = 31	0.36 (0.29–0.39); *n* = 4	0.35 (0.32–0.36); *n* = 10	0.3769	0.4 (0.34–0.48); *n* = 82	0.39 (0.34–0.43); *n* = 9	0.33 (0.31–0.38); *n* = 14	0.5403
Albumin, g/L	40.0 (37.0–42.45); *n* = 146	38.2 (36.5–42.37); *n* = 19	42.0 (38.5–45.1); *n* = 32	<0.0001***	39.78 (37.0–42.21); *n* = 494	40.95 (37.0–43.0); *n* = 68	40.0 (36.85–42.25); *n* = 48	<0.0001***
Na/Sodium, mmol/L	139.0 (137.0–142.0); *n* = 190	140.1 (137.5–142.5); *n* = 24	138.0 (137.0–140.7); *n* = 37	0.4646	139.4 (137.0–141.4); *n* = 621	140.0 (137.0–142.25); *n* = 87	138.0 (136.5–141.0); *n* = 65	0.1776
Urea, mmol/L	6.5 (5.2–8.8); *n* = 190	6.01 (4.88–9.71); *n* = 24	6.0 (4.4–7.28); *n* = 37	<0.0001***	6.9 (5.2–9.64); *n* = 619	6.82 (5.12–9.22); *n* = 87	6.6 (4.92–9.13); *n* = 65	<0.0001***
Protein, g/L	72.0 (67.9–76.0); *n* = 137	71.84 (66.5–79.0); *n* = 16	72.0 (69.5–77.0); *n* = 29	0.0005***	72.35 (68.45–77.0); *n* = 466	72.5 (68.25–77.2); *n* = 63	71.1 (68.25–75.8); *n* = 43	<0.0001***
Creatinine, umol/L	97.5 (73.6–134.0); *n* = 190	104.0 (71.5–125.0); *n* = 24	90.0 (67.5–126.0); *n* = 37	<0.0001***	99.0 (77.3–132.8); *n* = 621	93.9 (71.0–121.0); *n* = 87	94.7 (71.0–126.0); *n* = 65	<0.0001***
Alkaline phosphatase, U/L	76.0 (64.0–90.0); *n* = 146	70.0 (63.0–85.5); *n* = 19	69.85 (60.0–95.0); *n* = 32	0.0012**	76.0 (63.0–93.5); *n* = 495	72.35 (64.1–86.0); *n* = 68	71.0 (59.5–92.0); *n* = 48	<0.0001***
Aspartate transaminase, U/L	17.0 (13.0–23.0); *n* = 42	14.0 (13.0–17.95); *n* = 4	21.0 (16.5–25.0); *n* = 8	0.0196*	18.0 (13.0–25.0); *n* = 127	18.45 (13.0–25.5); *n* = 16	19.0 (13.5–25.0); *n* = 12	0.0003***
Alanine transaminase, U/L	16.0 (12.0–21.4); *n* = 120	14.0 (14.0–19.0); *n* = 17	25.0 (18.0–49.0); *n* = 25	<0.0001***	17.0 (12.0–24.5); *n* = 379	16.0 (13.0–20.45); *n* = 56	18.0 (11.0–34.0); *n* = 39	<0.0001***
Bilirubin, umol/L	10.0 (6.8–13.9); *n* = 146	9.9 (6.9–11.1); *n* = 19	11.9 (8.55–15.0); *n* = 32	0.0409*	9.0 (6.6–12.7); *n* = 493	9.0 (6.52–11.35); *n* = 68	9.7 (6.0–13.55); *n* = 48	<0.0001***
Glycemic and lipid profiles								
Triglyceride, mmol/L	1.22 (0.86–1.66); *n* = 165	1.22 (0.85–1.61); *n* = 19	1.25 (0.94–1.98); *n* = 32	<0.0001***	1.29 (0.95–1.78); *n* = 522	1.1 (0.88–1.58); *n* = 70	1.15 (0.83–1.84); *n* = 60	0.0077**
Total cholesterol, mmol/L	3.85 (3.34–4.8); *n* = 166	3.8 (3.49–4.47); *n* = 19	3.98 (2.58–4.61); *n* = 33	0.069	3.9 (3.27–4.6); *n* = 523	3.78 (2.91–4.31); *n* = 70	3.91 (3.1–4.51); *n* = 61	0.0009***
Low–density lipoprotein (LDL), mmol/L	2.21 (1.79–2.7); *n* = 144	2.17 (1.61–2.35); *n* = 17	1.98 (1.75–2.76); *n* = 25	0.4206	2.15 (1.72–2.72); *n* = 449	1.87 (1.59–2.42); *n* = 61	2.14 (1.75–2.71); *n* = 49	0.0068**
High–density lipoprotein (LDL), mmol/L	1.21 (1.03–1.5); *n* = 144	1.2 (1.01–1.53); *n* = 17	1.2 (1.08–1.4); *n* = 26	<0.0001***	1.17 (0.99–1.45); *n* = 454	1.2 (1.0–1.5); *n* = 61	1.18 (1.02–1.53); *n* = 50	0.0283*
Fast glucose, mmol/L	7.54 (6.0–10.87); *n* = 170	7.13 (6.1–9.73); *n* = 20	8.68 (6.95–10.52); *n* = 32	0.0667	7.99 (6.1–10.36); *n* = 522	7.26 (5.9–9.52); *n* = 64	8.14 (6.5–10.22); *n* = 54	0.9507
HbA1C, g/dL	11.9 (10.5–13.3); *n* = 131	12.2 (10.45–13.25); *n* = 15	12.45 (11.3–13.6); *n* = 26	<0.0001***	11.6 (9.7–13.0); *n* = 436	12.2 (10.2–13.5); *n* = 57	11.4 (10.4–12.94); *n* = 45	<0.0001***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; SGLT2I, Sodium–glucose cotransporter–2 inhibitors; DPP4I, Dipeptidyl peptidase–4 inhibitors; NLR, neutrophil–to–lymphocyte ratio; TIA, transient ischemic attack.*

with 1:2 ratio between SGLT2I and DPP4I users based on demographics, Charlson comorbidity index, prior comorbidities, non-SGLT2I/DPP4I medications, baseline fasting glucose, and HbA1c tests were performed using the nearest neighbor search strategy. Propensity score matching results between treatment-group (SGLT2I) vs. control-group (DPP4I) before and after matching are shown in **Supplementary Figure 1**. Propensity score matching adjustment approaches including propensity score stratification (26), propensity score matching with inverse probability weighting (27) and high-dimensional propensity score (28) were also performed.

Cox regression models were used to identify significant risk predictors for the study outcomes. Competing risk analysis models (cause-specific and sub-distribution) were considered. The hazard ratio (HR), 95% CI, and *P*-value were reported. Statistical significance is defined as $P < 0.05$. All statistical analyses were performed with R studio (Boston, MA, Version 1.1.456) and Python (Scotts Valley, CA, Version 3.6).

RESULTS

Baseline Characteristics Before and After Propensity Score Matching

The study cohort included 13,276 SGLT2I users and 36,544 DPP4I users (total $n = 51{,}460$; median age: 66.3 years old [IQR: 58–76], 55.65% men). After a mean follow-up of 472 days (IQR: 120–792), 724 (1.45%) developed new-onset dementia, 107 (0.21%) developed new-onset Alzheimer's disease, 77 (0.15%) developed with new onset Parkinson's disease, and in total, 5,687 (11.41%) died from all-causes in which 833 (1.67%) died with cardiovascular causes and 217 (0.43%) died with cerebrovascular causes.

The baseline and clinical characteristics of DPP4I and SGLT2I users before and after 1:2 propensity score matching are shown in **Table 1**. Both before and after 1:2 propensity score matching, SGLT2I users had lower incidences of new-onset dementia (0.19 vs. 0.78%, $p < 0.0001$), new onset Alzheimer's disease (0.01 vs. 0.1%, $p = 0.0047$), new onset Parkinson's disease (0.02 vs. 0.14%, $p = 0.0006$), all-cause mortality (5.48 vs. 12.69%, $p < 0.0001$), cardiovascular mortality (0.49 vs. 3.75%, $p < 0.0001$), and cerebrovascular mortality (0.88 vs. 3.88%, $p < 0.0001$) compared with DPP4I users. The balancing comparisons of treated (SGLT2I) and controls (DPP4I) after 1:2 propensity matching with nearest neighbor search strategy are shown in **Supplementary Table 2**. None of the confounding characteristics remained significant after propensity matching.

The baseline and clinical characteristics of patients with new-onset dementia, new-onset Alzheimer's, and new-onset Parkinson's before and after 1:2 propensity score matching are shown in **Table 2**. The cumulative incidence curves for new-onset cognitive dysfunction and mortality outcomes stratified by the drug use of SGLT2I and DPP4I after 1:2 propensity score matching are shown in **Figures 2**, **3**, respectively.

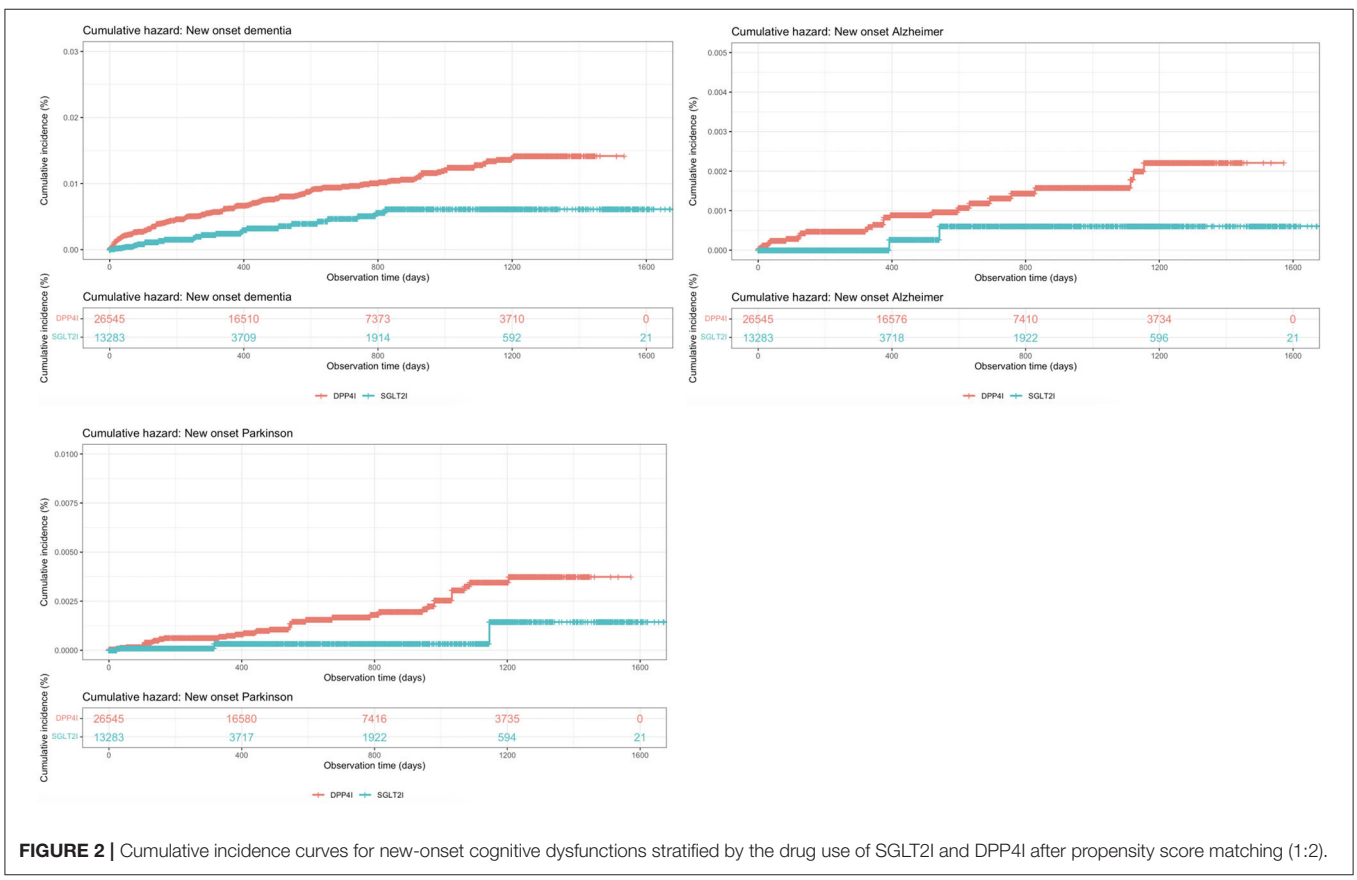

FIGURE 2 | Cumulative incidence curves for new-onset cognitive dysfunctions stratified by the drug use of SGLT2I and DPP4I after propensity score matching (1:2).

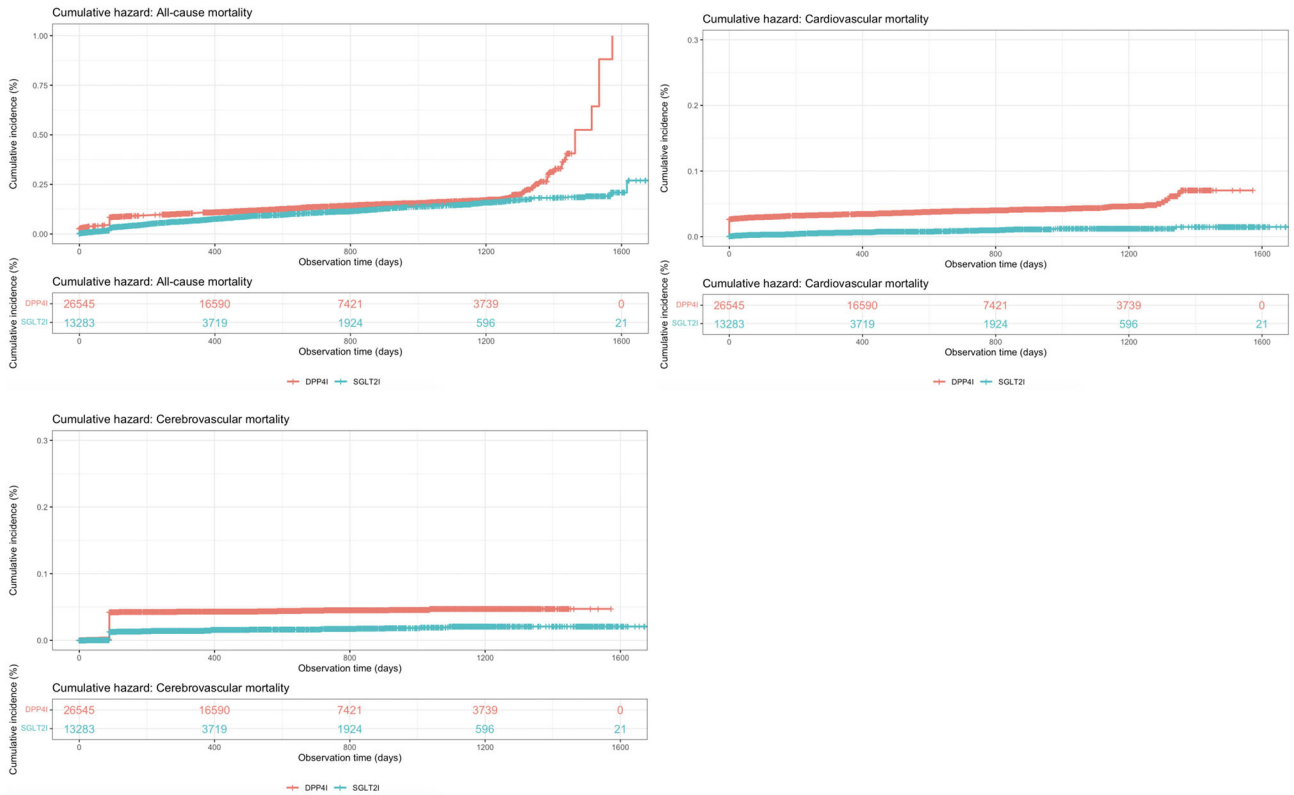

FIGURE 3 | Cumulative incidence curves for mortality outcomes stratified by the drug use of SGLT2I and DPP4I after propensity score matching (1:2).

Univariate Cox Regression Analyses

The univariate Cox analyses of significant risk factors for new-onset dementia, Alzheimer's, and Parkinson's disease are shown in **Supplementary Table 3** while the univariate Cox analyses of significant risk factors for all-cause, cardiovascular, and cerebrovascular mortality are shown in **Supplementary Table 4**. Compared with DPP4I, SGLT2I use demonstrated significant protective effects against new onset dementia (HR:0.41, 95% CI: [0.27, 0.61], $P < 0.0001$) and new onset Parkinson's disease (HR:0.28, 95% CI: [0.09, 0.91], $P = 0.0349$), but not new onset Alzheimer's disease (HR:0.25, 95% CI: [0.06, 1.04], $P = 0.0569$). SGLT2 use was also associated with significantly lower incidence of all-cause mortality (HR:0.84, 95% CI: [0.77, 0.91], $P < 0.0001$), cardiovascular mortality (HR:0.64, 95% CI: [0.49, 0.85], $P = 0.0017$), and cerebrovascular mortality (HR:0.36, 95% CI: [0.30, 0.43], $P < 0.0001$).

Sensitivity Analysis With Competing Risk Consideration

Competing for risk analyses using cause-specific and subdistribution hazard models were conducted on the matched cohorts as presented in **Table 3**. Both models confirmed the findings from the univariate Cox analyses that SGLT2I use is associated with lower incidence of new-onset dementia, new-onset Parkinson's, all-cause mortality, cardiovascular mortality, and cerebrovascular mortality, but not new-onset Alzheimer's disease compared with DPP4I use. In addition, sensitivity analyses were further conducted using Cox proportional hazard model on the matched cohorts with 1-year lag time, as presented in **Supplementary Table 5**.

Finally, different propensity score matching adjustment approaches were performed as presented in **Table 4**. Again, the three approaches confirmed the findings from the univariate Cox analyses that SGLT2I users have a lower risk of new-onset dementia, new-onset Parkinson's, all-cause mortality, cardiovascular mortality, and cerebrovascular mortality, but not new-onset Alzheimer's disease compared with DPP4I users.

Subgroup Analysis

A subgroup analysis was performed on SGLT2I and DPP4I users with concurrent type-2 diabetes and cardiovascular disease (defined as heart failure, myocardial infarction, ischemic heart disease, peripheral vascular disease, atrial fibrillation, or cardiovascular medication use) (**Table 5**). Patients with new-onset cardiovascular disease after SGLT2I/DPP4I use were excluded.

After propensity-score matching, SGLT2I users had a median follow-up time of 459 days (IQR: 42–849) while DPP4I users had a median follow-up time of 522 days (IUQ: 74–1,004). SGLT2I users had a significantly lower risk of new-onset dementia (HR:0.2, 95% CI: [0.09, 0.45], $P < 0.0001$) but not new-onset Alzheimer's disease (HR:0.27, 95% CI: [0.03, 2.16], $P = 0.2155$)

and Parkinson's disease (HR:0.42, 95% CI: [0.09, 1.96], $P = 0.2706$) compared with DPP4I users.

DISCUSSION

This study demonstrated several major findings. Firstly, SGLT2I users had a lower risk of new-onset dementia, Alzheimer's disease, and Parkinson's disease compared with DPP4I users. Secondly, SGLT2I users had a lower risk of all-cause mortality, as well as cerebrovascular and cardiovascular mortality. All of these were confirmed by univariate Cox regression analysis and competing risk analysis models apart from the association with Alzheimer's disease, which was not significantly reduced in SGLT2I users compared with DPP4I users.

The superior protective effect of DPP4I on dementia compared with other second-line anti-diabetic medication has been demonstrated by multiple studies (29–32). To our knowledge, no study so far has attempted a direct head-to-head between DPP4I and SGLT2I users for new-onset dementia, although a recent case-control study indirectly compared them when considering the risk of dementia associated with different antidiabetic medications (22). They found that while both DPP4I and SGLT2I were associated with lower odds of dementia, the odds ratio for dementia were 0.8 and 0.58 for DPP4I and SGLT2I, respectively. This is consistent with our findings that SGLT2I is superior to DPP4I in lowering dementia risk in diabetic patients. There are several possible explanations for the superior dementia-protective effects of SGLT2I. Firstly, both obesity and diabetes are independent risk factors for dementia due to shared pathophysiological mechanisms such as oxidative stress, inflammation, and insulin resistance (33, 34). Therefore, the increased reduction in weight and HbA1c observed in SGLT2I compared with DPP4I may account for the greater reduction in dementia risk (35, 36). Secondly, animal studies have proposed different neuroprotective mechanisms of SGLT2I and DPP4I which may account for their different efficacy in reducing dementia

TABLE 3 | HRs (and 95% CIs) of SGLT2I vs. DPP4I from cause–specific and subdistribution hazard models for cognitive dysfunction and mortality risks after 1:2 propensity score matching.

Model	Adverse outcomes	SGLT2I vs. DPP4I (After 1:2 matching) HR [95% CI]; *P*-value
Cause–specific model	New onset Parkinson's	0.28 [0.09–0.91]; 0.0347*
	New onset Alzheimer's	0.25 [0.06–1.04]; 0.0567.
	New onset dementia	0.43 [0.28–0.66]; 0.0002***
	Cerebrovascular mortality	0.55 [0.29–0.73]; <0.0001***
	Cardiovascular mortality	0.45 [0.31–0.59]; <0.0001***
	All–cause mortality	0.54 [1.45–0.78]; <0.0001***
Sub–distribution model	New onset Parkinson's	0.32 [0.12–0.89]; 0.0209*
	New onset Alzheimer's	0.29 [0.09–1.05]; 0.0502.
	New onset dementia	0.48 [0.31–0.72]; 0.0001***
	Cerebrovascular mortality	0.39 [0.22–0.59]; <0.0001***
	Cardiovascular mortality	0.55 [0.23–0.71]; <0.0001***
	All–cause mortality	0.54 [0.38–0.69]; <0.0001***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; SGLT2I, Sodium–glucose cotransporter–2 inhibitors; DPP4I, Dipeptidyl peptidase–4 inhibitors; HR, hazard ratio; CI, confidence interval.*

TABLE 5 | Subgroup analysis: Treatment effects of SGLT2I vs. DPP4I for incident adverse cognitive dysfunction events, and mortality outcomes in patients with both type−2 diabetes mellitus and cardiovascular diseases before and after propensity score matching (1:2).

Outcome	Before matching ($N = 39,828$) [95% CI]; *P*-value	After matching ($N = 49,830$) [95% CI]; *P*-value
All–cause mortality	0.60 [0.55–0.65]; <0.0001***	0.45 [0.41–0.51]; <0.0001***
Cardiovascular mortality	0.63 [0.51–0.77]; <0.0001***	0.47 [0.33–0.65]; <0.0001***
Cerebrovascular mortality	0.40 [0.24–0.65]; 0.0003***	0.18 [0.15–0.22]; <0.0001***
New onset dementia	0.53 [0.42–0.68]; <0.0001***	0.20 [0.09–0.45]; 0.0001***
New onset Alzheimer's	0.62 [0.34–1.14]; 0.1262	0.27 [0.03–2.16]; 0.2155
New onset Parkinson's	0.77 [0.39–1.50]; 0.4429	0.42 [0.09–1.96]; 0.2706

TABLE 4 | Risk of incident adverse cognitive dysfunction events, and mortality outcomes in matched cohorts associated with the treatment of SGLT2I vs. DPP4I using different matching approaches.

Outcome	HR after PS stratification [95% CI]; *P*-value	HR after HDPS matching [95% CI]; *P*-value	HR after PS IPTW [95% CI]; *P*-value
New onset Parkinson's	0.3 [0.13–0.9]; 0.0343*	0.25 [0.08–0.92]; 0.0357*	0.31 [0.09–0.87]; 0.0357*
New onset Alzheimer's	0.26 [0.08–1.02]; 0.0564.	0.28 [0.05–1.04]; 0.0557.	0.21 [0.01–1.02]; 0.0553.
New onset dementia	0.41 [0.29–0.75]; 0.0003***	0.46 [0.31–0.79]; 0.0014**	0.46 [0.3–0.75]; 0.0007***
Cerebrovascular mortality	0.42 [0.3–0.83]; <0.0001***	0.43 [0.29–0.8]; <0.0001***	0.47 [0.26–0.84]; <0.0001***
Cardiovascular mortality	0.61 [0.32–0.9]; <0.0001***	0.57 [0.32–0.89]; <0.0001***	0.43 [0.29–0.87]; <0.0001***
All–cause mortality	0.78 [0.62–0.84]; <0.0001***	0.72 [0.63–0.89]; <0.0001***	0.78 [0.62x−0.87]; <0.0001***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; SGLT2I, Sodium–glucose cotransporter–2 inhibitors; DPP4I, Dipeptidyl peptidase–4 inhibitors; HR, hazard ratio; CI, confidence interval; PS, propensity score; HDPS, high dimensional propensity score; IPTW, inverse probability of treatment weighting.*

risk. DPP4I predominantly reduced amyloid deposition, tau phosphorylation, while increased GLP-1 and stromal-derived factor-1 which promoted neurogenesis (16, 37). In contrast, SGLT2I improved brain mitochondrial function, hippocampal synaptic plasticity and inhibited acetylcholinesterase (18, 20, 38).

Alzheimer's disease and diabetes are closely linked by mechanisms such as oxidative stress, amyloid deposition, and tau hyperphosphorylation, so much so that some have termed Alzheimer's as "Type-3 diabetes" (39, 40). There has been growing interest in DPP4I as a potential new therapy against Alzheimer's, with animal studies showing that it reduces amyloid β protein, tau phosphorylation, inflammatory cytokines, and neuronal cell apoptosis in the brain (37, 41–43). This is consistent with clinical studies which found that DPP4I use is associated with the reduced rate of memory decline and increased mini-mental state examination (MMSE) score in Alzheimer's patients compared with metformin use (44, 45). Research on the role of SGLT2I in Alzheimer's disease so far has been based predominantly on animal models, with promising studies suggesting that SGLT2 reduces the amyloid burden, tau pathology, and brain atrophy volume (46). Our finding that SGLT2I use was associated with lower or similar risks of Alzheimer's compared with DPP4I suggested that both may have potential roles as novel therapeutic approaches for Alzheimer's patients and the role of SGLT2I in Alzheimer's should be further explored. The subgroup analysis on patients with both type 2 diabetes and cardiovascular disease showed SGLT2I did not significantly reduce the risk of Alzheimer's disease and Parkinson's disease. This could be a reflection of the equally strong association between cardiovascular disease and such cognitive pathologies (47, 48), as well as their link with type-2 diabetes.

Parkinson's disease is another neurodegenerative disorder closely associated with diabetes, sharing pathophysiological mechanisms such as insulin dysregulation, amyloid deposition, microglial activation, and mitochondrial dysfunction (49). This has been confirmed clinically by several cohort studies which demonstrate type 2 diabetes is associated with an increased risk of Parkinson's (50, 51). Whilst the interest in this is much lower than that of Alzheimer's, several recent studies have suggested beneficial effects of DPP4I in diabetic patients with Parkinson's. A retrospective longitudinal cohort study found a strong protective association between DPP4I and GLP-1 agonist use and Parkinson's disease while another retrospective study found that DPP4I use was associated with increased dopamine transporter availability, slower increase in levodopa dose, and lower risk of levodopa-induced dyskinesia in diabetic patients with Parkinson's disease (52, 53). Our study is the first to compare DPP4I and SGLT2I in their associated Parkinson's risk and demonstrated that SGLT2I has superior protective effects against Parkinson's. Due to the close association and overlapping pathophysiology between Parkinson's disease and dementia with Lewy bodies, it could be inferred that SGLT2I also has superior protective effects against dementia with Lewy Bodies and Parkinson's disease dementia compared with DPP4I (54–56). To date, no study has examined the role of SGLT2I in Parkinson's disease or dementia with Lewy Bodies and our finding suggests that this is an exciting area of research that warrants further investigation.

Limitations

Several limitations should be noted for the present study. First, given its observational nature, there was inherent information bias due to under-coding, coding errors, and missing data. Additionally, the drug compliance of the patient can only be assessed indirectly through prescription refills, which were ultimately not a direct measurement of drug exposure. Second, residual and post-baseline confounding might be present despite robust propensity-matching, particularly with the unavailability of information on lifestyle cardiovascular risk factors, e.g., smoking. The drug exposure duration among the patients has not been controlled, which might affect their risk against the study outcomes. Finally, the occurrence of cognitive dysfunction outcomes out of the hospital was not accounted for.

CONCLUSIONS

The use of SGLT2I is associated with a significantly lower risk of dementia, Parkinson's disease, all-cause mortality, cardiovascular mortality, and cerebrovascular mortality compared with DPP4 use.

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 the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JM and JZ: conception of study and literature search, preparation of figures, study design, data collection, data contribution, statistical analysis, data interpretation, manuscript drafting, and critical revision of manuscript. SL, KL, TLe, OC, ST, AW, TLi, WW, CC, GT, and QZ: conception of study and literature search, study design, data collection, data analysis, data contribution, manuscript drafting, critical revision of manuscript, and study supervision. 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.2021.747620/full#supplementary-material

REFERENCES

1. Fung ACH, Tse G, Cheng HL, Lau ESH, Luk A, Ozaki R, et al. Depressive symptoms, co-morbidities, and glycemic control in Hong Kong Chinese elderly patients with Type 2 diabetes mellitus. *Front Endocrinol.* (2018) 9:261. doi: 10.3389/fendo.2018.00261
2. Lee S, Zhou J, Wong WT, Liu T, Wu WKK, Wong ICK, et al. Glycemic and lipid variability for predicting complications and mortality in diabetes mellitus using machine learning. *BMC Endocr Disord.* (2021) 21:94. doi: 10.1186/s12902-021-00751-4
3. Kovesdy CP, Isaman D, Petruski-Ivleva N, Fried L, Blankenburg M, Gay A, et al. Chronic kidney disease progression among patients with type 2 diabetes identified in US administrative claims: a population cohort study. *Clin Kidney J.* (2021) 14:1657–64. doi: 10.1093/ckj/sfaa200
4. Folkerts K, Kelly AMB, Petruski-Ivleva N, Fried L, Blankenburg M, Gay A, et al. Cardiovascular and renal outcomes in patients with type-2 diabetes and chronic kidney disease identified in a United States administrative claims database: a population cohort study. *Nephron.* (2021) 145:342–52. doi: 10.1159/000513782
5. Shin JY, Roh SG, Lee NH, Yang KM. Influence of epidemiologic and patient behavior-related predictors on amputation rates in diabetic patients: systematic review and meta-analysis. *Int J Low Extrem Wounds.* (2017) 16:14–22. doi: 10.1177/1534734617699318
6. van Sloten TT, Sedaghat S, Carnethon MR, Launer LJ, Stehouwer CDA. Cerebral microvascular complications of type 2 diabetes: stroke, cognitive dysfunction, and depression. *Lancet Diabetes Endocrinol.* (2020) 8:325–36. doi: 10.1016/S2213-8587(19)30405-X
7. Cukierman T, Gerstein HC, Williamson JD. Cognitive decline and dementia in diabetes–systematic overview of prospective observational studies. *Diabetologia.* (2005) 48:2460–9. doi: 10.1007/s00125-005-0023-4
8. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: the Rotterdam Study. *Neurology.* (1999) 53:1937–42. doi: 10.1212/WNL.53.9.1937
9. Emerging Risk Factors C, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* (2010) 375:2215–22. doi: 10.1016/S0140-6736(10)60484-9
10. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev.* (2008) 29:494–511. doi: 10.1210/er.2007-0034
11. Munshi MN. Cognitive dysfunction in older adults with diabetes: what a clinician needs to know. *Diabetes Care.* (2017) 40:461–7. doi: 10.2337/dc16-1229
12. Crane PK, Walker R, Larson EB. Glucose levels and risk of dementia. *N Engl J Med.* (2013) 369:1863–4. doi: 10.1056/NEJMc1311765
13. Liu S, Lu Y, Cai X, Cong R, Li J, Jiang H, et al. Glycemic control is related to cognitive dysfunction in elderly people with Type 2 diabetes mellitus in a rural chinese population. *Curr Alzheimer Res.* (2019) 16:950–62. doi: 10.2174/1567205016666191023110712
14. Kim JY, Ku YS, Kim HJ, Trinh NT, Kim W, Jeong B, et al. Oral diabetes medication and risk of dementia in elderly patients with type 2 diabetes. *Diabetes Res Clin Pract.* (2019) 154:116–23. doi: 10.1016/j.diabres.2019.07.004
15. Zheng F, Yan L, Yang Z, Zhong B, Xie W. HbA(1c), diabetes and cognitive decline: the english longitudinal study of ageing. *Diabetologia.* (2018) 61:839–48. doi: 10.1007/s00125-017-4541-7
16. Chen S, Zhou M, Sun J, Guo A, Fernando RL, Chen Y, et al. DPP-4 inhibitor improves learning and memory deficits and AD-like neurodegeneration by modulating the GLP-1 signaling. *Neuropharmacology.* (2019) 157:107668. doi: 10.1016/j.neuropharm.2019.107668
17. Ide M, Sonoda N, Inoue T, Kimura S, Minami Y, Makimura H, et al. The dipeptidyl peptidase-4 inhibitor, linagliptin, improves cognitive impairment in streptozotocin-induced diabetic mice by inhibiting oxidative stress and microglial activation. *PLoS One.* (2020) 15:e0228750. doi: 10.1371/journal.pone.0228750
18. Lin B, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol.* (2014) 13:148. doi: 10.1186/s12933-014-0148-1
19. Wang S, Liu Y, Zhang H, Guo Y, Li M, Gao W, et al. Effects of an SGLT2 inhibitor on cognition in diabetes involving amelioration of deep cortical cerebral blood flow autoregulation and pericyte function. *Alzheimer's Dementia.* (2020) 16:e037056. doi: 10.1002/alz.037056
20. Sa-Nguanmoo P, Tanajak P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, et al. SGLT2-inhibitor and DPP-4 inhibitor improve brain function via attenuating mitochondrial dysfunction, insulin resistance, inflammation, and apoptosis in HFD-induced obese rats. *Toxicol Appl Pharmacol.* (2017) 333:43–50. doi: 10.1016/j.taap.2017.08.005
21. Perna S, Mainardi M, Astrone P, Gozzer C, Biava A, Bacchio R, et al. 12-month effects of incretins versus SGLT2-Inhibitors on cognitive performance and metabolic profile. a randomized clinical trial in the elderly with Type-2 diabetes mellitus. *Clin Pharmacol.* (2018) 10:141–51. doi: 10.2147/CPAA.S164785
22. Wium-Andersen IK, Osler M, Jørgensen MB, Rungby J, Wium-Andersen MK. Antidiabetic medication and risk of dementia in patients with type 2 diabetes: a nested case-control study. *Eur J Endocrinol.* (2019) 181:499–507. doi: 10.1530/EJE-19-0259
23. Lee S, Liu T, Zhou J, Zhang Q, Wong WT, Tse G. Predictions of diabetes complications and mortality using hba1c variability: a 10-year observational cohort study. *Acta Diabetol.* (2021) 58:171–80. doi: 10.1007/s00592-020-01605-6
24. Zhou J, Lee S, Guo CL, Chang C, Liu T, Leung KSK, et al. Anticoagulant or antiplatelet use and severe COVID-19 disease: a propensity score-matched territory-wide study. *Pharmacol Res.* (2021) 165:105473. doi: 10.1016/j.phrs.2021.105473
25. Zhou J, Wang X, Lee S, Wu WKK, Cheung BMY, Zhang Q, et al. Proton pump inhibitor or famotidine use and severe COVID-19 disease: a propensity score-matched territory-wide study. *Gut.* (2020) 70:gutjnl-2020-323668. doi: 10.1136/gutjnl-2020-323668
26. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res.* (2011) 46:399–424. doi: 10.1080/00273171.2011.568786
27. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. *Stat Med.* (2015) 34:3661–79. doi: 10.1002/sim.6607
28. Schneeweiss S, Rassen JA, Glynn RJ, Avorn J, Mogun H, Brookhart MA. High-dimensional propensity score adjustment in studies of treatment effects using health care claims data. *Epidemiology.* (2009) 20:512–22. doi: 10.1097/EDE.0b013e3181a663cc
29. Kim YG, Jeon JY, Kim HJ, Kim DJ, Lee KW, Moon SY, et al. Risk of dementia in older patients with type 2 diabetes on dipeptidyl-peptidase IV inhibitors versus sulfonylureas: a real-world population-based cohort study. *J Clin Med.* (2018) 8:28. doi: 10.3390/jcm8010028
30. Kim WJ, Noh JH, Han K, Park CY. The association between second-line oral antihyperglycemic medication on types of dementia in Type 2 diabetes: a nationwide real-world longitudinal study. *J Alzheimers Dis.* (2021) 81:1263–72. doi: 10.3233/JAD-201535

31. Rizzo MR, Barbieri M, Boccardi V, Angellotti E, Marfella R, Paolisso G. Dipeptidyl peptidase-4 inhibitors have protective effect on cognitive impairment in aged diabetic patients with mild cognitive impairment. *J Gerontol A Biol Sci Med Sci.* (2014) 69:1122–31. doi: 10.1093/gerona/glu032
32. Zhou JB, Tang X, Han M, Yang J, Simó R. Impact of antidiabetic agents on dementia risk: a Bayesian network meta-analysis. *Metabolism.* (2020) 109:154265. doi: 10.1016/j.metabol.2020.154265
33. Baglietto-Vargas D, Shi J, Yaeger DM, Ager R, LaFerla FM. Diabetes and Alzheimer's disease crosstalk. *Neurosci Biobehav Rev.* (2016) 64:272–87. doi: 10.1016/j.neubiorev.2016.03.005
34. Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kåreholt I, Winblad B, et al. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol.* (2005) 62:1556–60. doi: 10.1001/archneur.62.10.1556
35. Bailey CJ, Del Prato S, Wei C, Reyner D, Saraiva G. Durability of glycaemic control with dapagliflozin, an SGLT2 inhibitor, compared with saxagliptin, a DPP4 inhibitor, in patients with inadequately controlled type 2 diabetes. *Diabetes Obes Metab.* (2019) 21:2564–9. doi: 10.1111/dom.13841
36. Pinto LC, Rados DV, Remonti LR, Kramer CK, Leitao CB, Gross JL. Efficacy of SGLT2 inhibitors in glycemic control, weight loss and blood pressure reduction: a systematic review and meta-analysis. *Diabetol Metab Syndr.* (2015) 7(Suppl. 1):A58. doi: 10.1186/1758-5996-7-S1-A58
37. Kosaraju J, Gali CC, Khatwal RB, Dubala A, Chinni S, Holsinger RM, et al. Saxagliptin: a dipeptidyl peptidase-4 inhibitor ameliorates streptozotocin induced Alzheimer's disease. *Neuropharmacology.* (2013) 72:291–300. doi: 10.1016/j.neuropharm.2013.04.008
38. Shaikh S, Rizvi SM, Shakil S, Riyaz S, Biswas D, Jahan R. Forxiga (dapagliflozin): plausible role in the treatment of diabetes-associated neurological disorders. *Biotechnol Appl Biochem.* (2016) 63:145–50. doi: 10.1002/bab.1319
39. Nguyen TT, Ta QTH, Nguyen TKO, Nguyen TTD, Giau VV. Type 3 diabetes and its role implications in Alzheimer's disease. *Int J Mol Sci.* (2020) 21:3165. doi: 10.3390/ijms21093165
40. Kandimalla R, Thirumala V, Reddy PH. Is Alzheimer's disease a Type 3 diabetes? A critical appraisal. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:1078–89. doi: 10.1016/j.bbadis.2016.08.018
41. Kosaraju J, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, et al. Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. *J Pharm Pharmacol.* (2013) 65:1773–84. doi: 10.1111/jphp.12148
42. D'Amico M, Di Filippo C, Marfella R, Abbatecola AM, Ferraraccio F, Rossi F, et al. Long-term inhibition of dipeptidyl peptidase-4 in Alzheimer's prone mice. *Exp Gerontol.* (2010) 45:202–7. doi: 10.1016/j.exger.2009.12.004
43. Kosaraju J, Holsinger RMD, Guo L, Tam KY. Linagliptin, a dipeptidyl Peptidase-4 inhibitor, mitigates cognitive deficits and pathology in the 3xTg-AD mouse model of Alzheimer's disease. *Mol Neurobiol.* (2017) 54:6074–84. doi: 10.1007/s12035-016-0125-7
44. Isik AT, Soysal P, Yay A, Usarel C. The effects of sitagliptin, a DPP-4 inhibitor, on cognitive functions in elderly diabetic patients with or without Alzheimer's disease. *Diabetes Res Clin Pract.* (2017) 123:192–8. doi: 10.1016/j.diabres.2016.12.010
45. Wu CY, Ouk M, Wong YY, Anita NZ, Edwards JD, Yang P, et al. Relationships between memory decline and the use of metformin or DPP4 inhibitors in people with type 2 diabetes with normal cognition or Alzheimer's disease, and the role APOE carrier status. *Alzheimers Dement.* (2020) 16:1663–73. doi: 10.1002/alz.12161
46. Wiciński M, Wódkiewicz E, Górski K, Walczak M, Malinowski B. Perspective of SGLT2 inhibition in treatment of conditions connected to neuronal loss: focus on Alzheimer's disease and ischemia-related brain injury. *Pharmaceuticals.* (2020) 13:379. doi: 10.3390/ph13110379
47. Potashkin J, Huang X, Becker C, Chen H, Foltynie T, Marras C. Understanding the links between cardiovascular disease and Parkinson's disease. *Mov Disord.* (2020) 35:55–74. doi: 10.1002/mds.27836
48. Stampfer MJ. Cardiovascular disease and Alzheimer's disease: common links. *J Intern Med.* (2006) 260:211–23. doi: 10.1111/j.1365-2796.2006.01687.x
49. Cheong JLY, de Pablo-Fernandez E, Foltynie T, Noyce AJ. The Association between Type 2 diabetes mellitus and Parkinson's disease. *J Parkinsons Dis.* (2020) 10:775–89. doi: 10.3233/JPD-191900
50. Hu G, Jousilahti P, Bidel S, Antikainen R, Tuomilehto J. Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care.* (2007) 30:842–7. doi: 10.2337/dc06-2011
51. Xu Q, Park Y, Huang X, Hollenbeck A, Blair A, Schatzkin A, et al. Diabetes and risk of Parkinson's disease. *Diabetes Care.* (2011) 34:910–5. doi: 10.2337/dc10-1922
52. Brauer R, Wei L, Ma T, Athauda D, Girges C, Vijiaratnam N, et al. Diabetes medications and risk of Parkinson's disease: a cohort study of patients with diabetes. *Brain.* (2020) 143:3067–76. doi: 10.1093/brain/awaa262
53. Jeong SH, Chung SJ, Yoo HS, Hong N, Jung JH, Baik K, et al. Beneficial effects of dipeptidyl peptidase-4 inhibitors in diabetic Parkinson's disease. *Brain.* (2021) 144:1127–37. doi: 10.1093/brain/awab015
54. Aarsland D. Cognitive impairment in Parkinson's disease and dementia with Lewy bodies. *Parkinsonism Relat Disord.* (2016) 22(Suppl. 1):S144–8. doi: 10.1016/j.parkreldis.2015.09.034
55. McKeith IG, Mosimann UP. Dementia with Lewy bodies and Parkinson's disease. *Parkinsonism Relat Disord.* (2004) 10(Suppl. 1):S15–8. doi: 10.1016/j.parkreldis.2003.12.005
56. Weil RS, Lashley TL, Bras J, Schrag AE, Schott JM. Current concepts and controversies in the pathogenesis of Parkinson's disease dementia and Dementia with Lewy Bodies. *F1000Res.* (2017) 6:1604. doi: 10.12688/f1000research.11725.1

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Targeted Genetic Reduction of Mutant Huntingtin Lessens Cardiac Pathology in the BACHD Mouse Model of Huntington's Disease

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Individuals affected by Huntington's disease (HD) present with progressive degeneration that results in a wide range of symptoms, including cardiovascular (CV) dysfunction. The huntingtin gene (*HTT*) and its product are ubiquitously expressed, hence, the cardiomyopathy could also be driven by defects caused by its mutated form (*mHTT*) in the cardiomyocytes themselves. In the present study, we sought to determine the contribution of the *mHTT* expressed in the cardiomyocytes to CV symptoms. We utilized the BACHD mouse model, which exhibits many of the HD core symptoms, including CV dysfunction. This model allows the targeted genetic reduction of *mHTT* expression in the cardiomyocytes while maintaining the expression of the *mHTT* in the rest of the body. The BACHD line was crossed with a line of mice in which the expression of Cre recombinase is driven by the cardiac-specific alpha myosin-heavy chain (*Myh6*) promoter. The offspring of this cross (BMYO mice) exhibited a dramatic reduction in *mHTT* in the heart but not in the striatum. The BMYO mice were evaluated at 6 months old, as at this age, the BACHD line displays a strong CV phenotype. Echocardiogram measurements found improvement in the ejection fraction in the BMYO line compared to the BACHD, while hypertrophy was observed in both mutant lines. Next, we examined the expression of genes known to be upregulated during pathological cardiac hypertrophy. As measured by qPCR, the BMYO hearts exhibited significantly less expression of *collagen1a* as well as *Gata4*, and *brain natriuretic peptide* compared to the BACHD. Fibrosis in the hearts assessed by Masson's trichrome stain and the protein levels of fibronectin were reduced in the BMYO hearts compared to BACHD. Finally, we examined the performance of the mice on CV-sensitive motor tasks. Both the overall activity levels and grip strength were improved in the BMYO mice. Therefore, we conclude that the reduction of *mHtt* expression in the heart benefits CV function in the BACHD model, and suggest that cardiomyopathy should be considered in the treatment strategies for HD.

Keywords: Huntington's disease, cardiac dysfunction, BACHD mouse model, rescue, hypertrophy, cardiomyopathy, cardiovascular function

INTRODUCTION

Patients with Huntington's disease (HD), a progressive degenerative disease, present with cognitive, psychiatric and motor dysfunctions (1, 2). HD is caused by mutations within the first exon of the *huntingtin* (*Htt*) gene located on Chromosome 4, which produce a CAG repeat expansion. Translation of such repeats leads to a polyglutamine (polyQ) repeat with consequent protein misfolding, production of soluble aggregates and inclusion bodies throughout the body (3, 4). The normal function(s) of the HTT protein is still largely unknown and under study; albeit it is thought to be critical for a large range of cellular processes including cytoskeletal organization, protein folding, and metabolic processes (5). Cardiovascular (CV) dysfunction and (mal)events have been reported to play a role in HD progression, and likely to be, among others, one of the leading causes of early death in HD individuals [(6, 7); also see (8, 9)]. The clinical etiology in HD appears to be similar to Parkinson's disease where dysautonomia is a prominent early symptom of the disease (10–12). Furthermore, the mutant huntingtin gene (*mHTT*) is ubiquitously expressed raising the possibility that the observed CV symptoms could be driven by deficits in the cardiomyocytes themselves (13). The relative contribution of the *mHTT* in the heart and brain is difficult to tease apart in a clinical population thus, in the present study, we turned to a model organism to address this issue.

Several animal models of HD, which recapitulate important aspects of the human disease including CV dysfunction (9, 14–16), are available. For example, a classic physiological test of the autonomic nervous system (ANS) function is to measure the baroreceptor reflex in which changes in blood pressure evoke alterations in the heart rate. Both the BACHD (17) and the Q175 (18) lines show clear deficits in such response. Heart rate variability (HRV), a measure of the variation in the beat-to-beat interval, reflects the equilibrium between the sympathetic and parasympathetic systems in the control of heart function(s). Traditionally, it is considered as an indicator of CV health, with low HRV being predictive of CV disease and possibly, mortality (19, 20). Reduced HRV has been observed in the R6/1 (21), BACHD (22), and Q175 (18) models. Therefore, these animal models clearly display the dysautonomia seen in the HD patients.

In addition, there is evidence for direct cardiomyopathy in several of the preclinical models, as shown by the reduced contractility and cardiac output (18, 22–25). Cardiomyocytes appear to have metabolic dysfunction (26) as well as structural abnormalities in the mitochondria (27) that are not likely driven by the ANS. Finally, in *Drosophila*, heart specific expression of *mHtt* resulted in cardiac hypertrophy and decreased contractility (28). Similarly, in mice, when transgenic polyQ expression was driven by a cardiomyocyte specific promotor (α-myosin heavy chain promoter, *Myh6*), heart failure and premature death were observed (29). These two studies provide a proof-of-principle that *mHTT* can drive cardiomyopathy without central nervous system (CNS) involvement, but are subject to the concern that the expression levels were much higher than what is normally observed in HD.

Therefore, as an alternative strategy to explore the impact of heart specific *mHTT* expression on CV pathophysiology, we utilized the BACHD line of mice, which has been engineered to express the human mutation in the *HTT* gene, in a way that it can be excised by Cre recombinase (Cre) in a tissue specific manner (30, 31). Our previous studies showed structural and functional CV changes in the BACHD mice by 6 months of age (17, 22, 32). Therefore, the BACHD mouse was crossed with a line in which the cardiac-specific *Myh6* promoter drives Cre expression only in the cardiomyocytes with the expectation that *mHTT* would be selectively reduced in this cell type. Using this genetically targeted reduction (BACHD X cardiac-specific *Myh6*-Cre: BMYO line), we assessed cardiac function and structure with echocardiograms as well as gene expression of a set of markers associated with pathological hypertrophy in the heart at 6 months of age. Finally, we examined the impact of specific cardiac reduction of *mHTT* on motor behaviors sensitive to CV function. Taken together, our study provides important insights into cardiovascular pathophysiology in HD.

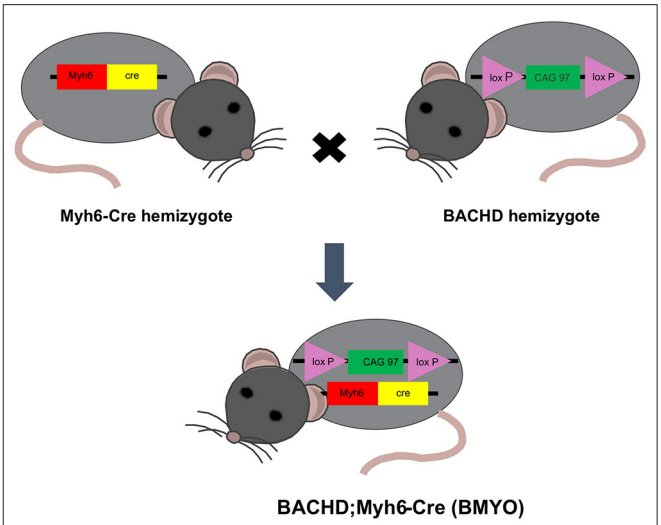

FIGURE 1 | Schematic representation of the breeding strategy used in the present study. Genetic reduction of *mHTT* expression in the heart of BACHD mice was achieved by crossing this transgene (hemizygous) with mice hemizygous for the cardiomyocyte-specific Myh6-Cre transgene. The full-length m*HTT* with two *loxP* sites enables cell specific reduction of m*HTT* using Cre-recombinase (Cre). Approximately 25% of the offspring were double transgenics (BACHD; Myh6-Cre), BMYO, with the *mHTT* floxed out in the cardiomyocytes.

METHODS

Generation of BMYO Mice

BACHD mice on the C57BL6/J background along with littermate wild-type (WT) controls were acquired from the mouse mutant resource at The Jackson Laboratory, (JAX, Bar Harbor, Maine) in a colony maintained by the CHDI Foundation. αMHC^{Cre} (Myh6-Cre) were obtained from the Jackson Laboratory (JAX). BACHD and Myh6-Cre mice on a C57BL/6 background were bred to generate a double transgenic: BACHD; Myh6-Cre (BMYO), with the *mHTT* floxed out in the cardiomyocytes (**Figure 1**). The following three genotypes were used in this study: WT, BACHD, and BMYO. Genotypes were confirmed by PCR of tail snips.

WT ($n = 8$), BACHD ($n = 8$), and BMYO ($n = 8$) male mice were group-housed and kept in a 12 h:12 h light/dark (LD) cycle with rodent chow provided *ad libitum*. Mice were examined with echocardiograms at 3 and 6 months of age. After the last echocardiogram measurement, the heart and the brain region, striatum, were rapidly dissected on ice and properly stored to be used for subsequent histological and biochemical measurements. The left and right striati were frozen separately to be used for whole tissue protein lysate or total RNA extraction. All procedures followed guidelines of the National Institutes of Health and were approved by the UCLA Animal Research Committee.

Quantitative Real-Time PCR

WT, BACHD and BMYO mice (3 and 6 months-old; $n = 3$–4 per genotype, per age) were deeply anesthetized with isoflurane, the hearts and striati rapidly dissected, and frozen. Total RNA was extracted using TRIzol® (ThermoFisher; Carlsbad, CA) and following the manufacturer's protocol. The samples were further treated with the Ambion® TURBO DNA-*free*™ (Life Technologies; Waltham, MA), followed by a second extraction with phenol/chloroform. Concentration and purity of the samples were assessed using a ThermoScientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Canoga Park, CA). Total RNA (600 ng) was reverse transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA) then analyzed for various transcripts on a CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories). Reactions were setup using the iQ™ SYBR® Green Supermix (Bio-Rad Laboratories) and the QuantiTect® Primer Assay (Qiagen, Valencia, CA). To detect the human *mHTT* expression, primers were designed as previously published (30). The forward primer 5′-ATC TTG AGC CAC AGC TCC AGC CA-3′ recognized both the endogenous mouse HD gene homolog and the exogenous *mHTT*, whilst two reverse primers were designed to be species-specific (human): 5′-GGC CTC CGA GGC TTC ATC AGG-3′; reverse (murine): 5′-TCT GAA AAC ATC TGA GAC TTC ACC AGA-3′. Assays were performed in triplicate following the manufacturer's directions. Negative controls (samples in which reverse transcriptase was omitted) were amplified individually using the same primer sets to ensure the absence of genomic DNA contamination. Amplification specificity was assessed by melting curve. Standard curves were prepared using serial dilutions of control RNA and used for quantification as well as to determine the efficiency of each run. Expression standardization was done using as housekeeping gene *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase; Gene ID 14433; Qiagen). The average values from three technical replicates per sample were normalized to those obtained for *Gapdh* in the same sample and the transcript levels expressed as the mean ± SEM.

Western Blotting

WT, BACHD and BMYO mice (3 and 6 months-old; $n = 3$–4 per genotype, per age) were deeply anesthetized with isoflurane, the hearts and striati rapidly dissected, and frozen. Whole tissue lysates from the heart and striatum were prepared as previously described with minor modifications (22). Briefly, hearts and striati were homogenized in lysis buffer [50 mM Tris/HCl, 0.25% (w/v) sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1nM EGTA, 1% (w/v) Nonidet-P40, 1 mM sodium orthovanadate, 1 mM AEBSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin and 4 μM sodium fluoride]. Total protein concentration in cleared extracts was estimated using the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA USA). Twenty or thirty-five μg of total proteins were resolved onto a 4–12% Tris-glycine gel (Invitrogen, Carlsbad, CA). Equal protein loading was verified by Ponceau S solution (Sigma, Saint Louis, MO) reversible staining of the membranes as well as later by the relative protein levels of GAPDH (1:3,000, GeneTex, Irvine, CA). Membranes were blocked for 1 hr at room temperature and incubated overnight with the primary antibody diluted in blocking solution [5% (w/v) non-fat milk in PBS containing 0.5% (v/v) Tween-20]. Extracts were analyzed for the relative protein levels of the anti-polyglutamine (polyQ)-expansion disease marker, clone 5TF1-1C2 (mouse monoclonal, 1:1,000, Millipore-Sigma, Temecula, CA), and fibronectin (rabbit polyclonal, 1:500, Millipore-Sigma). Protein bands were detected by chemiluminescence using the ThermoScientific™ Pierce™ SuperSignal West Pico or ECL 2 Western Blotting Substrate with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling, Danvers, MA). Relative intensities of the protein bands were quantified by scanning densitometry using the NIH Image Software (Image J, http://rsb.info.nih.gov/ij/). Each background-corrected value was normalized to the according GAPDH levels of the sample and are shown as the mean ± SEM.

Echocardiogram

WT, BACHD, and BMYO mice ($n = 8$ per genotype) were group housed and kept in a 12:12 LD cycle with rodent chow provided *ad libitum*. Mice were examined with echocardiograms at 3 and 6 months of age. After the last echocardiogram measurement, the heart and brain tissues of the mice were collected and morphologic and histological measurements were taken.

Echocardiograms were obtained using a Siemens Acuson Sequoia C256 instrument equipped with a 15L8 15MHz probe (Siemens Medical Solutions, Mountain View, CA) as previously described (18, 32). Briefly, two-dimensional, M-mode echocardiography and spectral Doppler images enabled measurements of heart dimension and function: [Left Ventricular (LV) Mass], end-diastolic dimension (EDD), end-systolic dimension (ESD), posterior wall thickness (PWT), ventricular septal thickness (VST), LV Ejection Fraction (LV EF). The mice were lightly sedated with 1% isoflurane vaporized in oxygen (Sommi Scientific, Foster City, CA) and heart rate (HR) was monitored using electrocardiogram to maintain physiological levels (between 450 and 600 beats per min).

Immunofluorescence and Cyto-Histomorphometrical Analyses

Heart samples were harvested from 6 months-old BACHD, perfused with 1x PBS, fixed in 4% paraformaldehyde overnight at 4°C, and cryoprotected in 15% sucrose. After embedding the

hearts in Tissue Tek O.C.T. (Sakura Finetek, Torrance, CA), frozen coronal sections (10 μm) were cut on a cryostat (Leica, Wetzlar, GR), sequentially collected on Superfrost Plus glass slides (Thermo Fisher Scientific, Waltham, MA), and stored at −20°C until processed for either immunofluorescence or Masson's Trichrome staining. Mid-ventricular sections slices were identified and paired based on the visual presence of the papillary muscles in the left ventricle.

For immunofluorescence, sections were stained as previously reported with minor modifications (33). Briefly, sections (n = 2 animals/genotype) were blocked in carrier solution [1% (w/v) BSA and 0.3% (w/v) Triton X-100 in 1x PBS] containing 20% (v/v) normal goat serum for 1 h and incubated overnight at 4°C with a rabbit polyclonal antibody against collagen I (abcam, Waltham, MA) diluted in carrier solution containing 5% (v/v) normal goat serum. Sections were washed in carrier solution and then incubated with a goat anti-rabbit secondary antibody conjugated to Cy3 (Jackson ImmunoResearch Laboratories, Bar Harbor, ME). Coverslips applied with a drop of Vectashield mounting medium with DAPI (49,6-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA). Immunostained sections were visualized on a Zeiss Axio Imager 2 equipped with an AxioCam MRm and the ApoTome imaging system (Carl Zeiss, Germany) using the Zen software (Zeiss).

Masson's Trichrome staining (Sigma-Aldrich, St Louis, MI) was performed as previously reported (18, 22). The presence or absence of fibrotic staining was visually scored by two researchers masked to the experimental conditions in 3 sections/animal. Trichrome stained slices were used for the following cyto- and histo-morphological analyses:

(1) Heart dimensions were measured using 2 sections from each Masson's Trichrome-stained heart. Images were acquired on a Zeiss Stereomicroscope (StemiSV11Apo) equipped with a Zeiss color camera (Axiocam 208 color) using the Zen software (Zeiss). The thickness of the left and right ventricular walls was measured in three non-septal parts to obtain an average thickness. The thickness of the intraventricular septum was determined in three middle parts (non-ventricular). Values from 2 sections/animal (n = 3 per genotype) were averaged.
(2) To measure the cardiomyocytes' cross-sectional area, images from multiple fields of the same 2 sections were acquired at the levels of the papillary of the Lv (10–15 images/animal), from non-septal parts of the Rv wall and the middle intraventricular septum (non-ventricular part; 5–10 images) on a Zeiss upright microscope (Axioskop; 20× objective) equipped with a Zeiss color camera (Axiocam 208 color) and the Zen software (Zeiss). The cross-sectional areas of at least 5–6 cells/image/animal were averaged and analyzed for statistical difference ($n = 3$ per genotype).
(3) The number of cells was determined in the same images acquired for the analysis of the cross-sectional areas, using the cell counting plugin/feature of ImageJ by an observer masked to the genotype. Cells were counted in 2–3 ROI (regions of interest) of equal size (153 μm × 153 μm) in each image. The number of cells/image was summed, and then divided by the total area of the 2 or 3 ROIs. The values from 10–15 images (Lv) or 5–10 images (Rv and Septum)/animal were averaged and analyzed for statistical difference. The number of cells/μm^2 is reported ($n = 3$ per genotype).

All the morphological assessments were performed using Image J by an observer masked to the genotype.

Behavioral Studies

Locomotor activity was recorded for 2 weeks from single-caged mice with infrared motion sensors using our established protocols (34). Locomotor activity was recorded in 3-min bins, and 10 days of data were averaged for analysis. The amount of cage activity over a 24-h period was averaged over 10 days and reported here as the hourly arbitrary units (au/day). The activity data was also analyzed for possible differences in diurnal rhythmicity. The period and rhythmic strength was determined as previously described (34, 35). The periodogram analysis used a χ^2 test with a threshold of 0.001 significance, from which the amplitude of the periodicities was determined at the circadian harmonic to obtain the rhythm power. The number of activity bouts and the average length of bouts were determined using Clocklab (Actimetrics, Wilmette, IL), where a bout consists of a duration in which activity never falls below the criterion (3 counts/min) for longer than the maximum gap (21 min).

The grip strength test was used to measure neuromuscular function as maximal muscle strength of forelimbs. The grip strength ergometer (Santa Cruz Biotechnology, Santa Cruz, CA) was set up on a flat surface with a mouse grid firmly secured in place. Peak mode was utilized to enable measurement of the maximal strength exerted. Mice were tested in their active phase under dim red light (3 lux), and prior to testing, mice were acclimated to the testing room. Mice underwent five trials with an inter-trial interval of at least 2 mins. For each trial, a mouse was lowered slowly over the grid, and only its forepaws were allowed to grip the grid. While the mouse was steadily pulled back, the experimenter ensured the mouse remained horizontal until the mouse was no longer able to grip the grid. The maximal grip strength value of the mouse was recorded each trial. The apparatus was cleaned with 70% ethanol and allowed to dry before testing each mouse cohort. The maximum value obtained per animal is reported as maximal strength divided by bodyweight.

Statistics

We were interested in determining if the reduction of *mHTT* would improve the symptoms reported in the BACHD mouse model; therefore, the BMYO mice were compared to age-matched BACHD and WT mice in all the experiments. The sample size per group was determined by both our empirical experience with the variability in the prior measures in the BACHD mice. In addition, for the echocardiograms and behavioral analysis, we carried out a power analysis that assumed a power of 0.8 and an alpha of 0.05. One-way ANOVA along with Holm-Sidak or Tukey's *post-hoc* test was used to evaluate the statistical significance of the findings. Data were examined

for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe test). If normality or equal variance tests failed, then the Kruskal-Wallis one-way analyses of variance (ANOVA) on ranks followed by Dunn's Multiple comparison test was used instead. Statistical analysis was performed using SigmaPlot (SYSTAT Software, San Jose, CA) or Prism 9 (Version 9.2.0; GraphPad Software, La Jolla, CA). Between-group differences were determined significant if $P < 0.05$. All values are reported as group mean ± standard error of the mean (SEM).

RESULTS

Genetic Reduction of *mHTT* in the Cardiomyocytes

To determine whether genetically reducing *mHTT* expression from the cardiomyocytes would rescue the cardiovascular phenotypes observed in the BACHD mice, we crossed BACHD mice with Myh6-Cre mice and generated the BACHD;Myh6-Cre (BMYO) (**Figure 1**). A significant reduction in the *mHTT* transcript (67% as compared to the BACHD; Tukey: $q = 18.584$, $P < 0.001$) expression levels was observed in the heart of 3 months old BMYO animals compared to the BACHD, but not in the striatum (**Figure 2A**). No differences were found in the expression of the endogenous *Htt* in the heart (**Figure 2B**) between the three mouse groups. Furthermore, we assessed the protein levels of mHTT in whole heart and striatum tissue lysate by using an antibody that recognizes the polyglutamine-expansion (polyQ) present in the pathological form of this protein. Significant differences in the polyQ expression levels were observed among the genotypes, with the BMYO displaying a significant reduction (65% as compared to the BACHD; Tukey: $q = 20.801$, $P < 0.001$; **Figures 2C,D**). In the BMYO striatum, the polyQ expression was unaffected and at a similar expression level as in the BACHD (**Figure 2C**). In summary, the BMYO hearts displayed a significant reduction in mHtt at both the transcript and protein level as compared to the BACHD hearts, while its expression was not affected in the striatum.

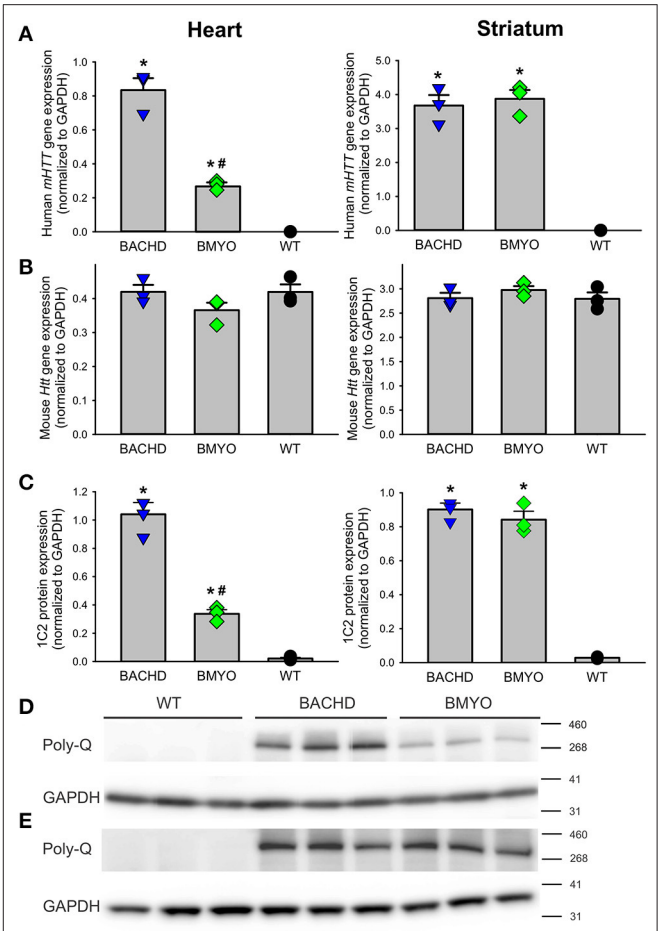

FIGURE 2 | Genetic targeting of human mHTT elicits a selective reduction of its transcript and protein expression levels in the heart, but not the striatum of 3 months old BMYO mice. **(A)** Quantification of the human *mHTT* transcript levels in the heart and striatum shows a significant and selective decrease in the heart of the BMYO line [$F_{(2,8)} = 125.5$, $P < 0.001$]; while, striatal levels were comparable in the two mutant lines [$F_{(2,8)} = 87.706$, $P < 0.001$]. **(B)** The levels of the endogenous mouse *Htt* were unchanged among genotypes in both the heart [$F_{(2,8)} = 2.045$, $P = 0.210$] and striatum [$F_{(2,8)} = 0.852$, $P = 0.473$]. **(C)** PolyQ protein expression levels were detected in the BACHD heart and were greatly reduced in the BMYO [$F_{(2,8)} = 162.549$, $P < 0.001$], but not in the striatum of both mutants. These data demonstrate that the genetic cross was successful, and the *mHTT* levels were selectively reduced in the BMYO heart, while maintaining "normal" expression in the striatum. Representative immunoblots for the heart **(D)** and **(E)** striatum. Values derived from the densitometric analysis were corrected for the background and normalized to GAPDH. The vertical bar plots represent the group means and SEM. The symbols show the values for each individual animal (n = 3) per genotype. Asterisks represent significant differences ($P < 0.05$) compared to WT while crosshatch indicates significant differences ($P < 0.05$) between BMYO and BACHD. One way ANOVA followed by Tukey's multiple comparison test was used to evaluate the possible significance of the findings.

Genetic Reduction of the *mHTT* Expression Improves Some Cardiovascular Functions in BMYO Mice

The effects elicited by the reduction of *mHTT* on the cardiac structure and functions were assessed by echocardiogram at 3 and 6-month age. Consistent with our earlier data (22) showing little loss of cardiac function in the BACHD mice at 3 months of age, few significant differences could be observed among the three genotypes (**Table 1**). At this age, the Lv of both mutants exhibited an increased mass (not significant), but only the BACHD exhibited a reduced ejection fraction (Tukey: $q = 3.941$, $P = 0.029$) compared to WT. The fractional shortening (FS, %) was just under with the BACHD showing reduced values compared to the BMYO and WT groups. At 6 months of age (**Figure 3**; **Table 1**), the BACHD (Tukey: $q = 5.809$, $P = 0.002$) and the BMYO (Tukey: $q = 3.664$, $P = 0.043$) exhibited significantly enlarged left ventricles compared to WT. Even though such feature clearly was not altered by the removal of the *mHTT*, still it is notable that the mass in the BMYO mass did not further increase compared to the measurement at 3 months old. Most of the other parameters did not vary among the genotypes. On the other hand, the ejection fraction (EF) was significantly reduced compared to WT in the BACHD (Tukey: $q = 4.889$, $P = 0.006$), while did not significantly change in the BMYO. The BMYO mice

TABLE 1 | Echocardiographic parameters in BACHD, BMYO and WT animals at 3 and 6 months of age.

	BACHD	BMYO	WT	Stats
	Mean ± SEM			
Age (months)	**3**	**3**	**3**	
Lv mass (mg)	60 ± 5	63 ± 4	52 ± 4	$F_{(2,23)} = 1.784$; $P = 0.193$
FS (%)	30 ± 2	35 ± 1	37 ± 2	$F_{(2,23)} = 3.186$; $P = 0.062$
E/A	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	$F_{(2,23)} = 0.027$; $P = 0.973$
Lv EF	63 ± 2	68 ± 1	71 ± 3	**$F_{(2,23)} = 3.952$; $P = 0.035$**
Age (months)	**6**	**6**	**6**	
Lv mass (mg)	69 ± 3	64 ± 2	55 ± 2	**$F_{(2,23)} = 5.210$; $P = 0.015$**
FS (%)	28 ± 2	32 ± 1	34 ± 1	$F_{(2,23)} = 3.148$; $P = 0.064$
E/A	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	$F_{(2,23)} = 0.027$; $P = 0.973$
Lv EF	63 ± 2	69 ± 2	71 ± 2	**$F_{(2,23)} = 6.689$; $P = 0.006$**

Two-dimensional, M-mode echocardiography and spectral Doppler images enabled measurement of heart dimension and function including Left ventricle mass (Lv mass), ratio of the early (E) to late (A) ventricular filling velocities (E/A ratio), fractional shortening (FS%), Lv Ejection Fraction (Lv EF). For these data and those shown in the other tables, one-way ANOVA was used to evaluate the possible significance of the findings. If normality or equal variance tests failed, then a Kruskal-Wallis one way ANOVA on ranks was used instead. Bold values indicate statistically significant genotypic differences. N = 7–8 per genotype, all males.

display a heart functionality closer to the WT. Overall, at the two ages points examined, most of the echocardiogram parameters did not vary significantly. The measure that appeared to be the most sensitive to the levels of *mHTT* in the cardiomyocytes was the EF, which refers to the percentage of blood that is pumped out of a filled ventricle with each heartbeat.

The Reduction of mHTT Impacted Gene Expression for Cardiovascular Disease Markers Within the BMYO Hearts

Along with abnormal echocardiogram parameters, a pathophysiological feature of the middle-aged (>12 months) BACHD hearts is to display extensive fibrotic tissue (22); however, it is unknown how early such process presents. Thus, the levels of transcripts known to be specific markers for cardiovascular disease were analyzed in the mutant hearts at 6 months of age (**Figure 4**; **Table 2**). The expression levels of the marker for cardiac fibrosis collagen 1a (*Col1a*) were found significantly augmented in the BACHD hearts as compared to the WT (Tukey: $q = 4.710$, $P = 0.03$), while the BMYO showed no significant difference from the other groups (**Figure 4A**; **Table 2**). The ventricular/slow myosin heavy chain isoform (*Myh7*), a gene associated with increased fibrosis and hypertrophic cardiomyopathy (36), showed a trend to increase in the BACHD (**Figure 4B**), with no significant changes in the BMYO in comparison to the WT. It is well established that the expression of genes involved in cardiomyocyte development is up-regulated in response to injury or myocardial remodeling in response to physiological and pathological events (e.g. 32–34). We found a significant increase in the expression of the GATA binding protein 4 (*Gata4*) in the hearts of the BACHD (Dunn's Method: Q = 2.697, $P = 0.021$ vs. WT) which was absent in the BMYO line (**Figure 4C**; **Table 2**). In addition, the BACHD mice

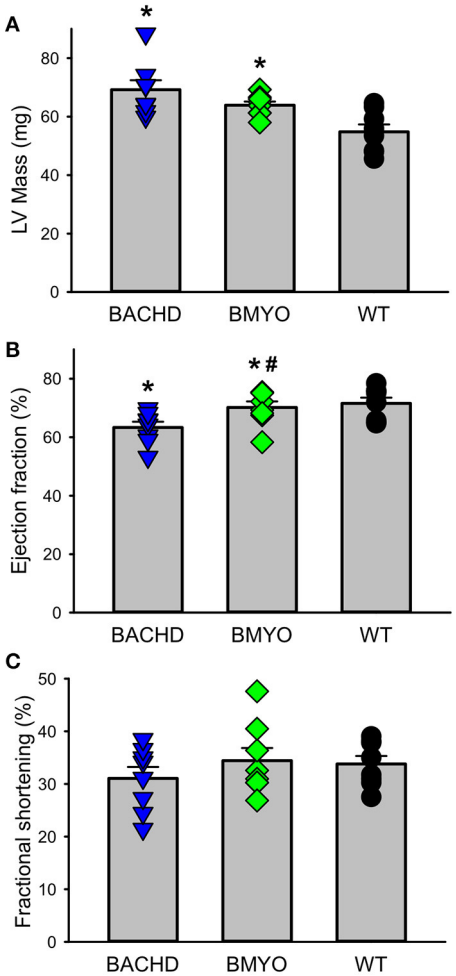

FIGURE 3 | Echocardiographic measurements of BACHD, BMYO, and WT animals at 6 months of age. Two-dimensional, M-mode echocardiography and spectral Doppler images enabled measurement of heart dimension and function including Left ventricle mass (LV mass), fractional shortening (FS%), Lv Ejection Fraction (Lv EF), and heart rate. Most parameters did not vary between the genotypes (**Table 1**). **(A)** Lv mass was increased in both the BACHD and BMYO mice compared to WT controls, while **(B)** the ejection fraction was reduced in BACHD compared to BMYO and WT hearts. **(C)** The fractional shortening followed a similar pattern although the differences were not significant by our criterion. The selective reduction of mHtt from the heart did not reduce the hypertrophy in the BMYO heart but seemed to improve the ejection fraction. The vertical bar plots show group means and SEM. The symbols show the values from individual animals ($n = 7$–8 per genotype). Asterisks represent significant differences ($P < 0.05$) compared to WT controls while crosshatch indicates significant differences ($P < 0.05$) between BMYO and BACHD. One way ANOVA followed by Tukey's multiple comparison test was used to evaluate the possible significance of the findings (see **Table 1**).

exhibited elevated transcript levels of brain natriuretic peptide (*Bnp*, **Figure 4D**; **Table 2**; Dunn's Method: Q = 2.562, $P = 0.031$ vs. WT), a hormone secreted by cardiomyocytes in the ventricles in response to stretching. Again, the BMYO line showed a much lower expression. In agreement, increased Collagen I immunoreactivity could be observed in mid-ventricular coronal sections of 6 months of age BACHD, in particular along the septal and Rv walls, with the BMYO hearts showing a much lower

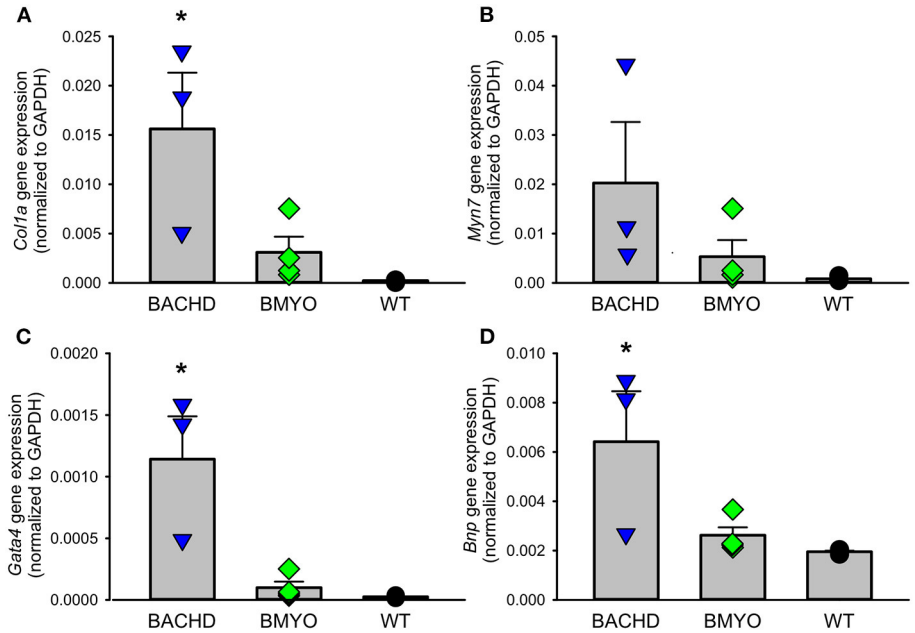

FIGURE 4 | Gene expression levels of molecular markers for cardiovascular injury and repair were significantly lowered by the specific removal of *mHtt* from the heart. **(A)** The expression levels of collagen 1a (*Col1a*) were significantly lower in the BMYO animals compared to the BACHD, while **(B)** the expression of ventricular/slow myosin heavy chain isoform (*Myh7*) did not significantly change. **(C)** GATA binding protein 4 (*GATA4*) and **(D)** brain natriuretic peptide (*BNP*) were both significantly diminished in the BMYO heart at 6 months of age as compared to BACHD (see also **Table 2** for values derived from the statistical analyses). Together these data suggest that changes in molecular markers of CV injury were reversed by selective removal of the *mHTT* in the BMYO line. The vertical bar plots show group means and SEM. The symbols show the values from individual animals (n = 3–4 per genotype). Asterisks represent significant differences ($P < 0.05$) compared to WT controls. One way ANOVA followed by Tukey's multiple comparison test was used to evaluate the possible significance of the findings. If normality or equal variance tests failed, then Kruskal-Wallis one-way ANOVA on ranks followed by Dunn's multiple comparison test was used instead (see also **Table 2**).

TABLE 2 | expression levels of selected genes in the hearts of BACHD, BMYO and WT animals at 6 months of age.

Gene	Gene ID	BACHD	BMYO	WT	Stats
		mean ± SEM (normalized to Gapdh)			
Col1a	12842	0.0156 ± 0.005	0.0031 ± 0.003	0.0002 ± 0.0001	**$F_{(2,9)} = 6.410$; $P = 0.026$**
Myh7	140781	0.020 ± 0.012	0.005 ± 0.003	0.001 ± 0.0003	$F_{(2,9)} = 2.095$; $P = 0.194$
Gata4	14463	0.0011 ± 0.0003	0.00001 ± 0.00004	0.00002 ± 0.000006	**$H = 7.318$; $P = 0.004$**
Bnp	18158	0.006 ± 0.002	0.003 ± 0.0003	0.002 ± 0.0003	**$H = 6.745$; $P = 0.010$**

Primers targeting the collagen 1a (Col1a); the ventricular/slow myosin heavy chain isoform (Myh7); the GATA binding protein 4 (Gata4), and the brain natriuretic peptide (Bnp) were used. For each gene, the average values from three technical replicates per sample were normalized to those obtained for Gapdh in the same sample. Bold values indicate statistically significant genotypic differences. N = 3–4 per genotype, all males.

staining (**Figure 5A**). Finally, the protein levels of fibronectin were significantly elevated in the BACHD heart in comparison to the WT ($H = 6.003$; $P = 0.042$, Kruskal-Wallis one-way ANOVA on ranks followed by Dunn's multiple comparison test, $P = 0.045$), and consistently reduced in the BMYO heart lysates (**Figure 5B**). Altogether these findings suggest that the BACHD hearts are under stress and such changes appear to be prevented by the selective deletion of the human *mHTT* from the cardiomyocytes of the BMYO mice.

Reduction of *mHTT* Lessens Fibrosis

As mentioned above, we have previously reported that 12 months old BACHD mice (22) present with an enlarged and fibrotic heart. Hence, in the present study, Masson's Trichrome staining was used to investigate the presence of fibrotic tissue, as well as cyto-histomorphological abnormalities in 6 months old BACHD and BMYO hearts. Whilst fibrotic staining was almost undetectable in the WT hearts (1/3 mice), all the BACHD hearts (3/3 mice) exhibited some pockets of fibrosis of variable extension located mostly in correspondence of the septal parts of the Rv wall (**Figures 6B,C**). The BMYO hearts (3/3 mice) appeared to have fewer and quite small fibrotic areas compared to what observed in the BACHD (**Table 3**).

To further assess possible morphological variations, including changes in cell numbers or cell morphology, which could account for the enlarged heart observed in BACHD mice, we performed a

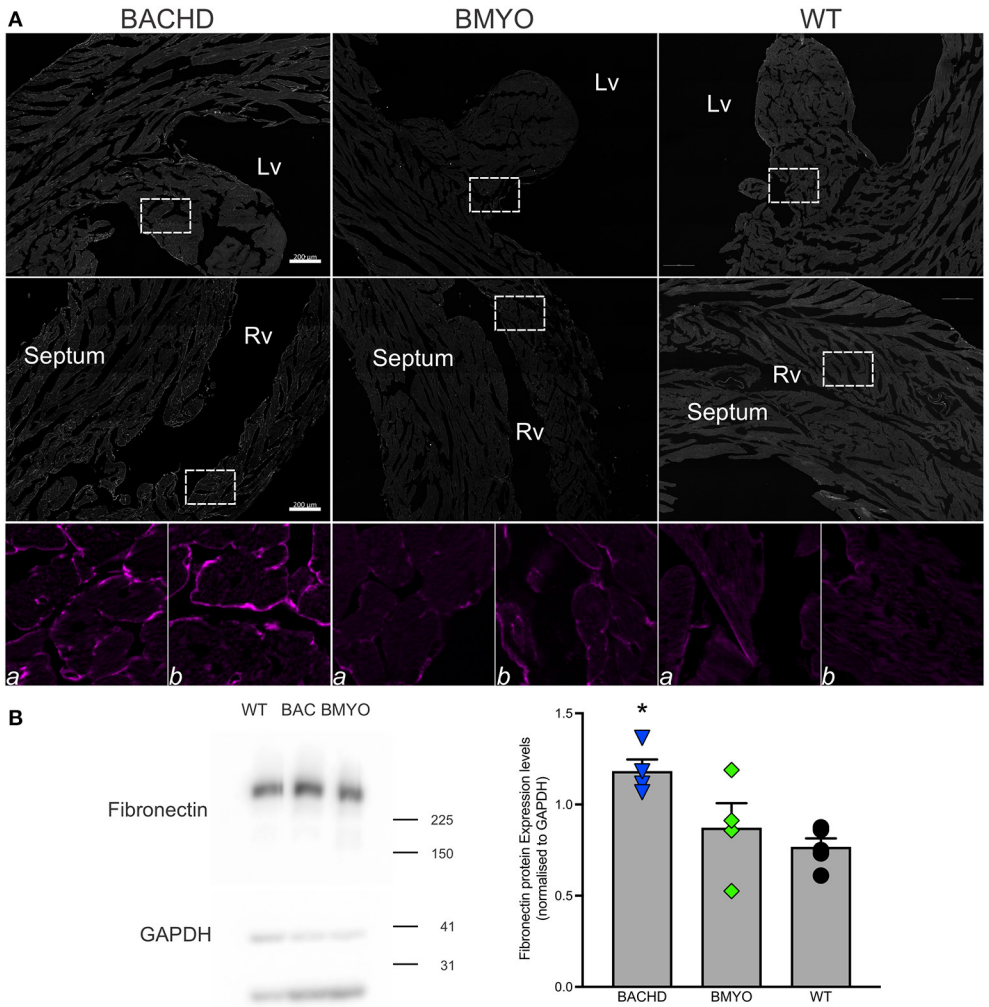

FIGURE 5 | The expression of fibrotic markers was diminished by the selective removal of human *mHTT* from the cardiomyocytes. **(A)** Representative images of immunostained mid-ventricular coronal heart sections showing stronger and diffuse expression of Collagen I in 6 months-old BACHD, but not in age-matched BMYO and WT mice ($n = 2$ per genotype). The white rectangles delineate the areas where the enlarged images were obtained: (a) left ventricular wall-papillary muscle; (b) right ventricular wall. Intense collagen I immunoreactivity can be observed around the cardiomyocytes in the BACHD heart (left panels), but at a lower level in the BMYO and WT (middle and right panels, respectively). Lv, left ventricle; Rv, right ventricle. Scale bars = 200 μm. **(B)** Representative western blotting of whole-tissue lysates of hearts from 6 months-old BACHD, BMYO and WT mice. The total protein levels of fibronectin displayed a significant increase in the BACHD mice as compared to WT. Each value derived from the densitometric analysis was corrected for background, and then divided by the sum of the background-corrected signals obtained in the same immunoblot. These values were then normalized to the respective relative GAPDH levels, which were corrected in the same manner. The vertical bar plots represent the group means and SEM. The symbols show the values for each individual animal (n = 4–5) per genotype. Kruskal-Wallis one-way ANOVA on ranks ($H = 6.003$; $P = 0.042$) followed by Dunn's multiple comparison test ($P = 0.045$) was used to evaluate the possible significance of the findings. Asterisks represent significant differences ($P < 0.05$) compared to WT mice.

series of measurements. Albeit not significant, the BACHD hearts appeared enlarged while the BMYO hearts were sized similarly to WT (**Table 4**). Whilst no differences were observed in the Lv of the three genotypes, strikingly, the Rv of the BACHD, but not of the BMYO, displayed a thinner wall and an enlarged lumen (**Figure 6A**; **Table 4**). The cross-sectional area or any other cytological measurement were not different among the three groups; however, an increased number of cardiomyocytes was observed in the Rv of the BACHD (**Table 4**). Hence, the BACHD mice exhibited cyto-histopathological changes also early in disease progressions, with a larger and thinner heart compared to the BMYO as well as the WT mice. Thus, genetically removing the *mHTT* from the cardiomyocytes may improve the defects observed in the BACHD hearts.

Reduction of *mHTT* in the Heart Improved Some Aspects of Motor Behavior

There is compelling evidence that a reduction in physical activity is a sensitive biomarker for chronic CV disease (37, 38). Therefore, in our final experiment, we sought to determine whether the genetic reduction of *mHTT* expression in the

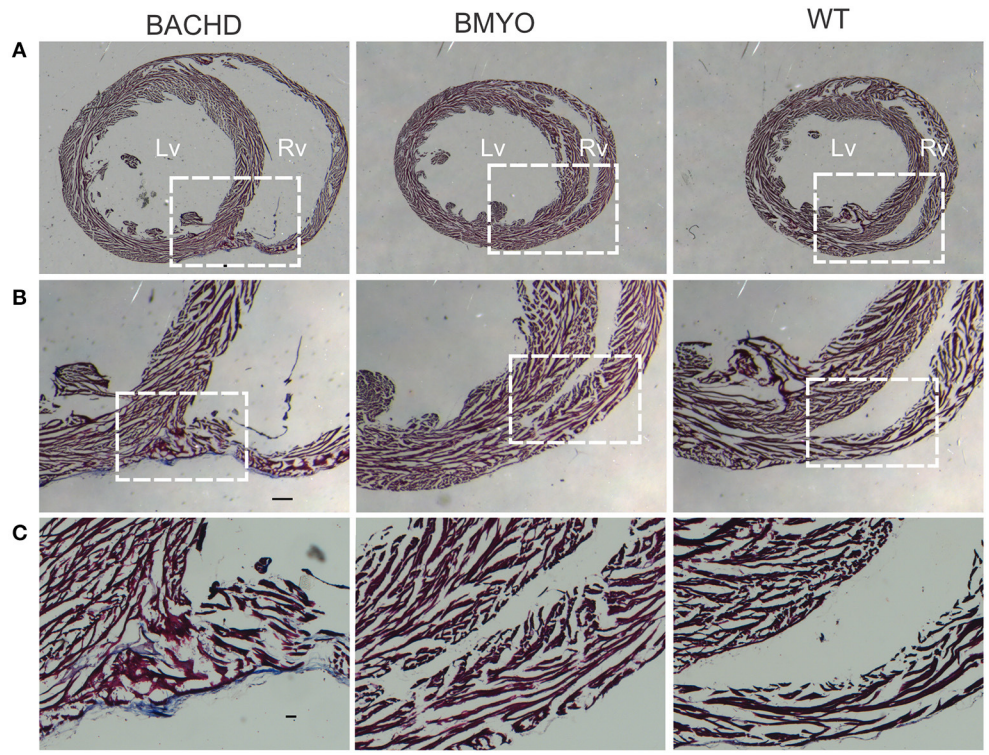

FIGURE 6 | Selective removal of the human *mHtt* lessened the fibrosis in the hearts of 6 months old BMYO mice. Representative photomicrographs of mid-ventricular heart sections stained with Masson's trichrome at different magnifications. For all three genotypes, a representative stained section is shown at three progressively higher magnifications. **(A)** Lower magnification images showing the enlarged BACHD heart, such phenotype was less evident in the BMYO mice (scale bar = 200 μm). The white rectangles delineate the areas where the higher magnification images were acquired. **(B,C)** Higher magnification images showing pockets of fibrotic tissue of variable extension in the septal part of the right ventricular wall of BACHD hearts. Some fibrotic areas could be also seen along the outer surface of the ventricular walls near the interventricular septum. Conversely, such areas were qualitatively judged to be almost inexistent in the BMYO (**Table 3**). At this age, WT hearts did not show strong evidence of fibrosis. Scale bars = 100 and 50 μm in the middle and lower panels, respectively. $n = 3$ per genotype. Lv, left ventricle; Rv, right ventricle.

TABLE 3 | Masson's Trichrome staining was used to investigate the presence of fibrotic tissue in heart sections from WT, BACHD and BMYO mice at 6 months of age.

	Lv wall and Papillary muscles	Septum	Rv wall
BACHD	+	++	++
BACHD	+	++	++
BACHD	+	++	++
BMYO	+	+	+
BMYO	−−	+	+
BMYO	+	+	++
WT	−−	−−	−−
WT	−−	−−	−−
WT	+	+	+

Sections (3/animal) at the mid-ventricular level were chosen based on the visual presence of the papillary muscles in the left ventricle (Lv). Two observers independently evaluated the presence and degree of fibrotic staining in the left ventricular wall and papillary muscles, right ventricular wall (Rv) as well as in the interventricular septum. The degree of fibrotic staining was classified as moderate (++), weak (+), or none (−−) in 3 sections/animal; n = 3 animals of each genotype.

cardiomyocytes had beneficial impact on motor function in the BACHD mice using two measures of motor function: grip strength and overall physical activity in the 24-hr cycle (**Figure 7**; **Table 5**). Grip strength was improved in the 6 months old BMYO mice compared to BACHD alone (**Figure 7A**; Holm-Sidak: $t = 3.262$, $P = 0.011$ BACHD vs. WT). In addition, both BACHD and BMYO exhibited a significant reduction in activity over 24 h compared to WT (Tukey: BACHD, $q = 8.850$, $P < 0.001$; BMYO, $q = 6.613$, $P < 0.001$), although the BMYO mice still exhibited more activity than the BACHD (**Figure 7B**). The strength of the daily rhythms as measured by power of the periodograms was similar in both mutant lines, so the rhythmicity of the activity data was not impacted by the reduction in *mHTT* in the heart (**Figure 7C**). BACHD mice have been shown to be more active in the day, during their normal rest time than WT (32, 39). Unexpectedly, such inappropriate activity in the day was improved in the BMYO line compared to BACHD (**Figure 7D**; Holm-Sidak: $t = 3.348$, $P = 0.007$). Overall, the performance of the mice on two motor tasks (grip strength, total activity) was improved by reducing *mHTT* in the heart.

DISCUSSION

Both clinical and preclinical research indicate that cardiovascular dysfunction should be considered a core symptom of at least

TABLE 4 | Gross morphological measurements were performed in heart coronal sections from WT, BACHD and BMYO mice at 6 months of age.

	BACHD	BMYO	WT	Stats
Whole heart				
Cross sectional area (mm^2)	36.9 ± 4.31	33.0 ± 1.40	32.5 ± 2.37	$H = 0.932$; $P = 0.667$
Circumference (mm)	22.3 ± 1.92	20.8 ± 0.75	20.7 ± 0.77	$H = 0.800$; $P = 0.721$
Left ventricle				
Wall thickness (mm)	0.88± 0.01	0.80 ± 0.06	0.87 ± 0.01	$H = 0.621$; $P = 0.829$
Lumen cross-sectional area (mm^2)	11.5 ± 1.65	12.6 ± 1.20	11.1 ± 0.51	$H = 1.867$; $P = 0.438$
Lumen perimeter (mm)	17.4 ± 1.48	17.6 ± 0.72	15.3 ± 1.34	$H = 1.689$; $P = 0.511$
Lumen diameter (mm)	4.52 ± 0.23	4.73 ± 0.25	4.42 ± 0.10	$H = 1.156$; $P = 0.629$
Average Cell number/μm^2	0.0029 ± 0.00027	0.0033 ± 0.00009	0.0032 ± 0.00018	$H = 1.867$; $P = 0.439$
Total Cell number/μm^2	0.020 ± 0.004	0.026 ± 0.006	0.024 ± 0.001	$H = 0.622$; $P = 0.829$
Cell cross-sectional area (μm^2)	99.9 ± 3.55	95.0 ± 2.56	123.4 ± 12.7	$H = 5.067$; $P = 0.086$
Cell diameter (μm)	4.83 ± 0.09	4.55 ± 0.03	5.30 ± 0.29	**$H = 5.956$; $P = 0.025$**
Cell perimeter (μm)	13.7 ± 0.38	13.3 ± 0.35	16.2 ± 0.92	**$H = 5.956$; $P = 0.025$**
Septum				
Wall thickness (mm)	0.81 ± 0.07	0.71 ± 0.01	0.80 ± 0.06	$H = 1.689$; $P = 0.511$
Average Cell number/μm^2	0.0033 ± 0.00013	0.0034 ± 0.00006	0.0035 ± 0.00018	$H = 0.356$; $P = 0.879$
Total cell number/μm^2	0.020 ± 0.004	0.026 ± 0.006	0.024 ± 0.001	$H = 0.622$; $P = 0.829$
Cell cross-sectional area (μm^2)	108.8 ± 3.06	108.2 ± 4.18	110.5 ± 3.44	$H = 0.089$; $P = 0.993$
Cell diameter (μm)	16.2 ± 0.35	16.2 ± 0.75	16.6 ± 0.18	$H = 1.689$; $P = 0.511$
Cell perimeter (μm)	44.6 ± 0.60	44.6 ± 0.61	45.2 ± 0.19	$H = 0.267$; $P = 0.929$
Right ventricle				
Wall thickness (mm)	0.31 ± 0.04	0.40 ± 0.04	0.42 ± 0.08	$H = 2.489$; $P = 0.338$
Lumen cross-sectional area (mm^2)	6.61 ± 2.85	3.12 ± 1.75	3.09 ± 1.97	$H = 1.689$; $P = 0.511$
Lumen perimeter (mm)	17.9 ± 2.28	14.8 ± 0.81	13.6 ± 1.84	$H = 2.222$; $P = 0.381$
Average cell number/μm^2	0.0033 ± 0.00018	0.0029 ± 0.00004	0.0028 ± 0.00003	**$H = 5.535$; $P = 0.050$**
Total Cell number/μm^2	0.025 ± 0.008	0.022 ± 0.003	0.020 ± 0.003	$H = 0.157$; $P = 0.950$
Cell cross-sectional area (μm^2)	108.9 ± 1.20	108.1 ± 8.26	98.7 ± 14.7	$H = 0.267$; $P = 0.929$
Cell diameter (μm)	16.7 ± 0.45	15.9 ± 1.05	15.7 ± 0.71	$H = 1.156$; $P = 0.629$
Cell perimeter (μm)	44.7 ± 1.08	43.2 ± 2.12	42.9 ± 2.28	$H = 0.267$; $P = 0.929$

Sections (2/animal) at the mid-ventricular level were chosen based on the visual presence of papillary muscles in the left ventricle (Lv). Since the left and right ventricles (Rv) were not perfectly round, we refer to the length of the lumen as perimeter. Cells were counted in 2-3 ROI of equal size (153.21 μm ×153.21 μm)/image and summed. The sums from 10-15 images (Lv) or 5-10 images (Rv and Septum)/animal were averaged and then divided by the ROI total area. Values are presented as the Mean ± SEM, statistical differences were determined using the Kruskal-Wallis one way ANOVA on ranks followed by Dunn's multiple comparisons test. Bold values indicate statistically significant genotypic differences. n=3 animals per genotype, all males.

in a subset of HD patients (9, 12). Mutant huntingtin (*mHTT*) is ubiquitously expressed so the cardiomyopathy could be the result of deficits in the cardiomyocytes themselves and/or in the dysautonomia driven by the nervous system. In this study, we utilized the BACHD mouse model that allows specific reduction of the *mHTT* expression in the cardiomyocytes when crossed with the *Myh6-cre* mouse line (**Figure 1**). As shown in **Figure 2**, we achieved a successful reduction in the *mHTT* mRNA and protein levels in the BMYO hearts. The cre-driven recombination would have occurred early in development so that the BMYO mice that we evaluated would have had greatly reduced mHtt from the heart with still high levels of *mHTT* present in the brain. This genetic construct provides a unique model to evaluate the contribution of the *mHTT* in the cardiomyocytes to the CV disease associated with HD.

We examined the CV function and structure using echocardiograms in all three genotypes at 6 months of age. Most of the functional echo parameters did not vary between the genotypes (**Table 1**). There were two notable exceptions. As previously observed (22), the LV mass of the BACHD hearts was larger than WT (**Figure 3A**). Hypertrophy is an unusual feature of the BACHD model and contrasts with the reduction in size seen in other models (18, 21, 23, 24). The divergence between the genotypes was already seen at 3 months but was not significant until 6 months of age. This hypertrophy was also observed in the BMYO hearts suggesting that the driver was not the *mHTT* in the cardiomyocytes. In addition, LV ejection fraction (EF) was reduced in the BACHD while the EF of the BMYO heart was comparable to WT levels (**Figure 3B**). The EF refers to the percentage of blood that is pumped out of a filled ventricle with each heartbeat. It is the relation between the amounts of blood expelled during each cardiac cycle relative to the size of the ventricle. It is important to note that an LV EF of 55% or higher is considered normal under physiologic loading conditions, with an EF of 50% or lower being considered reduced. Thus, the BACHD hearts were still working at functional levels at

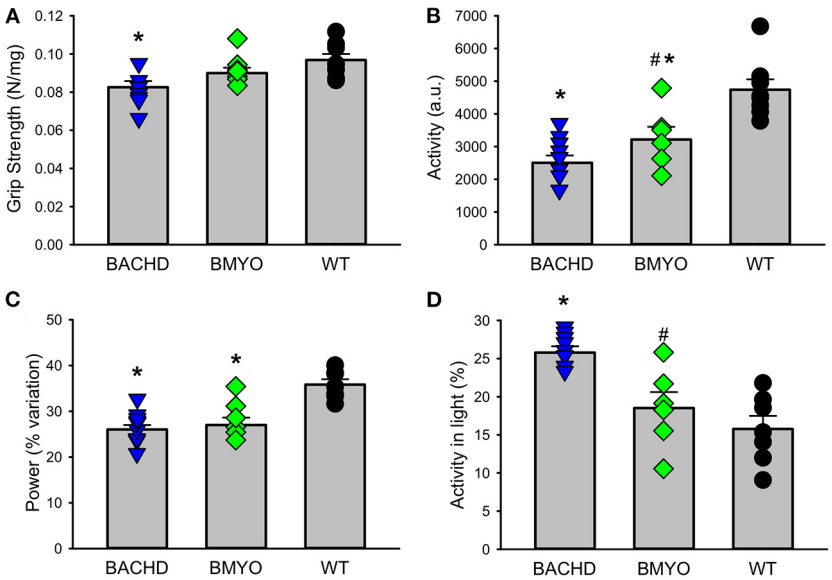

FIGURE 7 | Behavioral biomarkers of cardiovascular function were improved in the BMYO line. Behavior measures including grip strength, and diurnal rhythms of activity of the three lines were measured between 6 and 7 months of age. **(A)** Grip strength was reduced in the BACHD compared to BMYO and WT lines. **(B)** The average total activity over 24 h as reduced in both the BACHD and BMYO compared to WT. **(C)** Power, one measure of circadian rhythmicity, was reduced in both the BACHD and BMYO compared to WT controls. **(D)** The % of activity measured in the light was reduced in the BMYO. The vertical bar plots show group means and SEM. The symbols show the values from individual animals (n = 8 per genotype) in each group. Asterisks represent significant differences ($P < 0.05$) compared to WT mice while crosshatch indicates significant differences ($P < 0.05$) between BMYO and BACHD mice. One way ANOVA followed by Holm-Sidak or Tukey's multiple comparison test was used to evaluate the possible significance of the findings. If normality or equal variance tests failed, then Kruskal-Wallis one way ANOVA on ranks followed by Dunn's multiple comparison test was used instead (see **Table 5**).

TABLE 5 | Behavioral measurements of motor function from BACHD, BMYO and WT animals at 6 months of age.

	BACHD	BMYO	WT	Stats
		Mean ± SEM		
Body weight	25.1 ± 0.7	25.4 ± 2.4	26.7 ± 1.7	$F_{(2,23)} = 0.245$; $P = 0.785$
Grip strength (N/g)	0.083 ± 0.003	0.090 ± 0.002	0.097 ± 0.003	**$F_{(2,23)} = 5.323$; $P = 0.013$**
Activity (a.u)/ 24 h	2504 ± 224	2884 ± 227	4741 ± 317	**$F_{(2,23)} = 21.440$; $P < 0.001$**
Power (% variation)	26.0 ± 0.9	27.0 ± 1.9	35.8 ± 1.1	**$F_{(2,23)} = 17.053$; $P < 0.001$**
Onset (ZT)	12.39 ± 0.19	11.87 ± 0.16	11.93 ± 0.09	$F_{(2,23)} = 3.496$; $P = 0.052$
Activity in light (%)	25.8 ± 0.9	18.5 ± 1.9	15.8 ± 1.7	**$F_{(2,23)} = 12.451$; $P < 0.001$**

Maximal forelimb grip strength was measured by a meter during the night and normalize to body weight. Infrared monitors were used to measure total cage activity over a 10-day duration. The average activity levels during a 24-h cycle as shown as a measure of physical activity. The strength of diurnal rhythms in cage activity were assessed using periodogram including power (% variation), onset of activity as well as the % of total activity during the light. Bold values indicate statistically significant genotypic differences. n = 8 per genotype, all males.

the 6 month time point. The findings with LV EF were closest to our predictions for a cardiomyopathy driven by *mHTT* in the heart. Perhaps, we needed to wait to an older age to see more robust differences with the echocardiogram. Prior work indicated that the EF of the BACHD hearts steadily declined with age after the 6 months-time point (22) so the present measurements of cardiac output were done at the age of peak performance. Reduced cardiac output are observed in other models including the R6/2 (24) and R6/1 (21) and thus appears to be a general feature of the models (see 8 for review). Recent work described electrocardiogram (ECG) abnormalities in the BACHD line (40) and found several abnormalities associated with increased risk of sudden arrhythmic death in humans (41). Taken together, these data suggest that reduced *mHTT* expression in the cardiomyocytes help improve LV EF in the BMYO mice.

In prior work examining the hearts of middle-aged BACHD mice (>12 months), we found striking fibrosis (22). In the present study, *Col1a* mRNA expression in the BMYO hearts was significantly reduced compared to BACHD (**Figure 4A**). Similarly, we found that Collagen I immunoreactivity as well as Fibronectin protein expression was highest in the BACHD and reduced in the BMYO heart (**Figure 5**). In regard to *Mhy7* expression (**Figure 4B**), the trends are consistent with increased

fibrosis as well as an enlarged heart (36, 42, 43) exhibited by the BACHD, and previously described (22). However, the differences were not significant as measured by the one-way ANOVA (**Table 2**) and the power of the performed test (0.173) is below the desired power of 0.800. Already at this early age, we observed the presence of fibrotic regions in the BACHD and BMYO mouse hearts, but not in the WT (**Figure 6**). An increase in fibrosis in the heart could explain the reduction in the EF found in the echo data. Histological studies have suggested that myocardial fibrosis can be an important driver of a reduction in EF (44, 45). We also looked at the expression of genes involved in ventricular remodeling including *Gata4* (**Figure 4C**). These genes are known to be up-regulated in response to injury or cardiomyocyte remodeling (46–48). We found a significant increase in the expression of *Gata4* in the hearts of the BACHD but not in the BMYO line (**Table 2**). Finally, we examined the expression of *Bnp* (**Figure 4D**), which is a hormone secreted by cardiomyocytes in the heart ventricles in response to stretching (49, 50). Again, the BACHD mutants exhibited higher expression while the BMYO line showed a much lower expression. Together these data indicate that, at least transcriptionally, the BACHD hearts were under stress that was reduced in the BMYO line.

Finally, we looked at the impact of the cross on motor behaviors that are thought to be reflective of cardiovascular health. In humans, there is strong evidence that grip strength can provide indications of cardiovascular (mal)function (37, 38, 51). For example, a recent study examined the association of objectively measured grip strength (GS) with incident heart failure (HF) (52). Using the UK Biobank dataset, the authors evaluated data from 374,493 individuals. They found that higher GS was associated with 19% lower incidence of HF risk. While these data are correlational, the literature indicates that objective measurements of physical function (GS) are strongly associated with lower HF incidence. In this study, we found that grip strength was reduced in the BACHD compared to WT and that this measure was improved by the excision of the *mHTT* (**Figure 7A**). Overall, physical activity in this case measured by total activity over 24-hr followed the same pattern (**Figure 7B**). On the other hand, both of the mutant groups exhibited the reduction in the strength of the rhythm as measured by power (**Figure 7C**). Most other rhythmic parameters were not improved by the reduction of the *mHTT* from the heart. The central circadian clock is located in the hypothalamus thus improvements in circadian output were not expected from this cross. One unexpected finding is that the inappropriate day-time activity seen in the BACHD line (32, 39) was improved by the cross (**Figure 7D**). Taken at face value, this result suggests that some of the negative circadian phenotype seen in HD models could be a reflection of compromised CV function. This issue deserves further study.

We were forced to bring this study to a premature closure because of COVID-19. Due to the research stoppage, this work presents several weaknesses. First, while the study was originally designed to longitudinally follow the mice until 12 months of age, we had to terminate the experiments at 6 months. Therefore, we were only able to investigate the role of *mHTT* at early stages of disease progression and, based on our prior work (22), we expected more dramatic effects and differences among genotypes at a later age. In addition, as our colony had to be culled, the sample size for some of the measurements and assays is limited to 3–4 mice per genotype, about half of our original intent. Although we previously showed sex difference in the BACHD mice (53), with the female presenting less severe symptoms at early stages of disease, another limitation of the present study is the usage of only male mice. Future work will need to determine whether the reduction in *mHTT* in the cardiomyocytes differentially affects male and female mice at later stages of the disease, and may ameliorate mitochondrial dysfunction, protein aggregation, ER stress, and/or facilitates autophagy. In the present study, we were only able to interrogate a limited number of pathways. Obviously, because of all the above mentioned limitations, caution should be exerted when interpreting our findings.

In summary, the clinical and preclinical research indicate that cardiovascular dysfunction should be considered a core symptom of at least a subset of HD patients. As established by prior work, there is strong evidence for dysautonomia that can be detected early in the HD progression. In addition, there is evidence for cardiomyopathy and our results suggest that there is cardiac specific pathology in the BACHD pre-clinical model. Reducing *mHTT* specifically from cardiomyocytes did improve the LV EF as measured by the echocardiogram, reduced the expression of several markers of heart disease, and improved a behavioral marker of cardiovascular health. We believe that these findings argue that cardiomyopathy should be considered as part of the consequences of the HD mutation in clinical practice.

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 animal study was reviewed and approved by UCLA Animal Research Committee.

AUTHOR CONTRIBUTIONS

SP, CG, and CC conceived the hypothesis and experimental design of this study. SP, SL, RB, DW, EC, and MJ performed the experiments. SP, KR, CG, and CC analyzed the data. SP wrote for the first draft. CG and CC edited, wrote, and compiled the final version paper with contribution of the other authors. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Ross CA, Aylward EH, Wild EJ, Langbehn DR, Long JD, Warner JH, et al. Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat. Rev. Neurol.* (2014) 10:204–16. doi: 10.1038/nrneurol.2014.24
2. Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR. Huntington disease. *Nat Rev Dis Primers.* (2015) 1:15005. doi: 10.1038/nrdp.2015.5
3. Saft C, Zange J, Andrich J, Müller K, Lindenberg K, Landwehrmeyer B. (2005) Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. *Mov Disord.* (2005) 20:674–9. doi: 10.1002/mds.20373
4. Ciammola A, Sassone J, Alberti L, Meola G, Mancinelli E, Russo MA. Increased apoptosis, huntingtin inclusions and altered differentiation in muscle cell cultures from Huntington's disease subjects. *Cell Death Differ.* (2006) 13:2068–78. doi: 10.1038/sj.cdd.4401967
5. Saudou F, Humbert S. The biology of Huntingtin. *Neuron.* (2016) 89:910–26. doi: 10.1016/j.neuron.2016.02.003
6. Lanska DJ, Lavine L, Lanska MJ, Schoenberg BS. Huntington's disease mortality in the United States. *Neurology.* (1988) 38:769–72. doi: 10.1212/WNL.38.5.769
7. Sørensen SA. Causes of death in patients with Huntington's disease and in unaffected first degree relatives. *J Med Genet.* (1992) 29:911–4. doi: 10.1136/jmg.29.12.911
8. Melkani GC. Huntington's disease-induced cardiac disorders affect multiple cellular pathways. *React Oxygen Species*. (2016) 2, 325–338. doi: 10.20455/ros.2016.859
9. Critchley BJ, Isalan M, Mielcarek M. Neuro-cardio mechanisms in Huntington's disease and other neurodegenerative disorders. *Front Physiol.* (2018) 9:559. doi: 10.3389/fphys.2018.00559
10. Goldstein DS. Dysautonomia in Parkinson disease. *Compr Physiol.* (2014) 4:805–26. doi: 10.1002/cphy.c130026
11. Norcliffe-Kaufmann L, Kaufmann H, Palma JA, Shibao CA, Biaggioni I, Peltier AC. Orthostatic heart rate changes in patients with autonomic failure caused by neurodegenerative synucleinopathies. *Ann Neurol.* (2018) 83:522–31. doi: 10.1002/ana.25170
12. Park S, Colwell CS. Do disruptions in the circadian timing system contribute to autonomic dysfunction in Huntington's disease? *Yale J Biol Med.* (2019) 92:291–303.
13. Strong TV, Tagle DA, Valdes JM, Elmer LW, Boehm K, Swaroop M. Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nat Genet.* (1993) 5:259–65. doi: 10.1038/ng1193-259
14. Pouladi MA, Morton AJ, Hayden MR. Choosing an animal model for the study of Huntington's disease. *Nat Rev Neurosci.* (2013) 14:708–21. doi: 10.1038/nrn3570
15. Farshim PP, Bates GP. Mouse models of huntington's disease. *Methods Mol Biol.* (2018) 1780:97–120. doi: 10.1007/978-1-4939-7825-0_6
16. Mielcarek M, Inuabasi L, Bondulich MK, Muller T, Osborne GF, Franklin SA. Dysfunction of the CNS-heart axis in mouse models of Huntington's disease. *PLoS Genet.* (2014) 10:e1004550. doi: 10.1371/journal.pgen.1004550
17. Schroeder AM, Loh DH, Jordan MC, Roos KP, Colwell CS. Baroreceptor reflex dysfunction in the BACHD mouse model of Huntington's disease. *PLoS Curr.* (2011) 3:RRN1266. doi: 10.1371/currents.RRN1266
18. Cutler TS, Park S, Loh DH, Jordan MC, Yokota T, Roos KP. Neurocardiovascular deficits in the Q175 mouse model of Huntington's disease. *Physiol Rep.* (2017) 5. doi: 10.14814/phy2.13289
19. Shaffer F. McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front Psychol.* (2014) 5:1040. doi: 10.3389/fpsyg.2014.01040
20. Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB. Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality. *J Clin Endocrinol Metab.* (2015) 100:2443–8. doi: 10.1210/jc.2015-1748
21. Kiriazis H, Jennings NL, Davern P, Lambert G, Su Y, Pang T. Neurocardiac dysregulation and neurogenic arrhythmias in a transgenic mouse model of Huntington's disease: neurocardiac phenotype in Huntington's disease mice. *J Physiol.* (2012) 590:5845–60. doi: 10.1113/jphysiol.2012.238113
22. Schroeder AM, Wang HB, Park S, Jordan MC, Gao F, Coppola G. (2016) Cardiac dysfunction in the BACHD mouse model of Huntington's disease. *PLOS One.* (2016) 11:e0147269. doi: 10.1371/journal.pone.0147269
23. Mihm MJ, Amann DM, Schanbacher BL, Altschuld RA, Bauer JA, Hoyt KR. Cardiac dysfunction in the R6/2 mouse model of Huntington's disease. *Neurobiol Dis.* (2007) 25:297–308. doi: 10.1016/j.nbd.2006.09.016
24. Wood NI, Sawiak SJ, Buonincontri G, Niu Y, Kane AD, Carpenter TA. Direct evidence of progressive cardiac dysfunction in a transgenic mouse model of Huntington's disease. *J Huntingtons Dis.* (2012) 1:57–64. doi: 10.3233/JHD-2012-120004
25. Buonincontri G, Wood NI, Puttick SG, Ward AO, Carpenter TA, Sawiak SJ. Right ventricular dysfunction in the R6/2 transgenic mouse model of Huntington's disease is unmasked by dobutamine. *J Huntingtons Dis.* (2014) 3:25–32. doi: 10.3233/JHD-130083
26. Toczek M, Zielonka D, Zukowska P, Marcinkowski JT, Slominska E, Isalan M. An impaired metabolism of nucleotides underpins a novel mechanism of cardiac remodeling leading to Huntington's disease related cardiomyopathy. *Biochem Biophys Acta.* (2016) 1862:2147–57. doi: 10.1016/j.bbadis.2016.08.019
27. Joviano-Santos JV, Santos-Miranda A, Botelho AFM, Jesus DE, Andrade ICG, de Oliveira Barreto JN. Increased oxidative stress and CaMKII activity contribute to electro-mechanical defects in cardiomyocytes from a murine model of Huntington's disease. *FEBS J.* (2019) 286, 110–123. doi: 10.1111/febs.14706
28. Melkani GC, Trujillo AS, Ramos R, Bodmer R, Bernstein SI, Ocorr K. Huntington's disease induced cardiac amyloidosis is reversed by modulating protein folding and oxidative stress pathways in the Drosophila heart. *PLoS Genet.* (2013) 9:e1004024. doi: 10.1371/journal.pgen.1004024
29. Pattison JS, Sanbe A, Maloyan A, Osinska H, Klevitsky R, Robbins J. Cardiomyocyte expression of a polyglutamine preamyloid oligomer causes heart failure. *Circulation.* (2008). 117:2743–51. doi: 10.1161/CIRCULATIONAHA.107.750232
30. Gray M, Shirasaki DI, Cepeda C, André VM, Wilburn B, Lu X-H. Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. *J Neurosci.* (2008) 28:6182–6195. doi: 10.1523/JNEUROSCI.0857-08.2008
31. Wang N, Gray M, Lu XH, Cantle JP, Holley SM, Greiner E. Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of Huntington's disease. *Nat Med.* (2014) 20:536–41. doi: 10.1038/nm.3514
32. Kudo T, Schroeder A, Loh DH, Kuljis D, Jordan MC, Roos KP, et al. Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. *Exp Neurol.* (2011) 228:80–90. doi: 10.1016/j.expneurol.2010.12.011
33. Ghiani CA, Mattan NS, Nobuta H, Malvar JS, Boles J, Ross MG. Early effects of lipopolysaccharide-induced inflammation on fetal brain development in rat. *ASN Neuro.* (2011) 3:e00068. doi: 10.1042/AN20110027
34. Wang H-B, Whittaker DS, Truong D, Mulji AK, Ghiani CA, Loh DH. Blue light therapy improves circadian dysfunction as well as motor symptoms in two mouse models of Huntington's disease. *Neurobiol Sleep Circad Rhythms.* (2017) 2:39–52. doi: 10.1016/j.nbscr.2016.12.002
35. Loh DH, Jami SA, Flores RE, Truong D, Ghiani CA, O'Dell TJ, et al., (2015). Misaligned feeding impairs memories. *Elife* 4:e09460. doi: 10.7554/eLife.09460
36. Pandya K, Kim HS, Smithies O. Fibrosis, not cell size, delineates beta-myosin heavy chain reexpression during cardiac hypertrophy and normal aging *in vivo*. *Proc Natl Acad Sci USA.* (2006) 103:16864–9. doi: 10.1073/pnas.0607700103
37. Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J. Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. *BMJ.* (2018) 361:k1651.
38. Bohannon RW. Grip strength: an indispensable biomarker for older adults. *Clin Interv Aging.* (2019) 14:1681–91. doi: 10.2147/CIA.S194543
39. Whittaker DS, Loh DH, Wang H-B, Tahara Y, Kuljis D, Cutler T. Circadian-based treatment strategy effective in the BACHD mouse model of Huntington's disease. *J Biol Rhythms.* (2018) 33, 535–54. doi: 10.1177/0748730418790401

40. Zhu Y, Shamblin I, Rodriguez E, Salzer GE, Araysi L, Margolies KA. Progressive cardiac arrhythmias and ECG abnormalities in the Huntington's disease BACHD mouse model. *Hum Mol Genet.* (2020) 29:369–81. doi: 10.1093/hmg/ddz295
41. Stephen CD, Hung J, Schifitto G, Hersch SM, Rosas HD. Electrocardiogram abnormalities suggest aberrant cardiac conduction in Huntington's disease. *Mov Disord Clin Pract.* (2018) 5:306–11. doi: 10.1002/mdc3.12596
42. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circ Res.* (2000) 86:386–39. doi: 10.1161/01.RES.86.4.386
43. Pandya K, Smithies O. β-MyHC and cardiac hypertrophy: size does matter. *Circ Res.* (2011) 109:609–10. doi: 10.1161/CIRCRESAHA.111.252619
44. Krayenbuehl HP, Hess OM, Monrad ES, Schneider J, Mall G, Turina M. Left ventricular myocardial structure in aortic valve disease before, intermediate, and late after aortic valve replacement. *Circulation.* (1989) 79:744–55. doi: 10.1161/01.CIR.79.4.744
45. Hein S, Arnon E, Kostin S, Schönburg M, Elsässer A, Polyakova V. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation.* (2003) 107:984–91. doi: 10.1161/01.CIR.0000051865.66123.B7
46. Tomita-Mitchell A, Stamm KD, Mahnke DK, Kim M-S, Hidestrand PM, Liang HL. Impact of MYH6 variants in hypoplastic left heart syndrome. *Physiol Genomics.* (2016) 48:912–1. doi: 10.1152/physiolgenomics.00091.2016
47. Hayashi K, Teramoto R, Nomura A, Asano Y, Beerens M, Kurata Y. Impact of functional studies on exome sequence variant interpretation in early-onset cardiac conduction system diseases. *Cardiovasc Res.* (2020). 116:2116–30. doi: 10.1093/cvr/cvaa010
48. Whitcomb J, Gharibeh L, Nemer M. From embryogenesis to adulthood: critical role for GATA factors in heart development and function. *IUBMB Life.* (2020) 72:53–67. doi: 10.1002/iub.2163
49. Cao Z, Jia Y, Zhu B. BNP and NT-proBNP as diagnostic biomarkers for cardiac dysfunction in both clinical and forensic medicine. *Int J Mol Sci.* (2019) 20:1820. doi: 10.3390/ijms20081820
50. Matsuo A, Nagai-Okatani C, Nishigori M, Kangawa K, Minamino N. Natriuretic peptides in human heart: Novel insight into their molecular forms, functions, and diagnostic use. *Peptides.* (2019) 111:3–17. doi: 10.1016/j.peptides.2018.08.006
51. Gubelmann C, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. *Eur J Prev Cardiol.* (2017) 24:514–21. doi: 10.1177/2047487316680695
52. Sillars A, Celis-Morales CA, Ho FK, Petermann F, Welsh P, Iliodromiti S. Association of fitness and grip strength with heart failure: findings from the uk biobank population-based study. *Mayo Clin Proc.* (2019) 94:2230–40. doi: 10.1016/j.mayocp.2019.04.041
53. Kuljis DA, Gad L, Loh DH, MacDowell Kaswan Z, Hitchcock ON, Ghiani CA, et al. (2016) Sex differences in circadian dysfunction in the bachd mouse model of huntington's disease. *PLoS ONE.* 11:e0147583. doi: 10.1371/journal.pone.0147583

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Effects of Enhanced External Counterpulsation With Different Sequential Levels on Lower Extremity Hemodynamics

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Objective: This study aimed to investigate acute hemodynamics of lower extremities during enhanced external counterpulsation with a three-level sequence at the hips, thighs, and calves (EECP-3), two-level sequence at the hips and thighs (EECP-2), and single leg three-level sequence (EECP-1).

Methods: Twenty healthy volunteers were recruited in this study to receive a 45-min EECP intervention. Blood flow spectrums in the anterior tibial artery, posterior tibial artery, and dorsalis pedis artery were imaged by Color Doppler ultrasound. Mean flow rate (FR), area, pulsatility index (PI), peak systolic velocity (PSV), end-diastolic velocity (EDV), mean flow velocity (MV), and systolic maximum acceleration (CCAs) were sequentially measured and calculated at baseline during EECP-3, EECP-1, and EECP-2.

Results: During EECP-3, PI, PSV, and MV in the anterior tibial artery were significantly higher, while EDV was markedly lower during EECP-1, EECP-2, and baseline (all $P < 0.05$). Additionally, ACCs were significantly elevated during EECP-3 compared with baseline. Moreover, FR in the anterior tibial artery was significantly increased during EECP-3 compared with baseline ($P = 0.048$). During EECP-2, PI and MV in the dorsalis pedis artery were significantly higher and lower than those at baseline, (both $P < 0.05$). In addition, FR was markedly reduced during EECP-2 compared with baseline ($P = 0.028$). During EECP-1, the area was significantly lower, while EDV was markedly higher in the posterior tibial artery than during EECP-1, EECP-2, and baseline (all $P < 0.05$). Meanwhile, FR of the posterior tibial artery was significantly reduced compared with baseline ($P = 0.014$).

Conclusion: Enhanced external counterpulsation with three-level sequence (EECP-3), EECP-2, and EECP-1 induced different hemodynamic responses in the anterior tibial artery, dorsalis pedis artery, and posterior tibial artery, respectively. EECP-3 acutely improved the blood flow, blood flow velocity, and ACCs of the anterior tibial artery.

In addition, EECP-1 and EECP-2 significantly increased the blood flow velocity and peripheral resistance of the inferior knee artery, whereas they markedly reduced blood flow in the posterior tibial artery.

Keywords: enhanced external counterpulsation, lower extremity arteries, blood flow, hemodynamic responses, sequential level

INTRODUCTION

Enhanced external counterpulsation (EECP) is a noninvasive treatment for patients with cardiovascular disease (1–3). Studies have also reported that EECP not only alleviates symptoms of angina and reduces myocardial ischemia (4–6) but that it is also beneficial to peripheral vascular function (7–9). Braith et al. found that EECP significantly increases peripheral artery flow-mediated dilation (FMD) and promotes endothelial-derived vasoactive agents (9). Nichols et al. demonstrated that EECP reduces arterial stiffness and improves wave reflection characteristics (7). Zhang et al. also found that EECP decreases resistance index in the common carotid artery (10). Gurovich et al. demonstrated that EECP-induced blood flow patterns improve endothelial function in peripheral muscular conduit arteries (11). Avery et al. also found that EECP significantly elevates peak limb blood flow and improves endothelium-dependent vasodilation in calf resistance arteries (12).

However, there were some controversial studies. Werner et al. found that EECP significantly reduces flow volumes of the posterior tibial artery (13). Dockery *et al.* demonstrated that EECP cannot reduce arterial stiffness (14). It showed that EECP is unlikely to be influenced by the alterations in mechanical properties of the arterial wall (14). Martin *et al.* reported that EECP does not significantly improve resistance arterial function in the calf (15). Whether EECP may provide vascular medicine to the peripheral arterial tree needed to be verified (12). In addition, Hashemi *et al.* found that endothelial function is not significantly improved after EECP intervention (16). Meanwhile, we found that peripheral hemodynamic responses induced by EECP highlighted personalized plans in patients with different cardiovascular risk factors (10). Furthermore, our study demonstrated that EECP creates different responses of vascular and blood flow characteristics in the carotid and peripheral arteries. More importantly, beneficial effects on the inner diameter, blood flow velocity, resistance index, and blood flow after 45-min-EECP were shown only in patients with coronary artery disease (17).

Based on the above-mentioned issues, Buschmann et al. proposed an improved treatment for patients with peripheral vascular disease (PAD), individual shear rate therapy (ISRT), like EECP-2, which includes two cuffs wrapped around the hip and thighs for ensuring adequate calf perfusion compared to EECP-3 with the three-cuff system (18–20). It is evaluated with real-time Doppler-derived variables of calf perfusion during counterpulsation (18). Studies have reported that ISRT improves endothelial function and increases lower limb walking distance (19). A study showed that ISRT can improve the degree of peripheral arteriosclerosis, increase exercise capacity, and reduce arterial blood pressure (21). However, a study reported that ISRT cannot reduce ankle brachial index and pulse wave velocity (22).

Currently, only few effective treatments for the improvement of lower limb hemodynamics have been proposed. Additionally, their clinical evidence is insufficient, and a related hemodynamic mechanism is not clear (13, 23, 24). Furthermore, to our knowledge, there is no effective treatment for lower limb stenosis in different parts (e.g., anterior tibial artery, posterior tibial artery, or dorsalis pedis artery). However, the pathogenesis of patients with PAD is too complex to investigate detailed hemodynamic changes (e.g., unilateral, bilateral, and multi-vascular). Therefore, based on the aforementioned EECP technology platform, we changed the sequential level, and set the monitoring scheme of EECP with a three-level sequence (EECP-3), the two-level sequence at the hips and thighs (EECP-2), and single leg three-level sequence (EECP-1). The ultrasonic blood flow spectrum of inferior knee arteries (anterior tibial artery, posterior tibial artery, and dorsalis pedis artery) was analyzed during EECP-3, EECP-2, and EECP-1. On the one hand, this study can clarify the acute responses of these treatment schemes on lower extremity hemodynamics. On the other hand, it may provide a theoretical basis of exercise physiology for lower extremity arteriosclerosis, that is, personalized treatment schemes for patients with a different lower limb arterial stenosis.

MATERIALS AND METHODS

Subjects

Twenty young men ($n = 20$), ranging from 24 to 30 years old, were enrolled from the Health Examination Center of the Eighth Affiliated Hospital of Sun Yat-sen University (SYSU). All the participants were healthy and had no cardiovascular disease or related risk factors. Exclusion criteria consisted of exercising three times per week or more, known cardiovascular diseases, and contraindications of EECP and medication. Before the experiment, informed consent forms were signed by all the healthy participants. It was approved by the local medical ethics committee of the Eighth Affiliated Hospital of SYSU (2021-020-02).

Experiment Protocol

Hemodynamic data on lower extremities were collected by echo investigation at 5 p.m. every day. Before the experiment, all the healthy participants were required to not eat any food or drink alcohol or caffeine, and avoid EECP or exercise for at least 24 h prior to measurements. The flowchart of the whole experimental scheme is illustrated in **Figure 1**. The baseline measurements were performed for each group in the supine position after

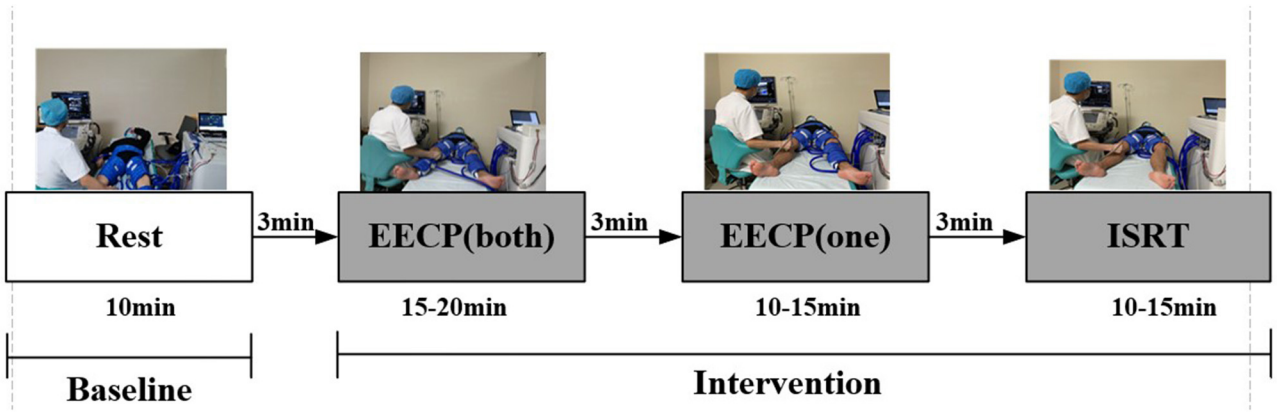

FIGURE 1 | Flowchart of the whole experimental scheme.

10 min of relaxation. Both blood pressure and heart rate were measured at baseline.

All the subjects first received EECP-3 intervention with the PSK P-ECP/TM Oxygen Saturation Monitoring EECP Instrument (Made in Chongqing, China). These healthy participants lay supine on the treatment bed with their legs and buttocks wrapped in cuffs, which were sequentially inflated from the lower thigh to the upper thigh and buttocks at the beginning of the diastolic phase, followed by quick, simultaneous deflation of all the cuffs just prior to the onset of systole. Second, all the healthy participants were conducted EECP-1 treatment. They lay supine on the treatment bed with one leg and buttocks wrapped in cuffs. Finally, EECP-2 was performed with no cuffs in the calves. The ultrasonic blood flow spectrum of inferior knee arteries (anterior tibial artery, posterior tibial artery, and dorsalis pedis artery) was monitored in each stage. A color Doppler ultrasound device (Toshiba Aplio 500 TUS-A500; Toshiba, Japan) was used to measure the hemodynamic information at baseline, during EECP- 3, EECP-2, and EECP-1.

Parameter Calculation

Parameters, namely, PSV, end-diastolic velocity (EDV), mean flow velocity (MV), pulsatility index (PI), area, flow rate (FR), and systolic maximum acceleration (CCAs) were continuously recorded for 5 s, and then were calculated for mean value.

The mean PI of all the arteries was calculated as:

$$PI = \frac{PSV - EDV}{TAMAX} \tag{1}$$

where $TAMAX = \frac{VTI}{T}$.

Flow rate (FR) was calculated from vessel diameter, cardiac period, and velocity-time integral as:

$$FR = \frac{S \times VTI}{T} \tag{2}$$

where VTI is the averaged velocity-time integral, and T is the averaged cardiac cycle time. S is a vascular area.

TABLE 1 | Basic characteristics of the participants.

Characteristics	Subjects ($n = 20$)
Age, year	25.51 ± 2.28
Height, cm	173.20 ± 4.74
Weight, kg	66.83 ± 7.38
Smoking	0
Drinking	0
Exercise habits, *n* (%)	6 (30)
Sleep disorders, *n* (%)	5 (25)
Family history, *n* (%)	3 (15)
SBP (mmHg)	118.80 ± 7.92
DBP (mmHg)	72.35 ± 4.52
HR (bpm)	64.75 ± 8.93

Statistical Analysis

All the variables were the mean value of the area under the envelope curve in a cardiac cycle. Results are shown as means ± SD. Normal distribution for all the lower limb hemodynamic variables was evaluated by the Kolmogorov-Smirnov test (at least one test $P > 0.05$). Basic characteristics were determined by descriptive analysis. A repeated ANOVA comparing the parameters of inferior knee arteries (anterior tibial artery, posterior tibial artery, and dorsalis pedis artery) was performed at baseline, during EECP-3, EECP-2, and EECP-1. Additionally, a one-way ANOVA comparing hemodynamic variables among the three arteries was performed. Fisher's least significant difference test was conducted as a *post-hoc* analysis. All the statistical tests were conducted with SPSS version 20.0 (IBM SPSS Statistics, United States), and $p < 0.05$ was taken as a measure of statistical significance.

RESULTS

The baseline information, including age, gender, height, weight, risk factors (smoking, drinking, family history and sleep

disorders, and exercise habits) were shown as below at resting conditions before EECP intervention (**Table 1**).

Original ultrasonic pictures and the Doppler spectrum of the anterior tibial artery at baseline, during EECP-3, EECP-2 and EECP-1, are illustrated in **Figure 2**. Different hemodynamic responses (e.g., blood flow, blood flow velocity, and PI) among the anterior tibial artery, posterior tibial artery, and dorsalis pedis artery are shown in **Figure 3**. FR, area, and PSV in the anterior tibial artery were significantly higher than in the other arteries (all $P < 0.01$). MV of the anterior tibial artery was also significantly higher than that of the other arteries during EECP-3 and EECP-2 (both $P < 0.01$). However, PI of the dorsalis pedis artery was significantly higher than that of the other arteries during EECP-2 (both $P < 0.01$).

Additionally, in order to clearly show hemodynamic response in each participant, the effects of EECP on the hemodynamic variables varied in each subject are illustrated in **Figures 4–10** and summarized in **Supplementary Table 2**.

Blood Flow

Flow rate (FR) of the posterior tibial artery was significantly decreased during EECP-2 and EECP-1 compared with baseline (both $P < 0.05$, **Figure 4A**). However, FR of the anterior tibial artery was significantly increased during EECP-3 ($P = 0.048$, **Figure 4B**). FR of the dorsalis pedis artery during EECP-2 was higher than that during EECP-3. In addition, the area, which is used to calculate FR in the posterior tibial artery during EECP-1, was significantly lower than that at baseline, during EECP-3 and EECP-2 (all $P < 0.01$, **Figure 5A**). However, there was no significant difference in the area of the anterior tibial artery and dorsalis pedis artery at baseline, during EECP-3, EECP-1, and EECP-2 (all $P > 0.05$, **Figures 5B,C**).

Pulsatility Index

The pulsatility index (PI) of the posterior tibial artery during EECP-3, EECP-2, and EECP-1 was significantly higher than the baseline (all $P < 0.01$, **Figure 6A**). PI of the anterior tibial artery was also significantly higher during EECP-3 than during EECP-1 ($P = 0.015$) and EECP-2 ($P = 0.049$). Moreover, PI of the dorsalis pedis artery was markedly higher during EECP-2 than during EECP-1 ($P = 0.003$, **Figure 6**).

Blood Flow Velocity

Peak systolic velocity (PSV) in the inferior knee artery was significantly increased during EECP-3, EECP-2, and EECP-1 compared with baseline (all $P < 0.01$, **Figure 7**). In addition, PSV of the anterior tibial artery was significantly higher during EECP-3 than during EECP-1 ($P = 0.002$) and EECP-2 ($P = 0.005$).

Compared with baseline, EDV of the inferior knee artery was significantly increased during EECP-3, EECP-2, and EECP-1 (all $P < 0.01$, **Figure 8**). EDV of the posterior tibial artery and dorsalis pedis artery during EECP-1 was higher than during EECP-3 (both $P < 0.01$, **Figure 8A**). Additionally, EDV of the anterior tibial artery and the posterior tibial artery was also significantly higher during EECP-2 than during EECP-3 in this study (both $P < 0.01$, **Figures 8A,B**).

Mean flow velocity (MV) of the anterior tibial artery during EECP-3 was markedly higher than the baseline ($P = 0.004$), during EECP-1 ($P = 0.012$) and EECP-2 [($P = 0.012$), **Figure 9**], while MV of the dorsalis pedis artery was markedly lower during EECP-2 than during EECP-3 ($P = 0.001$) and EECP-1 ($P = 0.045$, **Figure 9C**). There was no significant change in MV of the posterior tibial artery in each stage (all $P > 0.05$, **Figure 9**).

Systolic Maximum Acceleration (CCAs)

During EECP-3, CCAs in the anterior tibial artery, posterior tibial artery, and dorsalis pedis artery was significantly increased compared with the baseline (all $P < 0.01$, **Figure 10**). However, there was no significant difference in CCAs at baseline during EECP-1 and EECP-2 (all $P > 0.05$).

DISCUSSION

This study was designed to investigate lower extremity hemodynamics during EECP-3, EECP-2, and EECP-1, and determine acute hemodynamic effects on ultrasonic blood flow spectrum data. The major findings in this study are 2-fold: first, EECP-3 immediately improved the blood flow, blood flow velocity, and ACCs of the anterior tibial artery; second, EECP-1 and EECP-2 significantly increased the blood flow velocity and peripheral resistance of the inferior knee artery, whereas they markedly reduce the blood flow in the posterior tibial artery.

In this study, we found that EECP significantly increased the FR of the anterior tibial artery and that the FR of the anterior tibial artery was significantly higher than that of the other arteries. Few studies have investigated the acute effects of EECP on lower extremity hemodynamics. Studies have reported that inflation of the EECP-3 cuffs creates the high-pressure retrograde blood flow in the femoral arteries (11). Cai et al. (23) also found that EECP could improve blood circulation in the lower extremities by combining animal and human experiments. In addition, our previous study has shown that EECP increases the FR of the femoral artery (17). Meanwhile, Avery et al. found that EECP significantly increased FR and improved endothelium-dependent vasodilation in calf resistance arteries. EECP may provide a kind of "massage" on the peripheral function, starting with the elastic conduit arteries, muscular conduit arteries, and extending to the skeletal muscle resistance arteries (12). The diastolic inflation/systolic deflation sequence of EECP-3 results in diastolic augmentation/systolic unloading and leads to increased blood flow (8, 25).

However, our results are inconsistent, at least in part with the above findings. After changing the limb cuffs, FR in the posterior tibial artery was significantly decreased during both EECP-2 and EECP-1. Werner et al. found that the FR of the posterior tibial artery decreased to 69 ± 23% during EECP (13). Studies found that reduced peripheral flow during EECP may have similar physiologic effects like an exercise in patients with symptomatic PAD. More importantly, it is contributed by changes in MV and PI (13). PI of the posterior tibial and dorsalis pedis arteries markedly increased compared with baseline. In addition, MV of

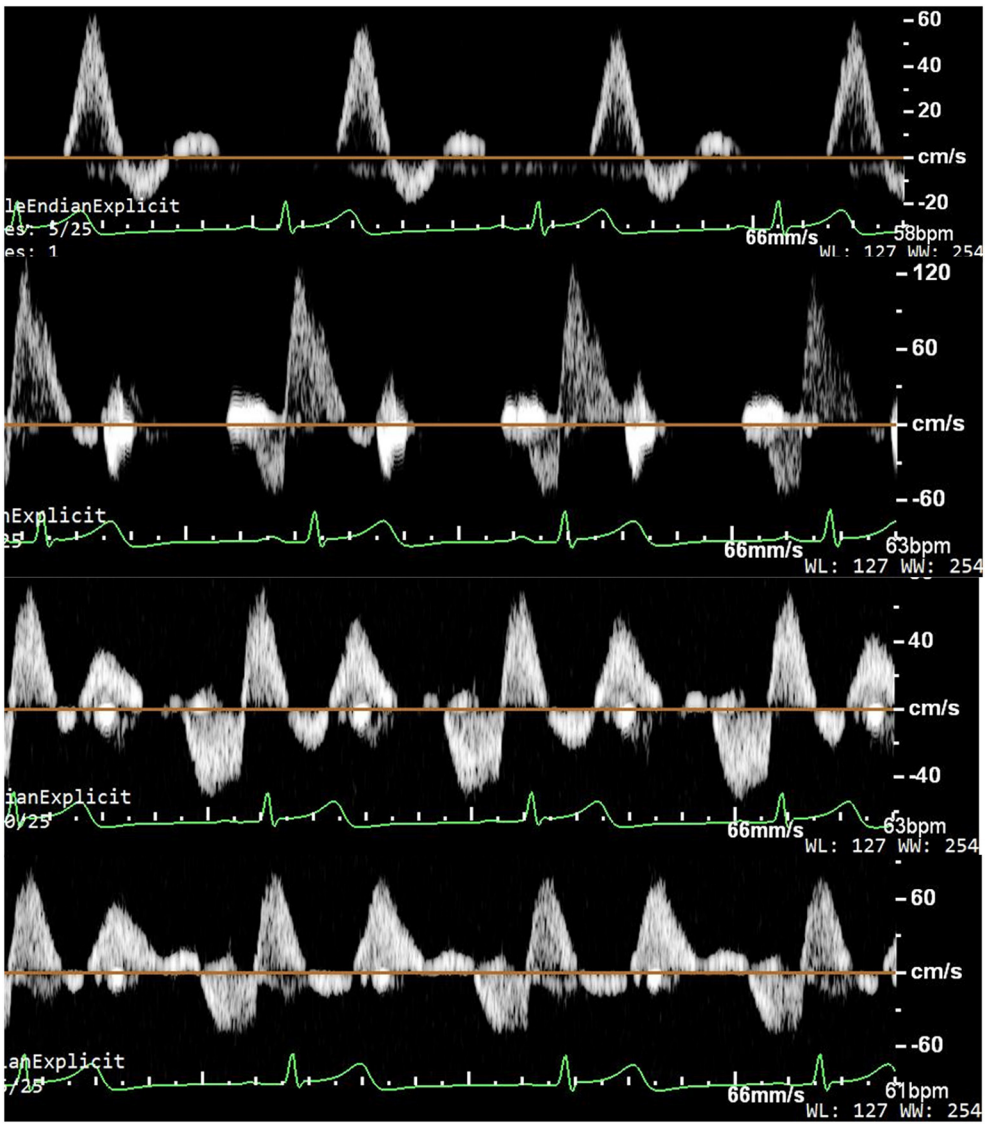

FIGURE 2 | Ultrasound pictures and Doppler spectrum of the anterior tibial artery at baseline, during EECP-3, EECP-1, and EECP-2.

the anterior tibial artery during EECP-3 was markedly higher than the baseline (P =0.004), during EECP-1 (P =0.012) and EECP-2 (P = 0.012), while MV of the dorsalis pedis artery during EECP-2 was markedly lower than during EECP-3 (P = 0.001) and EECP-1 (P = 0.045). Werner et al. assessed the blood flow velocity of the posterior tibial artery during EECP (13). They found a significant increase in the MV and PI, showing a marked increase in retrograde blood flow. EECP creates a second diastolic pulse wave in all arterial vessels (26).

In this study, during EECP-2, PI and MV of the dorsalis pedis artery were significantly increased and decreased, respectively. In addition, PI of the dorsalis pedis artery was significantly higher than that of the other arteries during EECP-2. The findings of this study support a previous study, which show that the PI of the posterior tibial artery has a 4-fold increase (13). Studies have reported that PI is an important indicator of peripheral resistance (27–29), which can be associated with postural changes, physiologic fluctuations, and vascular disease (30–32). A second diastolic pulse wave in the lower extremity artery was created by EECP-2. Additionally, changes in peripheral flow patterns during EECP-2 were also characterized by elevated PI.

Studies have also reported that regulation of FR is associated with vascular diameters and blood flow velocity (33). In this study, blood flow velocity, like PSV and EDV, in the inferior knee artery significantly increased. Few studies investigated the PSV of the inferior knee artery during EECP. Zhang et al. also found that vascular diameter and PSV were significantly increased after EECP intervention. Besides that, the acceleration of systolic peak velocity also increased. A study showed that the ACC of systolic peak velocity in the lower limbs is an effective marker of peripheral artery disease (34, 35). Cai et al. observed a 1.2-fold increase in femoral artery retrograde

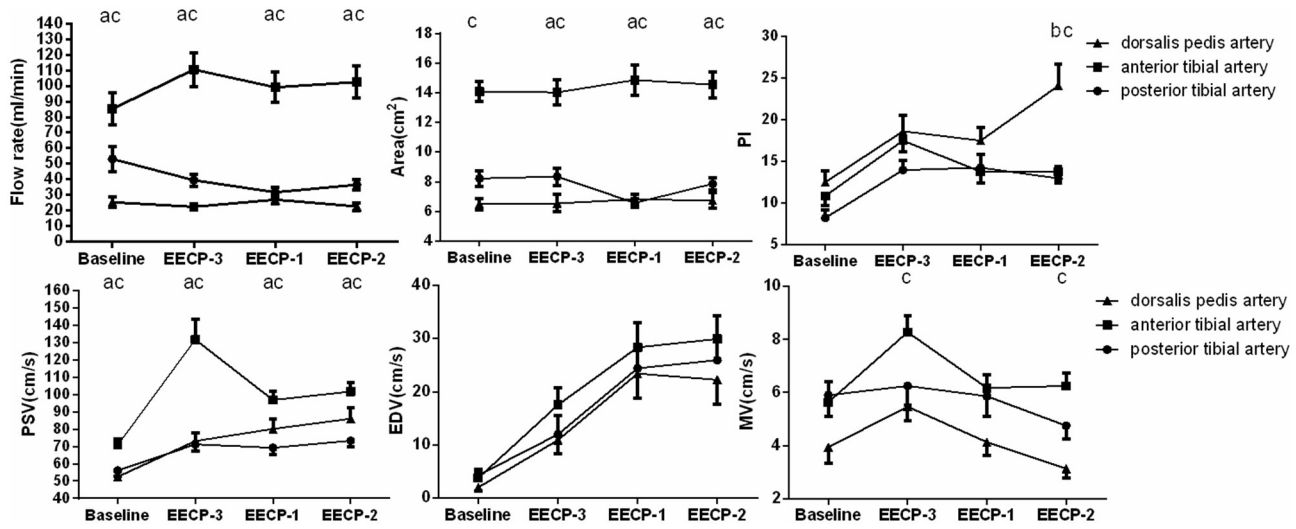

FIGURE 3 | Significant changes in the anterior tibial artery, posterior tibial artery, and dorsalis pedis artery at baseline, during EECP-3, EECP-2, and EECP-1. a. significant differences between the anterior tibial artery and posterior tibial artery; b. significant difference between dorsalis pedis artery and posterior tibial artery; c. significant differences between the anterior tibial artery and dorsalis pedis artery.

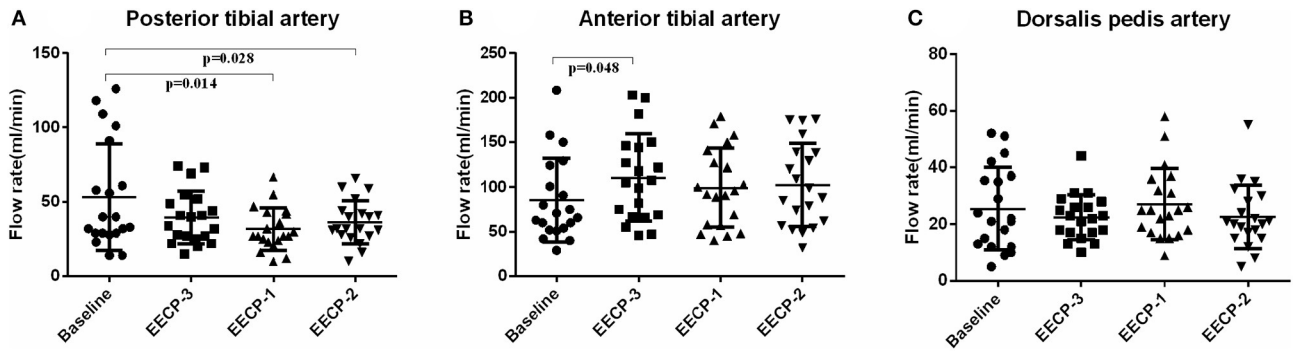

FIGURE 4 | Effect of EECP-3, EECP-2 and EECP-1 on the flow rate (FR) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

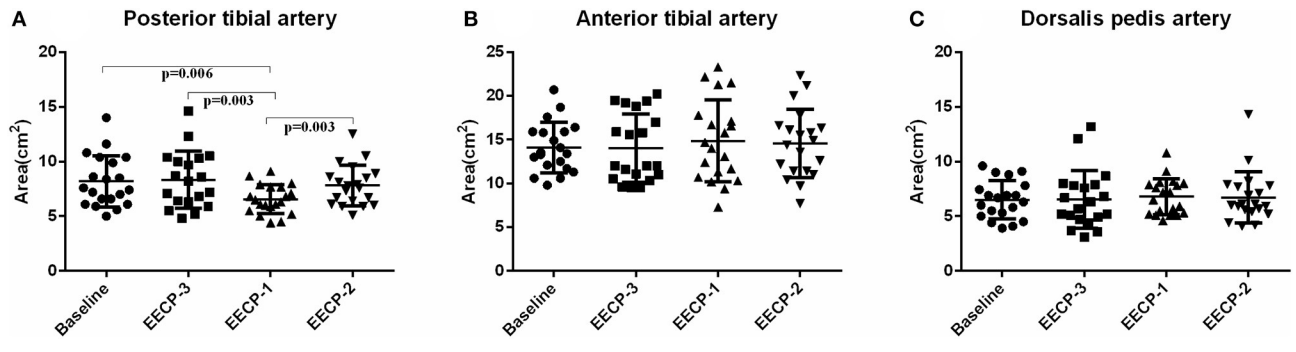

FIGURE 5 | Effect of EECP-3, EECP-2 and EECP-1 on the area of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

blood flow velocity (23). Based on a porcine EECP model, Zhang *et al.* demonstrated that blood flow velocity and wall shear stress of the peripheral artery increased by 1.3- and 2.1-fold, respectively, during EECP (8). Additionally, EECP increases flow pulsatility and shear stress (13). Gurovich et al. also found that EECP significantly increased retrograde

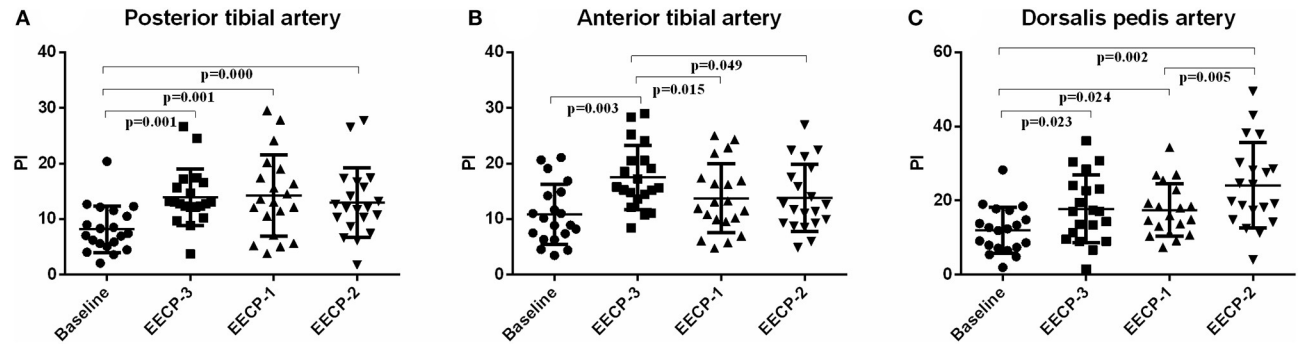

FIGURE 6 | Effect of EECP-3, EECP-2 and EECP-1 on the pulsatility index (PI) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

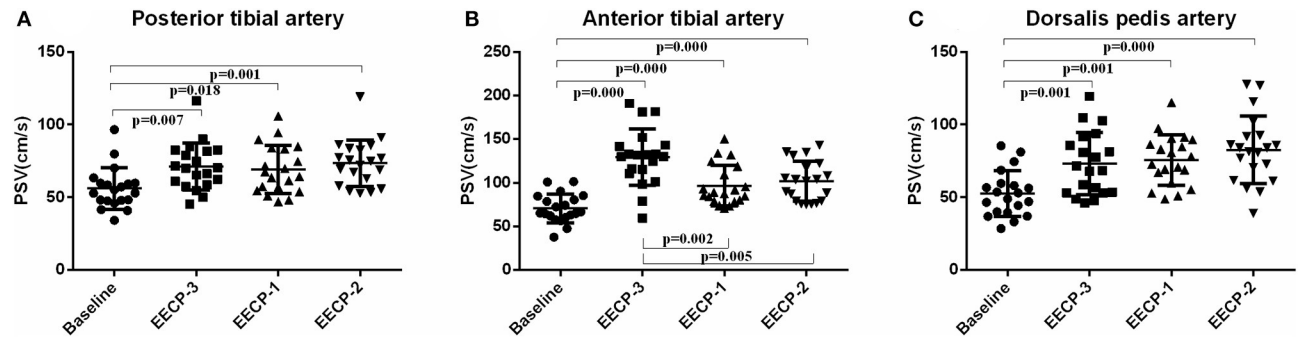

FIGURE 7 | Effect of EECP-3, EECP-2 and EECP-1 on the peak systolic velocity (PSV) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

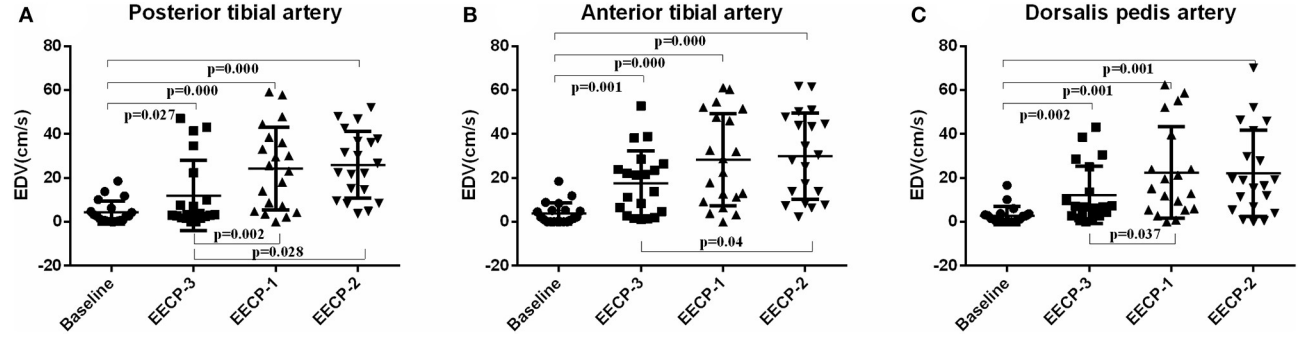

FIGURE 8 | Effect of EECP-3, EECP-2 and EECP-1 on the end-diastolic velocity (EDV) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

shear stress and retrograde-turbulent FR in the femoral artery (11). The mechanism responsible for this phenomenon is increased endothelial shear stress, which leads to vascular anti-inflammatory changes in human umbilical vein endothelial cells (36).

Moreover, they showed that changes in shear rate, led by femoral artery vascular tone, elicited increased femoral baseline diameter after EECP intervention (11). Werner et al. reported that the diameter of the posterior tibial artery was significantly decreased after EECP intervention (13). Sonka et al. demonstrated that femoral peak diameters were regarded as the single peak diameter investigated during the plateau phase after cuff deflation (37). Dopheide et al. found that femoral artery diameter and vascular shear stress were significantly increased after supervised exercise training (38). Nevertheless, in this study, the area of the posterior tibial artery was significantly reduced during EECP-1, while there was no significant difference in the area during EECP-3 and EECP-2. Studies have reported that both flow- and pressure-induced forces play an important role in vessel wall diameter (39–41). Decreased area induced by EECP-1 may be associated with different changes of pressure in both legs.

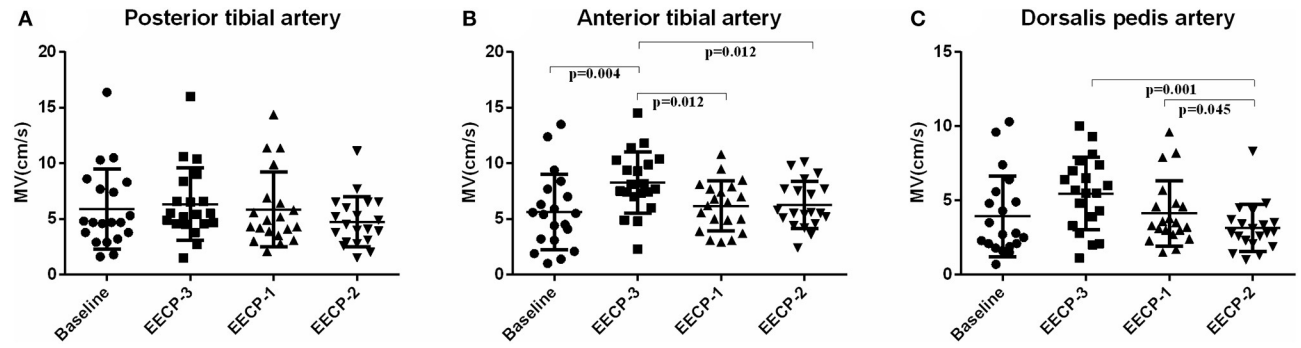

FIGURE 9 | Effect of EECP-3, EECP-2 and EECP-1 on the mean flow velocity (MV) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

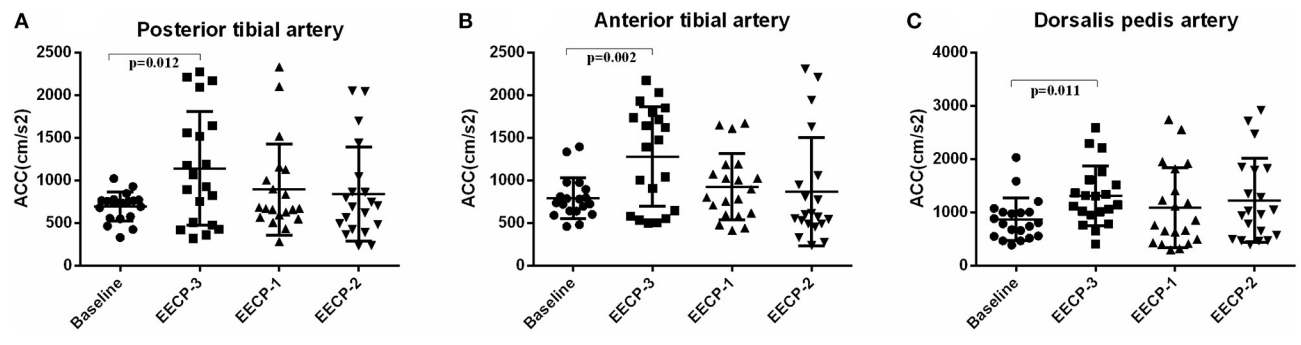

FIGURE 10 | Effect of EECP-3, EECP-2 and EECP-1 on the systolic maximum acceleration (CCAs) of posterior tibial artery **(A)**, anterior tibial artery **(B)** and dorsalis pedis artery **(C)**.

Limitations

Some limitations of this study should be emphasized. First, in order to explore physiological changes in lower limb hemodynamics, the participants in this study are healthy, young individuals, whereas EECP is normally prescribed for patients with cardiovascular disease. Second, we just investigate the acute effects of EECP on the lower extremity vascular parameters due to obtaining each immediate response of hemodynamics. Finally, we did not measure lower limb FMD, so we were not able to assess endothelial function after EECP in these arteries.

Future Direction

Further studies that will investigate EECP with different sequence level-induced lower limb hemodynamics in patients with PAD are appropriate. In addition, the long-term effects of EECP with different sequence levels on lower limb vascular function in patients with PAD will be explored in the future.

CONCLUSION

This study demonstrated that sensitive parameters in the anterior tibial artery, dorsalis pedis artery, and posterior tibial artery are highlighted during EECP-3, EECP-2, and EECP-1, respectively. EECP-3 immediately improved the blood flow, blood flow velocity, and ACCs of the anterior tibial artery. EECP-1 mainly regulated the hemodynamic indexes of the posterior tibial artery, such as FR and area. In contrast, EECP-2 significantly regulated the PI and MV of the dorsalis pedis artery. This study will be beneficial to realize the personalized and precise treatment of PAD with external counterpulsation. EECP-3 may be recommended for patients with anterior tibial artery stenosis. EECP-2 may be recommended to improve lower artery hemodynamics of patients with dorsal foot artery stenosis. On the contrary, EECP-2 and EECP-1 may be not suitable for the treatment of the posterior tibial artery.

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 the Local Medical Ethics Committee of The Eighth Affiliated Hospital of SYSU (2021-020-02). The

patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YuZ, CZ, and GW proposed the scientific problems. YuZ and CZ designed the experiments. YuZ, YaZ, WW, YW, XX, and JJ collected the experimental data. YuZ, WZ, and XZ processed and calculated the data. YuZ conducted the statistical analysis and wrote the draft manuscript. CZ and GW contributed to the revision and final version of 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.2021.795697/full#supplementary-material

REFERENCES

1. Masuda D, Nohara R, Hirai T, Kataoka K, Chen LG, Hosokawa R, et al. Enhanced external counterpulsation improved myocardial perfusion and coronary flow reserve in patients with chronic stable angina; evaluation by(13)N-ammonia positron emission tomography. *Eur Heart J.* (2001) 22:1451–8. doi: 10.1053/euhj.2000.2545
2. Fihn SD, Gardin JM, Abrams J, Berra K, Blankenship JC, Dallas AP, et al. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol.* (2012) 60:e44–e164. doi: 10.1016/j.jacc.2012.07.013
3. Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J.* (2013) 34:2949–3003. doi: 10.1093/eurheartj/eht296
4. Wu E, Desta L, Broström A, Mårtensson J. Effectiveness of enhanced external counterpulsation treatment on symptom burden, medication profile, physical capacity, cardiac anxiety, and health-related quality of life in patients with refractory angina pectoris. *J Cardiovasc Nurs.* (2020) 35:375–85. doi: 10.1097/JCN.0000000000000638
5. Caceres J, Atal P, Arora R, Yee D. Enhanced external counterpulsation: A unique treatment for the "No-Option" refractory angina patient. *J Clin Pharm Ther.* (2021) 46:295–303. doi: 10.1111/jcpt.13330
6. Wu E, Mårtensson J, Desta L, Broström A. Predictors of treatment benefits after enhanced external counterpulsation in patients with refractory angina pectoris. *Clin Cardiol.* (2021) 44:160–7. doi: 10.1002/clc.23516
7. Nichols WW, Estrada JC, Braith RW, Owens K, Conti CR. Enhanced external counterpulsation treatment improves arterial wall properties and wave reflection characteristics in patients with refractory angina. *J Am Coll Cardiol.* (2006) 48:1208–14. doi: 10.1016/j.jacc.2006.04.094
8. Zhang Y, He X, Chen X, Ma H, Liu D, Luo J, et al. Enhanced external counterpulsation inhibits intimal hyperplasia by modifying shear stress responsive gene expression in hypercholesterolemic pigs. *Circulation.* (2007) 116:526–34. doi: 10.1161/CIRCULATIONAHA.106.647248
9. Braith RW, Conti CR, Nichols WW, Choi CY, Khuddus MA, Beck DT, et al. Enhanced external counterpulsation improves peripheral artery flow-mediated dilation in patients with chronic angina. *Circulation.* (2010) 122:1612–20. doi: 10.1161/CIRCULATIONAHA.109.923482
10. Zhang Y, Mai Z, Du J, Zhou W, Wei W, Wang H, et al. Acute effect of enhanced external counterpulsation on the carotid hemodynamic parameters in patients with high cardiovascular risk factors. *Front Physiol.* (2021) 12:615443. doi: 10.3389/fphys.2021.615443
11. Gurovich AN, Braith RW. Enhanced external counterpulsation creates acute blood flow patterns responsible for improved flow-mediated dilation in humans. *Hypertens Res.* (2013) 36:297–305. doi: 10.1038/hr.2012.169
12. Avery JC, Beck DT, Casey DP, Sardina PD, Braith RW. Enhanced external counterpulsation improves peripheral resistance artery blood flow in patients with coronary artery disease. *Appl Physiol Nutr Metab.* (2014) 39:405–8. doi: 10.1139/apnm-2013-0309
13. Werner D, Michalk F, Hinz B, Werner U, Voigt JU, Daniel WG. Impact of enhanced external counterpulsation on peripheral circulation. *Angiology.* (2007) 58:185–90. doi: 10.1177/0003319707300013
14. Dockery F, Rajkumar C, Bulpitt CJ, Hall RJ, Bagger JP. Enhanced external counterpulsation does not alter arterial stiffness in patients with angina. *Clin Cardiol.* (2004) 27:689–92. doi: 10.1002/clc.4960271206
15. Martin JS, Beck DT, Braith RW. Peripheral resistance artery blood flow in subjects with abnormal glucose tolerance is improved following enhanced external counterpulsation therapy. *Appl Physiol Nutr Metab.* (2014) 39:596–9. doi: 10.1139/apnm-2013-0497
16. Hashemi M, Hoseinbalam M, Khazaei M. Long-term effect of enhanced external counterpulsation on endothelial function in the patients with intractable angina. *Heart Lung Circ.* (2008) 17:383–7. doi: 10.1016/j.hlc.2008.02.001
17. Zhang Y, Chen Z, Zhouming M, Zhou W, Wang H, Zhang X, et al. (2021a). Acute hemodynamic responses to enhanced external counterpulsation in patients with coronary artery disease. *Front Cardiovasc Med.* (1542). doi: 10.3389/fcvm.2021.721140
18. Buschmann I, Pries A, Styp-Rekowska B, Hillmeister P, Loufrani L, Henrion D, et al. Pulsatile shear and Gja5 modulate arterial identity and remodeling events during flow-driven arteriogenesis. *Development.* (2010) 137:2187–96. doi: 10.1242/dev.045351
19. Buschmann EE, Brix M, Li L, Doreen J, Zietzer A, Li M, et al. Adaptation of external counterpulsation based on individual shear rate therapy improves endothelial function and claudication distance in peripheral artery disease. *Vasa.* (2016) 45:317–24. doi: 10.1024/0301-1526/a000544
20. Zietzer A, Buschmann EE, Janke D, Li L, Brix M, Meyborg H, et al. Acute physical exercise and long-term individual shear rate therapy increase telomerase activity in human peripheral blood mononuclear cells. *Acta Physiol (Oxf).* (2017) 220:251–62. doi: 10.1111/apha.12820
21. Picard F, Panagiotidou P, Wolf-Putz A, Buschmann I, Buschmann E, Steffen M, et al. Usefulness of individual shear rate therapy, new treatment option for patients with symptomatic coronary artery disease. *Am J Cardiol.* (2018) 121:416–22. doi: 10.1016/j.amjcard.2017.11.004
22. Picard F, Panagiotidou P, Wolf-Putz A, Buschmann I, Buschmann E, Steffen M, et al. Individual shear rate therapy (ISRT)-further development of external counterpulsation for decreasing blood pressure in patients with

symptomatic coronary artery disease (CAD). *Hypertens Res.* (2020) 43:186–96. doi: 10.1038/s41440-019-0380-x
23. Cai D, Wu R, Shao Y. Experimental study of the effect of external counterpulsation on blood circulation in the lower extremities. *Clin Invest Med.* (2000) 23:239–47. doi: 10.1038/sj.cgt.7700222
24. Thakkar BV, Hirsch AT, Satran D, Bart BA, Barsness G, McCullough PA, et al. The efficacy and safety of enhanced external counterpulsation in patients with peripheral arterial disease. *Vasc Med.* (2010) 15:15–20. doi: 10.1177/1358863X09106549
25. Ozawa ET, Bottom KE, Xiao X, Kamm RD. Numerical simulation of enhanced external counterpulsation. *Ann Biomed Eng.* (2001) 29:284–97. doi: 10.1114/1.1359448
26. Werner D, Schneider M, Weise M, Nonnast-Daniel B, Daniel WG. Pneumatic external counterpulsation: a new noninvasive method to improve organ perfusion. *Am J Cardiol 84(8) 950-952.* (1999) a957–958. doi: 10.1016/S0002-9149(99)00477-4
27. Gosling RG, Dunbar G, King DH, Newman DL, Side CD, Woodcock JP, et al. The quantitative analysis of occlusive peripheral arterial disease by a non-intrusive ultrasonic technique. *Angiology.* (1971) 22:52–5. doi: 10.1177/000331977102200109
28. Aleksic M, Heckenkamp J, Gawenda M, Brunkwall J. Pulsatility index determination by flowmeter measurement: a new indicator for vascular resistance? *Eur Surg Res.* (2004) 36:345–9. doi: 10.1159/000081642
29. Liang HL. Doppler flow measurement of lower extremity arteries adjusted by pulsatility index. *AJR Am J Roentgenol.* (2020) 214:10–7. doi: 10.2214/AJR.19.21280
30. Delis KT, Nicolaides AN, Stansby G. Effect of posture on popliteal artery hemodynamics. *Arch Surg.* (2000) 135:265–9. doi: 10.1001/archsurg.135.3.265
31. Ascher, E., Hingorani, A. P., and Marks, N. A. (2007). Popliteal artery volume flow measurement: a new and reliable predictor of early patency after infrainguinal balloon angioplasty and subintimal dissection. *J Vasc Surg 45(1)* 17–23; discussion 23-14. doi: 10.1016/j.jvs.2006.09.042
32. Villar R, Hughson RL. Lower limb vascular conductance and resting popliteal blood flow during head-up and head-down postural challenges. *Clin Physiol Funct Imaging.* (2013) 33:186–91. doi: 10.1111/cpf.12008
33. Levenson JA, Simon AC, Safar ME. Vasodilatation of small and large arteries in hypertension. *J Cardiovasc Pharmacol.* (1985) 7 Suppl 2:S115–120. doi: 10.1097/00005344-198507002-00022
34. Li, L. (2017). Li, L. (2017). *Measuring Fluid Shear Stress With a Novel Doppler-derived Relative Pulse Slope Index and Maximal Systolic Acceleration Approach to Detect Peripheral Arterial Disease and to Modulate Arteriogenesis [D].* Berlin: Charité - Universitätsmedizin.
35. Peltokangas M, Vakhitov D, Suominen V, Korhonen J, Huotari M, Verho J, et al. Lower limb pulse rise time as a marker of peripheral arterial disease. *IEEE Trans Biomed Eng.* (2019) 66:2596–603. doi: 10.1109/TBME.2019.2892407
36. Duchene J, Cayla C, Vessillier S, Scotland R, Yamashiro K, Lecomte F, et al. Laminar shear stress regulates endothelial kinin B1 receptor expression and function: potential implication in atherogenesis. *Arterioscler Thromb Vasc Biol.* (2009) 29:1757–63. doi: 10.1161/ATVBAHA.109.191775
37. Sonka M, Liang W, Lauer RM. Automated analysis of brachial ultrasound image sequences: early detection of cardiovascular disease via surrogates of endothelial function. *IEEE Trans Med Imaging.* (2002) 21:1271–9. doi: 10.1109/TMI.2002.806288
38. Dopheide JF, Rubrech J, Trumpp A, Geissler P, Zeller GC, Schnorbus B, et al. Supervised exercise training in peripheral arterial disease increases vascular shear stress and profunda femoral artery diameter. *Eur J Prev Cardiol.* (2017) 24:178–91. doi: 10.1177/2047487316665231
39. Helisch A, Schaper W. Arteriogenesis: the development and growth of collateral arteries. *Microcirculation.* (2003) 10:83–97. doi: 10.1080/mic.10.1.83.97
40. Schaper W, Scholz D. Factors regulating arteriogenesis. *Arterioscler Thromb Vasc Biol.* (2003) 23:1143–51. doi: 10.1161/01.ATV.0000069625.11230.96
41. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res.* (2004) 95:449–58. doi: 10.1161/01.RES.0000141145.78900.44

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Optimal Time of Collapse to Return of Spontaneous Circulation to Apply Targeted Temperature Management for Cardiac Arrest: A Bayesian Network Meta-Analysis

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Background: Both the American Heart Association (AHA) and European Resuscitation Council (ERC) have strongly recommended targeted temperature management (TTM) for patients who remain in coma after return of spontaneous circulation (ROSC). However, the role of TTM, especially hypothermia, in cardiac arrest patients after TTM2 trials has become much uncertain.

Methods: We searched four online databases (PubMed, Embase, CENTRAL, and Web of Science) and conducted a Bayesian network meta-analysis. Based on the time of collapse to ROSC and whether the patient received TTM or not, we divided this analysis into eight groups (<20 min + TTM, <20 min, 20–39 min + TTM, 20–39 min, 40–59 min + TTM, 40–59 min, ≥60 min + TTM and ≥60 min) to compare their 30-day and at-discharge survival and neurologic outcomes.

Results: From an initial search of 3,023 articles, a total of 9,005 patients from 42 trials were eligible and were included in this network meta-analysis. Compared with other groups, patients in the <20 min + TTM group were more likely to have better survival and good neurologic outcomes (probability = 46.1 and 52.5%, respectively). In comparing the same time groups with and without TTM, only the survival and neurologic outcome of the 20–39 min + TTM group was significantly better than that of the 20–39 min group [odds ratio = 1.41, 95% confidence interval (1.04–1.91); OR = 1.46, 95% CI (1.07–2.00) respectively]. Applying TTM with < 20 min or more than 40 min of collapse to ROSC did not improve survival or neurologic outcome [<20 min vs. <20 min + TTM: OR = 1.02, 95% CI (0.61–1.71)/OR = 1.03, 95% CI (0.61–1.75); 40–59 min vs. 40–59 min + TTM: OR = 1.50, 95% CI (0.97–2.32)/OR = 1.40, 95% CI (0.81–2.44); ≧60 min vs. ≧60 min + TTM: OR = 2.09, 95% CI (0.70–6.24)/OR = 4.14, 95% CI (0.91–18.74), respectively]. Both survival and good neurologic outcome were closely related to the time from collapse to ROSC.

Conclusion: Survival and good neurologic outcome are closely associated with the time of collapse to ROSC. These findings supported that 20–40 min of collapse to ROSC should be a more suitable indication for TTM for cardiac arrest patients. Moreover, the future trials should pay more attention to these patients who suffer from moderate injury.

Systematic Review Registration: [https://inplasy.com/?s=202180027], identifier [INPLASY202180027]

Keywords: cardiac arrest, targeted temperature management, return of spontaneous circulation (ROSC), survival, good neurological outcome

INTRODUCTION

Cardiac arrest (CA) has a variety of causes and severely threatens human life. Even when cardiopulmonary resuscitation (CPR) is performed promptly and with high quality under the latest guidelines, only ∼10.4% of patients survive after out-of-hospital cardiac arrest (OHCA), and 25.8% survive after in-hospital cardiac arrest (IHCA) (1). Even with such a low survival rate, only 8–21% of patients have good neurologic outcome at discharge (2). Therefore, improving survival and neurologic outcome has remained a challenge in clinical practice.

The term *hypothermia* can be traced back to an ancient "neoteric" technology first used by ancient Egyptians, Greeks, and Romans to induce cooling for battle-inflicted trauma and a variety of cerebral disturbances (3). In recent decades, this technology has been applied to improve survival and neurologic outcome and has given rise to a new concept, targeted temperature management (TTM), for patients with CA (4). However, there is a debate about TTM that has not stopped. Two early randomized controlled trials (RCTs), as well as a more recent trial, have consistently shown that TTM could improve clinical outcomes for CA (5–7). Additionally, TTM has been recommended by both the American Heart Association (AHA) and European Resuscitation Council (ERC) in their latest guidelines for adults who do not follow physicians' orders after return of spontaneous circulation (ROSC) from OHCA or IHCA with any initial rhythm and control the temperature within 32–36°C (2, 8). TTM might improve ischemia-perfusion by reducing cell metabolism and thus improve clinical outcomes (9). While clinicians firmly believe that targeted hypothermia is beneficial for eligible patients, a recent blockbuster study has disrupted that belief. A recent RCT showed that targeted hypothermia did not improve clinical outcomes compared with targeted normothermia, whether survival or neurologic (10). Therefore, should we apply TTM? Or does it matter when we apply TTM? The results of the latest trial are difficult to deny. Thus, we conducted the current network meta-analysis to identify the suitable time of collapse to ROSC, which is an optimal indication for TTM to improve survival and neurologic outcome.

METHODS

Protocol Registration

We registered the protocol for this systematic review with the International Platform of Registered Systematic Review and Meta-analysis Protocols (registration number: INPLASY202180027).

Databases and Search Strategy

We performed this network meta-analysis by searching four online databases (PubMed, Embase, CENTRAL, and Web of Science). References of relevant meta-analyses, letters, editorials, reviews, and eligible trials were also screened. The initial search was broad, and no limitations were made regarding publication type, study data type, language, or species. Our detailed search strategy is shown in the **Supplementary Material**.

Initially, two trained investigators screened the titles and abstracts of all articles independently. If they were of different opinions, a third person intervened to settle the disagreements. Second, after preliminary screening, the remaining studies were further screened by reading the full text. Finally, the eligibility of all included trials was confirmed by contacting the corresponding authors.

Criteria for Inclusion and Exclusion

After the screening, trials that met the following criteria were included: (a) patients in the trial underwent CA, whether IHCA or OHCA; (b) valid 30-day or at-discharge survival neurologic outcome data could be extracted; (c) the time of collapse to ROSC could be extracted so we could divide patients into groups based on that data; (d) patients who received TTM should have a temperature below 36°C.

If a trial met any one of the following criteria, it was excluded from our analysis: (a) only special populations were included in the study, such as pediatric or obstetric; (b) full text was not available; (c) the article was written in a language other than English; (d) the trial performed on animals or cells; (e) collapse with no witness and without reliable approach to recode the time of collapse to ROSC. We did not limit inclusion to randomized controlled trials; if a retrospective or cohort trial met the inclusion criteria, it was included in our analysis. While meta-analyses and reviews were not included, we reviewed their references and included trials that met the inclusion criteria.

Data Extraction and Quality Assessment

Published data from the included trials were pooled in this network meta-analysis, and a standard method was used to extract patients' demographics and trial characteristics. Finally, two pre-specified endpoints, 30-day or at-discharge survival and neurologic outcome, were collected from the included trials.

Cochrane Handbook version 5.1.0 was used to assess the quality of each RCT and its risk of bias (**Supplementary Figures 9, 10**). The Newcastle–Ottawa Assessment Scale (NOS) for control and cohort studies was used to assess the quality and risk of bias of each retrospective and cohort study, respectively (**Supplementary Tables 5, 6**). We considered that a NOS score greater than seven was high quality (11).

Endpoints and Assignment Definitions

We distributed the studies in this analysis according to two endpoints: 30-day or at-discharge survival and good neurologic outcome. Based on the Cerebral Performance Category (CPC) scale (which ranges from 1 to 5, with higher scores indicating greater disability), a good neurologic outcome was defined as CPC 1–2 (12, 13). The center temperature controlled below 36°C was considered to be receiving TTM.

Based on the time of collapse to ROSC and whether a patient received TTM, we assigned patients to eight groups (<20 min + TTM, <20 min, 20–39 min + TTM, 20–39 min, 40–59 min + TTM, 40–59 min, ≥60 min + TTM and ≥60 min) to compare them and determine an optimal indication for TTM.

Synthesis and Analysis

We conducted this analysis based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A Bayesian random effects survival model for analyzing multiple treatment groups was built to compare the different groups regarding their 30-day or at-discharge survival endpoints and neurologic outcome endpoints. We used a Bayesian extension of the hierarchical random effects model proposed by Lumley for networks of multiarm trials. A Bayesian network meta-analysis can make direct or indirect comparisons to determine whether there is a direct comparison between two groups. Pooled data were analyzed *via* STATA/MP 16.0 (Stata Corp LP, College Station, Texas, USA). Markov chain Monte Carlo samplers were run in Stata, and 4 chains were run with different starting values. Vague, non-informative prior distributions with very small precision were given. A burn-in phase of 20,000 iterations was used to ensure convergence. The convergence was checked by running 4 chains at different starting values using the Gelman-Rubin methods, which were stable in all instances. For inference, 50,000 iterations were used. A fixed effect model was used in traditional frequentist meta-analysis to analyze pooled odds ratios (ORs), so a traditional frequentist

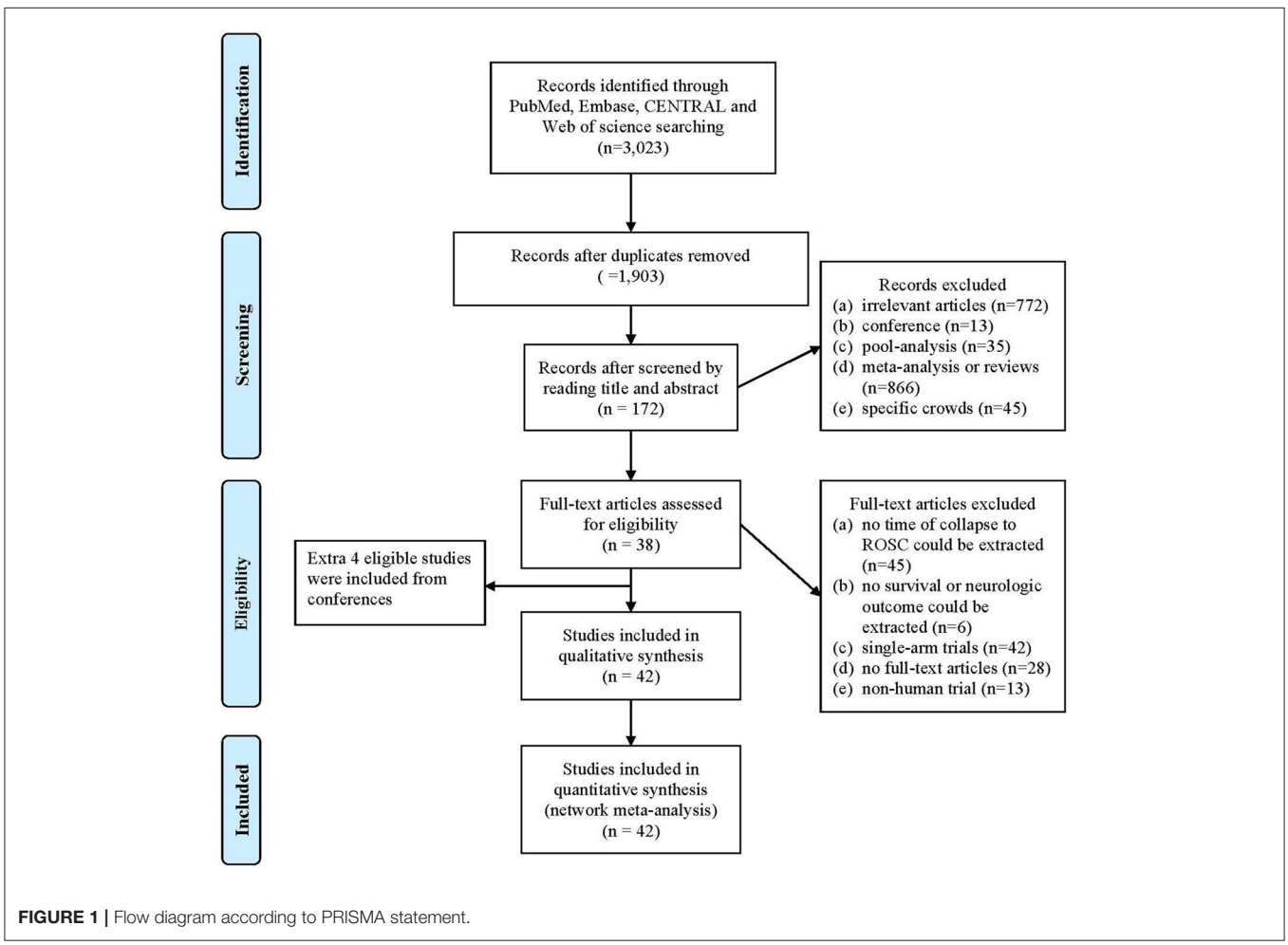


FIGURE 1 | Flow diagram according to PRISMA statement.

meta-analysis was performed in this study using STATA. Pairwise ORs were estimated from the median of the posterior distribution with credible intervals (CIs) taken from the 2.5 and 97.5% percentiles. The results are considered significantly different when CI did not include 1 and the OR > 1 means favor last or OR <1 means favor first. Markov chain Monte Carlo (MCMC) modeling was used to calculate the relative ranking probability of each intervention. "Rankograms" along with the surface under the cumulative ranking curve (SUCRA) were employed to compare the hierarchy of efficacy and safety of the interventions (14). SUCRA is a numeric representation of the overall ranking and assigns a single number associated with each treatment. SUCRA values range from 0 to 100%. The higher the SUCRA value, and the closer to 100%, the higher the likelihood that a group is in the top rank, or highly effective; the closer the SUCRA value is to 0, the more likely the group is in the bottom rank, or ineffective. Additionally, a funnel plot was constructed to assess the trials' publication bias (**Supplementary Figures 4, 8**). If the

TABLE 1 | Characteristics of included trials (RCT: randomized controlled trial).

Author name	Year	Site	Design of trial	Follow-up (day)	Survival outcome	Neurologic outcome
Agarwal et al.	2018	US	Retrospective	365	Yes	Yes
Arrich et al.	2007	Europe	Retrospective	At discharge	Yes	Yes
Blumenstein et al.	2015	Germany	Retrospective	365	Yes	Yes
Castren et al.	2010	Sweden	RCT	At discharge	Yes	Yes
Chen et al.	2008	China	Prospective	365	Yes	No
Choi et al.	2016	Korea	Cohort	30	Yes	Yes
Chou et al.	2012	China	Prospective	365	Yes	No
De Fazio et al.	2019	Belgium	Retrospective	180	Yes	Yes
Dankiewicz et al.	2021	Multicenter	RCT	180	Yes	Yes
Ferreira et al.	2009	Netherland	Retrospective	At discharge	Yes	Yes
Fink et al.	2008	Germany	Retrospective	30	Yes	No
Fjølner et al.	2016	Denmark	Retrospective	At discharge	Yes	No
Gillies et al.	2010	UK	Retrospective	At discharge	No	Yes
Goto et al.	2018	Japan	Retrospective	30	Yes	Yes
Hachimi-Idrissi et al.	2005	Belgium	RCT	30	Yes	Yes
Han et al.	2015	Korea	Retrospective	30	Yes	Yes
Holzer et al.	2002	Multicenter	RCT	180	Yes	Yes
Jouffroy et al.	2017	France	Prospective	28	Yes	Yes
Kagawa et al.	2010	Japan	Retrospective	365	Yes	Yes
Kagawa et al.	2012	Japan	Retrospective	30	Yes	Yes
Kagawa et al.	2015	Japan	Prospective	90	Yes	Yes
Kamarainen et al.	2009	Finland	RCT	At discharge	Yes	Yes
Kim et al.	2007	US	Prospective	At discharge	Yes	No
Kim et al.	2014	Korea	Retrospective	180	Yes	Yes
Kim et al.	2018	Korea	Retrospective	At discharge	Yes	Yes
Look et al.	2017	Singapore	RCT	30 or discharge	Yes	Yes
Maekawa et al.	2013	Japan	Prospective	180	Yes	Yes
Mecklenburg et al.	2020	Germany	Retrospective	28	Yes	No
Nagao et al.	2010	Japan	Retrospective	365	Yes	Yes
Nielsen et al.	2013	Multicenter	RCT	180	Yes	Yes
Okada et al.	2011	Japan	Retrospective	At discharge	No	Yes
Otani et al.	2018	Japan	Retrospective	At discharge	Yes	Yes
Pang et al.	2016	Singapore	RCT	180	Yes	Yes
Pang et al.	2017	Singapore	Retrospective	At discharge	Yes	Yes
Ryu et al.	2019	Korea	Retrospective	30	Yes	Yes
Scales et al.	2017	Canada	RCT	At discharge	Yes	Yes
Schenfeld et al.	2015	US	Retrospective	365	Yes	Yes
Schober et al.	2017	Austria	Cohort	At discharge	Yes	Yes
Shin et al.	2013	Korea	Cohort	730	Yes	No
Sonder et al.	2018	Multicenter	Prospective	At discharge	Yes	Yes
Tømte et al.	2011	Norway	Cohort	365	Yes	Yes
Yukawa et al.	2017	Japan	Retrospective	At discharge	No	Yes

TABLE 2 | Characteristics of comparison arms (A: <20 min + TTM; B: <20 min; C: 20–39 min + TTM; D: 20–39 min + TTM; E: 20–39 min; F: 40–59 min + TTM; G: ≥60 min + TTM; H: ≥60 min.

Author name	Time of collapse to ROSC of comparison arm (min)	Comparison arm	TTM case	Method of cooling	Temperature control (°C)	Male *n* (%)	Mean age (years)	OHCA *n* (%)	Witnessed arrest *n* (%)	Shockable rhythm *n* (%)	Bystander CPR *n* (%)	ACS cause CA *n* (%)
Agarwal et al.	15 vs. 20	A vs. C	385 (100)	Core	33–36	206 (54)	65	280 (73)	100 (26)	80 (21)	88 (23)	72 (19)
Arrich et al.	27 vs. 23	C vs. D	462 (79)	Core or surface	32–34	433 (74)	60	484 (83)	531 (90)	366 (62)	283 (48)	446 (76)
Blumenstein et al.	33 vs. 40	D vs. F	N/A	N/A	N/A	195 (60)	75	0	324 (100)	9 (3)	324 (100)	225 (69)
Castren et al.	18 vs. 30	A vs. C	93 (48)	Core	34	146 (75)	65	194 (100)	194 (100)	59 (30)	79 (41)	164 (85)
Chen et al.	53 vs. 43	E vs. F	59 (34)	Core	34	123 (72)	59	0	172 (100)	55 (32)	172 (100)	117 (68)
Choi et al.	16 vs. 13 vs. 22 vs. 20	A vs. B vs. C vs. D	16 (27)	Core	33	45 (75)	59	60 (100)	60 (100)	16 (27)	49 (82)	N/A
Chou et al.	15 vs. 30 vs. 42 vs. 66	B vs. D vs. F vs. H	N/A	N/A	N/A	57 (86)	64	0	66 (100)	35 (53)	66 (100)	66 (100)
De Fazio et al.	19 vs. 20	A vs. C	352 (100)	Core or surface	32–34	293 (83)	62	352 (100)	323 (92)	312 (89)	293 (83)	191 (54)
Dankiewicz et al.	35 vs. 35	C vs. D	1,861 (100)	Core	33	1,477 (79)	63	1,861 (100)	1,702 (91)	1,371 (74)	1,487 (80)	N/A
Ferreira et al.	8 vs. 10	A vs. B	49 (65)	Core or surface	32	25 (33)	64	75 (100)	N/A	25 (33)	55 (73)	25 (33)
Fink et al.	18 vs. 22	A vs. C	59 (100)	Surface	33	29 (59)	63	49 (100)	42 (86)	35 (71)	40 (82)	40 (82)
Fjølner et al.	54 vs. 70	F vs. H	N/A	N/A	N/A	12 (57)	48	21 (100)	21 (100)	14 (67)	21 (100)	12 (57)
Gillies et al.	19 vs. 22	A vs. C	34 (100)	Core or surface	32–36	63 (76)	61	73 (88)	83 (100)	53 (64)	83 (100)	N/A
Goto et al.	57 vs. 59	E vs. F	63 (44)	Core	34	122 (85)	63	144 (100)	25 (18)	88 (61)	54 (38)	100 (69)
Hachimi-Idrissi et al.	35 vs. 34	C vs. D	16 (48)	Surface	33	21 (64)	73	33 (100)	18 (55)	28 (85)	5 (15)	N/A
Han et al.	76 vs. 64	G vs. H	26 (26)	Core	32–34	74 (74)	55	75 (75)	86 (86)	54 (54)	73 (73)	N/A
Holzer et al.	21 vs. 22	C vs. D	137 (50)	Surface	32–34	210 (76)	59	275 (100)	273 (99)	275 (100)	127 (46)	51 (19)
Jouffroy et al.	36 vs. 40	C vs. E	39 (100)	Core	32–34	30 (65)	52	46 (100)	N/A	N/A	N/A	27 (59)
Kagawa et al. (2010)	17 vs. 22 vs. 43 vs. 40	B vs. C vs. E vs. F	25 (32)	Core	33–34	55 (71)	62	39 (51)	67 (87)	29 (38)	63 (82)	43 (56)
Kagawa et al. (2012)	45 vs. 55	E vs. F	32 (37)	Core	34	70 (81)	63	42 (49)	77 (90)	46 (53)	67 (80)	86 (100)
Kagawa et al. (2015)	32 vs. 43	C vs. E	237 (100)	Core	32–36	180 (76)	61	193 (81)	193 (81)	126 (53)	127 (54)	76 (32)
Kamarainen et al.	22 vs. 23	C vs. D	19 (51)	Core	33–36	35 (95)	61	37 (100)	29 (78)	28 (76)	15 (41)	32 (86)
Kim et al. (2007)	47 vs. 51	E vs. F	63 (50)	Core	33–36	88 (70)	66	125 (100)	88 (70)	51 (41)	54 (43)	N/A
Kim et al. (2014)	37 vs. 21	C vs. D	88 (18)	Core	33	326 (65)	67	499 (100)	371 (74)	116 (23)	174 (35)	226 (45)
Kim et al. (2018)	47 vs. 44	E vs. F	25 (25)	Surface	33–34	69 (68)	55	22 (22)	101 (100)	45 (45)	98 (97)	N/A
Look et al.	26 vs. 24	C vs. D	45 (52)	Core	34	69 (79)	64	72 (83)	65 (75)	9 (10)	25 (29)	21 (24)
Maekawa et al.	49 vs. 56	E vs. F	33 (20)	Core	34	123 (76)	64	162 (100)	162 (100)	56 (35)	71 (44)	N/A
Mecklenburg et al.	16 vs. 12	A vs. B	36 (55)	Core	32–34	46 (70)	51	N/A	N/A	N/A	N/A	27 (41)
Nagao et al.	58 vs. 64	E vs. G	177 (100)	Core	34	148 (84)	59	177 (100)	94 (53)	143 (81)	94 (53)	131 (74)
Nielsen et al.	25 vs. 25	C vs. D	473 (50)	Core	33 or 36	761 (81)	64	939 (100)	838 (89)	729 (78)	683 (73)	N/A
Okada et al.	17 vs. 35	A vs. C	40 (100)	Surface	34.5–35.5	53 (80)	59	66 (100)	57 (86)	52 (79)	27 (41)	44 (68)
Otani et al.	23 vs. 25 vs. 40 vs. 44	C vs. D vs. E vs. F	28 (21)	Core	34	115 (85)	65	135 (100)	135 (100)	87 (64)	74 (55)	64 (47)
Pang et al. (2016)	30 vs. 22	C vs. D	9 (43)	Core	34	17 (81)	53	2 (8)	19 (91)	7 (33)	21 (100)	N/A
Pang et al. (2017)	31 vs. 35	C vs. D	14 (18)	Core	34	62 (79)	50	6 (7)	73 (92)	33 (42)	79 (100)	62 (79)
Ryu et al.	19 vs. 38	B vs. D	N/A	N/A	N/A	174 (64)	63	24 (8)	272 (99)	79 (29)	266 (97)	104 (38)

(Continued)

TABLE 2 | Continued

Author name	Time of collapse to ROSC of comparison arm (min)	Comparison arm	TTM case	Method of cooling	Temperature control (°C)	Male *n* (%)	Mean age (years)	OHCA *n* (%)	Witnessed arrest *n* (%)	Shockable rhythm *n* (%)	Bystander CPR *n* (%)	ACS cause CA *n* (%)
Scales et al.	16 vs. 16	A vs. B	279 (48)	Core	33–36	380 (65)	68	582 (100)	351 (60)	258 (44)	270 (46)	N/A
Schenfeld et al.	17 vs. 20	A vs. C	132 (100)	Core	33	82 (62)	58	132 (100)	111 (84)	83 (63)	94 (75)	11 (8)
Schober et al.	17 vs. 17 vs. 55 vs. 55	A vs. B vs. E vs. F	51 (21)	Core	34–36	178 (74)	60	239 (100)	210 (88)	138 (58)	73 (31)	N/A
Shin et al.	32 vs. 31	C vs. D	85 (21)	Core	34	254 (63)	61	406 (100)	406 (100)	98 (24)	406 (100)	120 (30)
Sonder et al.	18 vs. 23	A vs. C	7 (6)	Core or surface	33–34	55 (46)	60	89 (74)	100 (83)	54 (45)	65 (54)	37 (31)
Tømte et al.	25 vs. 28	C vs. D	73 (45)	Core or surface	34	137 (85)	58	162 (100)	141 (87)	120 (74)	125 (77)	131 (71)
Yukawa et al.	33 vs. 45	D vs. F	N/A	N/A	N/A	65 (82)	59	79 (100)	21 (27)	58 (73)	46 (58)	39 (49)

CA, cardiac arrest; TTM, targeted temperature management; core cooling involved endovascular cooling, intravenous cold fluids, automated peritoneal lavage, any dialysis technique, extracorporeal membrane oxygenation, esophageal or transnasal; surface cooling involved skin exposure, cooling beds, iced packs, cooling pads, air-circulating or water-circulating blankets, water-filled blankets, air-filled blankets.

funnel plot was not evenly distributed, we considered publication bias to be evident in those trials. Nodesplitting models were constructed to assess the level of inconsistency between the direct and indirect evidence estimates (**Supplementary Figures 2, 6**). Global inconsistency was used to assess global inconsistency. When $P > 0.05$, we considered that there was no inconsistency (**Supplementary Figures 1, 5**). Moreover, the loop inconsistency test was used to assess the inconsistency of every closed loop. When the 95% confidence interval included 1, we considered that there was no inconsistency in this closed loop (**Supplementary Tables 2, 4**). A netweight plot was used to assess the weight of pairwise direct and indirect comparisons between different interventions (**Supplementary Figures 3, 7**).

RESULTS

Following our search strategy, an initial 3,023 articles were identified from four online databases. A total of 1,120 duplicate articles were removed. The remaining 1,903 articles were screened by reading the titles and abstracts, after which 937 additional articles were removed because they did not meet the inclusion and exclusion criteria. The full texts of 172 articles were acquired and further screened based on the inclusion and exclusion criteria. Finally, with the addition of 4 studies acquired by reviewing the articles' references, 42 studies were included in this analysis (**Figure 1**).

Characteristics of Included Trials

A total of 9,005 patients from 42 trials were included in this network meta-analysis to compare their 30-day or at-discharge survival and their neurologic outcome. Only nine of the total included trials were RCTs, with the others being retrospective or cohort trials. Of the 9,005 patients, 5,622 (62%) received TTM, of which the main cooling method was core cooling. The temperature of patients who received TTM ranged between 32 and 36°C. The vast majority of all patients were male (7,103/9,005, 79%). Survival data were available for 8,878 of the 9,005 patients, and neurologic outcome was available for 8,123 patients. The survival rate of all patients was 37% (3,315/8,878), and the rate of good neurologic outcome was 33% (2,712/8,123). Other detailed characteristics of the included trials are shown in **Tables 1**, **2**.

Survival Outcome

A total of 8,778 patients from 39 trials were included to compare survival (958 patients in the <20 min + TTM group from 11 trials; 713 patients in <20 min from 8 trials; 2,792 patients in 20–39 min + TTM from 23 trials; 2,941 patients in 20–39 min from 17 trials; 464 patients in 40–59 min + TTM from 12 trials, 609 patients in 40–59 min from 12 trials, 185 patients in ≧60 min + TTM from 2 trials and 116 patients in ≧60 min from 3 trials (**Figure 2A**).

Comparing the influence of the application of TTM in patients with the same time of collapse to ROSC, the 20–39 min + TTM group showed a significant difference from the 20–39 min [OR = 1.41, 95% CI (1.04–1.91)] group, but TTM resulted in no significant difference among the other groups (**Figure 3A**). Based

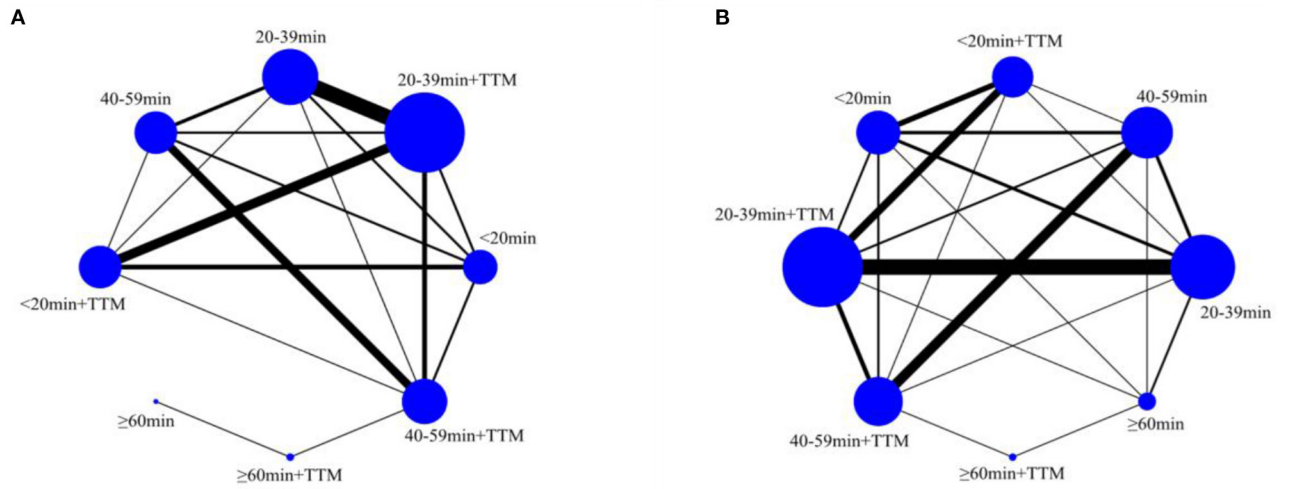


FIGURE 2 | Network plot for 30-day or at-discharge survival **(A)** and good neurologic outcome **(B)**.

on a forest plot of survival, we found a stepped comparative distribution among the different groups (which are the same background color in **Figure 5** forest plot), with the survival of patients related to the time of collapse to ROSC. Based on the rank and cumulative probability, patients in the <20 min + TTM group had the best probability of survival outcome (probability = 46.1%, SUCRA = 89.2) (**Figure 4**; **Supplementary Table 1**). We also found that there were significant differences among the other non-TTM groups in their comparisons with <20 min + TTM [20–39 min: OR = 1.82, 95% CI (1.11–2.98); 40–59 min: OR = 2.81, 95% CI (1.43–5.51); ≥60 min: OR = 6.33, 95% CI (1.90–21.11)]. When the time of collapse to ROSC exceeds 40 min, applying TTM might not improve survival, as only ≥60 min + TTM vs. 40–59 min showed a difference [OR = 3.38, 95% CI (1.07–10.66)] (**Figure 5**).

Good Neurologic Outcome

A total of 8,123 patients from 35 trials were included to compare neurologic outcome: 969 patients in the <20 min + TTM group from 11 trials; 671 patients in <20 min from 6 trials; 2,744 patients in 20–39 min + TTM from 23 trials; 2,589 patients in 20–39 min from 16 trials; 342 patients in 40–59 min + TTM from 10 trials, 549 patients in 40–59 min from 9 trials, 185 patients in ≧60 min + TTM from 2 trials and 74 patients in ≧60 min from 1 trial (**Figure 2B**).

Comparing the influence of the application of TTM in patients with the same time of collapse to ROSC, the 20–39 min + TTM group showed a significant difference from the 20–39 min [OR = 1.46, 95% CI (1.07–2.00)] group, but TTM resulted in no significant difference among the other groups (**Figure 3B**). Based on a forest plot of neurologic outcome, we found a stepped comparative distribution among the different groups (the same background color in **Figure 7** forest plot), with the neurologic outcome of patients related to the time of collapse to ROSC. Based on the rank and cumulative probability, patients in the <20 min + TTM group had the best probability of good neurologic outcome (probability = 52.5%, SUCRA = 92.2) (**Figure 6**; **Supplementary Table 3**). There were significant differences between the <20 min + TTM group and the other groups [20–39 min: OR = 1.90, 95% CI (1.18–3.06); 40–59 min + TTM: OR = 3.69, 95% CI (1.85–7.38); 40–59 min: OR = 2.63, 95% CI (1.34–5.18); ≥60 min + TTM: OR = 51.97, 95% CI (5.40–500.13); ≥60 min: OR = 12.56, 95% CI (2.32–67.83)] (**Figure 7**).

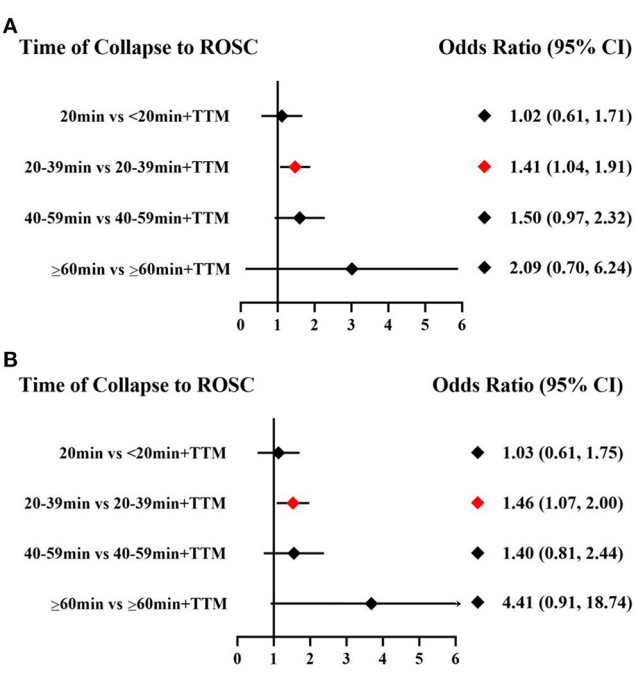


FIGURE 3 | Forest plot for survival **(A)** and good neurologic outcome **(B)** with the same time of collapse to ROSC.

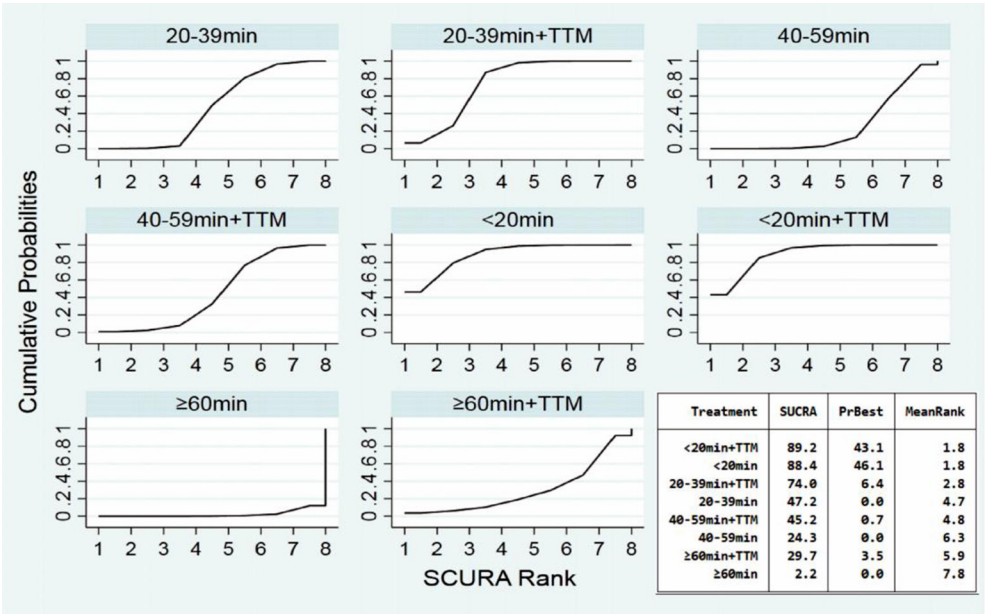


FIGURE 4 | SUCRA plot for survival (the area of SUCRA is shown in the lower right corner).

Sensitive Analysis

To detect the potential bias in this network meta-analysis, we conducted extra sensitivity analysis (15). First, we noticed that three trials included only IHCA (16–18). Thus, we consider that patients in these trials might receive CPR of higher quality by medical care personnel than OHCA by bystanders. In addition to the three trials, one trial with no record of OHCA and two trials with <10% OHCA were excluded from the sensitivity analysis (19–21). From this sensitivity analysis, we reached similar results of survival and good neurologic outcome: patients in 20–39+min TTM group had better clinical outcomes than patients in the 20–39 min without TTM group [survival: OR = 1.48, 95% CI (1.06–2.07); good neurologic outcome: OR = 1.38, 95% CI (1.01–1.88)] (**Supplementary Figures 11, 12**). Acute coronary syndrome (ACS) is one of most common causes of CA, and a previous trial showed that it might have better clinical outcomes than other diseases causing CA (22). To eliminate the potential bias that this factor may cause, we excluded 6 trials that included only ACS patients or did not record the cause of CA, which were not declared in the trial or protocol (18, 20, 23–26). From this sensitivity analysis, we reached similar results of survival and good neurologic outcome: patients in the 20–39 min TTM group had better clinical outcomes than patients in the 20–39 min without TTM group [survival: OR = 1.46, 95% CI (1.05–2.05); good neurologic outcome: OR = 1.35, 95% CI (1.01–1.80)] (**Supplementary Figures 13, 14**).

DISCUSSION

From this network meta-analysis, we found that both survival and good neurologic outcome were related to the time of collapse to ROSC. However, as this has long been clinicians' consensus, the most significant finding is that TTM did improve short-term survival and neurologic outcome for patients with CA and that improvement is also related to the time of collapse to ROSC. The positive effect of TTM takes place between 20 and 40 min of collapse to ROSC. A shorter or longer interval of collapse to ROSC applying the procedure does not appear to significantly improve survival or neurologic outcome, especially within 20 min of collapse to ROSC. Additionally, from the forest plots, we noticed that the stepped distribution was more apparent in improving neurologic outcome than survival. Therefore, we speculated that the main effect of TTM might be to improve short-term neurologic outcome for patients with CA.

Although both the AHA and ERC have recommended TTM for patients who are still in comas after ROSC from OHCA or IHCA with any initial rhythm, in clinical practice (2, 8), clinicians still hesitate to apply TTM; in particular, the latest RCT did not support this recommendation (10). What, then, is causing this hesitation? There are two main ways to implement TTM: core and surface (27). Regardless of the cooling method, the ultimate goal is to keep the core temperature at a certain level (28). A recent systematic review showed that, compared with surface cooling, core cooling could improve neurologic outcome for patients with CA (29). However, based on evidence in the currently reviewed studies, core cooling methods do not improve either survival or neurologic outcome (28, 30) and might incur more frequent bleeding complications for patients with CA (31), as confirmed by a recent meta-analysis (32). Therefore, the cooling methods do not seem to affect the outcomes of TTM for CA.

Another question related to clinician hesitation: does a difference in temperature level have any effect on the CA patient's

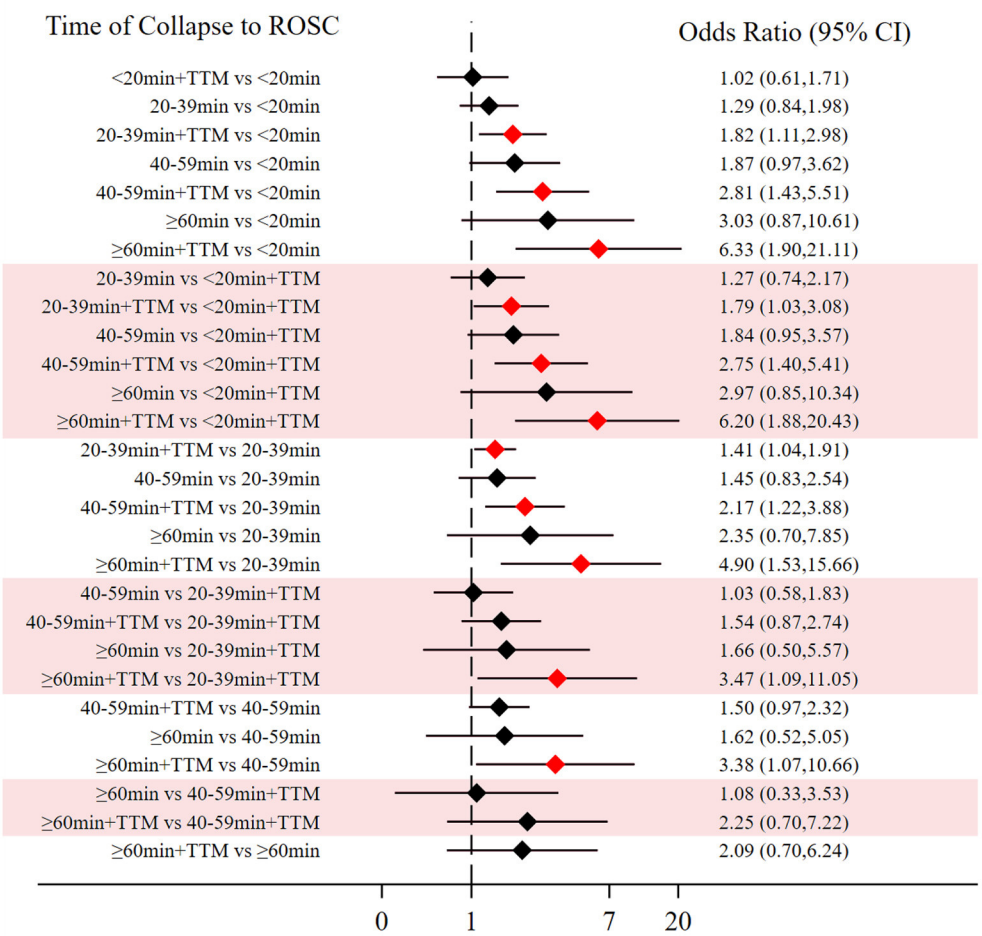
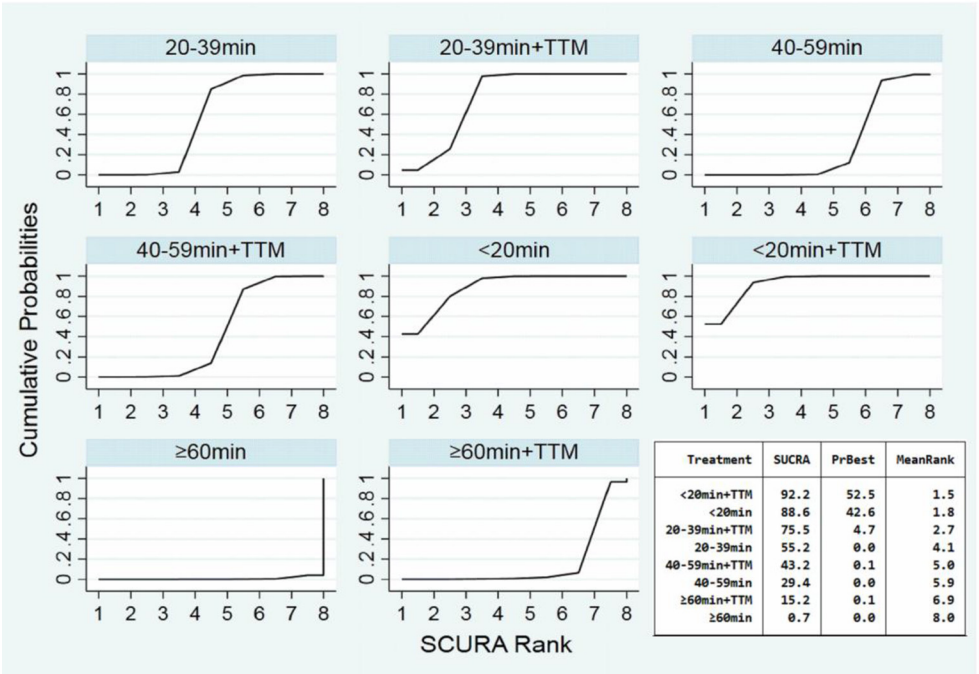


FIGURE 5 | Forest plot for survival (the stepped distributions of each group's comparison with the same given group are shown in the same background color).

clinical outcome? The recent RCT noted above has given us a definite answer. The researchers compared the mortality and neurologic outcome in CA patients at 33 and 36°C and found no significant difference at 180 days [mortality: hazard ratio = 1.06, 95% CI (0.89–1.28), $p = 0.51$; and poor neurologic outcome: risk ratio = 1.02, 95% CI (0.88–1.16), $p = 0.78$] (33). In addition to the two factors just discussed, temperature level and cooling method, there are other technological or methodological factors that might have influenced the clinical outcomes of applying TTM in the RCT, such as pre- or post-hospital cooling, local cooling, duration of TTM, and rate and extent of cooling and reheating. With regard to these multiple potential influences, it seems that the current evidence does not provide a very exact explanation (34–36), and AHA and ERC could therefore not make strong recommendations but could only offer guidance on some of these factors. Since the cooling method and temperature level did not affect the clinical outcome of CA patients in the RCT, we must conclude that it is difficult to improve the clinical outcome from either the TTM method or technology.

As we were at a loss and had to deny any benefit of TTM, we reviewed the above recent RCT again. We found that although this trial did not show a significant difference in survival and showed poor neurologic outcome at 6 months regardless of whether patients received TTM [RR = 1.04; 95% CI (0.94–1.14); $p = 0.37$ and RR = 1.00; 95% CI (0.92–1.09), respectively], the survival rate was surprisingly high in both groups [hypothermia group: 460/925 (50%); normothermia group: 479/925 (56%)] (10). in China, the survival rate of OHCA patients was ∼1% in 2018 (37). Moreover, the survival rate at discharge of OHCA with ROSC was <20% in China, and these data come from the standard cardiac arrest center (38). We did not question the methodology of this RCT, and we hypothesized that, in addition to TTM, there were other factors influencing the results. Many demographic characteristics, for example, can influence results, but the cause of CA is one of the most significant factors influencing clinical outcomes for patients. A recent meta-analysis showed that patients with CA caused by acute coronary syndromes, ventricular tachycardia, ventricular fibrillation, and other heart diseases had better survival outcomes than patients with CA from other causes [OR = 3.76, 95% CI (2.95–4.78), $p < 0.001$] (39). Moreover, an interesting trial showed that TTM might increase mortality for patients with non-shockable



Treatment	SUCRA	PrBest	MeanRank
<20min+TTM	92.2	52.5	1.5
<20min	88.6	42.6	1.8
20-39min+TTM	75.5	4.7	2.7
20-39min	55.2	0.0	4.1
40-59min+TTM	43.2	0.1	5.0
40-59min	29.4	0.0	5.9
≥60min+TTM	15.2	0.1	6.9
≥60min	0.7	0.0	8.0

FIGURE 6 | SUCRA plot for good neurologic outcome (the area of SUCRA is shown in the lower right corner).

rhythm. However, this trial did not match the time of ROSC, cause of CA and other baseline characteristics, which have a significant underlying influence on the prognosis of CA (40). In summary, it was associated with typical clinical manifestations, specific laboratory results, and mature removal techniques of etiology (41).

The other most important factor influencing patients' clinical outcomes is the time of collapse to ROSC. When the time of collapse to ROSC is extended, the patient's clinical outcome will deteriorate, despite an effective and high-quality implementation of CPR. However, concepts of pathology and physiopathology can explain the observation (42–44). Therefore, patient outcomes are based on the time of collapse to ROSC, regardless of whether any intervention is applied. If an intervention is applied for patients with shorter or longer intervals of collapse to ROSC, there may be no significant clinical benefit for patients with CA. Therefore, it is too early to completely deny the role of TTM. In *in vitro* and animal trials, TTM reduced injury to cells (45). We speculate that the level of cell injury or necrosis does not cause organ dysfunction or failure within 20 min of collapse to ROSC, so although TTM lessens the degree of cell damage, it does not show an improvement in clinical outcomes. In contrast, when TTM is applied for patients with a longer interval of collapse to ROSC (≥40 min in this meta-analysis), organ function damage has already appeared and is irreversible. Based on this network meta-analysis, patients with a time from collapse to ROSC < 20 min might have mild injury, and patients with a time from collapse to ROSC more than 40 min might have severe injury in organs. Because clinical outcomes in these patients may be predicted with a high probability, the role of TTM may be misestimated by studies including the above patients with shorter or longer times from collapse to ROSC. Most importantly, more attention should be given to patients with moderate injury (the time from collapse to ROSC within 20–40 min) who are at high risk of developing severe injury. Thus, just as with primary percutaneous coronary intervention or emergency thrombolysis for acute myocardial infarction or ischemic stroke, there might be an optimal time window to intervene for improving clinical outcomes for patients with CA (41, 46). If the results we reach may be confirmed in the future, we might be able to put an end to endless debate about TTM. In other words, avoiding ineffective application of TTM can not only reduce the cost burden but also avoid the occurrence of TTM-related adverse events, such as bleeding or bradyarrhythmia. Based upon time from collapse to ROSC, successful trials may be achieved by excluding patients who will become well regardless of what and those who are likely to become poorly regardless of what. Based on the above evidence and speculation, we conducted this network meta-analysis, and we did find an optimal time window, within 20–39 min of collapse to ROSC, in which to apply TTM. Moreover, we believe that the optimal time window may not only benefit patients but also provide a meaningful reference for future trials. Of course, TTM is only one of the most well-established interventions. There are certainly other interventions that are effective in improving patients' clinical outcomes, and it only remains for us to find evidence to support their application.

LIMITATIONS

This network meta-analysis has the following limitations: (a) since most of the included trials were retrospective, we could only cautiously summarize their conclusions; (b) we did not utilize a subgroup with which to compare clinical outcomes among

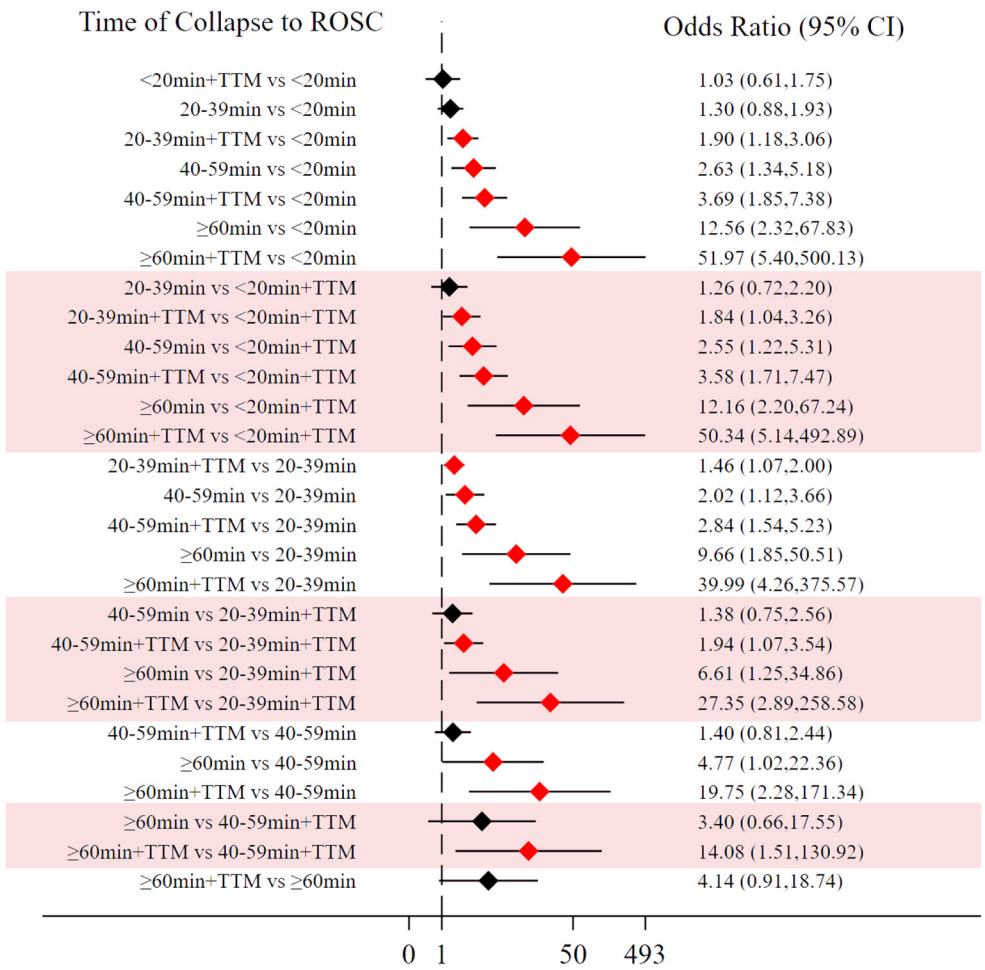


FIGURE 7 | Forest plot for good neurologic outcome (the stepped distributions of each group's comparison with the same given group are shown in the same background color).

different temperature levels; (c) the number of patients and trials was small, and might have biased the results; (d) both in survival and neurologic outcome, due to the lack of direct comparison between some of the groups, a complete closed loop could not be formed; there might therefore be some bias in the results of only indirect comparisons; (e) the 20-min interval might be too broad, and thus may mask some of the underlying factors; (f) we only compared short-term (30 days and at discharge) clinical outcomes.

CONCLUSION

From this network meta-analysis, we drew the following conclusions: (a) the survival and neurologic outcomes were related to the time of collapse to ROSC, and with the extension of time, these clinical outcomes would deteriorate for patients with CA; (b) TTM is still effective for CA patients to improve short-term clinical outcomes, however, this effect might be shown only within 20–40 min of collapse to ROSC; (c) the effectiveness of TTM might be inconclusive in improving survival, but its role in improving neurologic outcome should be recognized. Of course, based on our findings, further trials should pay more attention to patients with moderate time (20–39 min) from collapse to ROSC who suffer from moderate injury and intend to become worse.

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

JD: writing, searching, and statistics. QZ: draw pictures and table and collection. YS: screening articles. HG: screening articles and editing language. KZ: language editing and providing sensitive analysis. LD and BD: methodology. JY: methodology and editing language. QM: conceptualization and methodology. 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.2021.784917/full#supplementary-material

REFERENCES

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation.* (2020) 141:e139–e596. doi: 10.1161/CIR.0000000000000757
2. Panchal AR, Bartos JA, Cabañas JG, Donnino MW, Drennan IR, Hirsch KG, et al. Part 3: adult basic and advanced life support: 2020 American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. *Circulation.* (2020) 142 (16_suppl_2):S366–s468. doi: 10.1161/CIR.0000000000000916
3. Gunn AJ, Laptook AR, Robertson NJ, Barks JD, Thoresen M, Wassink G, et al. Therapeutic hypothermia translates from ancient history in to practice. *Pediatr Res.* (2017) 81:202–9. doi: 10.1038/pr.2016.198
4. Peberdy MA, Callaway CW, Neumar RW, Geocadin RG, Zimmerman JL, Donnino M, et al. Part 9: post-cardiac arrest care: 2010 American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. *Circulation.* (2010) 122 (18 Suppl 3):S768–86. doi: 10.1161/CIRCULATIONAHA.110.971002
5. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *New Engl J Med.* (2002) 346:557–63. doi: 10.1056/NEJMoa003289
6. Group HaCAS. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *New Engl J Med.* (2002) 346:549–56. doi: 10.1056/NEJMoa012689
7. Lascarrou JB, Merdji H, Le Gouge A, Colin G, Grillet G, Girardie P, et al. Targeted temperature management for cardiac arrest with nonshockable rhythm. *New Engl J Med.* (2019) 381:2327–37. doi: 10.1056/NEJMoa1906661
8. Perkins GD, Graesner JT, Semeraro F, Olasveengen T, Soar J, Lott C, et al. European Resuscitation council guidelines 2021: executive summary. *Resuscitation.* (2021) 161:1–60. doi: 10.1016/j.resuscitation.2021.02.003
9. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. *Crit Care Med.* (2009) 37 (7 Suppl.):S186–202. doi: 10.1097/CCM.0b013e3181aa5241
10. Dankiewicz J, Cronberg T, Lilja G, Jakobsen JC, Levin H, Ullén S, et al. Hypothermia versus normothermia after out-of-hospital cardiac arrest. *New Engl J Med.* (2021) 384:2283–94. doi: 10.1056/NEJMoa2100591
11. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
12. Haywood K, Whitehead L, Nadkarni VM, Achana F, Beesems S, Böttiger BW, et al. COSCA (core outcome set for cardiac arrest) in adults: an advisory statement from the International Liaison Committee on Resuscitation. *Circulation.* (2018) 137:e783–e801. doi: 10.1161/CIR.0000000000000562
13. Golan E, Barrett K, Alali AS, Duggal A, Jichici D, Pinto R, et al. Predicting neurologic outcome after targeted temperature management for cardiac arrest: systematic review and meta-analysis. *Crit Care Med.* (2014) 42:1919–30. doi: 10.1097/CCM.0000000000000335
14. Salanti G, Ades AE, Ioannidis JP. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J Clin Epidemiol.* (2011) 64:163–71. doi: 10.1016/j.jclinepi.2010.03.016
15. Kent DM, Rothwell PM, Ioannidis JP, Altman DG, Hayward RA. Assessing and reporting heterogeneity in treatment effects in clinical trials: a proposal. *Trials.* (2010) 11:85. doi: 10.1186/1745-6215-11-85
16. Blumenstein J, Leick J, Liebetrau C, Kempfert J, Gaede L, Groß S, et al. Extracorporeal life support in cardiovascular patients with observed refractory in-hospital cardiac arrest is associated with favourable short and long-term outcomes: a propensity-matched analysis. *Eur Heart J Acute Cardiovasc Care.* (2016) 5:13–22. doi: 10.1177/2048872615612454
17. Chen YS, Lin JW, Yu HY, Ko WJ, Jerng JS, Chang WT, et al. Cardiopulmonary resuscitation with assisted extracorporeal life-support versus conventional cardiopulmonary resuscitation in adults with in-hospital cardiac arrest: an observational study and propensity analysis. *Lancet.* (2008) 372:554–61. doi: 10.1016/S0140-6736(08)60958-7
18. Chou TH, Fang CC, Yen ZS, Lee CC, Chen YS, Ko WJ, et al. An observational study of extracorporeal CPR for in-hospital cardiac arrest secondary to myocardial infarction. *EMJ.* (2014) 31:441–7. doi: 10.1136/emermed-2012-202173
19. Mecklenburg A, Stamm J, Angriman F, Del Sorbo L, Fan E, Soeffker G, et al. Impact of therapeutic hypothermia on bleeding events in adult patients treated with extracorporeal life support peri-cardiac arrest. *J Crit Care.* (2021) 62:12–8. doi: 10.1016/j.jcrc.2020.11.008
20. Pang PYK, Wee GHL, Hoo AEE, Sheriff IMT, Lim SL, Tan TE, et al. Therapeutic hypothermia in adult patients receiving extracorporeal life support: early results of a randomized controlled study. *J Cardiothor Surg.* (2016) 11:43. doi: 10.1186/s13019-016-0437-8
21. Pang PYK, Wee GHL, Huang MJ, Hoo AEE, Sheriff IMT, Lim SL, et al. Therapeutic hypothermia may improve neurological outcomes in extracorporeal life support for adult cardiac arrest. *Heart Lung Circ.* (2017) 26:817–24. doi: 10.1016/j.hlc.2016.11.022
22. Omer MA, Tyler JM, Henry TD, Garberich R, Sharkey SW, Schmidt CW, et al. Clinical characteristics and outcomes of STEMI patients with cardiogenic shock and cardiac arrest. *JACC Cardiovasc Intervent.* (2020) 13:1211–9. doi: 10.1016/j.jcin.2020.04.004
23. Kagawa E, Dote K, Kato M, Sasaki S, Nakano Y, Kajikawa M, et al. Should we emergently revascularize occluded coronaries for cardiac arrest? Rapid-response extracorporeal membrane oxygenation and intra-arrest percutaneous coronary intervention. *Circulation.* (2012) 126:1605–+. doi: 10.1161/CIRCULATIONAHA.111.067538
24. Hachimi-Idrissi S, Zizi M, Nguyen DN, Schiettecate J, Ebinger G, Michotte Y, et al. The evolution of serum astroglial S-100 beta protein in patients with cardiac arrest treated with mild hypothermia. *Resuscitation.* (2005) 64:187–92. doi: 10.1016/j.resuscitation.2004.08.008
25. Schooer A, Sterz F, Herkner H, Wallmueller C, Weiser C, Huoner P, et al. Emergency extracorporeal life support and ongoing resuscitation: a retrospective comparison for refractory out-of-hospital cardiac arrest. *Emerg Med J.* (2017) 34:277–81. doi: 10.1136/emermed-2015-205232
26. Han KS, Kim SJ, Lee EJ, Jung JS, Park JH, Lee SW. Experience of extracorporeal cardiopulmonary resuscitation in a refractory cardiac arrest patient at the emergency department. *Clin Cardiol.* (2019) 42:459–66. doi: 10.1002/clc.23169
27. Polderman KH, Herold I. Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods. *Crit Care Med.* (2009) 37:1101–20. doi: 10.1097/CCM.0b013e3181962ad5
28. Look X, Li H, Ng M, Lim ETS, Pothiawala S, Tan KBK, et al. Randomized controlled trial of internal and external targeted temperature management methods in post- cardiac arrest patients. *Am J Emerg Med.* (2018) 36:66–72. doi: 10.1016/j.ajem.2017.07.017
29. Liao X, Zhou Z, Zhou M, Tang H, Feng M, Kou B, et al. Effects of endovascular and surface cooling on resuscitation in patients with cardiac arrest and a comparison of effectiveness, stability, and safety: a systematic review and meta-analysis. *Crit Care.* (2020) 24:27. doi: 10.1186/s13054-020-2731-z
30. Deye N, Cariou A, Girardie P, Pichon N, Megarbane B, Midez P, et al. Endovascular versus external targeted temperature

management for patients with out-of-hospital cardiac arrest: a randomized, controlled study. *Circulation.* (2015) 132:182–93. doi: 10.1161/CIRCULATIONAHA.114.012805
31. Pittl U, Schratter A, Desch S, Diosteanu R, Lehmann D, Demmin K, et al. Invasive versus non-invasive cooling after in- and out-of-hospital cardiac arrest: a randomized trial. *Clin Res Cardiol.* (2013) 102:607–14. doi: 10.1007/s00392-013-0572-3
32. Calabró L, Bougouin W, Cariou A, De Fazio C, Skrifvars M, Soreide E, et al. Effect of different methods of cooling for targeted temperature management on outcome after cardiac arrest: a systematic review and meta-analysis. *Crit Care.* (2019) 23:285. doi: 10.1186/s13054-019-2567-6
33. Nielsen N, Wetterslev J, Cronberg T, Erlinge D, Gasche Y, Hassager C, et al. Targeted temperature management at 33°C versus 36°C after cardiac arrest. *New Engl J Med.* (2013) 369:2197–206. doi: 10.1056/NEJMoa1310519
34. Kirkegaard H, Søreide E, de Haas I, Pettilä V, Taccone FS, Arus U, et al. Targeted temperature management for 48 vs 24 hours and neurologic outcome after out-of-hospital cardiac arrest: a randomized clinical trial. *JAMA.* (2017) 318:341–50. doi: 10.1001/jama.2017.8978
35. Donnino MW, Andersen LW, Berg KM, Reynolds JC, Nolan JP, Morley PT, et al. Temperature Management after cardiac arrest: an advisory statement by the advanced life support task force of the International Liaison Committee on Resuscitation and the American Heart Association Emergency Cardiovascular Care Committee and the Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation. *Circulation.* (2015) 132:2448–56. doi: 10.1161/CIR.0000000000000313
36. Scales DC, Cheskes S, Verbeek PR, Pinto R, Austin D, Brooks SC, et al. Prehospital cooling to improve successful targeted temperature management after cardiac arrest: a randomized controlled trial. *Resuscitation.* (2017) 121:187–94. doi: 10.1016/j.resuscitation.2017.10.002
37. Feng XF, Hai JJ, Ma Y, Wang ZQ, Tse HF. Sudden cardiac death in mainland china: a systematic analysis. *Circ Arrhythmia Electrophysiol.* (2018) 11:e006684. doi: 10.1161/CIRCEP.118.006684
38. Teng Y, Li Y, Xu L, Chen F, Chen H, Jin L, et al. Awareness, knowledge and attitudes towards cardiopulmonary resuscitation among people with and without heart disease relatives in South China: a cross-sectional survey. *BMJ Open.* (2020) 10:e041245. doi: 10.1136/bmjopen-2020-041245
39. Fernando SM, Tran A, Cheng W, Rochwerg B, Taljaard M, Vaillancourt C, et al. Pre-arrest and intra-arrest prognostic factors associated with survival after in-hospital cardiac arrest: systematic review and meta-analysis. *BMJ.* (2019) 367:l6373. doi: 10.1136/bmj.l6373
40. Khan MZ, Sulaiman S, Agrawal P, Osman M, Khan MU, Khan SU, et al. Targeted temperature management in cardiac arrest patients with a non-shockable rhythm: a national perspective. *Am Heart J.* (2020) 225:129–37. doi: 10.1016/j.ahj.2020.04.023
41. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J.* (2018) 39:119–77. doi: 10.1093/eurheartj/ehx393
42. Piper HM, Balser C, Ladilov YV, Schäfer M, Siegmund B, Ruiz-Meana M, et al. The role of Na+/H+ exchange in ischemia-reperfusion. *Basic Res Cardiol.* (1996) 91:191–202. doi: 10.1007/BF00788905
43. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol.* (2016) 13:193–209. doi: 10.1038/nrcardio.2016.5
44. Hearse DJ. Ischemia, reperfusion, and the determinants of tissue injury. *Cardiovasc Drugs Ther.* (1990) 4 (Suppl. 4):767–76. doi: 10.1007/BF00051274
45. Polderman KH. Application of therapeutic hypothermia in the intensive care unit. Opportunities and pitfalls of a promising treatment modality–Part 2: practical aspects and side effects. *Intens Care Med.* (2004) 30:757–69. doi: 10.1007/s00134-003-2151-y
46. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211

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Can Cardiopulmonary Rehabilitation Facilitate Weaning of Extracorporeal Membrane Oxygenation (CaRe-ECMO)? Study Protocol for a Prospective Multidisciplinary Randomized Controlled Trial

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Background: Mortality of patients suffering from critical illness has been dramatically improved with advanced technological development of extracorporeal membrane oxygenation (ECMO) therapy. However, the majority of ECMO-supported patients failed to wean from ECMO therapy. As one of several options, cardiopulmonary rehabilitation serves as effective intervention in the improvement of cardiovascular and respiratory function in various major critical illness. Nonetheless, its role in facilitating ECMO weaning has not yet been explored. The purpose of this study is to investigate the effectiveness of cardiopulmonary rehabilitation on rate of ready for ECMO weaning in ECMO-supported patients (CaRe-ECMO).

Methods: The CaRe-ECMO trial is a randomized controlled, parallel group, clinical trial. This trial will be performed in a minimum number of 366 ECMO-supported eligible patients. Patients will be randomly assigned to either: (1) the CaRe-ECMO group, which will be treated with usual care including pharmacotherapy, non-pharmacotherapy, and specific nursing for ECMO therapy and the CaRe-ECMO program; or (2) the control group, which will receive usual care only. The CaRe-ECMO program consists of protocolized positioning, passive range of motion (PROM) training, neuromuscular electrical stimulation (NMES), surface electrical phrenic nerve stimulation (SEPNS), and pulmonary rehabilitation. The primary outcome of the CaRe-ECMO trial is the rate of ready for ECMO weaning at CaRe-ECMO day 7 (refers to 7 days after the CaRe-ECMO program initiation). Secondary outcomes include rate of ECMO and mechanical ventilation weaning, total length in day of ready for ECMO weaning, ECMO weaning and mechanical ventilation, all-cause mortality, rate of major post-ECMO complications, ECMO unit length of stay (LOS) and hospital LOS, total cost for

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hospitalization, cerebral performance category (CPC), activities of daily living (ADL), and health-related quality of life (HRQoL).

Discussion: The CaRe-ECMO is designed to answer the question "whether cardiopulmonary rehabilitation can facilitate weaning of ECMO (CaRe-ECMO)." Should the implementation of the CaRe-ECMO program result in superior primary and secondary outcomes as compared to the controls, specifically the add-on effects of cardiopulmonary rehabilitation to the routine ECMO practice for facilitating successful weaning, the CaRe-ECMO trial will offer an innovative treatment option for ECMO-supported patients and meaningfully impact on the standard care in ECMO therapy.

Clinical Trial Registration: ClinicalTrials.gov, identifier: NCT05035797.

Keywords: cardiopulmonary rehabilitation, extracorporeal membrane oxygenation, weaning, multidisciplinary, randomized controlled trial, open-label

INTRODUCTION

Recent improvements in technology have created extra chances for lifesaving, i.e., using extracorporeal membrane oxygenation (ECMO) as a bridge to win over time for the body and medical strategies struggling with critical illness (1). Patients with acute cardiac, pulmonary, or cardiopulmonary failure who have failed conventional treatment appeared to benefit from ECMO therapy (2). However, this population is often exposed to prolonged immobilization, which may lead to multiple complications and impaired physical function (3). Shortening the sedative course and managing issues such as intensive care unit (ICU)-acquired weakness and critical illness-induced cardiopulmonary function impairment should take priority, as this can greatly impact the post-ECMO prognosis. In this context, attention toward enhancing ICU recovery, reducing complications, and improving functional prognosis has started to be shifted to ECMO-supported patients (4). Upon this perspective, early rehabilitation is recommended to be applied in this population.

The general aim of early rehabilitation for critical illness is to improve or maintain cardiopulmonary function, which encounters the requirement of ECMO-supported patients (5). There is a variety of early rehabilitation techniques that may benefit patients with critical illness (6). However, due to the huge difficulties in the application of early rehabilitation in ECMO-supported patients, only a few evidence has been published (4, 7). Considering their pathophysiological condition, regardless of venovenous ECMO (VV-ECMO) or venoarterial ECMO (VA-ECMO), cardiopulmonary rehabilitation is hypothesized to compensate cardiopulmonary function of patients and consequently speed up the recovery process after the emergency period (normally after 72 h) (8). For instance, cardiac output and maximal oxygen uptake were proven to increase after one subtype of cardiopulmonary rehabilitation in intubated, sedated patients confined to bed in the ICU (9).

Upon the above rationale, the upcoming question is that what benefits of cardiopulmonary rehabilitation can be delivered to ECMO-supported patients. For clinicians, ECMO weaning is particularly challenging and annual ECMO mortality has been reported to be approximately ranged 40–70% (10, 11). According to our experience, delayed ECMO weaning also demonstrated worse functional prognosis and impaired health-related quality of life (HRQoL). Previous studies have documented that rate of mechanical ventilation weaning was improved in patients received early rehabilitation intervention, while its effectiveness in ECMO weaning remains unclear (12). Indeed, the successful weaning of ECMO is complex and multifactorial (13). ECMO weaning at the earliest possible time would be expected to improve outcomes, reduce cost, and optimize functional prognosis (14). This inspires us to hypothesize that if the medical rationale is based on its assumed benefits on cardiac function and oxygenation, then cardiopulmonary rehabilitation may subsequently contribute to earlier weaning of ECMO. Our perspective is to provide solutions with the application of cardiopulmonary rehabilitation and convert the aforementioned physiological changes (e.g., increased cardiopulmonary function) into patient-oriented outcomes (e.g., earlier weaning of ECMO and increased rate of ECMO weaning).

Considering the limited evidence in this aspect, ongoing innovation must move from guessing to exploring the effectiveness of cardiopulmonary rehabilitation through an adequately powered trial. Therefore, we design this multidisciplinary randomized controlled trial (RCT) to answer the following clinical question—can cardiopulmonary rehabilitation facilitate weaning of ECMO (CaRe-ECMO)? The results of this trial may subsequently provide innovative treatment solution, specifically the add-on effects of cardiopulmonary rehabilitation to the routine clinical practice for further successful weaning of ECMO.

METHODS/DESIGN

Trial Design

The CaRe-ECMO trial is a pragmatic, multidisciplinary, randomized controlled, parallel group, clinical trial. **Table 1** shows the overview of the trial registration information. The trial protocol was developed according to the Consolidated Standards of Reporting Trials (CONSORT) statements for pragmatic trials and non-pharmacological treatment interventions (15, 16).

TABLE 1 | The WHO trial registration data set for the CaRe-ECMO trial.

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov Registry number: NCT05035797
Date of registration in primary registry	01 September 2021
Secondary identifying numbers	N/A
Trial protocol version	Version 1
Source(s) of monetary or material support	Nanjing Municipal Science and Technology Bureau
Primary sponsor	Nanjing Municipal Science and Technology Bureau
Secondary sponsor	N/A
Contact for public queries	XL, luxiao1972@163.com; JSZ, zhangjso@njmu.edu.cn; XFC, cxfyx@njmu.edu.cn
Contact for scientific queries	XL, luxiao1972@163.com; JSZ, zhangjso@njmu.edu.cn; XFC, cxfyx@njmu.edu.cn
Public title	Can cardiopulmonary rehabilitation facilitate weaning of extracorporeal membrane oxygenation (CaRe-ECMO)? A prospective multidisciplinary randomized controlled trial
Scientific title	Impact of cardiopulmonary rehabilitation on weaning of extracorporeal membrane oxygenation (CaRe-ECMO): A prospective multidisciplinary randomized controlled clinical trial
Countries of recruitment	China
Health condition(s) or problem(s) studied	Rate of ECMO weaning in critical care patients supported with ECMO
Intervention(s)	Active comparator: Usual care, ECMO and cardiopulmonary rehabilitation
Key inclusion and exclusion criteria	Placebo comparator: Usual care and ECMO
	Ages eligible for study: aged 18yr or order
	Sexes eligible for study: both
	Accepts health volunteers: No
	Inclusion criteria: see "Eligibility and withdrawal criteria" section, and **Tables 2**, **3**
	Exclusion criteria: see "Eligibility and withdrawal criteria" section, and **Tables 2**, **3**
Study type	Type: Pragmatic, multidisciplinary, randomized controlled, parallel group, clinical trial
	Allocation: Simple randomization
	Intervention model: Parallel assignment
	Masking: Assessor, physician, data analyst, and statistician blinded
	Primary purpose: Prevention and improvement
	Phase: N/A
Date of first enrollment	Not yet started
Target sample size	366
Recruitment status	Not yet started
Primary outcome(s)	Rate of ready for ECMO weaning at CaRe-ECMO Day 7
Key secondary outcomes	Rate of ECMO weaning, total length of ready for ECMO weaning, total length of ECMO weaning, rate of mechanical ventilation weaning, total length of mechanical ventilation, all-cause mortality, rate of major post-ECMO complications, ECMO Unit LOS, total hospital LOS, total cost for hospitalization, CPC, ADL, and HRQoL

CaRe-ECMO, cardiopulmonary rehabilitation on extracorporeal membrane oxygenation; LOS, length of stay; CPC, cerebral performance category; ADL, activities of daily living; HRQoL, health-related quality of life.

Trial Objectives

The primary objective of the CaRe-ECMO trial is to investigate the impact of cardiopulmonary rehabilitation combined with usual care on rate of ready for ECMO weaning at CaRe-ECMO day 7 (refers to 7 days after the CaRe-ECMO program initiation), when compared to usual care alone. Secondary objectives are to evaluate the effects of cardiopulmonary rehabilitation on rate of ECMO weaning, total length of ready for ECMO weaning, total length of ECMO weaning, rate of weaning of mechanical ventilation, total length of mechanical ventilation, all-cause mortality, rate of major post-ECMO complications, ICU length of stay (LOS), total hospital LOS, total cost for hospitalization, cerebral performance category (CPC), activities of daily living (ADL), and HRQoL.

Ethics Statement

The CaRe-ECMO trial has been prospectively registered at the ClinicalTrials.gov (https://register.clinicaltrials.gov/): NCT05035797, 1 September, 2021. The trial protocol has been reviewed and approved by the Research Ethics Committee at the First Affiliated Hospital of Nanjing Medical University. In accordance with the Declaration of Helsinki of 1964 (revised in 2013), a written informed consent will be obtained from the legal guardians of all the enrolled patients (17).

Trial Setting

The CaRe-ECMO trial will be mainly conducted in the Department of Emergency Medicine, the First Affiliated

Hospital of Nanjing Medical University (a 3,700-bed primary referral hospital in Nanjing, Eastern China), who is capable to provide ECMO therapy for ∼200 critical care patients annually. Clinicians from the Department of Emergency Medicine are required to have training certifications in ECMO therapy and at least 5-year therapeutic experience in management of critical care patients supported with ECMO. The Department of Rehabilitation Medicine will mainly be responsible for cardiopulmonary rehabilitation delivery. Clinicians from both the departments will collaborate on cardiopulmonary rehabilitation prescription. Therapists are required to have a certified degree in cardiopulmonary rehabilitation and at least 3-year therapeutic experience with critical care patients. The trial coordinating group will implement the CaRe-ECMO program guidelines and standard operation procedures (SOPs) for all the medical staffs via a kickoff meeting to be held in September 2021 (Nanjing, China).

Eligibility and Withdrawal Criteria

The consolidated criteria for patient enrollment are those: (1) aged 18 years or older; (2) eligible for receiving ECMO (VV or VA) therapy; (3) with mechanical ventilation; (4) with stable condition and eligible for cardiopulmonary rehabilitation after 72 h of ECMO; (5) with no contraindications for cardiopulmonary rehabilitation; (6) not pregnant; (7) with a life expectancy of more than 3 days; (8) wean from ECMO therapy within the first 3 days before the initiation of the CaRe-ECMO program; (9) not use ECMO as a bridge to recovery or definitive treatment (e.g., lung transplantation or heart transplantation); (10) not enrolled in another trial previously; and (11) sign informed consent form by the guardian (18). According to previously published guidelines, the specific indications and contraindications for VV-ECMO and VA-ECMO are given in **Tables 2**, **3**, respectively (19, 20).

Patients will be withdrawn from the trial if: (1) the guardian makes such a request with no reasons; (2) the patient develops adverse events due to cardiopulmonary rehabilitation (e.g., rib fracture, limb fracture); and (3) the patient develops ECMO-related complications (e.g., bleeding, thromboembolism, severe infection) and is ever unsuitable for participating in the trial according to the point of view by clinicians.

Enrollment, Randomization, and Allocation

Eligible patients who meet the eligibility criteria and are provided with ECMO therapy in our Department of Emergency Medicine will be enrolled in the CaRe-ECMO trial. Their guardians will receive a detailed explanation of trial purpose and procedures. The patients will have a same chance to be randomly assigned to the CaRe-ECMO group or the control group. They also will be informed that they have the right to withdraw from the trial at any time of the trial period.

Simple randomization (1:1) will be applied using a computer generated random sequence, which will be independently administered and concealed by the Clinical Research Board from the School of Public Health of Nanjing Medical University (from here on called allocation center). Once a patient has been deemed eligible and the informed consent form has been signed, an independent trial assistant will register the participant, record her/his group allocation, and reveal the group allocation to those delivering the interventions (21). **Figure 1** demonstrates the overview of the recruitment, randomization, and allocation based on the CONSORT principle.

TABLE 2 | Indications and contraindications for VV-ECMO.

Indications for VV-ECMO (one or more of the following)
1. Hypoxemic respiratory failure (PaO_2/FiO_2 < 80 mmHg), after optimal medical management (FiO_2 > 80%)
2. pH < 7.25 with $PaCO_2$ ≥ 60 mmHg for more than 6 h, despite optimal conventional mechanical ventilation (respiratory rate of 35 bpm and P_{plat} ≤ 30 cmH_2O)
3. Airway obstruction, unable to establish advanced airways
Contraindications for VV-ECMO
1. Irreversible non-cardiac organ failure limiting survival (e.g., severe anoxic brain injury or metastatic cancer)
2. Irreversible and incapacitating central nervous system pathology
3. Irreversible and incapacitating cardiovascular system pathology
4. Systemic bleeding
5. Mechanical ventilation for more than 7 days with P_{plat} > 30 cmH_2O and FiO_2 > 90%
6. Limited vascular access (severe peripheral arterial disease, extreme obesity, amputated limbs, among others)
7. Older age (increasing risk of death with increasing age)

VV-ECMO, venovenous extracorporeal membrane oxygenation; PaO_2, partial pressure of arterial oxygen; FiO_2, fraction of inspired oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; P_{plat}, plateau pressure.

TABLE 3 | Indications and contraindications for VA-ECMO.

Indications for VA-ECMO (one or more of the following)
1. Rapidly deteriorating or severe cardiogenic shock (cardiac Index < 2.2 L/min/m^2 with * VIS > 40) with LVEF < 35%
2. Rapidly deteriorating or severe cardiogenic shock (cardiac Index < 2.2 L/min/m^2 with * VIS > 40) with LVEF of 35–55%, and severe mitral regurgitation or aortic stenosis
3. Two consecutive lactate values ≥4 mmol/L (with at least 30 min interval between sampling), with non-decreasing trend on steady dose of inotropes and/or vasopressors
4. Witnessed cardiopulmonary resuscitation for more than 10 min or intermittent ROSC but unable to maintain
Contraindications for VA-ECMO
1. Irreversible non-cardiac organ failure limiting survival (e.g., severe anoxic brain injury or metastatic cancer)
2. Irreversible and incapacitating central nervous system pathology
3. Irreversible and incapacitating cardiovascular system pathology
4. Systemic bleeding
5. Aortic dissection
6. Limited vascular access (severe peripheral arterial disease, extreme obesity, amputated limbs, among others)
7. Older age (increasing risk of death with increasing age)

VA-ECMO, venoarterial extracorporeal membrane oxygenation; LVEF, left ventricular ejection fraction; SvO_2, mixed venous oxygen saturation; ROSC, return of spontaneous circulation; VAD, ventricular assist device.

**Vasoactive-inotrope score (VIS): (Epinephrine dose + Norepinephrine dose) μg/kg/min × 100 + (Dopamine dose + Dobutamine dose) μg/kg/min.*

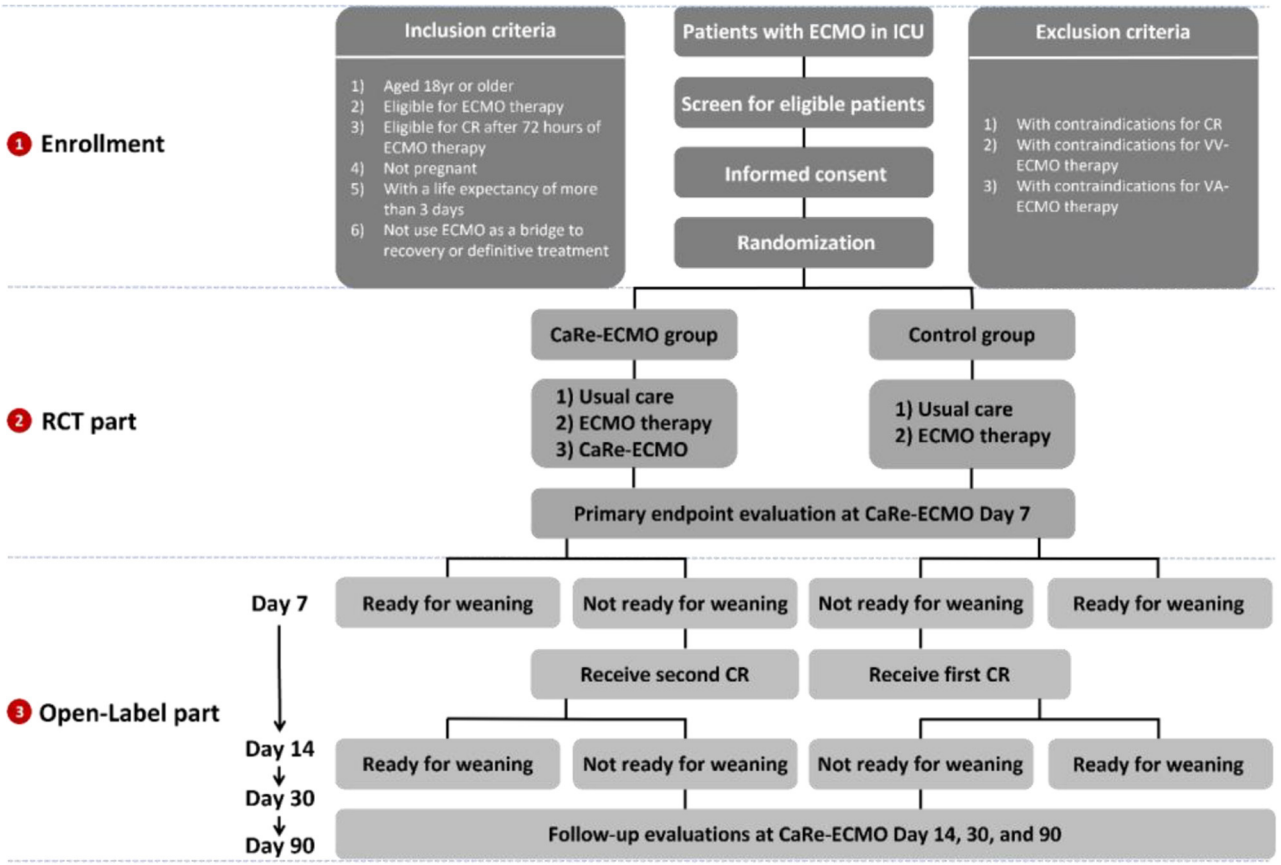

FIGURE 1 | Overview of trial recruitment, randomization, and allocation. ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; CaRe-ECMO, cardiopulmonary rehabilitation on ECMO; CR, cardiopulmonary rehabilitation.

Interventions

CaRe-ECMO Group

Patients in the CaRe-ECMO group will be treated with usual care, ECMO therapy, and cardiopulmonary rehabilitation program. Usual care normally comprises pharmacotherapy, mechanical ventilation, continuous renal replacement therapy (CRRT), intra-aortic balloon pump (IABP), and specific nursing for ECMO therapy and their original injuries, as appropriate. In addition, patients will receive cardiopulmonary rehabilitation from a multidisciplinary team (e.g., clinicians, rehabilitation physicians, nurses, and therapists) who were fully trained to provide care to every ECMO-supported patient when medically appropriate. A core group of clinicians and therapists (e.g., XL, ZS, XC, YZ, YC, and SW) are competent in the use of a screening procedure (e.g., stability of hemodynamic and vital signs, stability of blood coagulation, stability of homeostasis, stability of mechanical ventilation, stability of ECMO flows, and stability of cannulation position and tightness) to determine appropriateness and safety for cardiopulmonary rehabilitation delivery according to the experience from the University of Maryland Medical Center (4). Detailed daily eligibility screening flowchart for cardiopulmonary rehabilitation is shown in **Figure 2**. Once the patients pass the screen, the therapists will proceed to provide protocolized cardiopulmonary rehabilitation program, which encompasses five evidence-based components according to literature review: (1) positioning; (2) passive range of motion (PROM) training; (3) neuromuscular electrical stimulation (NMES); (4) surface electrical phrenic nerve stimulation (SEPNS); (5) respiratory proprioceptive neuromuscular facilitation (PNF) techniques; and (6) airway clearance techniques (22–24). Detailed content and protocol are given in **Table 4**. It should be emphasized that hemodynamic and vital signs, ECMO flows, and cannulation position and tightness will be continuously monitored during each rehabilitation session. In case any adverse events occur, cardiopulmonary rehabilitation session will be discontinued. Corresponding reasons for discontinuous and exact length of therapy will be recorded.

After randomized treatment, the primary outcome will be assessed at the CaRe-ECMO day 7 after last cardiopulmonary rehabilitation session. All the patients who are not responded (e.g., failure in fulfilling the criteria of ready for ECMO weaning and need continuous ECMO support), regardless of trial assignment, will be provided with open-label treatment of cardiopulmonary rehabilitation. Corresponding secondary

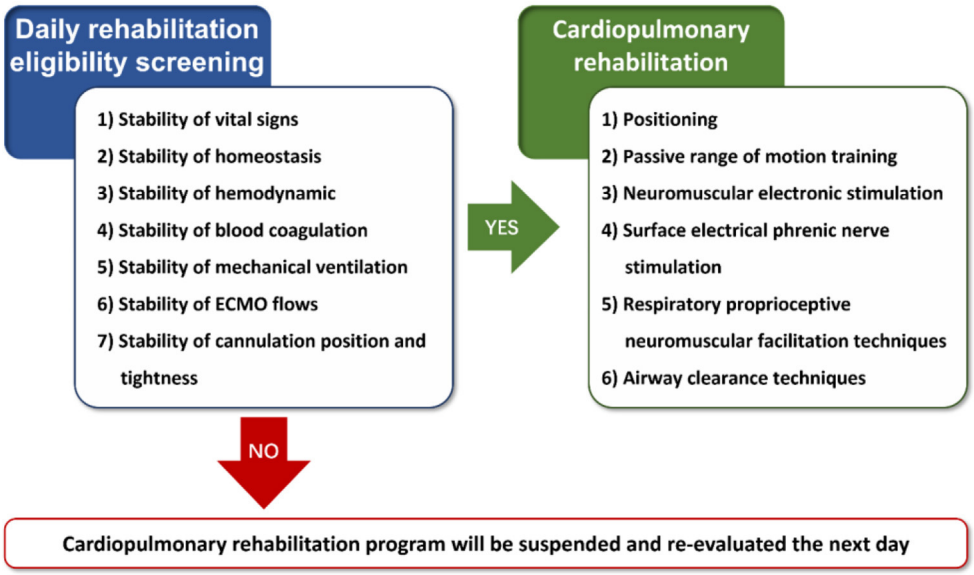

FIGURE 2 | Eligibility screening flowchat for cardiopulmonary rehabilitation. ECMO, extracorporeal membrane oxygenation.

outcomes will be assessed at the open-label stage according to the preplanned schedule.

Detailed procedure of the CaRe-ECMO program is shown in **Figure 3**.

Control Group

No additional rehabilitation intervention, except usual care and ECMO therapy, will be provided to patients in the control group.

Outcome Measures

Primary Outcome

The primary outcome of the CaRe-ECMO trial is the rate of ready for ECMO weaning at CaRe-ECMO day 7. It means that rate of ready for ECMO weaning will be calculated 7 days after cardiopulmonary rehabilitation delivery. Checklists to define ready for ECMO weaning (hereafter called ready for ECMO weaning checklists), either for VV-ECMO or VA-ECMO, have been prepared according to literature review and shown in **Tables 5**, **6** (20, 28). A fixed medical staff will be responsible for checkouts of listed items daily. Eligible patients defined as ready for ECMO weaning reveal the fulfillment of all the items in the checklists. Date of ready for ECMO weaning will be recorded and the rate of ready for ECMO weaning at CaRe-ECMO day 7 will be calculated.

Secondary Outcomes

Secondary outcomes are listed and elaborated as follows:

1) Rate of ECMO weaning will be calculated according to date of ECMO weaning fulfilled.
2) Total length of ready for ECMO weaning refers to exact length in day till patients fulfill all the criteria of ready for ECMO weaning according to daily checkout records (14).
3) Total length of ECMO weaning refers to exact length in day for patients treated with ECMO therapy (29).
4) Rate of mechanical ventilation weaning will be calculated according to date of mechanical ventilation weaning fulfilled. Daily screening of mechanical ventilation weaning will be strictly performed with checklist shown in **Table 7** according to the American Thoracic Society and the American College of Chest Physicians Clinical Practice Guideline (30).
5) Total length of mechanical ventilation refers to exact length in day for patients treated with mechanical ventilation.
6) All-cause mortality is defined as rate of death due to any causes and will be calculated according to date of death (31).
7) Rate of major post-ECMO complications refers to rate of complications occurred after ECMO including but not limited to ECMO-related complications (e.g., thromboembolism), mechanical ventilation-related complications (e.g., pneumonia), newly developed myocardial infarction, acute kidney injury, neurologic events (e.g., stroke, seizures), and multiple organ failure (32). Information of major post-ECMO complications will be collected in the Complication Recording Sheet (**Table 8**).
8) Diaphragmatic thickness and mobility refer to ultrasound-guided evaluation of diaphragmatic thickness and mobility under M-mode (33).
9) Extracorporeal membrane oxygenation unit LOS accounts for length in day for stay of patients in the ICU unit.
10) Total hospital LOS accounts for total hospital LOS in day for stay of patients in both the ECMO unit and other departments.
11) Total cost for hospitalization will be calculated by addition of the cost of all the units and department admission.

TABLE 4 | Detailed content and protocol of cardiopulmonary rehabilitation.

Cardiopulmonary rehabilitation	Components	Instructions	Duration and frequency	Rationale
Positioning	Semi-reclining positioning (30°) Left or right lateral positioning (45°) Prone positioning	Nurse-assisted position change	Every 2 h	Improve oxygenation (25) Improve ventilation/perfusion ratio of the lower part of lung (26) Improve oxygenation (27)
PROM training	PROM training for shoulder joint, elbow joint, wrist joint, hip joint, knee joint, and ankle joint	Therapist-assisted multidimensional movement of joints within normal ROM	20 min per session 1 session per day	1. Maintain joint mobility 2. Prevent contractures 3. Compress peripheral vessels to avoid thrombosis
NMES	NMES (Ruiyi S4, Nanjing Vishee Medical Technology Corporation, Nanjing, China) for triceps brachii, extensor carpal muscles, quadriceps, and tibialis anterior	1. Allocation of electrodes: surface electrodes attached to the motion points of muscles 2. Allocation confirmation: obtain a palpable muscle contraction of the targeted muscle 3. Stimulation parameters: pulse frequency of 40–60 Hz, pulse width of 0.2–0.4 ms, and rectangular, intermittent, bidirectional current	20 min per session 1 session per day	1. Maintain muscle mass, muscle strength 2. Prevent disuse atrophy 3. Compress peripheral vessels to avoid thrombosis
SEPNS	SEPNS (Diafun EDP, Arahelio Group, Guangzhou, China) for Diaphragm	1. Allocation of phrenic nerve: ultrasound scanning on supraclavicular region to identify phrenic nerve which is on the surface of anterior scalene muscle 2. Allocation confirmation: after the surface electrodes attachment, successful SEPNS can be confirmed with ultrasound imaging (M-mode) both at breathing and breath-hold phases reflecting changes of bilateral diaphragmatic thickness and mobility as compared to with no SEPNS 3. Stimulation principles and parameters: in synchrony with mechanical ventilation, pulse frequency of 40–60 Hz, pulse width of 0.2–0.4 ms, and rectangular, intermittent, bidirectional current	20 min per session 1 session per day	Prevent diaphragmatic ICU-acquired weakness
Respiratory PNF techniques	Manual thoracic expansion and compression	Therapist- or device-assisted pulmonary rehabilitation techniques	20 min per session 1 session per day	1. Improve chest wall compliance 2. Increase lung volume 3. Reduce and avoid atelectasis
Airway clearance techniques	Manual flutter mucus clearance High-frequency chest wall oscillation Endotracheal suctioning	Therapist-, nurse- or device-assisted airway clearance techniques	20 min per session 1 session per day	1. Assist airway clearance 2. Reduce post-ECMO pulmonary complications (e.g., infection)

PROM, passive range of motion; NMES, neuromuscular electrical stimulation; SEPNS, surface electrical phrenic nerve stimulation; ICU, intensive care unit; PNF, proprioceptive neuromuscular facilitation.

12) Cerebral performance category will be recorded, for those successfully weaning of ECMO, to reflect post-ECMO neurological status (34).

13) Activities of daily living will be evaluated, for those successfully weaning of ECMO, with Katz Index (35).

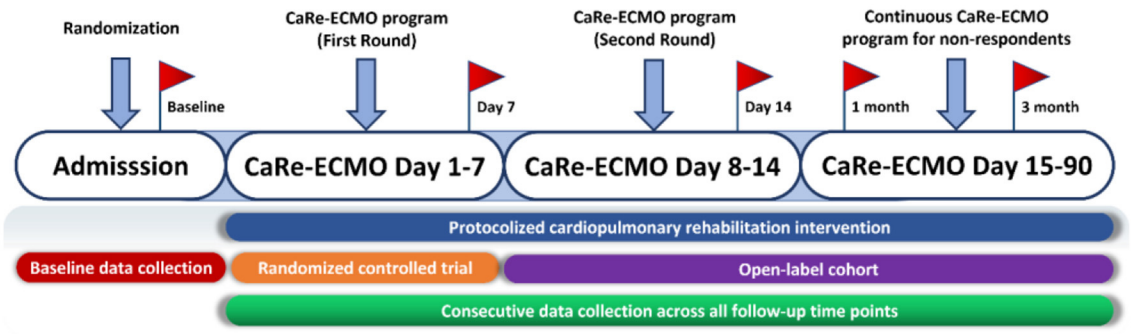

FIGURE 3 | Flow diagram of the CaRe-ECMO program. First round of the CaRe-ECMO program refers to the protocolized cardiopulmonary rehabilitation delivery in the RCT part; second round of the CaRe-ECMO program refers to the protocolized cardiopulmonary rehabilitation delivery in the RCT part. Red flags symbolize measurement points of selective outcomes across all the follow-up time points. CaRe-ECMO, cardiopulmonary rehabilitation on extracorporeal membrane oxygenation; RCT, randomized controlled trial.

TABLE 5 | Ready for VV-ECMO weaning checklist.

Ready for VV-ECMO weaning checklist	
Move forward to the nest step when the former step is fulfilled, should all of the steps be fulfilled indication the patient is ready for ECMO weaning	
Step 1	The primary disease has been controlled or improved pulmonary function assessment: If VT < 6 mL/kg, $FiO_2 \leq 60\%$, PEEP < 10 cmH_2O, PaO_2 > 70 mmHg, PaO_2/FiO_2 > 200 mmHg, $PaCO_2$ < 45 mmHg, pH 7.35–7.45, improved changes in X-ray/CT scanning, lung compliance >0.8 ml/kg*cmH_2O, then move to the next step
Step 2	Cardiac function assessment: If MAP > 70 mmHg, the heart rate increases over 20% of the baseline following the reduction of ECMO flow, then move to the next step
Step 3	Organ function and tissue perfusion assessment: If lactate < 2 mmol/L, SvO_2 > 70%, no symptoms of respiratory distress and improved function of other damaged organs, then move to the next step
Step 4	Patient must tolerate a full weaning trial: If disconnect oxygen supply of the ECMO with no reduction of ECMO flow, observation for 6 h, monitoring the patient to maintain parameters mentioned in Step 1–3, then move to the next step
Step 5	If Step 1, 2, 3, and 4 are validated then the patient is under minimal ECMO support: Be sure that there is no CO_2 retention and the patient can maintain normal oxygenation and normocarbia, and FiO_2 reaches 21%, ECMO flow becomes 3–4L per minute and the sweep should be <1 L per minute, then move to the next step
Step 6	The patient is ready for VV-ECMO weaning

VV-ECMO, venovenous extracorporeal membrane oxygenation; VT, tidal volume; FiO_2, fraction of inspired oxygen; PEEP, positive end-expiratory pressure; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; MAP, mean arterial pressure; SvO_2, mixed venous oxygen saturation.

14) Health-related quality of life will be measured, for those successfully weaning of ECMO, with SF-12 (36).

Data Collection

Data in terms of rate of ready for ECMO weaning, rate of ECMO weaning, rate of mechanical ventilation weaning, all-cause mortality, and rate of major post-ECMO complications will be calculated throughout the index hospitalization and follow-up period (e.g., CaRe-ECMO days 7, 14, 30, and 90) according to daily evaluations recorded in the medical records. Outcomes, for instance ready for ECMO weaning, will be treated as time-to-event outcomes; therefore, date and timing of events will be also recorded to facilitate statistical analysis. Total length of ready for ECMO weaning, total length in hour of ECMO weaning, total length in day of mechanical ventilation, ECMO unit LOS, and total hospital LOS will be collected according to the medical records. CPC index, ADL score, and HRQoL score will be selectively collected among those successfully weaning of ECMO across discharge day (discharge from ECMO unit) and all follow-up time points (e.g., CaRe-ECMO days 30 and 90). In addition, diaphragmatic thickness and mobility will be evaluated with ultrasound (M-mode) every 3 days for determining the allocation of phrenic nerve and the efficacy of SEPNS. The detailed data collection schedule is given in **Table 9**.

Baseline demographics and clinical characteristics, directly from the medical files at randomization, will also be collected including but not limited to the following information: age, gender, main diagnosis, comorbidities (diabetes mellitus, hypertension, respiratory infection, or others), the Acute Physiology and Chronic Health Evaluation II (APACHE II)

TABLE 6 | Ready for VA-ECMO weaning checklist.

Ready for VA-ECMO weaning checklist	
Move forward to the nest step when the former step is fulfilled, should all of the steps be fulfilled indicating the patient is ready for ECMO weaning	
Step 1	The etiology of cardiac failure must be compatible with myocardial recovery
Step 2	Pulmonary function should not be severely impaired: If $FiO_2 \leq 50\%$, PEEP < 10 cmH_2O, PIP < 30 cmH_2O, $PaO_2 > 70$ mmHg, $PaO_2/FiO_2 > 200$ mmHg, $PaCO_2 < 45$ mmHg, pH 7.35–7.45, no pulmonary edema in X-ray/CT scanning, then move to the next step
Step 3	Hemodynamic and cardiac functional assessment: If MAP > 70 mmHg and PPD > 30 mmHg for 24 h, the heart rate increases over 20% of the baseline following the reduction of ECMO flow, VIS of <10, LVEF of more than 40%, aortic VTI of more than 10 cm/s, decreasing trend of cTnT and BNP values, CVP < 12 cmH_2O, lactate < 2 mmol/L, $SvO_2 > 70\%$, with improved function of other damaged organs, then move to the next step
Step 4	The patient must tolerate a full weaning trial: Increase ACT to 200 s, then gradually reduce ECMO flow to 66% for 15 min, and then to 33% of its baseline value for 15 min, and finally a minimum of 1–1.5 L/min for 15 min. During the weaning trial, monitoring the patient to maintain parameters mentioned in Step 3
Step 5	The patient is ready for VA-ECMO weaning

VA-ECMO, venoarterial extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; PEEP, positive end-expiratory pressure; PIP, peak inspiratory pressure; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; MAP, mean arterial pressure; PPD, pulse pressure difference; VIS, vasoactive-inotrope score; VTI, velocity time integral; cTnT, cardiac troponin T; CVP, central venous pressure; SvO_2, mixed venous oxygen saturation; ACT, activated clotting time.

TABLE 7 | Mechanical ventilation weaning checklist.

Mechanical ventilation weaning checklist	
• Ready for mechanical ventilation weaning: Daily check prerequisite of primary disease controlled; $FiO_2 \leq 0.6$, $PaO_2/FiO_2 > 200$ with PEEP ≤ 5 cmH_2O; no or low dose vasopressor being used; continuous intravenous sedation minimized as appropriate; response to simple questions; cough during tracheal aspirations • Should the above ready for mechanical ventilation weaning criteria fulfilled, move through the following steps when the former step is fulfilled. Should all of the steps be fulfilled indicating weaning of mechanical ventilation	
Step 1	Weaning test • Gradually decrease PEEP to 5 cmH_2O with possible increase of FiO_2 to 0.6 (over 20–30 min) • If: desaturation occur ($SpO_2 < 88\%$) and persist (>5 min) during this trial, then fail • If not: blood gases determined 10–20 min after establishing PEEP at 5 cmH_2O • If: under these conditions (PEEP = 5 cmH_2O and FiO_2 at 30–60%), $PaO_2/FiO_2 < 200$, then fail • If not: weaning test passes, forward through the next step within 24 h
Step 2	Prolonged weaning test • Mechanical ventilation mode of volume-assist control ventilation or pressure support • Mechanical ventilation parameters of VT < 10 mL/kg; P_{plat} or pressure support < 30 cmH_2O; RR ≤ 35/min; PEEP = 5 cmH_2O; $FiO_2 \leq 50\%$ • If: desaturation occur ($SpO_2 < 88\%$) and persist (>15 min) during this trial, then failed • If not: prolonged weaning test passes, forward through the next step within 24 h
Step 3	Spontaneous breathing trial • T piece or PSV with pressure support at $+7$ cmH_2O
Step 4	Weaning of mechanical ventilation

PEEP, positive end-expiratory pressure; FiO_2, fraction of inspired oxygen; SpO_2, oxygen saturation; PaO_2, partial pressure of arterial oxygen; VT, tidal volume; P_{plat}, plateau pressure; RR, respiratory rate; PSV, pressure support ventilation.

score, ECMO mode (VV-ECMO or VA-ECMO), length in hour since mechanical ventilation, partial pressure of arterial oxygen/fraction of inspired oxygen (PaO_2/FiO_2) in mm Hg, positive end-expiratory pressure (PEEP), tidal volume in ml/kg of predicted body weight, respiratory rate in breaths/min, plateau pressure in cm of water, driving pressure in cm of water, arterial blood pH, PaO_2 in mm Hg, partial pressure of arterial carbon dioxide ($PaCO_2$) in mm Hg, N-terminal pro-B-type natriuretic peptide (NT-proBNP) in pg/ml, creatine kinase-MB (CK-MB) in ng/l, cardiac troponin T (cTnT) in ng/l, and ejection fraction (EF) in percentage. The above data will be recorded in the Baseline Data Collection Sheet (**Table 10**). Apart from that, daily clinical monitoring for both the condition and ECMO running status of patient will be performed. The detailed information will be recorded in the Daily Monitoring Sheet (**Table 11**).

TABLE 8 | Complication recording sheet.

Complication recording sheet

Name: ___ Gender: ___ Age: ___ Inpatient ID: ___ Bed number:___
Date of record: ___ / ___ / ___, ___: ___ Main diagnosis:

Neurological complications
☐ **No** ☐ **Yes (If yes, then fill the following items)**

Date and time: ___ / ___ / ___, ___: ___

☐ Cerebral hemorrhage ☐ Cerebral infarction ☐ Epileptic seizure

Cardiovascular complications
☐ **No** ☐ **Yes (If yes, then fill the following items)**

Date and time: ___ / ___ / ___, ___: ___

☐ Cardiac arrest ☐ Newly developed myocardial infarction ☐ Heart failure
☐ Myocardium stunning (Ultrasound) ☐ Newly developed hypertension requiring vasodilator therapy
☐ Shock (☐ Cardiogenic ☐ Hypovolemic ☐ Obstructive ☐ Distributive)
☐ North-south syndrome
☐ Tamponade (☐ Blood ☐ Plasma ☐ Air)

Respiratory complications
☐ **No** ☐ **Yes (If yes, then fill the following items)**

Date and time: ___ / ___ / ___, ___: ___

☐ Pneumothorax ☐ Pulmonary infection ☐ Pulmonary edema ☐ Pulmonary atelectasis
☐ Ventilator-associated lung injury

Other complications
☐ **No** ☐ **Yes (If yes, then fill the following items)**

Date and time: ___ / ___ / ___, ___: ___

Extremities complications:
☐ Distal ischemia ☐ Necrosis ☐ Deep venous thrombosis ☐ Osteofascial compartment syndrome ☐ Limb amputation
Infection:
Infection pathogen: ☐ G^+ bacteria ☐ G^- bacteria ☐ Mycobacteria ☐ Fungus ☐ Virus and prion ☐ Protozoon
Infection site: ☐ Blood ☐ Bone marrow ☐ Cerebrospinal fluid ☐ Ascites ☐ Hydrothorax ☐ Respiratory tract
☐ Skin, muscle, soft tissue ☐ Feces ☐ Urine ☐ Surgical site ☐ Wound site ☐ Other_____
Bleeding:
☐ Cerebral hemorrhage ☐ Gastrointestinal bleeding ☐ Respiratory tract bleeding ☐ Cannulation site bleeding ☐ Thrombocytopenia ☐ Surgical site bleeding
☐ Hemolysis ☐ DIC ☐ Other_____
Thrombosis:
☐ Pulmonary thromboembolism ☐ ECMO catheter thrombosis ☐ Limb thrombosis ☐ Mesenteric thrombosis ☐ Other_____

ECMO-related complications
☐ **No** ☐ **Yes (If yes, then fill the following items)**

Date and time: ___ / ___ / ___, ___: ___

☐ Oxygenator failure ☐ Raceway rupture ☐ Crack in connectors ☐ Heat exchanger malfunction
☐ Cannula problems ☐ Clots or thrombosis of circuit component ☐ Pump failure ☐ Clots hemofilter
☐ Circuit change ☐ Other_____

ID, identified number; DIC, disseminated intravascular coagulation; ECMO, extracorporeal membrane oxygenation.

Blinding

Trial assistants from the allocation center responsible for randomization will be blinded to patients in both the groups. Each cardiopulmonary rehabilitation session provided by the therapists will be sheltered with a curtain. In the control group, an optimization procedure will be imitated as closely as possible, while no cardiopulmonary rehabilitation will be provided. This action may be helpful to blind the clinicians and nurses and mitigate any bias. Assessors, data analysts, and statisticians will be blinded to group allocation.

Data Management and Monitoring

All the data will be entered into standardized electronic case report forms (eCRFs) and stored in a bespoke trial cloud database upon its collection. Data entry will be independently performed and dated by two trial assistants. Typos and missing data will be detected by this dual-track data entry mode. When discrepancies occur, consensus will be achieved by raw-data review or discussion. Confidentiality of data is assured by restricted access to the cloud database granted to authorized investigators only, for example, members of the Data and Safety Monitoring Committee (DSMC). The primary investigators, trial coordinators, the DSMC members, data analysts, statisticians, and trial assistants will meet periodically to: (1) monitor and review safety of patient; (2) request and perform interim data analyses; (3) review patient recruitment, accrual, and withdrawal; (4) discuss about continuing or modifying the trial; and (5) stop the trial upon any severe adverse events considered to have been caused by the CaRe-ECMO program (37).

TABLE 9 | Scheduled events and timeline of the CaRe-ECMO trial.

		Study period						
		Eligibility	**Allocation**	**Post-Allocation**			**Close-out**	
	Timepoint	$-t_1$	t_0	t_1	t_2	t_3	t_4	t_x
		Day of ECMO		**7 days after CaRe-ECMO**	**14 days after CaRe-ECMO**	**30 days after CaRe-ECMO**	**90 days after CaRe-ECMO**	**Discharge day***
Enrollment	Eligibility check	X						
	Informed consent	X						
	Randomization		X					
Assignment	CaRe-ECMO group		X					
	Control group		X					
Variables	Demographics		X					
	Clinical characteristics		X					
	Rate of ready for ECMO weaning			X	X	X	X	
	Rate of ECMO weaning			X	X	X	X	
	Total length of ready for ECMO weaning	←	←	←	←	←	←	→
	Total length of ECMO weaning	←	←	←	←	←	←	→
	Rate of mechanical ventilation weaning			X	X	X	X	
	Total length of mechanical ventilation	←	←	←	←	←	←	→
	All-Cause mortality			X	X	X	X	
	Rate of major post-ECMO complications			X	X	X	X	
	Diaphragmatic thickness and mobility							
	ECMO unit LOS							X
	Total hospital LOS	←	←	←	←	←	←	→
	Total cost for hospitalization	←	←	←	←	←	←	→
	CPC			X	X	X	X	X
	ADL			X	X	X	X	X
	HRQoL			X	X	X	X	X

CaRe-ECMO, cardiopulmonary rehabilitation on extracorporeal membrane oxygenation; LOS, length of stay; CPC, cerebral performance category; ADL, activities of daily living; HRQoL, health-related quality of life.
**Discharge day refers to discharge from ECMO unit and it may variate according to recovery process of patients.*

TABLE 10 | Baseline data collection sheet.

Baseline data collection sheet			
Basic information			
Name	Gender		Age
Height (cm)	Weight (kg)		Bed number
Inpatient ID	Phone number		BMI (kg/m^2)
Main diagnosis	APACHE II score		SOPA score
Date of admission	___ / ___ / ___, ___: ___	Date of ECMO admission	___ / ___ / ___, ___: ___
Date of record	___ / ___ / ___, ___: ___		
Medical history			
Cardiovascular system	☐ No ☐ Yes ☐ Cardiac surgery ☐ Cardiac intervention ☐ Myocardial infarction: ☐ Non ST-segment elevation ☐ ST-segment elevation ☐ Angina pectoris: ☐ Stable ☐ Unstable ☐ Structural heart disease ☐ Heart failure (☐ New York Heart Association: ☐ I ☐ II ☐ III ☐ IV) ☐ Atrial fibrillation ☐ Hypertension		
Respiratory system	☐ No ☐ Yes ☐ COPD ☐ Asthma ☐ Tuberculosis		
Cerebrovascular event and sequelae	☐ No ☐ Yes ☐ Cerebral hemorrhage ☐ Cerebral infarction ☐ Dyskinesia ☐ Dysphagia ☐ Cognitive disorder ☐ Other_____		
Diabetes	☐ No ☐ Yes ☐ Type 1 diabetic mellitus ☐ Type 2 diabetic mellitus ☐ Impaired glucose tolerance		
Malignant tumor	☐ No ☐ Yes ☐ Solid tumor ☐ Metastatic tumor ☐ Leukemia ☐ Lymphoma ☐ Other_____		
Organ transplantation	☐ No ☐ Yes		
Immune system disease	☐ No ☐ Yes		
Medication history	☐ Antiplatelet drugs: ☐ Aspirin ☐ Clopidogrel ☐ Dipyridamole ☐ Other_____ ☐ Anticoagulants: ☐ Warfarin ☐ Dabigatran ☐ Rivaroxaban ☐ Heparin ☐ Other_____ ☐ Antihyperlipidemic drugs: ☐ Statin ☐ Fibrates ☐ PCSK9 ☐ Other_____ ☐ Antihypertensive drug: ☐ β-receptor blocker ☐ ACEI ☐ ARB ☐ CCB ☐ Other_____ ☐ Hypoglycemic drugs: ☐ Insulin ☐ Sulphonylurea ☐ Metformin ☐ Other_____ ☐ Other_____		
Personal history	☐ Smoking history: Smoking index_____ (Smoking index is a number of cigarettes smoked per day * years of tobacco smoking) ☐ History of alcoholism: Drinking index_____g/day ☐ Dust and toxic exposure history		
Allergic history	☐ No ☐ Yes ☐ Food ☐ Drug_____		
Family history	☐ No ☐ Yes ☐ Cardiovascular disease ☐ Cerebral disease ☐ Immune disease ☐ Endocrine disease ☐ Malignant tumor ☐ Other_____		
ECMO-Related information			
Venue of ECMO operation	☐ Out-of-hospital ☐ In-hospital		
ECMO cannulation	Drainage cannula ☐ Left ☐ Right Site: ☐ Femoral vein ☐ Axilla vein ☐ Internal jugular vein ☐ Right atrium Perfusion cannula ☐ Left ☐ Right Site: ☐ Femoral vein or femoral artery☐ Axilla vein ☐ Internal jugular vein Whether additional drainage cannula is needed ☐ No ☐ Yes		
Running status of ECMO			
Indication	☐ Cardiac ☐ Pulmonary ☐ ECPR		
ECMO type	☐ VA-ECMO ☐ VV-ECMO ☐ Other_____		
ECMO cannulation sites	VV-ECMO: ☐ No ☐ Yes (If yes, then fill the following items) ☐ Right femoral vein ☐ Left femoral vein ☐ Right internal jugular vein ☐ Left internal jugular vein ☐ Other_____ VA-ECMO: ☐ No ☐ Yes (If yes, then fill the following items) ☐ Right femoral vein ☐ Left femoral vein ☐ Right internal jugular vein ☐ Left internal jugular vein ☐ Right femoral artery ☐ Left femoral artery ☐ Other_____		
Distal collateral circulation	☐ No ☐ Yes		
ECMO flow	ECMO blood flow_____L/min ECMO gas flow_____L/min ECMO FiO_2_____%		
Length from ECMO response to operation	_____mins		
Length of ECMO operation	_____mins		
Length of MV before ECMO	_____days _____hours _____mins		
First examination after ECMO			
Blood routine examination	WBC_____10^9/L NE_____% Hb_____g/L PLT_____10^9/L		
Arterial blood gases	FiO_2_____% pH_____ PO_2_____mmHg PCO_2_____mmHg BE_____mmol/L HCO_3^-_____mmol/L Lactate_____mmol/l		
Inflammatory factors	C-reactive protein_____mg/L Procalcitonin_____ug/L Tumor necrosis factor_____pg/mL IL-6_____pg/mL Serum amyloid A_____mg/L		

(Continued)

TABLE 10 | Continued

Baseline data collection sheet	
Basic information	
Biochemical examination	ALT_____U/L AST_____U/L TBIL_____μmol/L DBIL_____μmol/L ALP_____U/L ALB_____g/L BUN_____mmol/L Cr_____μmol/L Na^+_____mmol/L K^+_____mmol/L Cl^-_____mmol/L Ca^{2+}_____mmol/L
Anticoagulation monitoring	Loading dose of heparin_____IU/kg Maintenance dose of heparin_____IU/kg APTT_____s PT_____s D-Dimer_____ng/mL
Antibiotic prophylaxis	☐ No ☐ Yes_____
Cardiac function and myocardial injury markers	NT-proBNP_____pg/ml cTnT_____ng/L MyO_____ng/L CK-MB_____ng/L
Electrocardiogram	☐ Sinus arrhythmia ☐ Atrial arrhythmia ☐ Ventricular arrhythmia ☐ Supraventricular arrhythmia ☐ Junctional rhythm ☐ Other_____ Ventricular rate_____bpm
Radiology	☐ No ☐ Yes ☐ Pulmonary infection ☐ Pneumothorax ☐ Pulmonary edema ☐ Pulmonary atelectasis ☐ Pleural effusion/Hemothorax ☐ Cardiomegaly ☐ Pericardial effusion/Hemopericardium ☐ Cerebral edema ☐ Cerebral hemorrhage ☐ Ascites/Hemoperitoneum ☐ Ileus ☐ Other_____
Echocardiogram	EF_____% VTI_____ LVID_____mm RVID_____mm Valve ☐ Normal ☐ Abnormal ☐ Aortic valve (☐ Moderate to severe aortic stenosis ☐ Moderate to severe aortic insufficiency) ☐ Mitral valve (☐ Moderate to severe mitral stenosis ☐ Moderate to severe mitral insufficiency) ☐ Tricuspid valve (☐ Moderate to severe tricuspid stenosis ☐ Moderate to severe tricuspid insufficiency) Structural heart disease ☐ No ☐ Yes ☐ Atrial septal defect ☐ Ventricular septal defect ☐ Tetralogy of fallot ☐ Endocardial cushion defect ☐ Single ventricle ☐ Other_____ Pulmonary arterial hypertension ☐ No ☐ Yes Ventricular aneurysm ☐ No ☐ Yes
Coronary angiography	☐ No ☐ Yes Date of examination___ / ___ / ___ (If yes, then fill the following items) Stenosis ☐ No ☐ Yes Stenosis degree: I ≤ 25% II 26%-50% III 51%-74% IV ≥ 75% Left main artery: ☐ I ☐ II ☐ III ☐ IV Left anterior descending artery: ☐ I ☐ II ☐ III ☐ IV Left circumflex artery: ☐ I ☐ II ☐ III ☐ IV Right coronary artery: ☐ I ☐ II ☐ III ☐ IV Other_____: ☐ I ☐ II ☐ III ☐ IV
Cardiac arrest (If ECPR, then fill the following items)	
Venue of cardiac arrest	☐ Out-of-hospital ☐ In-hospital
Primary disease	☐ Pulmonary ☐ Cardiac ☐ Anesthesia ☐ Other_____
Initial heart rhythm (Before CPR)	☐ Ventricular fibrillation ☐ Pulseless ventricular tachycardia ☐ Pulseless electrical activity ☐ Cardiac standstill ☐ Other_____
Cardioversion or defibrillation	☐ No ☐ Yes ☐ Unclear (If yes, then fill the following items) Drugs for cardioversion: ☐ Lidocaine ☐ Amiodarone ☐ Other_____
Length from cardiac arrest to CPR	Time of cardiac arrest___ / ___ / ___, ___: ___ Time of CPR___ / ___ / ___, ___: ___ Length_____mins
Length from CPR to ECPR	Time of CPR___ / ___ / ___, ___: ___ Time of ECPR___ / ___ / ___, ___: ___ Length_____mins

ID, identified number; BMI, body mass index; ECMO, extracorporeal membrane oxygenation; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; ECPR, external cardiopulmonary resuscitation; VV-ECMO, venovenous ECMO; VA-ECMO, venoarterial ECMO; FiO_2, fraction of inspired oxygen; BE, base excess; MV, mechanical ventilation; CPR, cardiopulmonary resuscitation; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; PCSK9, proprotein convertase subtilisin/kexin type 9; COPD, chronic obstructive pulmonary disease; WBC, white blood cell; NE, neutrophil; Hb, hemoglobin; PLT, platelet; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; IL-6, interleukin-6; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALP, alkaline phosphatase; AIB, albumin; BUN, blood urea nitrogen; Cr, creatinine; APTT, activated partial thromboplastin time; PT, prothrombin time; ACT, activated clotting time; NT-proBNP, N-terminal pro-B-type natriuretic peptide; cTnT, cardiac troponin T; MyO, myoglobin; CK-MB, creatine kinase-MB; EF, ejection fraction; VTI, velocity-time integral; LVID, left ventricular internal dimension; RVID, right ventricular internal dimension.

Sample Size Calculation

We considered the rate of ready for ECMO weaning being conservatively 50% higher in the CaRe-ECMO group than in the control group at CaRe-ECMO day 7 as a clinically meaningful effect, implying a hazard ratio (HR) of 0.5. Accordingly, we performed sample size calculation for the Cox proportional hazards model with an alpha error of 5% and a power of 80%, effect size of HR = 0.5, and overall event (ready for weaning) probability of 30% at CaRe-ECMO day 7 (increases from 30 to 45%) according to our retrospective data analysis and literature review (38). The needed sample size was estimated as n = 348, i.e., 174 per group. Due to the relative short observational period and the real-world situation (e.g., patients treated with ECMO therapy generally will not discharge till stable or death), accounting for 5% attrition according to their failing for fulfill of daily eligibility screening for cardiopulmonary rehabilitation during the therapeutic period, the target sample size is set at 366 (183 per group).

Statistical Methods

The demographic and clinical characteristics collected at randomization will be presented as means with SDs or medians with interquartile ranges for continuous variables and as percentages for categorical variables, as appropriate.

For all the outcomes, we will estimate differences in effect size between the groups on both the intention-to-treat (ITT) and per protocol (PP) basis (39). Categorical variables will be compared with the chi-square or Fisher's exact tests and

TABLE 11 | Daily monitoring sheet.

Daily monitoring sheet

Name: ___ Gender: ___ Age: ___ Inpatient ID: ___ Bed number: ___
Date of record: ___ / ___ / ___, ___: ___ Main diagnosis:

Evaluation	Index	CaRe-ECMO Day 1	CaRe-ECMO Day 2	CaRe-ECMO Day 3	CaRe-ECMO Day 4	…	CaRe-ECMO Day X
Consciousness	RASS score						
	GCS score						
	BIS index						
	NSE						
	S100-β protein						
	EEG						
Temperature	Bladder temperature (°C)						
	Axillary temperature (°C)						
Fluid intake and output	Intake (ml)						
	Output (ml)						
	Output excess (ml)						
Nutrition	Enteral nutrition (Y/N)						
	Parenteral nutrition (Y/N)						
	Total calories (Kcal)						
Blood routine examination	WBC (10^9/L)						
	NE (%)						
	Hb (g/L)						
	PLT (10^9/L)						
Inflammatory factors	C-reactive protein (mg/L)						
	Procalcitonin (ug/L)						
	Tumor necrosis factor (pg/mL)						
	IL-6 (pg/mL)						
	Serum amyloid A (mg/L)						
Biochemical examination	ALT (U/L)						
	AST (U/L)						
	TBIL (μmol/L)						
	DBIL (μmol/L)						
	ALP (U/L)						
	ALB (g/L)						
	BUN (mmol/L)						
	Cr (μmol/L)						
	Na^+ (mmol/L)						
	K^+ (mmol/L)						
	Cl^- (mmol/L)						
	Ca^{2+} (mmol/L)						
Arterial blood gases	FiO_2 (%)						
	pH						
	PO_2 (mmHg)						
	PCO_2 (mmHg)						
	BE (mmol/l)						
	HCO_3^- (mmol/l)						
	Lactate (mmol/l)						
Anticoagulation monitoring	Maintenance dose of heparin (IU/kg)						

(Continued)

TABLE 11 | Continued

Daily monitoring sheet

Name: ___ Gender: ___ Age: ___ Inpatient ID: ___ Bed number: ___
Date of record: ___ / ___ / ___, ___: ___ Main diagnosis:

Evaluation	Index	CaRe-ECMO Day 1	CaRe-ECMO Day 2	CaRe-ECMO Day 3	CaRe-ECMO Day 4	…	CaRe-ECMO Day X
	APTT (s)						
	PT (s)						
	D-Dimer (ng/ml)						
	ACT (s)						
Cardiac function and myocardial injury markers	NT-proBNP (pg/ml)						
	cTnT (ng/L)						
	MyO (ng/L)						
	CK-MB (ng/L)						
Electrocardiogram	Heart rhythm						
	Ventricular rate (bpm)						
Echocardiogram	EF (%)						
	VTI						
	LVID (mm)						
	RVID (mm)						
Hemodynamics	Vasoactive agent (Y/N)						
	Type of vasoactive agent						
	Dose of vasoactive agent						
	Vasoactive inotropic score						
	Systolic blood pressure (mmHg)						
	Mean arterial pressure (mmHg)						
	Central venous pressure (mmHg)						
	Inferior vena cava (mm)						
	Inferior vena cava variability (%)						
Ventilator parameters	Mechanical ventilation (Y/N)						
	Mode						
	Tidal volume (ml)						
	Respiratory rate (bpm)						
	FiO_2 (%)						
	PEEP/CPAP (mmHg)						
	PIP (mmHg)						
	P_{plat} (mmHg)						
	Minute ventilation (L/min)						
	Pulmonary compliance (ml/cmH_2O)						
	Inspiratory resistance						
	Expiratory resistance						
Radiology	Pulmonary infection						
	Pneumothorax						
	Pulmonary edema						
	Pulmonary atelectasis						
	Pleural effusion						
	Cardiomegaly						

(Continued)

TABLE 11 | Continued

Daily monitoring sheet

Name: ___ Gender: ___ Age: ___ Inpatient ID: ___ Bed number: ___
Date of record: ___ / ___ / ___, ___: ___ Main diagnosis:

Evaluation	Index	CaRe-ECMO Day 1	CaRe-ECMO Day 2	CaRe-ECMO Day 3	CaRe-ECMO Day 4	…	CaRe-ECMO Day X
	Pericardial effusion						
	Cerebral edema						
	Cerebral hemorrhage						
	Ascites						
	Ileus						
Pathogenic microorganism	Bacteria (Y/N)						
	Fungus (Y/N)						
	Virus (Y/N)						
	Location of the microorganism						
	Name of the microorganism						
Running status of ECMO	ECMO mode						
	ECMO blood flow						
	ECMO gas flow						
	ECMO FiO_2 (%)						
Adjuvant therapy	CRRT (Y/N)						
	IABP (Y/N)						
	Other (Y/N)						

ID, identified number; ECMO, extracorporeal membrane oxygenation; RASS, Richmond Agitation Sedation Scale; GCS, Glasgow Coma Scale; BIS, bispectral index; NSE, neuron-specific enolase; EEG, electroencephalography; WBC, white blood cell; NE, neutrophils; Hb, hemoglobin; PLT, platelet; IL-6, interleukin-6; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALP, alkaline phosphatase; ALB, albumin; BUN, blood urea nitrogen; BE, base excess; Cr, creatinine; FiO_2, fraction of inspired oxygen; pO_{v2}, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; PIP, peak inflation pressure; Pplat, plateau pressure; APTT, activated partial thromboplastin time; PT, prothrombin time; ACT, activated clotting time; NT-proBNP, N-terminal pro-B-type natriuretic peptide; cTnT, cardiac troponin T; MyO, myoglobin; CK-MB, creatine kinase-MB; EF, ejection fraction; VTI, velocity-time integral; LVID, left ventricular internal dimension; RVID, right ventricular internal dimension; PEEP, positive end-expiratory pressure; CPAP, continuous positive airway pressure; CRRT, continuous renal replacement therapy; IABP, intra-aortic balloon pump.

continuous variables will be compared with the Student's *t*-test or the Wilcoxon signed-rank test, as appropriate. The time effect of day the time-to-event outcomes identified (e.g., rate of ready for ECMO weaning, rate of ECMO weaning, rate of mechanical ventilation weaning, all-cause mortality, and rate of major post-ECMO complications) between the groups will be estimated with the Cox proportional hazards regression analysis and graphically illustrated using the Kaplan–Meier methods until CaRe-ECMO days 7, 14, 30, and 90, respectively (40). Variables, including total length of ready for ECMO weaning, total length of ECMO weaning, total length of mechanical ventilation, ECMO unit LOS, and total hospital LOS, will be treated with time-to-event data and will be analyzed with mixed-effects ordered logistic regression (41). Secondary outcomes with normal and non-normal distributions, including CPC, ADL, HRQoL, and diaphragmatic thickness and mobility, will be estimated for group differences with mixed-effects linear regression (41).

Considering that part of patients will definitely not reach the primary endpoint, i.e., ready for ECMO weaning at CaRe-ECMO day 7 for sensitivity purpose, we will assess the heterogeneity of the treatment effect on primary outcome and secondary outcomes in pre-specified subgroups of interest as per baseline data to be collected at randomization (e.g., age, gender, main diagnosis, comorbidities, the APACHE II score, ECMO mode, length since mechanical ventilation) by using exploratory *post-hoc* sensitivity adjusted analyses for any corresponding statistical methods mentioned above.

Interim analysis will be scheduled halfway through enrollment to check that there are no serious issues with respect to sample handling or data collection.

All the analyses will be conducted at a two-sided alpha level of 5% and will be performed using the Stata version 16 (StataCorp, College Station, Texas, USA) or other software, as appropriate.

DISCUSSION

Cardiopulmonary rehabilitation was proven to be effective in the improvement of cardiovascular and respiratory function in various major diseases (42). However, its efficacy in ECMO-supported patients has not been well-verified. Its application in routine clinical practice always encounters a number of

challenges. Specifically, one of the major barriers is the multiple sites of cannulation, which restrict the mobilization of the body. This explained why most of the trials involved patients with VV-ECMO with cannulation sites in the upper body allowing for more chances of mobilization while less on VA-ECMO (4). Nonetheless, active cardiopulmonary rehabilitation is ever difficult to be performed for various reasons. In the emergency room, patients would always be sedated with the application of nitric oxide and neuromuscular blocking agents to promote oxygenation and improve ventilator synchrony. That is why, only few studies reported results with limited sample size in awake patients with ECMO (43, 44). Furthermore, active mobilization of the body may interrupt the ECMO flow (45). Therefore, the upcoming challenge is to explore a cardiopulmonary rehabilitation protocol, which can be safely delivered with no interruption of the routine clinical treatment and subsequently benefit all the types of ECMO-supported patients.

Despite the fact that most of cardiopulmonary rehabilitation carried out in the ICU and emergency room are low intensity, intensive rehabilitation has not been found to be superior to rehabilitation with relatively low intensity (46, 47). In addition, various types of cardiopulmonary rehabilitation have been documented as functional beneficial in the literature. For instance, prone positioning has been demonstrated to decrease mortality at day 28 (16.0 in the prone group vs. 32.8% in the supine group, $p < 0.001$) and day 90 (23.6 vs. 41.0%, $p < 0.001$) in patients with acute respiratory distress syndrome. Its application was strongly encouraged by several well-designed studies (27, 48). Physiotherapy such as NMES-induced muscle contraction may serve as muscle pump to increase ejection capacity and improve both the venous return and muscle perfusion, consequently compensate cardiac function (49). Moreover, ICU-acquired weakness was frequently observed in the ICU and diaphragm dysfunction developed more often than limb muscle weakness (50). Diaphragmatic weakness, occurs in as early as 18 h after mechanical ventilation, is attributed to proteolysis-induced muscle contractile dysfunction (51). It was proven to be associated with a higher rate of mechanical ventilation weaning failure and prolonged length of mechanical ventilation (52). Upon this condition, we also included electrical stimulation of phrenic nerve to minimize the reduction of diaphragm atrophy and strength over time, possibly contributing to speed up respiratory functional recovery and subsequently leading to increased rate of ECMO weaning and shortened length of ECMO therapy (53–55). Taken together, it is reasonable to hypothesize that our protocolized cardiopulmonary rehabilitation may contribute to internal pathophysiological changes (e.g., cardiac output and maximal oxygen uptake) and spread it to external clinical outcomes (e.g., earlier weaning of ECMO and increased rated of ECMO weaning).

Additional important considerations, which we addressed when preparing the CaRe-ECMO trial protocol, were the following. First, the current trial is consisting of a RCT part followed by an open-label cohort part. According to our clinical experience and retrospective data analysis, the median length of ECMO weaning varied ∼from 7 to 9 days in all the types of ECMO-supported patients. The design rationale of the RCT part is to power the primary outcome, while the following open-label cohort part is dedicated to benefit patients who do not respond to one cycle of cardiopulmonary rehabilitation. This action may impair the effects of RCT on long-term outcome observation; however, it is hypothesized to provide additional opportunity for those who are probably benefitted from active intervention (56). Second, a critical concern of the CaRe-ECMO trial design is the uncertainty of the effect size of the proposed interventions. Therefore, our attention on primary outcome selection has been shifted from the exact ECMO weaning to ready for ECMO weaning. If any significant results would be introduced in terms of earlier ready for ECMO weaning or increased rate of ready for ECMO weaning due to our proposed interventions, then it may satisfy the preliminary requirements when extending its effects to more consolidated outcomes (e.g., rate of ECMO weaning). To compensate the aforementioned risks, we introduce several evidence-based checklists to ensure the standardized definition and evaluation of ECMO or mechanical ventilation weaning (**Tables 5–7**) and to maximally avoid potential performance bias. Third, functional outcomes are essential to the long-term recovery; therefore, CPC index, ADL score, HRQoL score, and diaphragmatic thickness and mobility will be consecutively collected. In case when primary outcome of the CaRe-ECMO trial does not show a statistically significant difference, future trials may consider to analyze our secondary clinical and functional outcomes.

To sum up, the currently proposed CaRe-ECMO trial is a pragmatic, multidisciplinary, randomized controlled, parallel group, clinical trial, designed to answer the question whether cardiopulmonary rehabilitation can facilitate weaning of ECMO. Should the implementation of the CaRe-ECMO program result in superior primary and secondary outcomes as compared to the controls, the CaRe-ECMO trial will offer an innovative treatment option for ECMO-supported patients and meaningfully impact the standard care in ECMO therapy.

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 the First Affiliated Hospital of Nanjing Medical University (Reference number of 2021-SR-416). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL, JZ, XC, YZ, and HS conceived the design of the trial, prepared and drafted the study protocol, and planned the statistical analysis. XL and JZ contributed to critical revision of the protocol, training of medical staff, and coordinating

the trial. XC will conduct the eligibility screening for trial enrollment. YC, DP, LW, XZ, and SW are responsible for cardiopulmonary rehabilitation delivery. YM, JL, DH, FS, WL, GZ, and HZ are the clinical members of our ECMO therapy group and will be responsible for the medical care for ECMO-supported patients. YG is the leading nurse in the emergency room and will be responsible for coordination of providing nursing care for ECMO-supported patients. YZ and HS are responsible for data management and the communication with the DSMC. ZZ and BL are responsible for data acquisition and assist trial coordination. All the authors read and contributed intellectually important content and approved the final version of the manuscript.

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REFERENCES

1. Mosier JM, Kelsey M, Raz Y, Gunnerson KJ, Meyer R, Hypes CD, et al. Extracorporeal membrane oxygenation (ECMO) for critically ill adults in the emergency department: history, current applications, and future directions. *Crit Care.* (2015) 19:431. doi: 10.1186/s13054-015-1155-7
2. Combes A, Schmidt M, Hodgson CL, Fan E, Ferguson ND, Fraser JF, et al. Extracorporeal life support for adults with acute respiratory distress syndrome. *Intensive Care Med.* (2020) 46:2464–76. doi: 10.1007/s00134-020-06290-1
3. Truong AD, Fan E, Brower RG, Needham DM. Bench-to-bedside review: mobilizing patients in the intensive care unit–from pathophysiology to clinical trials. *Crit Care.* (2009) 13:216. doi: 10.1186/cc7885
4. Wells CL, Forrester J, Vogel J, Rector R, Tabatabai A, Herr D. Safety and feasibility of early physical therapy for patients on extracorporeal membrane oxygenator: university of maryland medical center experience. *Crit Care Med.* (2018) 46:53–9. doi: 10.1097/CCM.0000000000002770
5. Gosselink R, Bott J, Johnson M, Dean E, Nava S, Norrenberg M, et al. Physiotherapy for adult patients with critical illness: recommendations of the European respiratory society and European society of intensive care medicine task force on physiotherapy for critically ill patients. *Intensive Care Medicine.* (2008) 34:1188–99. doi: 10.1007/s00134-008-1026-7
6. Connolly B, O'Neill B, Salisbury L, Blackwood B, Enhanced Recovery After Critical Illness Programme G. Physical rehabilitation interventions for adult patients during critical illness: an overview of systematic reviews. *Thorax.* (2016) 71:881–90. doi: 10.1136/thoraxjnl-2015-208273
7. Abrams D, Madahar P, Eckhardt C, Short B, Yip N, Parekh M, et al. Early mobilization during ECMO for cardiopulmonary failure in adults: factors associated with intensity of treatment. *Ann Am Thorac Soc.* (2021) doi: 10.1513/AnnalsATS.202102-151OC. [Epub ahead of print].
8. Abrams D, Javidfar J, Farrand E, Mongero L, Agerstrand C, Ryan P, et al. Early mobilization of patients receiving extracorporeal membrane oxygenation: a retrospective cohort study. *Crit Care.* (2014) 18:R38. doi: 10.1186/cc13746
9. Medrinal C, Combret Y, Prieur G, Robledo Quesada A, Bonnevie T, Gravier FE, et al. Comparison of exercise intensity during four early rehabilitation techniques in sedated and ventilated patients in ICU: a randomised cross-over trial. *Crit Care.* (2018) 22:110. doi: 10.1186/s13054-018-2030-0
10. Smith M, Vukomanovic A, Brodie D, Thiagarajan R, Rycus P, Buscher H. Duration of veno-arterial extracorporeal life support (VA ECMO) and outcome: an analysis of the extracorporeal life support organization (ELSO) registry. *Crit Care.* (2017) 21:45. doi: 10.1186/s13054-017-1633-1
11. Barbaro RP, Odetola FO, Kidwell KM, Paden ML, Bartlett RH, Davis MM, et al. Association of hospital-level volume of extracorporeal membrane oxygenation cases and mortality. Analysis of the extracorporeal life support organization registry. *Am J Respir Crit Care Med.* (2015) 191:894–901. doi: 10.1164/rccm.201409-1634OC
12. Schweickert W, Pohlman M, Pohlman A, Nigos C, Pawlik A, Esbrook C, et al. Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomised controlled trial. *Lancet.* (2009) 373:1874–82. doi: 10.1016/S0140-6736(09)60658-9
13. Lusebrink E, Stremmel C, Stark K, Joskowiak D, Czermak T, Born F, et al. Update on weaning from veno-arterial extracorporeal membrane oxygenation. *J Clin Med.* (2020) 9:992–1009. doi: 10.3390/jcm9040992
14. Gannon W, Stokes J, Bloom S, Sherrill W, Bacchetta M, Rice T, et al. Safety and feasibility of a protocolized daily assessment of readiness for liberation from venovenous extracorporeal membrane oxygenation. *Chest.* (2021) 160:1693–703. doi: 10.1016/j.chest.2021.05.066
15. Zwarenstein M, Treweek S, Gagnier JJ, Altman DG, Tunis S, Haynes B, et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. *BMJ.* (2008) 337:a2390. doi: 10.1136/bmj.a2390
16. Boutron I, Altman DG, Moher D, Schulz KF, Ravaud P. CONSORT statement for randomized trials of nonpharmacologic treatments: a 2017 update and a CONSORT extension for nonpharmacologic trial abstracts. *Ann Intern Med.* (2017) 167:40–7. doi: 10.7326/M17-0046
17. Association WM. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA.* (2013) 310:2191–4. doi: 10.1001/jama.2013.281053
18. Munshi L, Kobayashi T, DeBacker J, Doobay R, Telesnicki T, Lo V, et al. Intensive care physiotherapy during extracorporeal membrane oxygenation for acute respiratory distress syndrome. *Ann Am Thorac Soc.* (2017) 14:246–53. doi: 10.1513/AnnalsATS.201606-484OC
19. Guglin M, Zucker MJ, Bazan VM, Bozkurt B, El Banayosy A, Estep JD, et al. Venoarterial ECMO for adults: JACC scientific expert panel. *J Am Coll Cardiol.* (2019) 73:698–716. doi: 10.1016/j.jacc.2018.11.038
20. Tonna JE, Abrams D, Brodie D, Greenwood JC, Rubio Mateo-Sidron JA, Usman A, et al. Management of adult patients supported with venovenous extracorporeal membrane oxygenation (VV ECMO): guideline from the extracorporeal life support organization (ELSO). *ASAIO J.* (2021) 67:601–10. doi: 10.1097/MAT.0000000000001432

21. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. *Lancet.* (2002) 359:614–8. doi: 10.1016/S0140-6736(02)07750-4
22. Ko Y, Cho YH, Park YH, Lee H, Suh GY, Yang JH, et al. Feasibility and safety of early physical therapy and active mobilization for patients on extracorporeal membrane oxygenation. *ASAIO J.* (2015) 61:564–8. doi: 10.1097/MAT.0000000000000239
23. Mao L, Luo L, Wang D, Yu Y, Dong S, Zhang P, et al. Early rehabilitation after lung transplantation with extracorporeal membrane oxygenation (ECMO) of COVID-19 patient: a case report. *Ann Transl Med.* (2021) 9:512. doi: 10.21037/atm-21-456
24. Reardon PM, Wong J, Fitzpatrick A, Goligher EC. Diaphragm function in acute respiratory failure and the potential role of phrenic nerve stimulation. *Curr Opin Crit Care.* (2021) 27:282–9. doi: 10.1097/MCC.0000000000000828
25. Llaurado-Serra M, Ulldemolins M, Fernandez-Ballart J, Guell-Baro R, Valenti-Trulls T, Calpe-Damians N, et al. Related factors to semi-recumbent position compliance and pressure ulcers in patients with invasive mechanical ventilation: an observational study (CAPCRI study). *Int J Nurs Stud.* (2016) 61:198–208. doi: 10.1016/j.ijnurstu.2016.06.002
26. Li J, Wang M, Peng L, Zhang H, He H, Zhao Y. Effect of care bundles in the nursing of severe pneumonia. *Am J Translational research.* (2021) 13:1757–1763.
27. Guerin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, et al. Prone positioning in severe acute respiratory distress syndrome. *N Engl J Med.* (2013) 368:2159–68. doi: 10.1056/NEJMoa1214103
28. Zwischenberger JB, Pitcher HT. Extracorporeal membrane oxygenation management: techniques to liberate from extracorporeal membrane oxygenation and manage post-intensive care unit issues. *Crit Care Clin.* (2017) 33:843–53. doi: 10.1016/j.ccc.2017.06.006
29. Ramanathan K, Shekar K, Ling R, Barbaro R, Wong S, Tan C, et al. Extracorporeal membrane oxygenation for COVID-19: a systematic review and meta-analysis. *Crit Care.* (2021) 25:211–22. doi: 10.1186/s13054-021-03634-1
30. Schmidt G, Girard T, Kress J, Morris P, Ouellette D, Alhazzani W, et al. Official executive summary of an american thoracic society/american college of chest physicians clinical practice guideline: liberation from mechanical ventilation in critically ill adults. *Am J Respir Crit Care Med.* (2017) 195:115–9. doi: 10.1164/rccm.201610-2076ST
31. Cho HW, Song IA, Oh TK. Quality of life and long-term mortality among survivors of extracorporeal membrane oxygenation: a nationwide cohort study in South Korea. *Crit Care Med.* (2021) 49:e771–80. doi: 10.1097/CCM.0000000000005015
32. Knudson KA, Gustafson CM, Sadler LS, Whittemore R, Redeker NS, Andrews LK, et al. Long-term health-related quality of life of adult patients treated with extracorporeal membrane oxygenation (ECMO): an integrative review. *Heart Lung.* (2019) 48:538–52. doi: 10.1016/j.hrtlng.2019.08.016
33. Matamis D, Soilemezi E, Tsagourias M, Akoumianaki E, Dimassi S, Boroli F, et al. Sonographic evaluation of the diaphragm in critically ill patients. Technique and clinical applications. *Intensive Care Med.* (2013) 39:801–10. doi: 10.1007/s00134-013-2823-1
34. Gravesteijn BY, Schluep M, Disli M, Garkhail P, Dos Reis Miranda D, Stolker RJ, et al. Neurological outcome after extracorporeal cardiopulmonary resuscitation for in-hospital cardiac arrest: a systematic review and meta-analysis. *Crit Care.* (2020) 24:505. doi: 10.1186/s13054-020-03201-0
35. McDonald MD, Sandsmark DK, Palakshappa JA, Mikkelsen ME, Anderson BJ, Gutsche JT. Long-Term outcomes after extracorporeal life support for acute respiratory failure. *J Cardiothorac Vasc Anesth.* (2019) 33:72–9. doi: 10.1053/j.jvca.2018.05.023
36. Kolaitis NA, Soong A, Shrestha P, Zhuo H, Neuhaus J, Katz PP, et al. Improvement in patient-reported outcomes after lung transplantation is not impacted by the use of extracorporeal membrane oxygenation as a bridge to transplantation. *J Thorac Cardiovasc Surg.* (2018) 156:440–8.e2. doi: 10.1016/j.jtcvs.2018.01.101
37. Grant AM, Altman DG, Babiker AG, Campbell MK, Clemens F, Darbyshire JH, et al. A proposed charter for clinical trial data monitoring committees: helping them to do their job well. *Lancet.* (2005) 365:711–22. doi: 10.1016/S0140-6736(05)17965-3
38. Fernando SM, Qureshi D, Tanuseputro P, Fan E, Munshi L, Rochwerg B, et al. Mortality and costs following extracorporeal membrane oxygenation in critically ill adults: a population-based cohort study. *Intensive Care Med.* (2019) 45:1580–9. doi: 10.1007/s00134-019-05766-z
39. Kamper S. Per-Protocol, intention-to-treat, and complier average causal effects analyses in randomized controlled trials: linking evidence to practice. *J Orthop Sports Phys Ther.* (2021) 51:314–5. doi: 10.2519/jospt.2021.0701
40. Sitlani C, Lumley T, McKnight B, Rice K, Olson N, Doyle M, et al. Incorporating sampling weights into robust estimation of cox proportional hazards regression model, with illustration in the multi-ethnic study of atherosclerosis. *BMC Med Res Methodol.* (2020) 20:62. doi: 10.1186/s12874-020-00945-9
41. Rabe-Hesketh S, Skrondal A. *Multilevel and Longitudinal Modeling Using Stata*. 2nd edn. College Station, TX: Stata Press (2008).
42. Lai CC, Chou W, Cheng AC, Chao CM, Cheng KC, Ho CH, et al. The effect of early cardiopulmonary rehabilitation on the outcomes of intensive care unit survivors. *Medicine.* (2019) 98:e14877. doi: 10.1097/MD.0000000000014877
43. Yu X, Gu S, Li M, Zhan Q. Awake extracorporeal membrane oxygenation for acute respiratory distress syndrome: which clinical issues should be taken into consideration. *Front Med.* (2021) 8:682526. doi: 10.3389/fmed.2021.682526
44. Salna M, Abrams D, Brodie D. Physical rehabilitation in the awake patient receiving extracorporeal circulatory or gas exchange support. *Ann Transl Med.* (2020) 8:834. doi: 10.21037/atm.2020.03.151
45. Turner DA, Cheifetz IM, Rehder KJ, Williford WL, Bonadonna D, Banuelos SJ, et al. Active rehabilitation and physical therapy during extracorporeal membrane oxygenation while awaiting lung transplantation: a practical approach. *Crit Care Med.* (2011) 39:2593–8. doi: 10.1097/CCM.0b013e3182282bbe
46. Morris PE, Berry MJ, Files DC, Thompson JC, Hauser J, Flores L, et al. Standardized rehabilitation and hospital length of stay among patients with acute respiratory failure: a randomized clinical trial. *JAMA.* (2016) 315:2694–702. doi: 10.1001/jama.2016.7201
47. Moss M, Nordon-Craft A, Malone D, Van Pelt D, Frankel SK, Warner ML, et al. A randomized trial of an intensive physical therapy program for patients with acute respiratory failure. *Am J Respir Crit Care Med.* (2016) 193:1101–10. doi: 10.1164/rccm.201505-1039OC
48. Combes A, Hajage D, Capellier G, Demoule A, Lavoue S, Guervilly C, et al. Extracorporeal membrane oxygenation for severe acute respiratory distress syndrome. *N Engl J Med.* (2018) 378:1965–1975. doi: 10.1056/NEJMoa1800385
49. Alberti L, Petroianu A, Corrêa D, Franco Silva T. The influence of physical activity on chronic venous insufficiency of the lower limbs. *Acta Med Port.* (2008) 21:215–20.
50. Dres M, Dubé B, Mayaux J, Delemazure J, Reuter D, Brochard L, et al. Coexistence and impact of limb muscle and diaphragm weakness at time of liberation from mechanical ventilation in medical intensive care unit patients. *Am J Respir Crit Care Med.* (2017) 195:57–66. doi: 10.1164/rccm.201602-0367OC
51. Levine S, Nguyen T, Taylor N, Friscia M, Budak M, Rothenberg P, et al. Rapid disuse atrophy of diaphragm fibers in mechanically ventilated humans. *N Engl J Med.* (2008) 358:1327–35. doi: 10.1056/NEJMoa070447
52. Kim WY, Suh HJ, Hong SB, Koh Y, Lim CM. Diaphragm dysfunction assessed by ultrasonography: influence on weaning from mechanical ventilation. *Crit Care Med.* (2011) 39:2627–30. doi: 10.1097/CCM.0b013e3182266408
53. Jaber S, Petrof BJ, Jung B, Chanques G, Berthet JP, Rabuel C, et al. Rapidly progressive diaphragmatic weakness and injury during mechanical ventilation in humans. *Am J Respir Crit Care Med.* (2011) 183:364–71. doi: 10.1164/rccm.201004-0670OC
54. O'Rourke J, Sotak M, Curley GF, Doolan A, Henlin T, Mullins G, et al. Initial Assessment of the percutaneous electrical phrenic nerve stimulation

system in patients on mechanical ventilation. *Crit Care Med.* (2020) 48:e362–70. doi: 10.1097/CCM.0000000000004256
55. Masmoudi H, Coirault C, Demoule A, Mayaux J, Beuvin M, Romero N, et al. Can phrenic stimulation protect the diaphragm from mechanical ventilation-induced damage? *Euro Respir J.* (2013) 42:280–3. doi: 10.1183/09031936.00045613
56. Megan B, Pickering RM, Weatherall M. Design, objectives, execution and reporting of published open-label extension studies. *J Eval Clin Pract.* (2012) 18:209–15. doi: 10.1111/j.1365-2753.2010.01553.x

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CASE REPORT
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Non-Compaction Cardiomyopathy and Multiple Sclerosis: Associated or Independent Diseases? A Case Report

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Non-compaction cardiomyopathy (NCCM) is associated with neuromuscular disorders; however, there has been little investigation on its association with other neurological diseases, such as multiple sclerosis. We present the case of a 46-year-old woman with a history of multiple sclerosis who developed heart failure and was diagnosed with non-compaction cardiomyopathy.

Keywords: heart failure, multiple sclerosis, myocardium, non-compaction cardiomyopathy, left ventricular dysfunction

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INTRODUCTION

Non-compaction cardiomyopathy (NCCM) is characterized by a bilayer myocardial structure, a thin compacted epicardial layer, and a thicker spongy endocardial layer with prominent trabeculations and deep intertrabecular recesses (1). There is an embryonic hypothesis that attributes the development of this pathology to arrested compaction of the loose meshwork of the myocardial primordium during embryonic development, causing the persistence of deep trabecular recesses in the myocardial wall (2, 3). However, there is not enough evidence to affirm this hypothesis, as argued by Jensen et al. (4), who found that adult hypertrabeculated left ventricles (LVs) were different from the embryo in that they were less trabeculated (15–40% vs. 55–80%) and had thicker trabeculae.

When NCCM is systematically investigated, neuromuscular disorders are found in 80% of cases, and associated mutated genes have also been described (5); unlike what happens with multiple sclerosis (MS), where the common pathogenesis is still unknown. To our knowledge, this is the second reported case of NCCM associated with MS (6).

CASE DESCRIPTION AND DIAGNOSTIC ASSESSMENT

A 46-year-old woman was admitted to our hospital for pelvic inflammatory disease complicated by pelvic peritonitis; she underwent exploratory laparotomy with right salpingectomy, and double antibiotic therapy was started. In the postoperative period, she presented with stage 2 acute kidney injury (creatinine: 2.45 mg/dl, urea: 74 mg/dl) and new-onset dyspnea. Her past medical history revealed a history of relapsing-remitting MS (**Figures 1A–C**) diagnosed 15 years ago and treated with azathioprine and prednisone. She had no previous history of hypertension, diabetes, or dyslipidemia. In the important family history, one of her sisters also has MS. She did not give detailed information about heart disease in her family. Physical examination showed spastic

paraparetic gait with little steps, and auscultation found inspiratory crackles in the lower third of both lungs, rhythmic heart sounds with no murmurs or gallops. Blood pressure was 120/80 mmHg, pulse rate was 120 beats/min, respiratory rate was 16 breaths/min, and oxygen saturation was 95% with nasal cannula set at 2 L/min. The electrocardiogram (ECG) showed sinus tachycardia and complete left bundle branch block (LBBB) (**Figure 1D**).

The Pro-B-type natriuretic peptide plasma level was elevated (Pro-BNP: 1,038 pg/ml, normal < 125 pg/ml). High-sensitivity troponin T and dimer D concentrations were within the normal range. The complete blood count showed a decrease in white blood cell count (11.39 10^3/ul; at the beginning 19.60 10^3/ul), and the acute kidney injury had resolved (creatinine: 0.98 mg/dl; urea: 44.5 mg/dl).

In the first postoperative evaluation, we observed signs and symptoms of volume overload, sinus tachycardia, and LBBB on the ECG. In this setting, the initial differential diagnosis was directed toward acute decompensated heart failure (ADHF) secondary to cardiomyopathy. The history of MS raised the suspicion of an undetected underlying structural heart disease. On the other hand, other differential diagnoses were considered, such as atelectasis that was ruled out in the chest X-ray, vascular causes like pulmonary embolism since the D-dimer was in the normal range, and systemic causes like sepsis of abdominal origin, because of the absence of fever and a marked decrease in infectious markers with double antibiotic therapy.

Transthoracic echocardiogram (TTE) demonstrated a left ventricular ejection fraction (LVEF) of 30%, mildly dilated, diffusely hypokinetic, prominent trabeculations of LV, and a non-compacted to compacted (NC/C) myocardium ratio of 2.75 in end-systole at inferolateral mid-ventricular level (**Figures 2A–D**), so the diagnosis of NCCM was realized. On the sixth hospital day, coronary angiography revealed that coronary arteries were normal (**Figures 2E,F**). Right-sided cardiac catheterization showed normal cardiac index and absence of pulmonary hypertension.

The clinical status was stabilized with diuretic therapy, enalapril 5 mg bid, spironolactone 25 mg OD, and bisoprolol 5 mg OD for heart failure, with reduced ejection fraction (HFrEF) were progressively initiated. During hospitalization, she was evaluated by neurology to regulate her treatment for MS and avoid any relapse crisis, which did not appear. The evolution of the patient was favorable, and she was discharged on the 14th day of hospitalization.

Cardiovascular magnetic resonance (CMR) imaging was performed 1 month after discharge to confirm the diagnosis and demonstrated positive diagnostic criteria for NCCM; additionally, the study did not show late gadolinium enhancement (LGE) (**Figure 3**). Currently, after 9 months of outpatient follow-up, the patient remains in optimal medical therapy. Bisoprolol was titrated to 5 mg bid, and dapagliflozin 10 mg OD was started. The LVEF improved slightly to ∼35%, in NYHA class II, and she did not require hospitalizations for ADHF. A timeline is showcased in **Figure 4**.

Abbreviations: ADHF, acute decompensated heart failure; CMR, cardiac magnetic resonance; CRT-D, cardiac resynchronization therapy with defibrillator; DCM, dilated cardiomyopathy; ECG, electrocardiogram; HCM, hypertrophic cardiomyopathy; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricular ejection fraction; MS, multiple sclerosis; NCCM, non-compaction cardiomyopathy; NC/C, non-compacted to compacted; NYHA, New York Heart Association; OMT, optimal medical therapy; TTE, transthoracic echocardiogram.

DISCUSSION

Non-compaction cardiomyopathy (NCCM) is classified as primary genetic cardiomyopathy by the American Heart Association (AHA) and unclassified cardiomyopathy by the European Society of Cardiology (ESC), with an adult prevalence of 0.014% (2). NCCM is associated with neuromuscular disorders; however, there has been little investigation on its association with other neurological diseases, such as MS. The first case report of NCCM and MS was published in 2009 (6). However, it should be noted that we are proposing for the first time a possible association pathway, which is through tenascin C, on which some research has already been conducted in NCCM and MS independently for now. This is a glycoprotein of the extracellular matrix that is temporarily expressed in the heart during embryonic development and regulates several cellular functions, including embryogenesis and nerve regeneration (7). In adults, this protein is reexpressed under pathological conditions regulated by inflammation, such as NCCM and MS, and is found in elevated serum levels (7, 8).

Momčilović et al. (9), in an experimental MS model with mice, evidenced that tenascin C is involved in the pathogenesis of central nervous system autoimmunity, through Th1 and Th17 cells.

The NCCM can be grouped into seven phenotypical subtypes (3), among which our case is associated with LV dilation and dysfunction at baseline. The interaction between MS relapses and myocardial stunning through a sympathetic outburst has been proposed, similar to what occurs in stress cardiomyopathy, where it has been found in common that demyelinating lesions compromise the medulla oblongata that controls the neurovegetative cardiovascular center (10). Unlike this, in our patient, the demyelinating lesions found (**Figures 1A–C**) did not affect the brainstem that includes the medulla oblongata; therefore, we rule out this alternative hypothesis to explain the reduced LVEF in our case, and we suggest that this is probably due to the advancement of the NCCM itself.

The TTE fulfills a fundamental value in the initial diagnostic approach, being Jenni's criteria the most widely used, and evaluates a two-layer ventricular myocardium with thick non-compacted and thinner compacted myocardium, generating an NC/C ratio >2 in end-systole on short-axis parasternal view (11). These alterations affect predominantly the mid-lateral, mid-inferior, mid-anterior, and apical LV areas (12). Finally, prominent excessive trabeculations and the flow between the deep intertrabecular recesses by color Doppler and excluded

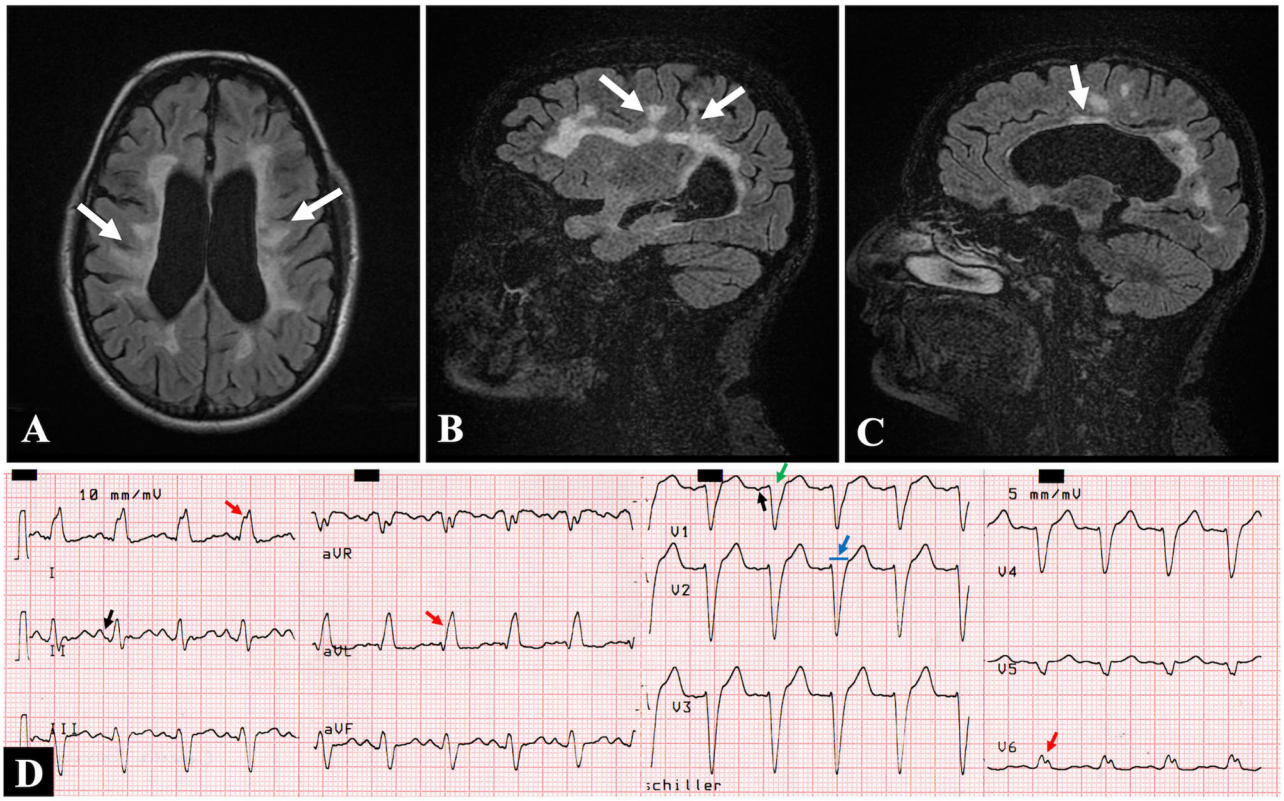

FIGURE 1 | Brain magnetic resonance imaging. **(A)** Axial and **(B,C)** sagittal views in FLAIR sequence showing demyelinating lesions consistent with multiple sclerosis: perpendicular to the lateral ventricles **(A)**, periventricular **(B)**, and corpus callosum **(C)** (white arrows). **(D)** Twelve-lead electrocardiogram showing sinus tachycardia (125 beats/min), left atrial dilatation [P mitrale in II, and deepening of the terminal negative portion of the P wave in V1 (black arrows)] and left bundle branch block [QRS duration of >120 ms (blue arrow), lead V1 with a dominant deep S wave (green arrow); broad, notched R wave in V6 and I, and monophasic R wave in aVL (red arrows)]. ms, milliseconds.

coexisting structural cardiac abnormalities are also considered (11). Cardiac magnetic resonance (CMR) allows us to confirm the diagnosis with 86% sensitivity and 99% specificity, following Petersen's criteria that consider an NC/C ratio >2.3 in end-diastole (11). Both criteria were met by our patient. The differential diagnosis by TTE was made with a normal variation of myocardial trabeculations, defined as less than 3 trabeculations located in the LV apex, also with LV apical thrombi, false tendons, aberrant chords, cardiac tumors, hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM) (12, 13). The LV apical thrombi are distinguished by their higher echogenicity compared to myocardium; false tendons and aberrant cords usually cross the LV cavity (13). None of these findings were observed in our patient, neither was any cardiac tumor. Recently, the characteristics of Speckle Tracking in NCCM began to be studied, evidencing a reduced global longitudinal strain, with greater involvement of the apical segments, generating a significant basal-to-apical gradient, useful to differentiate it from HCM and DCM (11, 13). Additionally, it differs from HCM, since trabeculations and crypts that can mimic NCCM are mainly limited to the basal ventricular septum or the posterior wall (13).

The ECG findings are non-specific and include left ventricular hypertrophy, inverted T waves, and different types of bundle branch blocks (2, 12). Currently, there is no specific therapy for patients with NCCM and HFrEF, so it is recommended to follow the heart failure (HF) management guidelines (12). Consequently, our patient received optimal medical therapy. According to the 2021 European HF guidelines, an implantable cardioverter-defibrillator (ICD) should be considered to reduce the risk of sudden death and all-cause mortality in patients with symptomatic HF (NYHA class II–III) of non-ischemic etiology and LVEF $\leq$ 35 % after at least 3 months of optimal medical therapy (OMT), and with a life expectancy greater than 1 year with good functional status (class IIa, level of evidence A). If a patient is scheduled to receive an ICD and is in sinus rhythm, with an LBBB, cardiac resynchronization therapy with defibrillator (CRT-D) is recommended if the QRS is $\geq$ 150 ms to improve symptoms and reduce morbidity and mortality (class I, level of evidence A) (14). In our case, we are still titrating OMT; and for ICD implantation, a minimum of 3 months of OMT is recommended to assess whether the LVEF fails to increase to >35%, so that in the following controls, according to the

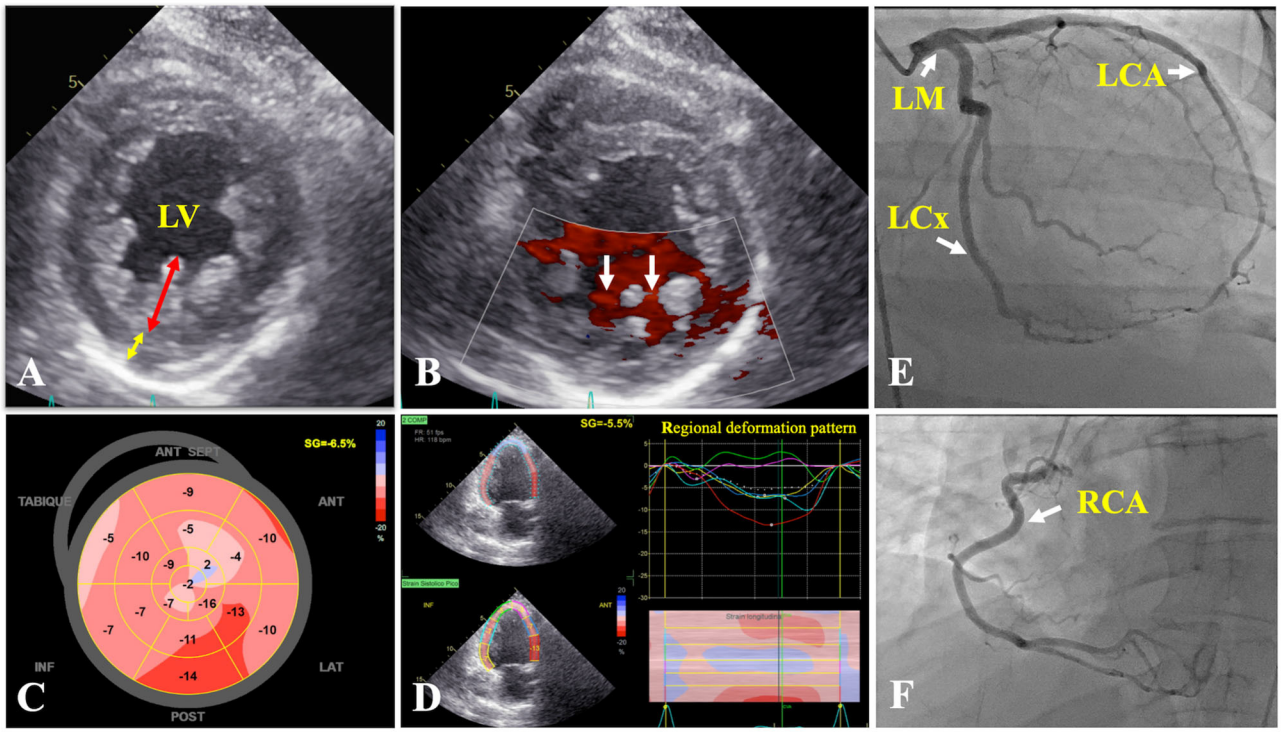

FIGURE 2 | Transthoracic echocardiogram. **(A)** Parasternal short axis of the mid left ventricle demonstrating two-layer myocardial structure, with a ratio of ticker non-compacted (redline) to thin compacted (yellow line) myocardium >2 at end-systole in the inferolateral segment. **(B)** Flow between intertrabecular recesses by intraventricular blood on color Doppler (white arrows). **(C)** Reduced global longitudinal strain (−6.5%). **(D)** Two-chamber view showing a regional deformation pattern with a markedly decreased myocardial deformation in apical segments (green and purple curves) in comparison to basal segments (red and yellow curves). Coronary angiography: coronary arteries without lesions **(E,F)** (white arrows). LCA, left coronary artery; LCx, left circumflex coronary artery; LM, left main coronary artery; LV, left ventricle; RCA, right coronary artery.

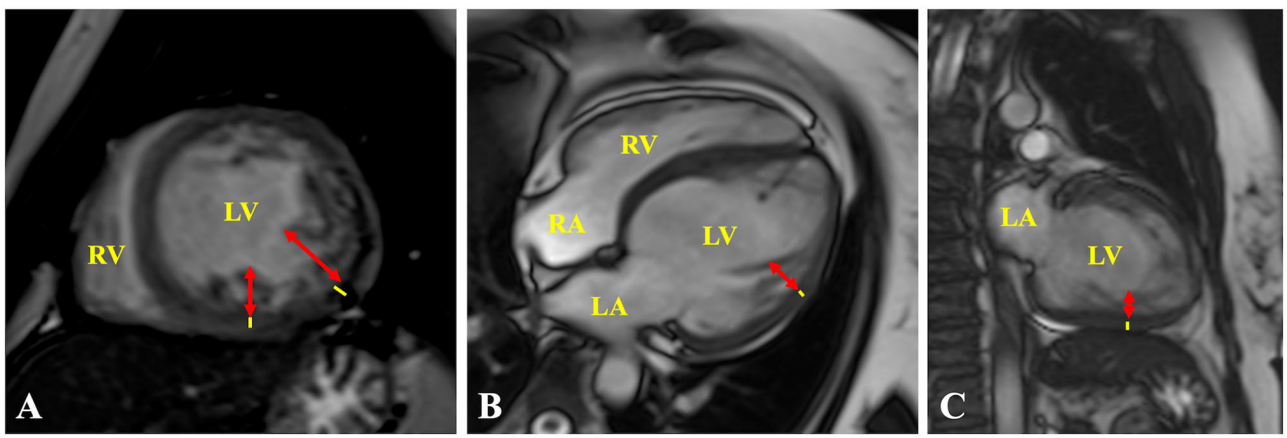

FIGURE 3 | Cardiac MRI. **(A)** Short axis, **(B)** 4-chamber, and **(C)** 2-chamber views showing representative measurements of the ratio of ticker non-compacted (redlines) to thin compacted (yellow lines) myocardium >2.3 at end-diastole, in the **(A)** mid-inferolateral, **(B)** mid-anterolateral, and **(A,C)** mid-inferior segments. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

evolution of the patient she will be reassessed for the need to implant an ICD and/or CRT-D.

Anticoagulation is mandatory in all patients with NCCM with atrial fibrillation, previous thromboembolic events, or LV thrombus (class I, level of evidence B), whereas in those with NCCM and only LV dysfunction, it may be considered (class IIb, level of evidence B) (1, 15). Patients with NCCM and LV dysfunction, presenting with LGE on CMR imaging, compared

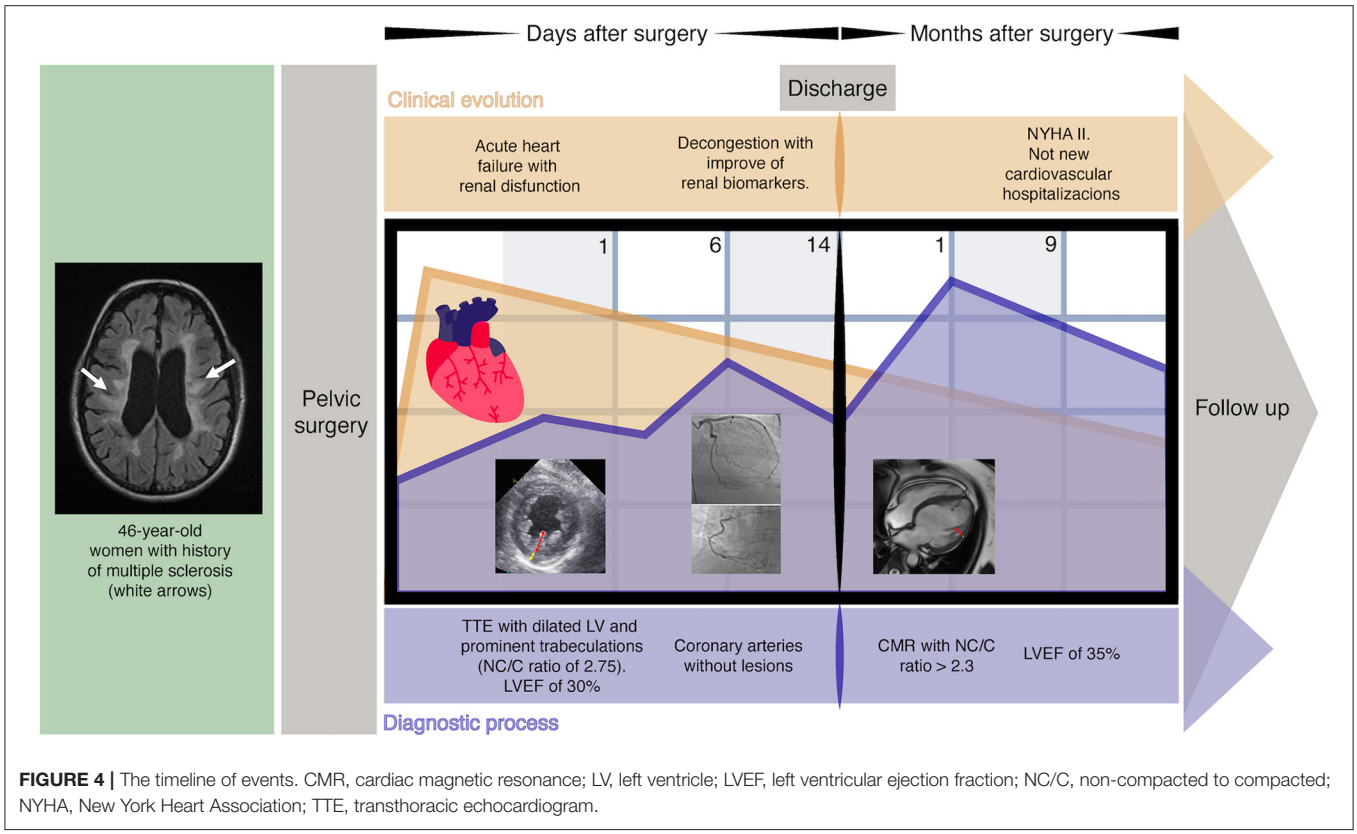

FIGURE 4 | The timeline of events. CMR, cardiac magnetic resonance; LV, left ventricle; LVEF, left ventricular ejection fraction; NC/C, non-compacted to compacted; NYHA, New York Heart Association; TTE, transthoracic echocardiogram.

to those without LGE, experienced stroke more frequently (1). Fortunately, in our case, the presence of LGE was not observed. Starting with direct oral anticoagulants was proposed to the patient and, as a second option, a vitamin K antagonist. However, both options were rejected because of economic issues and the risk of bleeding, respectively.

The prognosis depends on the development of HF or the need for heart transplantation; likewise, there is a variety of predictors associated with poor outcomes that include the presence of advanced age, inpatient's NCCM diagnosis, NYHA functional class III–IV, and LVEF < 31% (12, 16). Vaidya et al. (17) found that age, LVEF < 50%, and non-compaction extending from the apex to the mid or basal segments were associated with all-cause mortality.

It was suggested that first-degree relatives undergo echocardiographic screening; however, because of health insurance issues and living in another locality in Peru, this has not yet been possible. When we were evaluating the family history, the patient mentioned that one of her sisters also had a diagnosis of MS and was being evaluated in a private clinic.

LIMITATIONS AND STRENGTHS

Strengths

This case serves to propose a possible pathway of association between NCCM and MS, through Tenascin C. In addition, it highlights the importance of performing a cardiovascular evaluation that includes echocardiographic screening in all patients with MS to timely identify NCCM, and particularly the dilated LV phenotype with reduced LVEF, to initiate OMT and prevent HF progression.

Weaknesses

Because of the lack of advanced genetic studies, it was not possible to perform genetic screening on our patient. Tenascin C was not measured in our patient because of the lack of resources in our reality. It is necessary to carry out studies on large population groups to confirm this possible association between the pathologies described.

FUTURE DIRECTIONS

The NCCM is possibly associated with MS; however, the exact pathophysiological mechanisms that explain the association are not clear, and with the present case, we would like to encourage the research for evidence through studies with large population groups on Tenascin C.

CONCLUSION

Patients with NCCM with a phenotype of dilated LV with reduced LVEF must be diagnosed promptly to initiate OMT and avoid the progression of HF.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital Nacional Edgardo Rebagliati Martins. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM contributed to conception and design of the article. JM and CH wrote the first draft of the manuscript. JM, CH, and CL wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Chimenti C, Lavalle C, Magnocavallo M, Alfarano M, Mariani MV, Bernardini F, et al. A proposed strategy for anticoagulation therapy in noncompaction cardiomyopathy. *ESC Heart Fail.* (2022) 9:241–50. doi: 10.1002/ehf2.13694
2. Hussein A, Karimianpour A, Collier P, Krasuski RA. Isolated noncompaction of the left ventricle in adults. *J Am Coll Cardiol.* (2015) 66:578–85. doi: 10.1016/j.jacc.2015.06.017
3. Arbustini E, Favalli V, Narula N, Serio A, Grasso M. Left ventricular noncompaction: a distinct genetic cardiomyopathy? *J Am Coll Cardiol.* (2016) 68:949–66. doi: 10.1016/j.jacc.2016.05.096
4. Jensen B, Agger P, de Boer BA, Oostra RJ, Pedersen M, van der Wal AC, et al. The hypertrabeculated (noncompacted) left ventricle is different from the ventricle of embryos and ectothermic vertebrates. *BiochimBiophys Acta.* (2016) 1863:1696–706. doi: 10.1016/j.bbamcr.2015.10.018
5. Stöllberger C, Finsterer J. Understanding left ventricular hypertrabeculation/noncompaction: pathomorphologic findings and prognostic impact of neuromuscular comorbidities. *Expert Rev Cardiovasc Ther.* (2019) 17:95–109. doi: 10.1080/14779072.2019.1561280
6. Henderson MH, Ogden PE, Sibbitt SJ, Colbert CY, Puschett JB, Scott RC. 62-year-old-female with weakness and shortness of breath. *Am J Med Sci.* (2009) 337:126–30. doi: 10.1097/MAJ.0b013e31818bb0bf
7. Erer HB, Guvenc TS, Kemik AS, Yilmaz H, Kul S, Altay S, et al. Assessment of tenascin-C levels in ventricular noncompaction/hypertrabeculation patients: a cross-sectional study. *Echocardiography.* (2014) 31:203–8. doi: 10.1111/echo.12328
8. Harada M, Kamimura D, Arima Y, Kohsaka H, Nakatsuji Y, Nishida M, et al. Temporal expression of growth factors triggered by epiregulin regulates inflammation development. *J Immunol.* (2015) 194:1039–46. doi: 10.4049/jimmunol.1400562
9. Momčilović M, Stamenković V, Jovanović M, Andjus PR, Jakovčevski I, Schachner M, et al. Tenascin-C deficiency protects mice from experimental autoimmune encephalomyelitis. *J Neuroimmunol.* (2017) 302:1–6. doi: 10.1016/j.jneuroim.2016.12.001
10. Dell'Aquila A, Sciatti E, Vizzardi E, Metra M. The brain-heart connection: a multiple sclerosis relapse presenting as Takotsubo Syndrome. A case report and literature review. *Monaldi Arch Chest Dis.* (2020) 90:e1153. doi: 10.4081/monaldi.2020.1153
11. Rao K, Bhaskaran A, Choudhary P, Tan TC. The role of multimodality imaging in the diagnosis of left ventricular noncompaction. *Eur J Clin Invest.* (2020) 50:e13254. doi: 10.1111/eci.13254
12. Gerecke BJ, Engberding R. Noncompaction cardiomyopathy-history and current knowledge for clinical practice. *J Clin Med.* (2021) 10:2457. doi: 10.3390/jcm10112457
13. Di Fusco SA, Lucà F, Madeo A, Massimiliano Rao C, Iorio A, Rizzo M, et al. Left ventricular noncompaction: diagnostic approach, prognostic evaluation, and management strategies. *Cardiol Rev.* (2020) 28:125–34. doi: 10.1097/CRD.0000000000000251
14. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J.* (2021) 42:3599–726. doi: 10.1093/eurheartj/ehab368
15. Towbin JA, McKenna WJ, Abrams DJ, Ackerman MJ, Calkins H, Darrieux FC, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm.* (2019) 16:e301–72. doi: 10.1016/j.hrthm.2019.05.007
16. Kubik M, Dabrowska-Kugacka A, Lewicka E, Daniłowicz-Szymanowicz L, Raczak G. Predictors of poor outcome in patients with left ventricular noncompaction: review of the literature. *Adv Clin Exp Med.* (2018) 27:415–22. doi: 10.17219/acem/67457
17. Vaidya VR, Lyle M, Miranda WR, Farwati M, Isath A, Patlolla SH, et al. Long-term survival of patients with left ventricular noncompaction. *J Am Heart Assoc.* (2021) 10:e015563. doi: 10.1161/JAHA.119.015563

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Effects of BNP and Sacubitrilat/Valsartan on Atrial Functional Reserve and Arrhythmogenesis in Human Myocardium

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Background: Although the angiotensin receptor-neprilysin inhibitor (ARNI) sacubitril/valsartan started a new era in heart failure (HF) treatment, less is known about the tissue-level effects of the drug on the atrial myocardial functional reserve and arrhythmogenesis.

Methods and Results: Right atrial (RA) biopsies were retrieved from patients ($n = 42$) undergoing open-heart surgery, and functional experiments were conducted in muscle strips ($n = 101$). B-type natriuretic peptide (BNP) did not modulate systolic developed force in human myocardium during β-adrenergic stimulation, but it significantly reduced diastolic tension ($p < 0.01$) and the probability of arrhythmias ($p < 0.01$). In addition, patient's plasma NTproBNP positively correlated with isoproterenol-induced contractile reserve in atrial tissue *in vitro* ($r = 0.65$; $p < 0.01$). Sacubitrilat+valsartan (Sac/Val) did not show positive inotropic effects on atrial trabeculae function but reduced arrhythmogeneity. Atrial and ventricular biopsies from patients with end-stage HF ($n = 10$) confirmed that neprilysin (NEP) is equally expressed in human atrial and ventricular myocardium. RA NEP expression correlates positively with RA ejection fraction (EF) ($r = 0.806$; $p < 0.05$) and left ventricle (LV) NEP correlates inversely with left atrial (LA) volume ($r = -0.691$; $p < 0.05$).

Conclusion: BNP ameliorates diastolic tension during adrenergic stress in human atrial myocardium and may have positive long-term effects on the inotropic reserve. BNP and Sac/Val reduce atrial arrhythmogeneity during adrenergic stress *in vitro*. Myocardial NEP expression is downregulated with declining myocardial function, suggesting a compensatory mechanism in HF.

Keywords: BNP, sacubitrilat/valsartan (Sac/Val), atrial function, arrhythmias, heart failure, neprilysin, sacubitril/valsartan

INTRODUCTION

Heart failure (HF) is a major burden in Western societies, both economically and in terms of disability-adjusted life years lost. Its prevalence is increasing with age up to 10% in the 8th decade of life (1, 2). HF with reduced ejection fraction (EF) and HF with preserved EF are characterized by a reduced functional reserve to physiological stress, such as adrenergic stimulation (3). The heart's atria play an important role in HF, as they improve ventricular filling and contribute to ventricular stroke volume and cardiac output to up to 40% during periods of hemodynamic demand (4). Atrial dysfunction and remodeling are closely associated with new-onset HF, atrial fibrillation, and overall increased mortality (5–7). The atria also contribute to endocrine signaling in HF, especially by releasing natriuretic peptides (NPs), such as atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). NPs modulate the functional reserve of the ventricular myocardium; however, their role in modulating the atria's functional reserve is unknown (8).

One of the pivotal enzymes involved in NP degradation is neprilysin (NEP), an endopeptidase located on the extracellular portion of the cell membrane (9). The regulation of NEP expression in HF is part of an ongoing discussion, as an early study found NEP upregulation in human ventricular cardiomyocytes in patients with aortic stenosis and HF (10), while a more recent study in a porcine model of ischemic cardiomyopathy suggested a systemic downregulation of total NEP expression (11). The expression of NEP in human atrial tissue has not been reported yet.

In 2014, the PARADIGM-HF trial introduced the first of its kind angiotensin receptor-neprilysin inhibitor (ARNI), sacubitril/valsartan, which exerts its function by a dual mechanism, namely, angiotensin receptor blockage and NEP inhibition (12). Besides its beneficial effects on HF, evidence supports a reduced probability of (ventricular) arrhythmias in patients with HF on ARNI treatment (13–15). The exact mechanisms involved are unknown and supposedly multifactorial. By increasing the NP levels and stimulating the cyclic guanosine monophosphate/ protein kinase G (cGMP/PKG) pathway, sacubitrilat (Sac; the active metabolite of sacubitril) may improve cardiomyocyte function in (early) cardiac remodeling, as reported for ANP in a murine model of HF (8). Thus, we hypothesized that an increase in NP tissue concentration by Sac without or with angiotensin-receptor blockade by valsartan (Val) may exert beneficial effects on atrial myocardial function reserve and arrhythmias.

In this study, we, therefore, investigated the functional and antiarrhythmic effects of BNP and Sac without and with Val on human atrial myocardium. Furthermore, we also analyzed the expression of NEP in failing human left ventricular (LV) and right atrial (RA) myocardial samples.

METHODS

Patient Selection

The study was performed in line with the 1975 Declaration of Helsinki ethical guidelines. Sample acquisition at Charité Campus Virchow-Klinikum and German Heart Center Berlin (DHZB) was approved by the Local Ethics Committee of the Charité (EA2/167/15) and the Ethics vote for the Biobank at the DHZB (EA4/028/12). All patients were ≥18 years old and provided written informed consent prior to enrolment. Patients with active malignancy, congenital heart disease, and endocarditis were not enrolled in our study, and one patient was excluded due to reported chronic right coronary artery occlusion. RA myocardial tissue and peripheral blood serum samples were obtained as part of the routine surgical procedure for patients undergoing heart surgery with extracorporeal circulation (predominantly coronary artery bypass graft or aortic valve replacement surgeries; see data specified below). Relevant medical and drug history was obtained from the patients' files.

Analysis of Echocardiography Recordings

Transthoracic echocardiography was performed within a week before surgery by an experienced investigator, using an Epiq 7G station (Philips, Andover, MA, USA) with a 2.5-MHz probe, and loops were recorded using standardized protocols. Echo data sets on (preoperative) ventricular and atrial function were reviewed and reanalyzed as applicable regarding the relevant readouts by an independent examiner (U.P.). According to the local standard operating procedure, left atrial (LA) and RA speckle-tracking analysis was performed using TomTec software (TOMTEC Imaging Systems GmbH, Unterschleißheim, Germany).

Functional Human Myocardial Experiments

Human myocardium for functional measurements was collected as excess tissue from the RA appendage (RAA) as part of the standard surgical procedure for patients undergoing open-heart surgery with extracorporeal circulation (aortic valve replacement surgeries and coronary artery bypass grafts). RAA biopsies from 42 patients with cumulative 101 muscle strips were included in our study collected between December 2018 and April 2020. Trabeculae were excised using microsurgical scissors and forceps. Human atrial muscle strips (≥3 mm length >0.65 mm, and preferred <1 mm diameter) were isolated, as the risk of central ischemia increases with muscle diameter. Directly after the end of the functional measurements, all muscle strips were snap-frozen in liquid nitrogen and stored for further molecular biology analysis. Functional atrial myocardium measurements were performed with a force transducer that recorded every muscle contraction (developed force) and relaxation. Muscle strips were stimulated at 1 Hz and 5 ms rectangular field stimulation and then prestretched up to the point of maximal force development (L_{max}), as also described previously (16). The acquisition was performed using the software MyoDat (MyoTronic UG, Heidelberg, Germany). Analysis of systolic and diastolic functional parameters was performed using the program MyoViewer (MyoTronic UG, Heidelberg, Germany). Furthermore, the incidence of arrhythmias (=spontaneous aftercontractions) was determined (see **Supplementary Material** for detailed information).

Experimental Protocols and Pharmacological Interventions

We investigated the effects of BNP, Sac (the active metabolite of sacubitril), and the combination of Sac with Val (Sac/Val) on atrial functional reserve and arrhythmogenesis in the presence of physiological stressors and varying stimulation frequency (1, 2, 3, and 0.5 Hz) in 42 patients ($n = 101$ muscle strips). Atrial trabecula were all treated with the physiological stressor isoproterenol (ISO), which is an unspecific beta-adrenoreceptor (β-AR) agonist. A concentration of 20 nM was used, as this marks a half-maximal saturation of the protein kinase A (PKA)-mediated phosphorylation (17). To investigate the effect of BNP on atrial trabeculae, the intervention consisted of treatment with 100 nM BNP, which was found to be the dose of maximal BNP effect in a study conducted by Guo et al. (18). Furthermore, the effect of either 8.5 μg/ml Sac or the combination of Sac with 4.0 μg/ml Val was investigated on atrial functional reserve or arrhythmogenesis. Those concentrations were found to be the peak plasma concentrations of Sac/Val following oral treatment with an ARNI (19, 20). Finally, after the addition of BNP, Sac, or Sac/Val, different stimulation frequencies were tested beginning at 1 Hz and increasing to 2 and 3 Hz, followed by 0.5 Hz in the end.

Western Blot VASP-Ser239/-Ser157 Phosphorylation

Vasodilator-stimulated phosphoprotein (VASP)-Ser239 and -Ser157 phosphorylation was investigated in human atrial muscle strips (control strips and strips treated with Sac/Val) using Western blot analysis (see **Supplementary Material** for detailed information).

Neprilysin Enzyme-Linked Immunosorbent Assay

NEP levels were determined in LV and RA biopsies from patients ($n = 10$) with end-stage HF. Tissue was analyzed by the enzyme-linked immunosorbent assay (ELISA) kit (EHMME, Thermo Scientific, USA) according to the manufacturer's instruction. The total protein concentration in supernatants was determined *via* Pierce BCA assay (Thermo Scientific). Samples were then diluted in the ratio of 1:5 and assessed in duplicate. The concentration (ng/ml) was normalized to total protein content (ng/mg of total protein). See **Supplementary Material** for detailed information.

Statistical Analysis

Statistical analysis and figure design were performed using GraphPad Prism version 8.0.1. Data points in the graphs represent single muscle strip experiments, and error bars are presented as the standard error of means (S.E.M) if not stated otherwise. Multiple comparisons of functional parameters (developed systolic force and diastolic tension) were analyzed with a two-way analysis of variance (ANOVA) with repeated measures (RMs) and Sidak *post-hoc* test (**Figures 1C,G**, **2C,F,G**; **Supplementary Figures 1A, 2**). Comparison of two groups was done by unpaired two-sided *t*-test (**Figures 1B,D,F**, **2B,E,H**, **4A**; **Supplementary Figures 1B, 3A,B**), and arrhythmias were quantified by the two-sided chi-square test and additional calculation of odds ratio by the Baptista-Pike method (**Figures 3A–H**). Correlations were analyzed by either Pearson *r* (**Figures 1H**, **4B,C**; **Supplementary Figure 4**) or Spearman *r* test (**Figure 4D**). For all analyses, a two-sided *p*-value of $p < 0.05$ was considered to be significant.

RESULTS

Patient Characteristics and Muscle Strip Experiments

Overall, 42 patients were included in our study between December 2018 and April 2020 for functional measurements in atrial muscle strips ($n = 101$). Most patients suffered from at least one cardiovascular risk factor (**Table 1A**). Treatment included angiotensin-converting-enzyme (ACE) inhibitors/AT1 blockers blockers and beta-blockers in most patients. The biomarker analysis showed normal blood count and electrolytes, elevated NTproBNP levels, and, on median, a slightly reduced glomerular filtration rate (GFR) (**Table 1A**). The echocardiographic evaluation demonstrated a slightly reduced LVEF with a median of 53% overall. LV hypertrophy and mild diastolic dysfunction were observed in most patients (**Table 1B**).

Acute Effect of BNP on Atrial Inotropy and Lusitropy

We tested the time-depended effect of BNP (100 nM) during adrenergic stimulation with 20 nM ISO over 70 min on diastolic tension and developed systolic force ($n = 20$ strips from $N = 9$ patients, **Figure 1A**). BNP did not significantly increase systolic developed force in response to prolonged ISO treatment (70 min, 1 Hz stimulation, **Figure 1B**). BNP significantly lowered diastolic tension in ISO-treated atrial muscle strips (overall $p = 0.005$, two-way ANOVA with RM, **Figure 1C**), as also reflected by a higher negative slope in end-diastolic tension over time (**Figure 1D**).

Furthermore, we investigated the effects of BNP on frequency-dependent modulation of the functional response to ISO (**Figure 1E**, $n = 40$ muscle strips from 17 patients). Developed systolic force in the presence of ISO decreased with higher frequency stimulation (2 and 3 Hz) and recovered with 0.5 Hz (**Figures 1E–G**). Treatment with BNP did not alter the frequency-dependent atrial systolic force response or contraction kinetics at any frequency (**Figures 1F,G**). At 3 and 0.5 Hz, BNP furthermore significantly reduced end-diastolic tension (ISO+BNP vs. ISO: 3 Hz <0.05; 0.5 Hz: $p < 0.01$, overall $p = 0.01$, two-way ANOVA with RM) (**Supplementary Figure 1A**). The slope of end-diastolic tension during the frequency stair was positive in ISO but slightly negative in ISO+BNP (**Supplementary Figure 1B**, $p = 0.09$).

We found that the mean inotropic reserve of atrial muscle strips in response to isoproterenol *in vitro* correlated well with preoperative plasma NTproBNP values of the same patient (available from $n = 19$ patients without preincubation, **Figure 1H**).

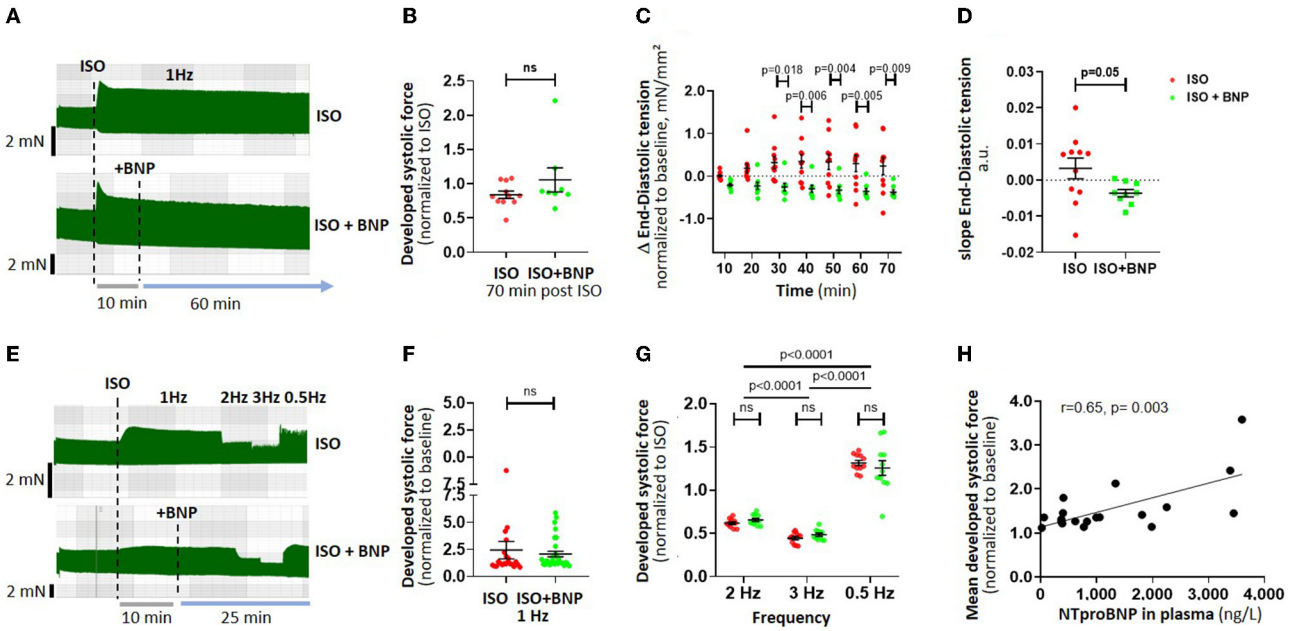

FIGURE 1 | Effect of BNP on atrial inotropy and lusitropy after adrenergic stimulation. Example traces of time-dependent analysis of BNP ($n = 20$ strips) are shown in **(A)**, with the corresponding data on the effect of BNP treatment on atrial systolic force **(B)**, and end-diastolic tension generation **(C)** after 70 min. The slope of end-diastolic tension over time is shown in **(D)**. The effects of a frequency-variation protocol [example traces in **(E)**, $n = 40$ strips] on systolic force at different stimulation frequencies are shown in **(F,G)**. Correlation of patient plasma NTproBNP and relative force increase after ISO treatment (values from $n = 19$ patients with available NTproBNP) is shown in **(H)**. Data are shown as mean ± SEM; each data point represents one muscle strip. In **(H)**, each point represents the mean developed systolic force per patient. ISO, isoproterenol; BNP, brain natriuretic peptide.

Effect of Sac/Val on Atrial Inotropy and Lusitropy

We evaluated the effects of the NEP inhibitor Sac on the atrial functional reserve. Preincubation (1 h) and the presence of Sac alone did not affect the developed force or diastolic tension after ISO at different stimulation frequencies (**Figures 2A–C**, $n = 8$ and 7 muscle strips, respectively, from 5 patients). Preincubation (1 h) and the presence of Sac in combination with Val in concentrations matching the peak plasma levels found in patients treated orally with an ARNI showed a slight trend toward an increased inotropy with ISO compared to control (**Figures 2D–F**) at 1 Hz, but otherwise had no significant effects on developed force or kinetics (time to peak, half time of relaxation, or relaxation constant tau; data not shown). Interestingly, despite the effects seen with BNP earlier, diastolic tension was unchanged with Sac/Val (**Figures 2G,H**; $n = 24$ muscle strips from 9 patients, overall $p = 0.3$, two-way ANOVA with RM).

Effect of BNP and Sac/Val on Atrial Arrhythmias During Cardiac Stress

During ISO treatment, some of the atrial muscle strips developed arrhythmias (aftercontractions, **Figure 3A**). In the protocol with prolonged ISO-treatment at 1 Hz, the addition of BNP tended to lower the incidence of arrhythmias (**Figure 3B**, $n = 20$ trabeculae from 9 patients). The antiarrhythmic effect of BNP was more pronounced in atrial muscle strips exposed to ISO and frequency stair ($n = 40$ trabeculae from 17 patients), with a pronounced reduction of arrhythmias in the recovery phase (0.5 Hz) following 3 Hz stimulation (**Figures 3C,D**). Overall the incidence of arrhythmias was reduced by 48% with BNP compared to the control group in this protocol [$p = 0.008$, two-sided chi-square test; odds ratio (OR) = 0.37; **Figure 3E**].

Sac/Val preincubation and presence tended to decrease overall arrhythmia burden during ISO and frequency stair ($p = 0.12$, two-sided chi-square test; OR = 0.48; **Figures 3F–H**) with a significant reduction of arrhythmias at higher stimulation frequencies (**Figure 3G**; $p = 0.04$, two-sided chi-square test; OR = 0.24, $n = 24$ trabeculae from 9 patients).

Sac treatment without Val had no impact on the prevalence of arrhythmias during adrenergic and frequency-dependent atrial stress (in $n = 15$ trabeculae from 5 patients, data not shown).

Effects of Sac/Val on VASP Phosphorylation

We probed signaling downstream of cGMP by measuring phosphorylation of VASP as a surrogate of PKG activity (21). pVASP-Ser239 is a known indicator for (A)NP-dependent increase in phosphorylation (22). In a subset of muscle strips ($n = 15$ from 5 patients) treated with either Sac/Val or vehicle (dimethyl sulfoxide, DMSO) and undergoing the frequency protocol, VASP phosphorylation at Ser239 and Ser157 was studied. We did not observe any difference in VASP

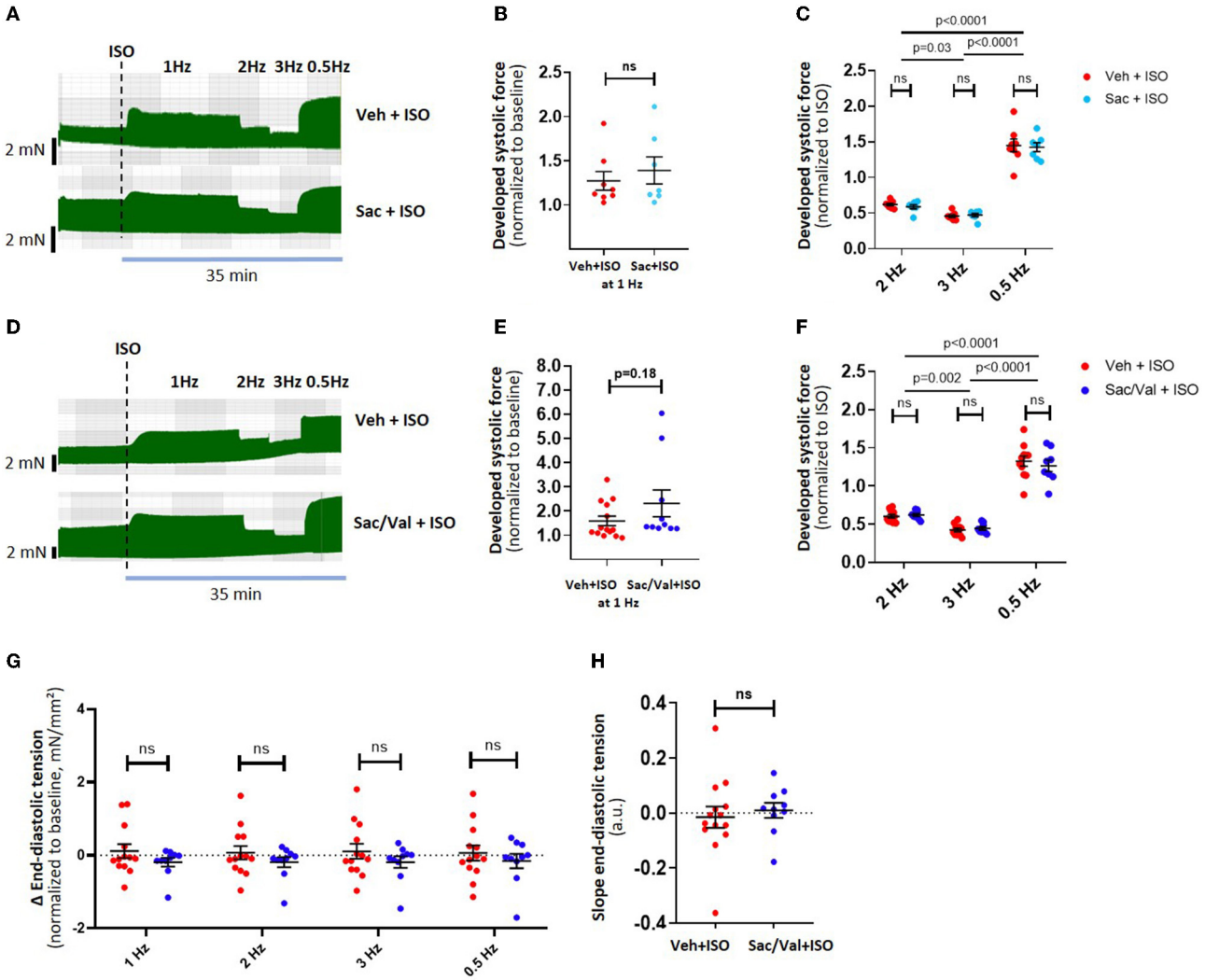

FIGURE 2 | Effect of Sac/Val on atrial inotropy and lusitropy after adrenergic stimulation. Example traces of a "frequency-variation protocol" in Sac-treated muscle strips ($n = 15$ strips) are shown in **(A)**, with its effects on developed force during different frequencies shown in **(B,C)**. Examples traces of a frequency-variation protocol in Sac/Val treated muscle strips ($n = 24$ strips) are shown in **(D)**, with the corresponding effects on developed force in **(E,F)**. The effects of Sac/Val on end-diastolic tension are shown as per stimulation frequency **(G)** and as the diastolic tension slope derived from linear regression **(H)**. Data are shown as mean ± SEM; each data point represents one muscle strip. ISO, isoproterenol, Sac: Sacubitrilat; Val: valsartan; Veh: vehicle (DMSO).

phosphorylation between Sac/Val-treated atrial muscle strips and control group (**Supplementary Figure 3**).

Neprilysin Expression in Human End-Stage HF

NEP protein expression was measured in RA biopsies from patients with end-stage HF ($n = 10$, median LVEF 20%) that either underwent heart transplantation (70%) or left ventricular assistant device implantation (30%). We also measured NEP in the LV of these patients. The detailed patient characteristics can be found in **Supplementary Table 1**. Plasma NTproBNP was elevated to >6,500 pg/ml on average as a marker of severe end-stage HF. NEP was equally expressed in RA and LV tissue (**Figure 4A**), with a significant positive correlation of RA and LV NEP expression within the same patient (**Figure 4B**), and RA NEP expression and RA EF (**Supplementary Figure 4**). Interestingly, also a strong negative correlation was found between LA volume and LV NEP (**Figure 4C**), and between patients' NYHA class and RA NEP ($r = -0.81$; $p < 0.01$, Spearman r test; **Figure 4D**).

DISCUSSION

This study investigated the effects of BNP and Sac/Val on the functional reserve of human atrial myocardium. To the best of our knowledge, this is the first study to show that (i) BNP ameliorates increased diastolic tension during adrenergic stress and alleviates stress-induced atrial arrhythmogeneity *in vitro*;

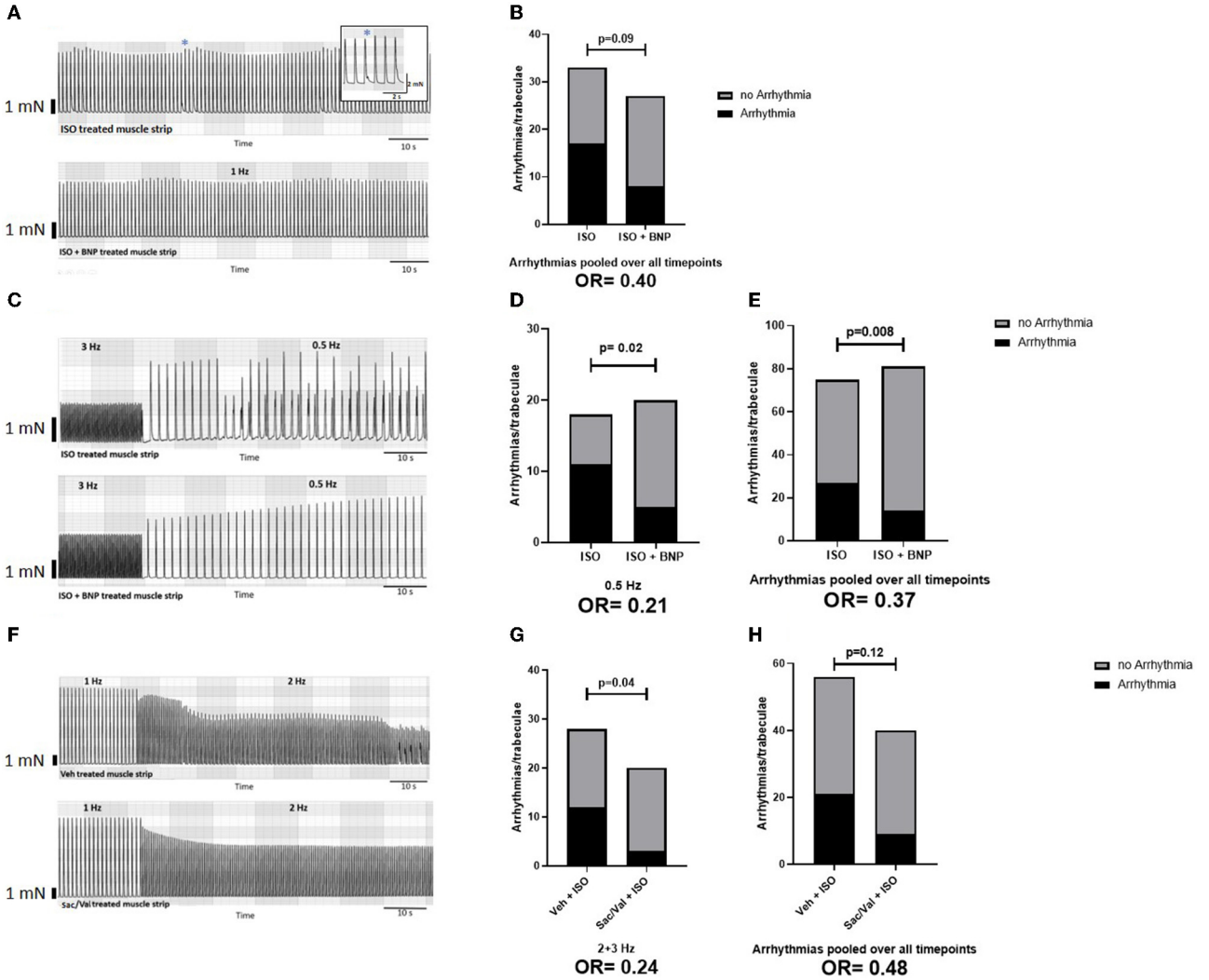

FIGURE 3 | Effect of BNP and Sac/Val on atrial arrhythmogenesis. **(A)** Representative traces of a protocol with constant stimulation at 1 Hz in strips treated with ISO ± BNP; the overall effect of BNP treatment on arrhythmogenesis in this protocol is shown in **(B)**. For this analysis, three time intervals (25–30, 45–50, and 65–70 min) after ISO have been screened for arrhythmias. **(C)** Example traces for muscle strips treated with ISO ± BNP during the switch from 3 Hz to 0.5 Hz stimulation frequency in a "frequency-variation protocol" protocol. The effects of BNP on arrhythmogenesis at 0.5 Hz **(D)** and pooled over all time points **(E)** are shown. For this analysis, the arrhythmias at 1 Hz (10 min) and 2, 3, and 0.5 Hz (each 5 min) have been included Finally, example traces of muscle strips treated with a vehicle (DMSO) or Sac/Val at the transition between 1 and 2 Hz stimulation frequency are shown in **(F)**, with the effects of Sac/Val treatment on arrhythmias at higher stimulation frequencies in **(G)** and the overall effect on arrhythmia in **(H)**. For this analysis, the arrhythmias at 1 Hz (10 min) and 2, 3, and 0.5 Hz (each 5 min) have been included. ISO, isoproterenol; BNP, brain natriuretic peptide; Veh, vehicle (DSMO); Sac, sacubitrilat; Val, valsartan.

(ii) NEP is equally present in human atrial and ventricular myocardium, but its expression is reduced with a progression of cardiac dysfunction; and (iii) the combination of Sac and Val does not influence the adrenergic functional reserve in isolated atrial muscle but reduces arrhythmias in response to adrenergic stimulation.

NEP Expression and Regulation

Even though ARNIs have been the subject of intense research during the past decade, only little attention has been paid to the expression of NEP in the failing human heart. Fielitz et al. (10) were the first to show that NEP expression and activity were altered in LV myocardium in HF. They reported an increase in NEP expression and activity in LV samples from patients with HF, which they suggested to contribute to increased local degradation of bradykinin and NPs (10). In contrast to that, Pavo et al. found a reduction in LV NEP expression, concentration, and activity in a porcine model of ischemic cardiomyopathy. They suggested that NEP downregulation might represent a counterregulatory mechanism to HF (11). In this study, we show NEP expression in human atrial myocardium in similar quantities as in human failing LV. The inverse correlation of atrial dilatation with LV NEP protein expression suggests that chronically increased cardiac pressures as reflected by atrial

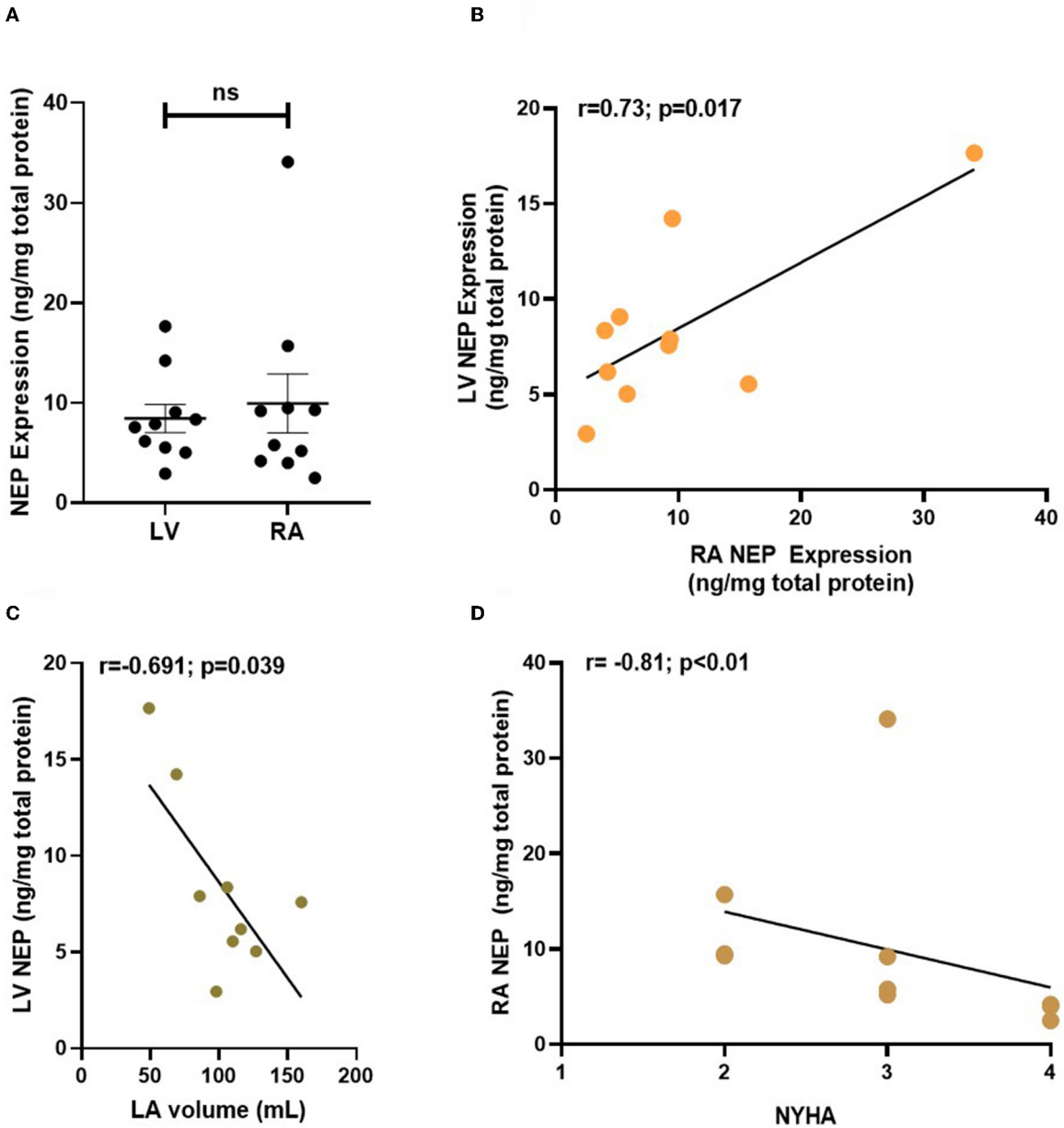

FIGURE 4 | NEP expression (in ng/mg of total protein) in human atrial and ventricular samples from patients with end-stage heart failure. NEP expression was analyzed in RA and LV biopsies from $n = 10$ patients with end-stage heart failure. NEP is equally expressed in the failing atrial and ventricular myocardium **(A)** and expression of atrial and ventricular NEP correlates in the patient cohort **(B)**. LV NEP expression correlates inversely with LA volume **(C)** and RA NEP negatively correlates with patient NYHA **(D)**. Each point represents data from one patient. NEP, Neprilysin; LV, left ventricle; LA, left atrium; RA, right atrium.

dilatation may be a trigger for NEP downregulation in the human heart. Indeed, NEP inhibition with candoxatril has been shown to reduce ventricular filling pressures (cardiac preload) (23, 24). Thus, downregulation of NEP may contribute to a compensatory reduction of preload in situations of increased wall strain in HF. However, candoxatril, in contrast, also increased systolic blood pressure, which is presumably due to increased angiotensin II and endothelin levels (23, 25). These effects diminish the positive effects of isolated NEP downregulation or inhibition (26, 27). The elevated levels of endothelin 1 or angiotensin II (both of which are synthesized within the myocardium, among others) also could explain why isolated NEP inhibition by Sac alone did not have any effects on functional parameters or arrhythmia in our *in vitro* study. The previously described mechanism of NEP downregulation in HF, therefore, may be, just like other compensatory mechanisms in HF [e.g., myocardial hypertrophy

TABLE 1 | Patient characteristics.

(A)			
Patient characteristics		**Medication**	
Age	71 (66–77)	ACE Inhibitors/ AT1–RB	76 % 32/42
Sex (male)	86 % 36/42	Beta–Blocker	64 % 27/42
BMI	27 (24–30)	Diuretics	36 % 15/42
Arterial hypertension	76 % 32/42	MRI	14 % 6/42
Dyslipidemia	45% 19/42	ARNI	2 % 1/42
Adipositas	26 % 11/42	Statine	64 % 27/42
Diabetes mellitus	33 % 14/42	Oral antidiabetic	26 % 11/42
Coronary artery disease	88 % 37/42	Insulin	5 % 2/42
Atrial Fibrillation	26 % 11/42		
COPD	14 % 6/42	**Type of surgery**	
Heart failure	29 % 12/42	Coronary Artery Bypass Graft	83 % 35/42
Chronic kidney disease	17 % 7/42	Aortic Valve Replacement or reconstruction	24 % 10/42
Smoker	33 % 14/42	Mitral Valve Replacement or reconstruction	5 % 2/42
Alcoholism	7 % 3/42	Aortic–Root–Replacement	12 % 5/42
Laboratory markers			
Hb (g/dl)	14 (13–15)	N = 42	
Na+ (mmol/l)	140 (138–143)	N = 42	
K+ (mmol/l)	4.2 (4.0–4.5)	N = 42	
NT–proBNP (ng/l)	769 (396–1984)	N = 23	
GFR (ml/min)	76 (61–90)	N = 42	
Creatinin (mg/dl)	0.95 (0.79–1.17)	N = 42	
CRP (mg/l)	2.75 (0.9–11.23)	N = 42	
HbA1c (mmol/mol)	41 (38–46)	N = 19	
(B)			
Echo data			
LVEF (%)	53 (45–57)	N = 42	
LVEDD (mm)	46 (44–52)	N = 41	
IVSd (mm)	13 (12–14)	N = 41	
PWd (mm)	12 (11–12)	N = 41	
LA volume biplan (ml)	61 (51–81)	N = 36	
LAVI (ml/m2)	31 (27–38)	N = 35	
LA diameter (mm)	38 (35–40)	N = 39	
LA emptying fraction (norm >37%)	50 (35–55)	N = 35	
LA strain (>23% norm)	19 (14–29)	N = 20	
RA area (cm2)	16 (14–20)	N = 36	
RA diameter (mm)	35 (28–38)	N = 38	
RA emptying fraction (norm >37%)	52 (33–59)	N = 32	
RA strain reservoir+conduit (ϵe)	42 (31–49)	N = 14	
E/É	11 (10–13)	N = 17	
TAPSE (mm)	19 (17–23)	N = 39	
RVEDD (mm)	35 (31–39)	N = 37	
sPAP (mmHg)	25 (24–31)	N = 21	

*Clinical characteristics of patient cohort included for in vitro experiments **(A)** and echo characterization of patient cohort **(B)**. Data are presented as "median (IQR)" or "percent (n patients/all patients)." MRI, mineralocorticoid receptor inhibitor; ARNI, angiotensin receptor-neprilysin inhibitor; LV, left ventricle; LA, left atrium; RA, right atrium; AT1-RB, Angiotensin II receptor blocker.*

(28) and renin-angiotensin-aldosterone system (RAAS) activation (29)], considered to be at least partly maladaptive. Not until NEP inhibition is combined with angiotensin II receptor blockage, the full potential of this mechanism can be therapeutically exploited.

BNP and Sac/Val Effect on Developed Systolic Force and Diastolic Tension

The ARNI treatment is accompanied by a significant increase in circulating BNP, as its degradation is partly inhibited (30). Gu et al. (19) could show that ANP levels are already increased as

short as 15 min after oral administration of an ARNI in a rat model. In combination with other studies showing that ARNI treatment increased the EF in patients with HFrEF (31), this raised the question if there are also short-term effects of NPs and NEP inhibition on the systolic or diastolic functional reserve in human atrial myocardium.

Perera et al. demonstrated that ANP increases contractility in mice ventricular cardiomyocytes in a phase of early cardiac hypertrophy already seconds after wash-in, but only if the cells were pretreated with ISO. This positive inotropic effect was explained by an augmentation of cAMP signaling in the hypertrophied myocardium exerted by a spatial redistribution of cGMP sensitive PDE2 and PDE3 (8). In this study, we did not observe an immediate positive inotropic effect of BNP in human atrial myocardium. The first reason for the lack of immediate positive inotropic effects may be that our patient cohort was rather suffering from chronic than acute conditions (CAD in 91%, aHT in 81%, NTproBNP 769 ng/L); LV hypertrophy was found to be in some extent (IVSd median diameter 13 mm), but RA diameter (median 34 mm) did not indicate atrial enlargement. These findings are also in line with other publications studying coronary artery bypass graft (CABG) patient characteristics and echocardiography (32, 33). As chronic HF is associated with a reduction in NP-A receptor sensitivity, immediate natriuretic signaling may have been attenuated in the patient group presented in this study (34, 35). Furthermore, the positive-inotropic spatial redistribution of PDE2 and PDE3 between β-ARs was only described in the early stages of hypertrophy by Perera et al. (8). A loss of this early compensatory mechanism during progression/duration of cardiac hypertrophy due to NP and β-AR desensitization and/or phosphodiesterases (PDE) reorganization is very likely and can also explain the observed loss of positive inotropic effects of NPs in chronic stages of cardiac disease (as seen in our patient cohort) (8). Finally, differences between atrial and ventricular adaptive mechanisms may also contribute to the observations made: it is unclear to the extent in which the mechanism of PDE reorganization can also be found in atrial tissue, but our results suggest that, at least in chronic LV hypertrophy, atrial tissue does not exhibit cellular adaptive processes associated with increased inotropy after NP incubation. Thus, patient and sample characteristics could explain why these effects were not seen in the present cohort. It is imaginable that BNP does not show these effects at an equivalent dose to ANP. However, as both NPs were administered in a concentration of 100 nM, also in accordance with previous experimental findings (18), and as ANP and BNP bind to the same receptors (NPR-As), this was not further explored in this study.

Interestingly, with the addition of BNP, we did observe a time- and frequency-dependent reduction of diastolic tension during adrenergic stress (ISO), thus establishing BNP's effectiveness in improving lusitropy in isolated atrial myocardium after ISO treatment.

Sac/Val inhibits the degradation of NPs in the myocardium and, therefore, increases the tissue concentration of ANP and BNP. In isolated atrial tissue as used in our study, however, Sac/Val did not reproduce the effect of supramaximal BNP treatment. We, therefore, conclude that the BNP levels intrinsically recruitable (by stretch and adrenergic stimulation) are not sufficient to see an acute effect of Sac/Val treatment on diastolic atrial tension.

Interestingly, we observed a strong correlation between plasma NTproBNP levels and the adrenergic atrial functional reserve *in vitro*, which suggests that long-term effects of (B)NP on adrenergic signaling may play a role in modulating the atrial myocardial functional reserve. Especially, the interplay of cAMP- and cGMP regulated by PDEs, such as PDE3, may serve as an explanation for this effect, as PDE3 degrades up to 50% of cellular cAMP, but can be inhibited by cGMP (cGMP inhibited cAMP PDE) (36). Constantly elevated tissue BNP and cGMP levels would, therefore, inhibit the degradation of cAMP and strengthen the intracellular pathway mediated by ISO, ultimately leading to a rise in relative force increase as a long-term effect.

BNP and LBQ/Val Effect on Atrial Arrhythmias

The PARADIGM-HF study did not only show a reduced risk of HF-related hospitalization and death in ARNI treatment but also a significant reduction in mortality by sudden cardiac death (SCD) (12, 37). For that reason, only recently the focus of ANRI research also shifted to study the effects of ARNIs on cardiac (ventricular) arrhythmias. de Diego et al. (31) published one of the first clinical studies assessing the influence of ARNI treatment on arrhythmia. While episodes of premature ventricular contractions (PVCs), non-sustained ventricular tachycardia (NSVT), and sustained ventricular tachycardia were significantly reduced in ARNI treatment, there was only a statistical trend in the reduction of atrial fibrillation episodes reported ($p = 0.07$; ns) (31). These findings were verified in another study with a 12-month follow-up (15).

Despite that, observational clinical data also suggested that ARNI treatment may lower the risk for the development of (recurring) atrial fibrillation and lower the disease burden and frequency of arrhythmic events in patients with non-permanent atrial fibrillation (38–41).

Interestingly, in our study, we could demonstrate that while Sac/Val did not have a significant influence on diastolic tension and, therefore, wall strain, it still reduced the probability of arrhythmias significantly in ISO-treated muscle strips. This suggests that the reduction of atrial arrhythmias is not only related to a reduction in strain (diastolic tension). In ventricular tissue, it has been shown that ANP (10 nM) significantly suppresses ISO-induced Ca^{2+} spark frequency (CaSF) and reactive oxygen species (ROS) production on a cellular level (42). Eiringhaus et al. demonstrated that in ventricular myocardium, Sac/Val decreases diastolic SR Ca^{2+} leak and CaSF. These effects were not visible under basal conditions but only after ISO treatment (43). Interestingly, in atrial myocardium, we could reproduce the antiarrhythmic effect at a nearly 50% lower Sac/Val dosage than Eiringhaus et al. (40 μmol). The dosage we used matched the peak plasma concentration

achieved in humans in Sac/Val treatment (≈22.17 μmol Sac) (19, 20). That indicates that the antiarrhythmic properties of Sac/Val are exerted at a dose attained in standard HF treatment. As Sac and Sac/Val did not affect developed force in atrial muscle but external BNP reproduced the reduction in atrial arrhythmia incidence, we propose that the increased (juxtacellular) concentration of NPs by NEP-inhibitor Sac/Val contributes to the antiarrhythmic effects in human atrial muscle strips. The exact downstream mechanisms of NP and Sac/Val's antiarrhythmic properties are yet to be studied. All in all, an antiarrhythmic effect of Sac/Val based on effects of cellular Ca^{2+} handling seems to be likely. We have probed VASP phosphorylation in trabeculae treated with Sac/Val, as an indicator of increased PKG activity. However, VASP phosphorylation is highly time-dependent and rapidly decreases over time, no longer being significant only 1 h after treatment with ANP (22).

In light of the potential antiarrhythmic properties of Sac/Val on atrial tissue, ARNI treatment should be evaluated in high-risk patients with simultaneous HF and atrial fibrillation or atrial remodeling in future studies. Especially, in this patient group with high morbidity and mortality, it is likely that early ARNI treatment could exert major beneficial effects beyond the classically known ARNI effects by suppressing the induction of atrial arrhythmias and, therefore, maintaining atrial function.

CONCLUSION

In this study, we could demonstrate that both BNP and Sac/Val exert beneficial effects on human atrial myocardium and that NEP expression in progressing HF with reduced EF is downregulated as part of an adaptive mechanism, and our data suggest that the favorable effects of Sac/Val are partly due to the increased concentration of NPs in the myocardium and that these effects are already achieved in therapeutic clinical doses.

Limitations

For this study, we were working with human myocardial samples obtained from routine heart surgeries. As these human samples are not available on a large scale, we had to work with rather small sample sizes. Furthermore, all patients come with a unique combination of comorbidities and genetic variations. Therefore, we had to deal with a heterogeneous group in terms of underlying conditions, which, on the positive side, realistically reflects the clinical variation of patients. For the experiments with Sac and Val, we had to use DMSO as a solvent. More recently, DMSO was identified to influence human cellular processes and to exert toxicity already on low doses (44, 45). To account for these factors, we added DMSO to our control groups.

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 Local Ethics Committee of the Charité (EA2/167/15) and the Ethics Vote for the Biobank at the DHZB (EA4/028/12). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FRH and UP conceived the study design and obtained patients informed consent. FRH obtained Ethics Committee approval and funding. UP collected the patients' clinical data and performed echocardiography analysis. FH, FB, CK, VF, and HG collected right atrial appendages from all patients. PD acquired and analyzed right atrial trabeculae function and assisted in molecular biology. KT contributed to acquisition and analysis of tissue preparation and right atrial trabeculae function. CK furthermore provided patient characteristics for molecular biology studies on NEP expression. DL provided methodological support and contributed to interpretation of the results. PW performed Western Blot and ELISA and interpretation of these results. UP and PD equally contributed to the analysis. FRH, UP, and PD drafted the manuscript and interpretation of all data. BP and FH provided valuable feedback to the manuscript draft. All authors contributed to the manuscript and approved the submitted version.

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REFERENCES

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. (2016) 37:2129–2200m. doi: 10.1093/eurheartj/ehw128
2. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* (2012) 380:2197–223. doi: 10.1016/S0140-6736(12)61689-4
3. Norman HS, Oujiri J, Larue SJ, Chapman CB, Margulies KB, Sweitzer NK. Decreased cardiac functional reserve in heart failure with preserved systolic function. *J Card Fail.* (2011) 17:301–8. doi: 10.1016/j.cardfail.2010.11.004
4. Rahimtoola SH, Ehsani A, Sinno MZ, Loeb HS, Rosen KM, Gunnar RM. Left atrial transport function in myocardial infarction. *Am J Med.* (1975) 59:686–94. doi: 10.1016/0002-9343(75)90229-6
5. Hoit BD. Left atrial size and function: role in prognosis. *J Am Coll Cardiol.* (2014) 63:493–505. doi: 10.1016/j.jacc.2013.10.055
6. Habibi M, Chahal H, Opdahl A, Gjesdal O, Helle-Valle TM, Heckbert SR, et al. Association of CMR-measured LA function with heart failure development. *JACC Cardiovasc Imaging.* (2014) 7:570–9. doi: 10.1016/j.jcmg.2014.01.016
7. Hohendanner F, Messroghli D, Bode D, Blaschke F, Parwani A, Boldt LH, et al. Atrial remodelling in heart failure: recent developments and relevance for heart failure with preserved ejection fraction. *ESC Hear Fail.* (2018) 5:211–21. doi: 10.1002/ehf2.12260
8. Perera RK, Sprenger JU, Steinbrecher JH, Hübscher D, Lehnart SE, Abesser M, et al. Microdomain switch of cGMP-regulated phosphodiesterases leads to ANP-induced augmentation of β-adrenoceptor-stimulated contractility in early cardiac hypertrophy. *Circ Res.* (2015) 116:1304–11. doi: 10.1161/CIRCRESAHA.116.306082
9. Turner AJ, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases : genomics and function. *BioEssays.* (2001) 23:261–9. doi: 10.1002/1521-1878(200103)23:3<261::AID-BIES1036>3.0.CO;2-K
10. Fielitz J, Dendorfer A, Pregla R, Ehler E, Zurbrügg HR, Bartunek J, et al. Neutral endopeptidase is activated in cardiomyocytes in human aortic valve stenosis and heart failure. *Circulation.* (2002) 105:286–9. doi: 10.1161/hc0302.103593
11. Pavo IJ, Pavo N, Kastner N, Traxler D, Lukovic D, Zlabinger K, et al. Heart failure with reduced ejection fraction is characterized by systemic NEP downregulation. *JACC Basic to Transl Sci.* (2020) 5:715–26. doi: 10.1016/j.jacbts.2020.05.011
12. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med.* (2014) 371:993–1004. doi: 10.1056/NEJMoa1409077
13. Castiello A, Sciatti E, Gavazzoni M, Vizzardi E, Metra M. The potential antiarrhythmic properties of sacubitril/valsartan: a case report. *J Cardiovasc Med.* (2019) 20:780–2. doi: 10.2459/JCM.0000000000000808
14. Huo JY, Jiang WY, Chen C, Chen R, Ge TT, Chang Q, et al. Effects of angiotensin receptor neprilysin inhibitors on inducibility of ventricular arrhythmias in rats with ischemic cardiomyopathy. *Int Heart J.* (2019) 60:1168–75. doi: 10.1536/ihj.19-065
15. Martens P, Nuyens D, Rivero-Ayerza M, Van Herendael H, Vercammen J, Ceyssens W, et al. Sacubitril/valsartan reduces ventricular arrhythmias in parallel with left ventricular reverse remodeling in heart failure with reduced ejection fraction. *Clin Res Cardiol.* (2019) 108:1074–82. doi: 10.1007/s00392-019-01440-y
16. Wen Y, Deißler PM, Primeßnig U, Dushe S, Falk V, Parwani AS, et al. Impaired relaxation and reduced lusitropic reserve in atrial myocardium in the obese patients. *Front Cardiovasc Med*. (2021) 8:1348. doi: 10.3389/fcvm.2021.739907
17. Negroni JA, Morotti S, Lascano EC, Gomes AV, Grandi E, Puglisi JL, et al. B-adrenergic effects on cardiac myofilaments and contraction in an integrated rabbit ventricular myocyte model. *J Mol Cell Cardiol.* (2015) 81:162–75. doi: 10.1016/j.yjmcc.2015.02.014
18. Guo S, Goetze JP, Jeppesen JL, Burnett JC, Olesen J, Jansen-Olesen I, et al. Effect of natriuretic peptides on cerebral artery blood flow in healthy volunteers. *Peptides.* (2015) 74:33–42. doi: 10.1016/j.peptides.2015.09.008
19. Gu J, Noe A, Chandra P, Al-Fayoumi S, Ligueros-Saylan M, Sarangapani R, et al. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting angiotensin receptor-neprilysin inhibitor (ARNi). *J Clin Pharmacol.* (2010) 50:401–14. doi: 10.1177/0091270009343932
20. Akahori M, Ayalasomayajula S, Langenickel T, Pal P, Zhou W, Sunkara G. Pharmacokinetics after single ascending dose, food effect, and safety of sacubitril/valsartan (LCZ696), an angiotensin receptor and neprilysin inhibitor, in healthy Japanese subjects. *Eur J Drug Metab Pharmacokinet.* (2017) 42:407–16. doi: 10.1007/s13318-016-0354-1
21. Borgognone A, Lowe KL, Watson SP, Madhani M. Natriuretic peptides induce weak VASP phosphorylation at Serine 239 in platelets. *Platelets.* (2014) 25:1–7. doi: 10.3109/09537104.2013.773969
22. Sartoretto JL, Jin BY, Bauer M, Gertler FB, Liao R, Michel T. Regulation of VASP phosphorylation in cardiac myocytes: differential regulation by cyclic nucleotides and modulation of protein expression in diabetic and hypertrophic heart. *Am J Physiol Heart Circ Physiol.* (2009) 297:H1697–710. doi: 10.1152/ajpheart.00595.2009
23. Ando SI, Rahman MA, Butler GC, Senn BL, Floras JS. Comparison of candoxatril and atrial natriuretic factor in healthy men: effects on hemodynamics, sympathetic activity, heart rate variability, and endothelin. *Hypertension*. (1995) 26:1160–6. doi: 10.1161/01.hyp.26.6.1160
24. Elsner D, Müntze A, Kromer EP, Riegger GA. Effectiveness of endopeptidase inhibition (candoxatril) in congestive heart failure. *Am J Cardiol.* (1992) 70:494–8. doi: 10.1016/0002-9149(92)91196-b
25. Richards AM, Wittert GA, Espiner EA, Yandle TG, Ikram H, Frampton C. Effect of inhibition of endopeptidase 24. 11 on responses to angiotensin II in human volunteers. *Circ Res.* (1992) 71:1501–7. doi: 10.1161/01.RES.71.6.1501
26. von Lewinski D, Kockskämper J, Rübertus SU, Zhu D, Schmitto JD, Schöndube FA, et al. Direct pro-arrhythmogenic effects of angiotensin II can be suppressed by AT1 receptor blockade in human atrial myocardium. *Eur J Heart Fail.* (2008) 10:1172–6. doi: 10.1016/j.ejheart.2008.09.014
27. Castro-Chaves P, Fontes-Carvalho R, Pintalhao M, Pimentel-Nunes P, Leite-Moreira AF. Angiotensin II-induced increase in myocardial distensibility and its modulation by the endocardial endothelium in the rabbit heart. *Exp Physiol.* (2009) 94:665–74. doi: 10.1113/expphysiol.2008.046458
28. Ritter O, Neyses L. The molecular basis of myocardial hypertrophy and heart failure. *Trends Mol Med.* (2003) 9:313–21. doi: 10.1016/S1471-4914(03)00114-X
29. Hartupee J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. *Nat Rev Cardiol.* (2018) 14:30–8. doi: 10.1038/nrcardio.2016.163.Neurohormonal
30. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation.* (2015) 131:54–61. doi: 10.1161/CIRCULATIONAHA.114.013748
31. de Diego C, Gonzalez-Torres L, Núñez JM, Inda RC, Martin-Langerwerf DA, Sangio AD, et al. Effects of angiotensin-neprilysin inhibition compared to angiotensin inhibition on ventricular arrhythmias in reduced ejection fraction patients under continuous remote monitoring of implantable defibrillator devices. *Hear Rhythm.* (2018) 15:395–402. doi: 10.1016/j.hrthm.2017.11.012
32. Gibson PH, Croal BL, Cuthbertson BH, Rae D, McNeilly JD, Gibson G, et al. Use of preoperative natriuretic peptides and echocardiographic parameters in predicting new-onset atrial fibrillation after coronary artery bypass grafting: a prospective comparative study. *Am Heart J.* (2009) 158:244–51. doi: 10.1016/j.ahj.2009.04.026
33. Koene RJ, Kealhofer J V, Adabag S, Vakil K, Florea VG. Effect of coronary artery bypass graft surgery on left ventricular systolic function. *J Thorac Dis.* (2017) 9:262–70. doi: 10.21037/jtd.2017.02.09
34. Forfia PR, Lee M, Tunin RS, Mahmud M, Champion HC, Kass DA. Acute phosphodiesterase 5 inhibition mimics hemodynamic effects of B-type natriuretic peptide and potentiates B-type natriuretic peptide effects in failing but not normal canine heart. *J Am Coll Cardiol.* (2007) 49:1079–88. doi: 10.1016/j.jacc.2006.08.066
35. Blanton RM. CGMP signaling and modulation in heart failure. *J Cardiovasc Pharmacol.* (2020) 75:385. doi: 10.1097/FJC.0000000000000749
36. Zaccolo M, Movsesian MA. cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology. *Circ Res.* (2007) 100:1569–78. doi: 10.1161/CIRCRESAHA.106.144501

37. Rohde LE, Chatterjee NA, Vaduganathan M, Claggett B, Packer M, Desai AS, et al. Sacubitril/Valsartan and sudden cardiac death according to implantable cardioverter-defibrillator use and heart failure cause: a PARADIGM-HF analysis. *JACC Hear Fail.* (2020) 8:844–55. doi: 10.1016/j.jchf.2020.06.015
38. De Vecchis R, Paccone A, Di Maio M. Favorable effects of sacubitril/valsartan on the peak atrial longitudinal strain in patients with chronic heart failure and a history of one or more episodes of atrial fibrillation: a retrospective cohort study. *J Clin Med Res.* (2020) 12:100–7. doi: 10.14740/jocmr4076
39. Guerra F, Pimpini L, Flori M, Contadini D, Stronati G, Gioacchini F, et al. Sacubitril/valsartan reduces atrial fibrillation and supraventricular arrhythmias in patients with HFrEF and remote monitoring: preliminary data from the SAVE THE RHYTHM. *Eur Heart J.* (2020) 41(Supplement_2): ehaa946.0926. doi: 10.1093/ehjci/ehaa946.0926
40. Lou Q, Liu GZ, Lv JC, Yun FX Li TK, Yang W, et al. Sacubitril/valsartan attenuates atrial electrical and structural remodelling in a rabbit model of atrial fibrillation. *Eur J Pharmacol.* (2020) 881:173120. doi: 10.1016/j.ejphar.2020.173120
41. Russo V, Bottino R, Rago A, Papa AA, Liccardo B, Proietti R, et al. The effect of sacubitril/valsartan on device detected arrhythmias and electrical parameters among dilated cardiomyopathy patients with reduced ejection fraction and implantable cardioverter defibrillator. *J Clin Med*. (2020) 9:1111. doi: 10.3390/jcm9041111
42. Murakami W, Kobayashi S, Susa T, Nanno T, Ishiguchi H, Myoren T, et al. Recombinant atrial natriuretic peptide prevents aberrant Ca^{2+} leakage through the ryanodine receptor by suppressing mitochondrial reactive oxygen species production induced by isoproterenol in failing cardiomyocytes. *PLoS ONE.* (2016) 11:1–16. doi: 10.1371/journal.pone.0163250
43. Eiringhaus J, Wünsche CM, Tirilomis P, Herting J, Bork N, Nikolaev VO, et al. Sacubitrilat reduces pro-arrhythmogenic sarcoplasmic reticulum Ca2+ leak in human ventricular cardiomyocytes of patients with end-stage heart failure. *ESC Hear Fail.* (2020) 7:2992–3002. doi: 10.1002/ehf2.12918
44. Galvao J, Davis B, Tilley M, Normando E, Duchen MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. *FASEB J.* (2014) 28:1317–30. doi: 10.1096/fj.13-235440
45. Verheijen M, Lienhard M, Schrooders Y, Clayton O, Nudischer R, Boerno S, et al. DMSO induces drastic changes in human cellular processes and epigenetic landscape *in vitro*. *Sci Rep.* (2019) 9:1–12. doi: 10.1038/s41598-019-40660-0

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Use of covered stents to treat complex cerebrovascular diseases: Expert consensus

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The treatment of complex cerebrovascular diseases (CCVDs) at the skull base, such as complex intracranial aneurysms, carotid-cavernous sinus fistulas, and intracranial artery traumatic injuries, is a difficult clinical problem despite advances in endovascular and surgical therapies. Covered stents or stent graft insertion is a new concept for endovascular treatment that focuses on arterial wall defect reconstruction, differing from endovascular lesion embolization or flow diverter therapies. In recent years, covered stents specifically designed for cerebrovascular treatment have been applied in the clinical setting, allowing thousands of patients with CCVDs to undergo intraluminal reconstruction treatment and achieving positive results, even in the era of flow diverters. Since there is no unified reference standard for the application of covered stents for treating CCVDs, it is necessary to further standardize and guide the clinical application of this technique. Thus, we organized authoritative experts in the field of neurointervention in China to write an expert consensus, which aims to summarize the results of covered stent insertion in the treatment of CCVDs and propose suitable standards for its application in the clinical setting. Based on the contents of this consensus, clinicians can use individualized intraluminal reconstruction treatment techniques for patients with CCVDs.

KEYWORDS

cerebrovascular disease, covered stent, intraluminal reconstruction treatment, expert consensus, endovascular treatment

Introduction

Endovascular therapy has been widely used in the treatment of various hemorrhagic cerebrovascular diseases. However, one class of complex cerebral artery lesions, represented by complex cerebral aneurysms, traumatic carotid cavernous sinus fistulas (CCFs), arterial injuries, dissections, and blister-like aneurysms, indicates a challenge both to endovascular treatment and surgery, and satisfactory treatment goals are not always met. As most complex cerebrovascular diseases (CCVDs) are a kind of parent artery wall defect disease, reconstruction treatment using stent graft repair and flow diversion treatment has overcome the deficiencies of traditional coil embolization to achieve positive outcomes in recent years (1–4).

Covered stents have been approved and widely applied in China to treat CCVDs at the cervical region or skull base (1, 2, 5–7) (Figure 1). Currently, there are no unified criteria for the application of covered stents for the treatment of CCVDs. Furthermore, unlike more conventional devices, they have some features rendering their use more challenging: difficult delivery, vessel wall injury can occur when deployed in curved vessels, poor membrane adhesion, and high thrombogenicity. The selection of cases and devices, the procedure itself, and the management of complications require standardization to be fully applicable to CCVDs. Consequently, we required authoritative experts with years of experience to review the literature, repeatedly discuss their findings, and form an expert consensus to summarize the current application of covered stents in the treatment of CCVDs and establish a suitable clinical reference standard for their application in clinical settings. Clinical doctors might use the information presented here to tailor individualized endovascular reconstruction therapy regimens for patients with CCVDs according to their specific situation.

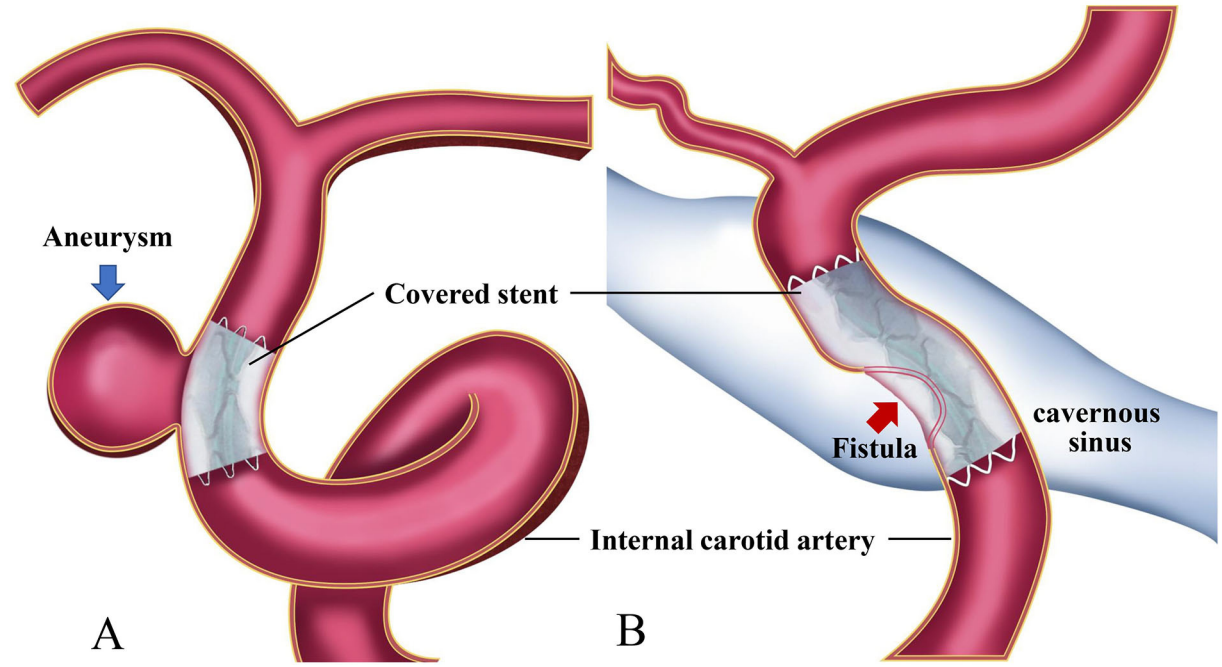

FIGURE 1
Diagrams of covered stent insertion in the treatment of a cerebral aneurysm **(A)** and a carotid cavernous fistula **(B)**.

Considerations for intraluminal reconstruction therapy

Lesion selection

1) Cerebral aneurysms include complex saccular aneurysms, recurrent aneurysms, pseudoaneurysms, dissecting aneurysms, blood blister-like aneurysms, multiple aneurysms at the same site, and so on (8–14).
2) Direct carotid-cavernous fistulas (15–17).
3) Internal carotid artery injuries, including traumatic injuries, iatrogenic injuries, tumor erosions, and so on (18, 19) (Table 1).

No important branches or perforators must be present in the covered artery segment, including the fetal-type posterior communicating artery, anterior choroidal artery, posterior inferior cerebellar artery, anterior spinal artery, and primitive trigeminal artery. For the ophthalmic segment of the internal carotid artery, a balloon occlusion test can be performed to assess the risk of blindness before considering the use of covered stents.

Parent artery selection

Evaluation of the parent artery before receiving covered stent insertion is more important than that of the disease itself. Generally, the parent artery must be relatively straight, including the petrous (C2), lacerum (C3), cavernous (C4), and ophthalmic (C6) segments of the internal carotid artery (ICA), and the V1–4 segments of the vertebral artery (VA), which are selected as the first choice. This requirement can also be selectively applied to other segments according to the specific condition of the patient.

Key points for the intraluminal reconstruction technique

Basic considerations for the lesion and its parent artery

Imaging evaluation of the diseased segment artery is important, including the diameter of the proximal and distal parts of the treated segment artery, the length of the lesion in the vessel wall, the tortuosity of the diseased artery, especially the treated segment artery, as well as calcification and plaque in the arterial wall. Arterial wall lesions should generally be <10 mm in length for treatment with a covered stent; if the length of the lesion exceeds 10 mm, overlapping multiple covered stents can be considered if technically feasible and the treatment segment of the parent artery is relatively straight (multiple stents are not recommended for tortuous vessels). If a potentially important perforator is suspected in the diseased segment of the vessel, a high-dose contrast

TABLE 1 Comparison of different techniques for endovascular treatment of cerebrovascular diseases.

	Covered stent insertion	Coil embolization	Flow diverter deployment
Indication	1) Complex cerebral aneurysms	1) Most of cerebral aneurysm	1) Unruptured side-wall giant or large cerebral aneurysms
	2) Direct carotid-cavernous fistulas 3) Internal carotid artery injuries	2) Some kinds of AVMs or fistulas	2) Fusiform or wide-necked aneurysms
Treatment conception	Diseased parent artery repair	Aneurysm sac occlusion	Blood flow diverting
Target lesion	Parent artery	Aneurysm sac	Impaired flow
Device navigation	Difficult	Easy	Moderate
Technical challenging	Easy for stent deployment	Higher skill required for coiling	Higher skill required for device deployment
Anatomic location	Distal ICA and VA	Most segments as long as the microcatheter can be in place	Distal ICA and VA
Outcomes evaluation	Endoleak type	Reymond classification	OKM scale
Complication	Parent artery injury, in-stent thrombosis, stenosis or occlusion	Aneurysm rupture, parent artery occlusion	Aneurysm rupture, parent artery thrombosis, stenosis or occlusion
Follow up images	DSA, CTA	DSA, MRA	DSA, CTA

AVM, arteriovenous malformation; ICA, internal carotid artery; VA, vertebral artery; DSA, digital substraction angiography; CTA, computerized tomography angiography; MRA, magnetic resonance angiography; OKM scale, O'Kelly-Marotta scale.

agent is recommended for use in angiography to further confirm its presence. Coverage of any perforating vessels should be avoided to prevent serious adverse consequences (Figure 2).

A relatively straight arterial segment is the ideal condition for the implantation of covered stents. If the artery requiring treatment is not straight, the curvature of tortuosity should not be sharp (generally $>130^{\circ}$ is recommended) and the lesion is located on the larger side of the curvature of the artery, a covered stent can be considered for treatment. For patients with excessive vascular tortuosity in the segment requiring stenting, stent release by balloon dilation is likely to cause arterial wall damage or intracranial hemorrhage. Similarly, if the lesion is located on the smaller side of the curvature of the tortuous vessel, the implantation of a covered stent may result in endoleak due to its poor membrane adherence to the arterial wall and is thus generally not recommended. The discrepancy between the distal and proximal ends of the treated segment of the artery should not be too large; in principle, if the difference is >0.5 mm, it should be regarded as a contraindication for the application of a covered stent.

Recommendations for stent size and length selection

It is generally recommended to measure the diameter of the aneurysm sac and parent artery on 2D angiography. Additionally, accurate 3D multiangle measurement is recommended for evaluating the angulation of the parent artery to prevent improper stent selection due to an incorrect measurement or evaluation of the parent artery. The diameter of the covered stent should be essentially the same as or slightly larger (no >0.5 mm) than the vessel diameter of the segment requiring treatment. When patients were in the stage of acute hemorrhage and vasospasm of the target artery was confirmed, the diameter selection of the covered stent should be referred to arterial diameter in its natural status or slightly larger than its currently measured diameter. If the treated artery is located epidurally or interdurally, the choice of diameter for the covered stent can be appropriately enlarged. When the diameters of the distal and proximal ends of the treated segment artery are different, the diameter of the covered stent should be selected according to the diameter of the wide end of the artery segment requiring treatment.

The length of the covered stent should be greater than that of the arterial lesion; generally, the stent should fully cover the diseased arterial wall, including the aneurysm neck, CCF fistula, and wall damage from other causes. A few points are worth noting. First, in the treatment of saccular aneurysms, the length should be 4–6 mm greater than that of the aneurysm neck visible on the best imaging projection. Second, the length of the stent should be chosen as short as possible while guaranteeing the coverage of the lesion in the tortuous vessel, which may prevent vessel wall damage caused by balloon dilation or endoleak caused by poor adherence of the stent membrane to the tortuous artery. Third, the length of the covered stent can be slightly increased if deployed epidurally or interdurally and the segmented artery to be treated is straight. When important branch vessels need to be preserved, if the end of the coated scaffold does not completely cover the branch opening, the undisturbed blood flow of the branch can be preserved in most cases.

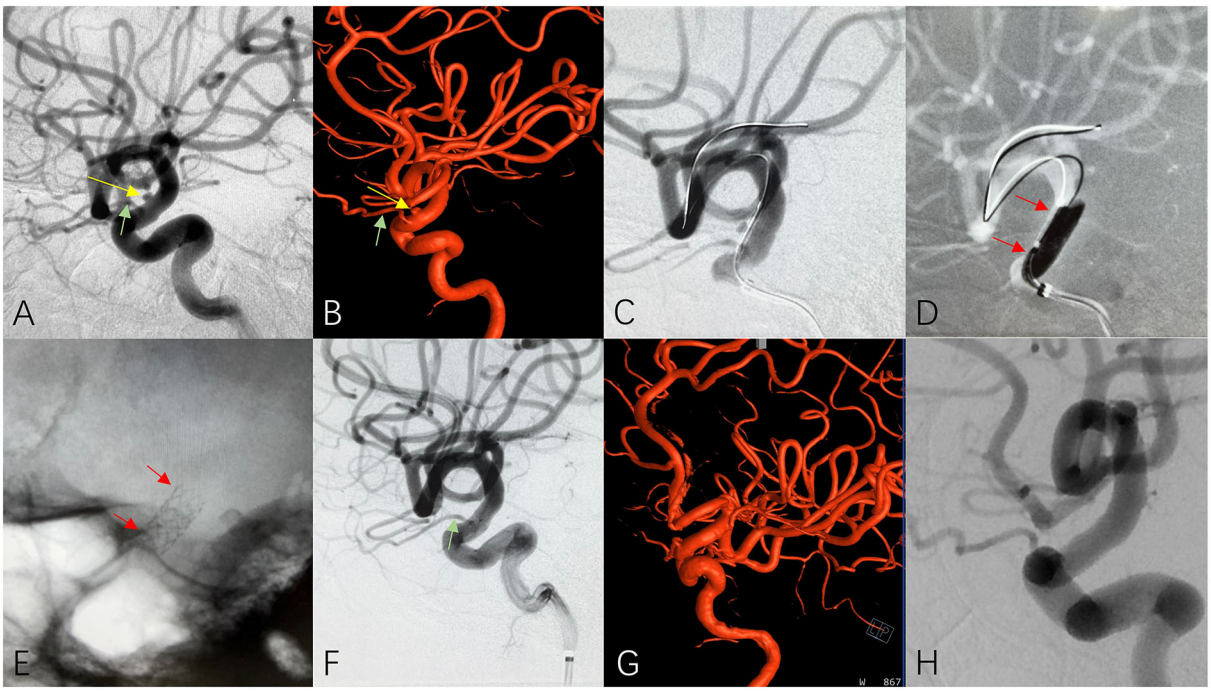

FIGURE 2
A patient with cerebral aneurysm received covered stent insertion. 2D **(A)** and 3D **(B)** cerebral angiograms reveled a unruptured tiny cerebral aneurysm (yellow arrow) and adjacent ophthalmic artery (green arrow) at the C6 segment of ICA. A covered stent (3.5 × 10 mm) was delivered (**C**, red arrows) released at the diseased parent artery (**D**, red arrows) and the plain film clearly showed well opened covered stent after deployment (**E**, red arrows). Immediate 2D **(F)**, 3D **(G)** and 4-months follow up **(H)** cerebral angiogram confirmed complete disappearance of aneurysm and excellent patency of the ophthalmic artery (green arrow) and parent artery.

Key technical notes for stent delivery

The stent system consists of a covered stent and a balloon catheter, which makes the whole system stiff runs the risk of failing to arrive at the target lesion, and may cause membrane damage during stent delivery. Therefore, for patients with tortuous pathways, proximal long-sheath support and distal placement of intermediate catheters can be combined to improve the success rate of stent system delivery. For patients with extreme vascular tortuosity, an intermediate catheter with a smaller profile (5 French) and better flexibility is recommended. In rare cases, multiple guidewire support, balloon catheter support, and distal stent deployment support can also be used to assist the intermediate catheter in crossing over the lesion to the distal part of the vessel. By establishing passage with an intermediate catheter, the stent system can be delivered and released smoothly.

Key technical notes for stent deployment

Negative pressure emptying of the balloon is not recommended until the covered stent is in the correct location. The balloon should be expanded slowly during the deployment of the covered stent to reduce damage to the vessel wall, improve the adhesion between the stent and the vessel wall, and minimize membrane damage. Additionally, attention should be given to controlling the tension of the whole delivery system to reduce the risk of stent migration during balloon expansion. Depending on the degree of vascular curvature, the time from the beginning of balloon dilation to the establishment of nominal pressure should be limited to 1–3 min. When balloon dilatation is sufficient to ensure full opening of the covered stent, it is not necessary to reach the named pressure in the tortuous vessel segment, by which the risk of vessel damage can be minimized. Additionally, slow balloon pressure relief is recommended to avoid poor stent adhesion or stent migration.

Covered stents and assistant technologies

Covered stents and coils

For high-flow CCFs, large/giant aneurysms, or aneurysms located at the curved segment of the vessel, intraluminal covered stent reconstruction treatment has a high risk of leakage due to poor stent adhesion or retrograde filling of the side branches; thus, it is necessary to consider the placement of some coils in

the aneurysm sac or within the cavernous sinus before covered stent insertion. For wide-necked aneurysms or high-flow CCFs, the placement of coils in the aneurysm sac or on the distal side of the fistula can support and stabilize the covered stent. Moreover, coiling may be of great help in reducing the flow of the CCF, allowing a clear display of the fistula and ensuring accurate coverage. It is generally recommended to perform coil embolization before deployment of the covered stent. If coil insertion is performed after the deployment of the stent, the risk of internal leakage will increase during coil packing or microcatheter withdrawal. If coil embolization should be performed after stent deployment, the balloon should be placed *in situ*, and rescue dilation should be performed after the microcatheter is removed if necessary.

Bare stent-assisted covered stent deployment

This technique is not generally recommended, especially in the era of flow diverters. For some wide-necked large aneurysms involving the parent artery fusiform or dissecting aneurysms, when multiple covered stents are planned to be inserted, the first placed covered stent risks collapsing into the aneurysm sac; thus, a long-bared stent (closed cell stent is preferred) can be placed to provide enough support. These procedures should only be considered for straight vessels, but nevertheless, they are associated with an increased risk of thrombosis or stenosis.

Flow diverter-assisted covered stent deployment

The implantation of a covered stent into a flow-diverting device is not recommended as a routine procedure. In a few special cases, if obvious inject flow is observed after immediate flow diverter insertion or the repair of the defect in the wall is not satisfactory after implantation of the flow diverting device after long-term follow-up and other techniques are ineffective, a covered stent can be implanted into the flow diverting device as a rescue method. Similarly, in cases where a non-healing, high-flow endoleak develops after covered stent implantation and rescue coiling or telescoping covered stent insertion is not feasible, a flow diverting device can also be considered as an option for treating the endoleak.

Management of complications

Endoleaks

Endoleak classification

Endoleaks that develop after implantation of covered stent insertion can be classified into four types. Type I: those arising due to poor stent apposition to the vessel wall or stent size mismatch; Type II: retrograde flow filling of the aneurysm sac *via* the collateral vessels; Type III: those arising due to defects of the stent itself; and Type IV: leakage due to membrane pores (Figure 3) (20).

General principles for endoleak management

i) Selecting the right size for the covered stent (or slightly larger stents released at low balloon pressure) is key to avoiding endoleaks or to determining whether the endoleak can be properly handled. A proximal endoleak in a straight parent artery should be actively managed, while a small amount of distal endoleakage can be observed and followed up (7). For ruptured aneurysms, endoleaks should be actively managed regardless of whether they are proximal or distal (Figure 4).

ii) The balloon should be dilated slowly and the pressure should be increased step by step. For stents that are significantly smaller in diameter than the parent artery, post-balloon dilatation is often ineffective.

iii) For aneurysms located in tortuous artery segments or large or wide-necked aneurysms, some coils should be placed into the aneurysm prior to stenting. Preset microcatheterization may be considered if necessary, or if the leakage is severe, coils or Onyx glue may be used to further occlude the aneurysm.

General techniques for treating endoleaks

After covered stent deployment, the balloon should be retained *in situ*. The classification and location of any internal leakage (e.g., distal or proximal end of the stent) should be carefully determined on angiography. If the amount of endoleakage is too large to be distinguished, the balloon can be dilated *in situ* with the same or higher pressure to reduce it. If the endoleak persists, post-dilation should be performed, targeted at the endoleak site by partially moving the balloon forward or backward so that the main body (not the tip or end of the balloon) is located at the endoleak site to increase the adherence of the stent membrane to the vessel wall. If the endoleak is not obvious, multiangle angiography should be performed and then repeated after a 10-min wait before a decision to follow-up observation. It is recommended that the balloon not be expanded more than three times and that the dilation pressure not be too high. When additional covered stent insertion is considered, the size of the stent should generally be the same as or slightly larger than that of the previously inserted stent.

If the above methods are adopted, but the endoleak persists, and there is a high risk of bleeding, occlusion of the parent artery on the affected side or surgical clipping can be considered following sufficient evaluation of the contralateral artery or compensation of the adjacent circulation.

Vasospasm

After delivery and release of the balloon-dilated covered stent, the target vessels and adjacent branches may spasm,

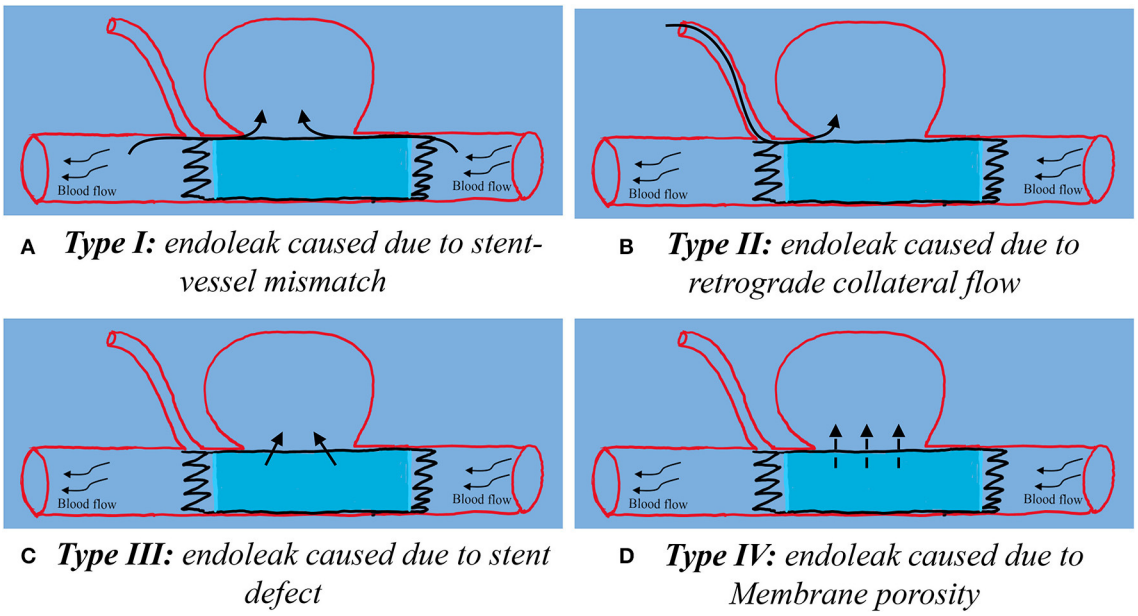

FIGURE 3
Classification of the covered stent-associated endoleaks (20).

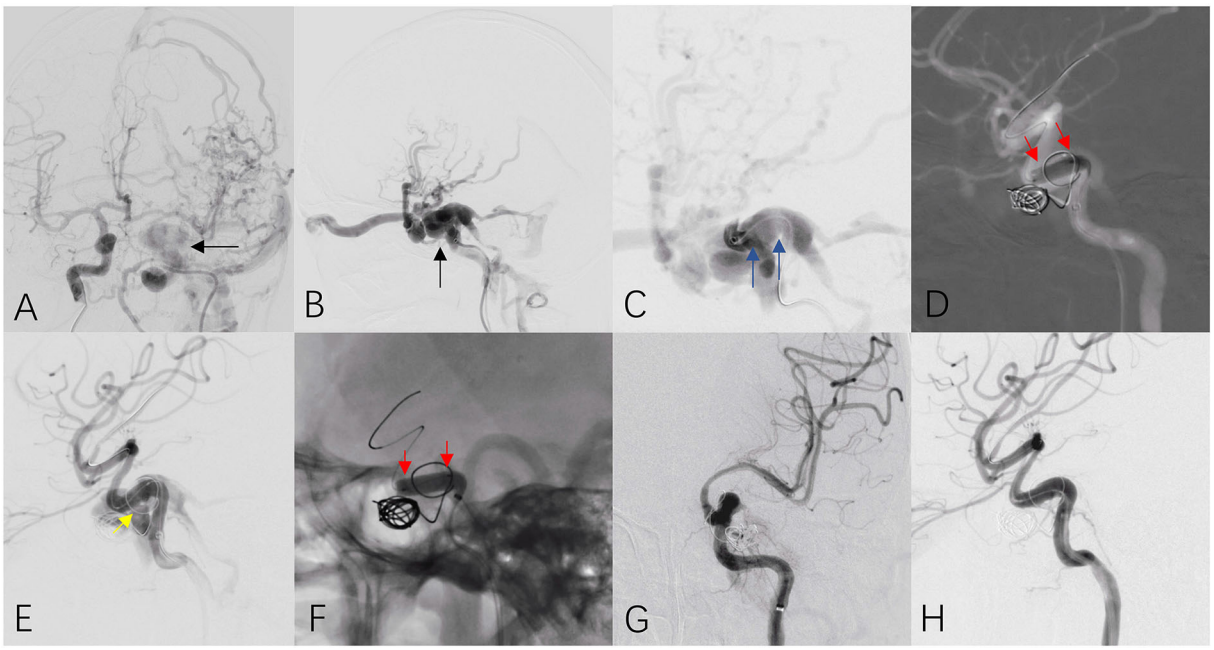

FIGURE 4
A patient with traumatic carotid cavernous sinus fistula received covered stent insertion. Anteroposterior **(A)** and lateral **(B)** cerebral angiograms revealed high volume of fistula (black arrow) at the C4 segment of the left ICA. Continuous angiogram from an intermediate catheter located the fistula at the horizontal segment at C4 (**C**, blue arrows). A covered stent (4.0 × 13 mm) was released at the diseased parent artery (**D**, red arrows) and immediate angiogram confirmed high volume of type I endoleak at the distal end of stent (**E**, yellow arrow). Post dilation within the stent was performed (**F**, red arrows) and final cerebral angiograms **(G,H)** confirmed complete disappearance of fistula and excellent patency of the parent artery.

resulting in the illusion of a complete occlusion image. However, after the vasospasm resolves, there is a possibility of endoleak recurrence. Therefore, angiography should be performed again after a period of time (or after the use of antivasospasm drugs).

Thrombotic events

In addition to a metal scaffold, the covered stent includes an outside membrane. After the covered stent is implanted into the curved segment of the vessel, the membrane can crumple and pile up, especially on the smaller side of the curvature, resulting in thrombosis. Similarly, incomplete opening or poor adherence of the covered stent after implantation at the curved segment can also demonstrate high thrombogenic properties (21).

Adequate pre- and post-operative double antiplatelet therapy, thromboelastography or genetic testing to determine whether there is resistance to drugs, intraoperative heparinization and postoperative anticoagulation are key to preventing thrombotic events after stent implantation. If acute in-stent thrombotic events occur during or after stent implantation, antegrade flow can be restored through the application of platelet GPIIb/IIIa receptor antagonists, balloon dilation, or other mechanical thrombectomy techniques. For some high-risk patients, a target vessel balloon occlusion (BOT) test is recommended before implantation of a covered stent.

Vessel rupture

Vascular rupture and bleeding caused by the release of covered stents are the most serious complications of their implantation. Improper stent size selection, tortuosity of the treated segment, and sclerosis of the arterial wall are risk factors for rupture of the parent artery. It should be noted that since the covered stent is a balloon-expandable stent, the balloon is longer than the stent, and the flexibility during balloon expansion is poor, arterial wall damage can easily develop in torturous vessels, leading to vessel rupture. Therefore, when choosing the length of the covered stent, it is necessary to consider the potential risk of a longer balloon length during dilation. Remedial measures after hemorrhage mainly include fast balloon re-expansion for hemostasis and occlusion of the parent artery.

Delayed aneurysm rupture

The main cause of delayed aneurysm rupture is the persistence of various types of endoleaks. Therefore, the main measure to avoid delayed aneurysmal bleeding is to resolve the leakage.

In-stent stenosis

The incidence of stenosis after covered stent implantation is affected by many factors, such as clinical factors, lumen diameter, vessel curvature, and hemodynamics. The late restenosis rate after covered stent insertion is low. The rates of lumen loss at the site of stent implantation at 2 and 6 years are only 18.0 ± 13.3 and 29.0 ± 18.5%, respectively, compared to those immediately after implantation (1). Stenosis at the site of covered stent implantation is closely related to delayed endothelialization. Studies have shown that covered stent endothelialization can be significantly delayed with respect to that of bare stents and can occur later in curved segments than in straight segments. Complete endothelialization of the covered stent usually takes 6–12 months, so a dual antiplatelet aggregation therapy of at least half a year is recommended (18). Smoking and stent angulation have been identified as risk factors for predicting late in-stent stenosis (1).

Covered membrane bulging

After the covered stent is implanted, local lumen dilation or bulging can be observed during noninvasive angiography or angiographic follow-up, which usually occurs in the neck of sidewall aneurysms or in the body of fusiform/dissected aneurysms, and it is necessary to identify whether an endoleak has developed. This angiographic finding may be related to the bulging of the local membrane of the covered stent, which is more likely to occur in the body of the covered stent and the parts lacking vessel wall support. A possible mechanism is a loose connection between the coated membrane and the scaffold by suturing; this design is intended to provide a redistribution of the membrane as the stent at the smaller side of the curved segment shrinks. This may be the primary cause of membrane bulging under continuous hemodynamics, a phenomenon that should be harmless and thus recommended for regular clinical follow-up.

Perioperative medication

Preoperative antiplatelet aggregation therapy

A combination of dual antiplatelet aggregation agents, clopidogrel (75 mg/day), and aspirin (100 mg/day) is recommended. Oral administration should begin 3–5 days before surgery. Routine preoperative thromboelastography is recommended. If clopidogrel resistance is confirmed, the more potent P2Y12 receptor inhibitor ticagrelor may be substituted; otherwise, the load dose, 300 mg clopidogrel and 300 mg aspirin, should be taken orally or by other means 4 h before surgery.

Intraoperative heparinization

Intraoperative heparin should be administered *via* intravenous injection with an initial dose of 4,000–5,000 U or 60–80 U/kg for a bolus injection, followed by a half-dose mass injection in the second hour, and so on, with at least 1,000 U added every hour to maintain the patient's systemic heparinized state (activated clotting time (ACT) maintained at more than two times the basal level).

Postoperative anticoagulation and antiplatelet aggregation therapy

Short-term anticoagulant therapy can be considered after covered stenting. A subcutaneous injection of low-molecular-weight heparin 4,000–5,000 U every 12 h for 3 days is recommended. Oral clopidogrel (75 mg/day) and aspirin (100 mg/day) should be taken for at least 6–12 months; long-term oral administration of a single antiplatelet aggregation agent is then recommended (22).

If an endoleak is obvious in a ruptured aneurysm or there is a high risk of hemorrhage after covered stenting for other lesions, the use of low-molecular-weight heparin can be reduced as appropriate.

For emergency stent implantation, the intraoperative use of platelet GPIIb/IIIa receptor antagonists (e.g., tirofiban) is recommended if unconditionally taken for 3–5 days as a dual antiplatelet aggregation agent before surgery. If postoperative CT confirms no increased hemorrhage, then an overlapping antiplatelet aggregation agent (aspirin 300 mg, clopidogrel 300 mg) can be given. The platelet GPIIb/IIIa receptor antagonist should be discontinued 4–6 h later.

If necessary, hormonal therapy can be used to reduce inflammatory reactions after treatment of a giant aneurysm with a covered stent. The main reason for such inflammatory reactions is an increase in the volume of the aneurysm sac after thrombus formation, resulting in the stimulation of the meninges and nerves and compression of the adjacent brain tissue, leading to the aggravation of patient symptoms. However, their clinical effects need to be further evaluated.

Follow-up protocol

Image follow-up protocol

Similar to the treatment of complex cerebrovascular diseases with other technologies, imaging follow-up after covered stent insertion is needed for a short period of 3–6 months, a medium period of 1–2 years, and a long period of more than 5 years. Catheterized angiography is preferred for imaging follow-up. However, the metal struts of the covered stent are thicker than those of intracranial stents and have better radiopacity. Therefore, for cases with covered stent implantation alone, both VR and MIP reconstruction of CTA can clearly show the stent, while multiplane reconstruction and curved reconstruction of thin-layer MIP-CTA, referring to thin-layer coronal images of CTA, can clearly show the patency of the covered stent and isolation of the aneurysm sac. Therefore, CTA can be used as an effective supplementary follow-up method. Magnetic resonance angiography (MRA), including TOF or contrast-enhanced MRA, shows almost complete loss of vascular signal in the stented segment and is therefore not recommended as an imaging follow-up method. In the case of combined coil embolization, catheterized angiography is preferred due to radiative artifacts caused by coils. For cases with sustained endoleak, close imaging follow-up is needed, and whether further intervention is required depends on the location and volume of the endoleak.

Clinical follow-up protocol

After stent implantation, short-term (1–3 months) and subsequent annual clinical follow-ups are routinely recommended. For cases with endoleak, it is necessary to adjust the use strategy of antiplatelet drugs in time according to the patient's symptoms and follow-up imaging results.

Economic analysis

By extracting and analyzing direct medical cost data, including the costs of drugs, medical devices, daily hospital beds, nursing, and examinations, a decision tree model was established to evaluate the health costs and efficacy of the treatment of intracranial aneurysms (diameter >7 mm) with covered stents and coil embolization. The results showed that the direct medical costs of covered stent insertion and coil embolization were 21,860.4 and 27,391.7 dollars, respectively, and the aneurysm recurrence rates were 0 and 28.9%, respectively. Therefore, the therapeutic effect of covered stents is better than that of coil embolization, with an incremental cost-effectiveness ratio (ICER) of −9,735,732.7 dollars/death averted. Thus, coated stents can improve the clinical efficacy and reduce the total medical cost for patients with intracranial aneurysms of diameter >7 mm (23).

Conclusion

Covered stents could become an effective treatment option for complex cerebrovascular diseases, such as complex aneurysms, direct carotid-cavernous fistulas, and internal carotid artery injuries. The requirement for the parent artery for covered stent treatment is higher than that of the disease itself,

which indicates that the parent artery must be relatively straight. Endoleak types should be carefully evaluated, and the proximal or high volume endoleaks should be managed properly.

Author contributions

YZhu: acquisition of data, analysis and interpretation of data and drafting of the manuscript. CF and ML: organize of this expert consensus, critical revision of the manuscript for important intellectual content, and supervision. HT, ZW, TieL, LM, JL, HZ, YG, TiaL, SG, XX, CJ, ZZ, CD, JWan, XZ, WF, XHe, HaS, QW, DL, QL, WJ, GM, SZ, EC, HuS, SR, DW, YL, ZL, JWu, FW, XHu, JWang, FZ, WC, DY, QZ, LW, BG, GC, and YZha: revision of the manuscript. All authors contributed to the article and approved the submitted version.

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.

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References

1. Zhu YQ, Li MH, Lin F, Song DL, Tan HQ, Gu BX, et al. Frequency and predictors of endoleaks and long-term patency after covered stent placement for the treatment of intracranial aneurysms: a prospective, non-randomised multicentre experience. *Eur Radiol.* (2013) 23:287–97. doi: 10.1007/s00330-012-2581-4

2. Li MH, Li YD, Tan HQ, Luo QY, Cheng YS. Treatment of distal internal carotid artery aneurysm with the Willis covered stent: a prospective pilot study. *Radiology.* (2009) 253:470–7. doi: 10.1148/radiol.2532090037

3. Chua MMJ, Silveira L, Moore J, Pereira VM, Thomas AJ, Dmytriw AA. Flow diversion for treatment of intracranial aneurysms: mechanism and implications. *Ann Neurol.* (2019) 85:793–800. doi: 10.1002/ana.25484

4. Walcott BP, Stapleton CJ, Choudhri O, Patel AB. Flow diversion for the treatment of intracranial aneurysms. *JAMA Neurol.* (2016) 73:1002–8. doi: 10.1001/jamaneurol.2016.0609

5. Zhu YQ, Li MH, Fang C, Tan HQ, Wang W, Zhang PL, et al. Application of the Willis covered stent in the treatment of aneurysm in the cisternal segment of the internal carotid artery: a pilot comparative study with midterm follow-up. *J Endovasc Ther.* (2010) 17:55–65. doi: 10.1583/09-2688.1

6. Ma L, Xu JC, Yan S, Feng H, Han HJ, Tan HQ, et al. single-center experience in the endovascular treatment of carotid siphon aneurysms using the Willis covered stent: a retrospective analysis. *J Neurointerv Surg.* (2018) 10:1197–202. doi: 10.1136/neurintsurg-2017-013695

7. Tan HQ, Li MH, Zhang PL, Li YD, Wang JB, Zhu YQ, et al. Reconstructive endovascular treatment of intracranial aneurysms with the Willis covered stent: medium-term clinical and angiographic follow-up. *J Neurosurg.* (2011) 114:1014–20. doi: 10.3171/2010.9.JNS10373

8. Liu Y, Yang HF, Xiong ZY, Zheng J, Liu CY, Zhao HY, et al. Efficacy and safety of Willis covered stent for treatment of complex vascular diseases of the internal carotid artery. *Ann Vasc Surg.* (2019) 61:203–11. doi: 10.1016/j.avsg.2019.05.027

9. Li MH, Zhu YQ, Fang C, Wang W, Zhang PL, Cheng YS, et al. The feasibility and efficacy of treatment with a Willis covered stent in recurrent intracranial aneurysms after coiling. *AJNR Am J Neuroradiol.* (2008) 29:1395–400. doi: 10.3174/ajnr.A1096

10. Fang C, Tan HQ, Han HJ, Feng H, Xu JC, Yan S, et al. Endovascular isolation of intracranial blood blister-like aneurysms with Willis covered stent. *J Neurointerv Surg.* (2017) 9:963–8. doi: 10.1136/neurintsurg-2016-012662

11. Liu LX, Zhang CW, Xie XD, Wang CH. Application of the Willis covered stent in the treatment of blood blister-like aneurysms: a single-center experience and systematic literature review. *World Neurosurg.* (2019) 123:e652–60. doi: 10.1016/j.wneu.2018.11.245

12. Wang JB, Li MH, Fang C, Wang W, Cheng YS, Zhang PL, et al. Endovascular treatment of giant intracranial aneurysms with Willis covered stents: technical case report. *Neurosurgery.* (2008) 62:E1176–7; discussion E1177. doi: 10.1227/01.NEU.0000312341.92946.A1

13. Li MH, Li YD, Gao BL, Fang C, Luo QY, Cheng YS, et al. new covered stent designed for intracranial vasculature: application in the management of pseudoaneurysms of the cranial internal carotid artery. *AJNR Am J Neuroradiol.* (2007) 28:1579–85. doi: 10.3174/ajnr.A0668

14. Xiang S, Li G, He C, Ren J, Zhang H. Reconstructive endovascular treatment of the V4 segment of a vertebral artery dissecting aneurysm with the Willis covered stent: a retrospective study. *Interv Neuroradiol.* (2019) 25:548–55. doi: 10.1177/1591019918824413

15. Wang W, Li YD, Li MH, Tan HQ, Gu BX, Wang J, et al. Endovascular treatment of post-traumatic direct carotid-cavernous fistulas: a single-center experience. *J Clin Neurosci.* (2011) 18:24–8. doi: 10.1016/j.jocn.2010.06.008

16. Wang W, Li MH, Li YD, Gu BX, Lu HT. Reconstruction of the internal carotid artery after treatment of complex traumatic direct carotid-cavernous fistulas with the Willis covered stent: a retrospective study with long-term follow-up. *Neurosurgery.* (2016) 79:794–805. doi: 10.1227/NEU.0000000000001266

17. Wang YL, Ma J, Li YD, Ding PX, Han XW, Wu G. Application of the Willis covered stent for the management of posttraumatic carotid-cavernous fistulas: an initial clinical study. *Neurol India.* (2012) 60:180–4. doi: 10.4103/0028-3886.96397

18. Cai MJ, Xu GZ, Yang M, Ma LT, Yang XJ, Shi BC, et al. Treatment strategies for traumatic cervico-cranial pseudoaneurysms: a single institution experience. *Neurol India.* (2018) 66:105–14. doi: 10.4103/0028-3886.222873

19. Pan L, Liu P, Yang M, Ma L, Li J, Chen G. Application of stent-graft is the optimal therapy for traumatic internal carotid artery pseudoaneurysms. *Int J Clin Exp Med.* (2015) 8:9362–7.

20. Hoit DA, Schirmer CM, Malek AM. Stent graft treatment of cerebrovascular wall defects: intermediate-term clinical and angiographic results. *Neurosurgery.* (2008) 62(5 Suppl 2):ONS380–8; discussion ONS388–9. doi: 10.1227/01.neu.0000326022.08973.b2

21. Zhu YQ, Li MH, Xie J, Tan HQ, Cheng YS, Wang JB. Treatment of carotid siphon aneurysms by use of the Willis stent graft: an angiographic and histopathological study. *Eur Radiol.* (2010) 20:1974–84. doi: 10.1007/s00330-010-1738-2

22. Lai XB, Li MH, Tan HQ, Luo BY, Zhu YQ, Wang J, et al. Predictors of in-stent stenosis and occlusion after endovascular treatment of intracranial vascular disease with the Willis covered stent. *J Clin Neurosci.* (2013) 20:122–7. doi: 10.1016/j.jocn.2012.01.051

23. Ting J, Liu Y, Petrillo J, Giannattasio G, Sabatella G. Treatment satisfaction with disease modifying therapies in multiple sclerosis: a systematic review of studies using the treatment satisfaction questionnaire for medication (Tsqm). *Value Health.* (2015) 18:A760–1. doi: 10.1016/j.jval.2015.09.2484

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Effects of high-intensity and moderate-intensity exercise training on cardiopulmonary function in patients with coronary artery disease: A meta-analysis

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Purpose: The study aims to evaluate the effects of high-intensity and moderate-intensity exercise training on cardiopulmonary function and exercise endurance in patients with coronary artery diseases (CAD).

Methods: We performed a systematic search of the English and Chinese databases from their inception to March 2022. Randomized controlled trials (RCTs) were included to compare high-intensity and moderate-intensity exercise training on cardiopulmonary function in patients with CAD. The primary outcomes included peak oxygen uptake (peak VO_2) and anaerobic threshold (AT). The secondary outcomes included left ventricular ejection fraction (LVEF), exercises duration (ED), respiratory exchange ratio (RER), resting heart rate (RHR), peak heart rate (PHR) and oxygen pulse (O_2 pulse). The continuous variables were expressed as mean differences (MD) along with their corresponding standard deviations (SD), and the I^2 test was applied in the assessment of heterogeneity.

Results: After systematically literature search, 19 studies were finally selected for our meta-analysis (n = 1,036), with 511 patients in the experimental group (high-intensity exercise) and 525 patients in the control group (moderate-intensity exercise). The results showed that high-intensity exercise significantly increased patients' Peak VO_2 [MD = 2.67, 95% CI (2.24, 3.09), $P < 0.00001$], LVEF [MD = 3.60, 95% CI (2.17, 5.03), $P < 0.00001$], ED [MD = 37.51, 95% CI (34.02, 41.00), $P < 0.00001$], PHR [MD = 6.86, 95% CI (4.49, 9.24), $P < 0.00001$], and O_2 pulse [MD = 0.97, 95% CI (0.34, 1.60), $P = 0.003$] compared with moderate-intensity exercise. However, there were no significant differences in AT [MD = 0.49, 95% CI (−0.12, 1.10), $P = 0.11$], RER [MD = 0.00, 95% CI (−0.01, 0.02), $P = 0.56$], and RHR [MD = 1.10, 95% CI (−0.43, 2.63), $P = 0.16$].

Conclusion: Our results show that high-intensity exercise training has more significant positive effects compared with moderate-intensity exercise training in improving peak VO_2, LVEF, ED, PHR and O_2 pulse in patients with CAD, while no significant differences were observed in AT, RER and RHR. To sum up,

high-intensity exercise training is better than moderate-intensity exercise training in improving cardiopulmonary function and exercise endurance in patients with CAD.

Systematic review registration: PROSPERO (CRD42022328475), https://www.crd.york.ac.uk/PROSPERO/.

KEYWORDS

coronary artery disease, exercise intensity, peak oxygen uptake, anaerobic threshold, meta-analysis

Introduction

Cardiovascular diseases are the leading cause of death among non-communicable diseases worldwide (1), in which CAD is known having the highest occurrence (2). Thus, CAD has been recognized as a major public health issue worldwide, causing a heavy economic burden to the society (3). Cardiac rehabilitation is internationally recognized as a Class 1A recommendation to improve the prognosis and quality of life in patients with cardiovascular diseases, including patients who have undergone percutaneous coronary implantation (PCI) and coronary artery bypass grafting (CABG). Exercise training is the principal component of cardiac rehabilitation (4). Studies have shown that regular exercise can effectively control the risk factors associated with cardiovascular diseases, such as lowering blood pressure (5), controlling blood glucose (6), controlling body fat, and weight loss (7). Furthermore, it can also improve cardiopulmonary function in patients with CAD (8), improve vascular endothelial function (9), improve ventricular filling, and reverse ventricular remodeling (10). Thereby, it can reduce the incidence of cardiovascular diseases, improve the quality of life of patients, and reduce all-cause mortality (11). Exercise training mainly includes endurance exercise, resistance exercise and aerobic exercise (12). Aerobic exercise training is further classified into three types of exercise intensities: low, medium and high. Until now, there is no international consensus on the choice of exercise intensity. The United States and Canada prefer medium or high-intensity sports training, while Australia and Japan support low or moderate-intensity exercise training (13). The 2016 European Guidelines for the Prevention of Cardiovascular Disease recommended moderate-intensity continuous aerobic training with high safety as an exercise method for patients with cardiovascular diseases (14). Recently, an increasing number of studies have shown that high-intensity aerobic training has the advantages of short training time and high efficiency compared with long and boring moderate-intensity aerobic training (15). However, high-intensity exercise may trigger cardiac arrest in individuals with cardiovascular disease, especially in sedentary patients or those who have advanced cardiovascular diseases (16, 17). Therefore, it is pertinent and urgent to systematically evaluate the effects of the duration and intensity of exercise training and evaluate its effects on patients, and thus establish an optimal exercise training intensity prescription that optimizes the synergy between the rewards and safety (18).

The cardiopulmonary function is a powerful indicator of body's ability to exercise, diagnose diseases and efficiently evaluate prognosis. It can reflect the ability of the circulatory, respiratory and muscular systems to supply oxygen during continuous physical activity (19). Research evidence suggests that improvements in cardiorespiratory fitness are strongly associated with a reduction in all-cause mortality and cardiovascular mortality. And this is why the American Heart Association (AHA) has identified cardiorespiratory fitness as an important landmark indicator in the assessment and intervention of cardiovascular risks and cardiovascular mortality (20). Randomized controlled trials and meta-analyses have demonstrated that both high and moderate exercise intensity can improve cardiopulmonary function in patients with cardiovascular diseases, enhance exercise tolerance, and improve their quality of life compared with drug therapy alone. However, there are numerous controversies regarding the efficacy and safety of different exercise intensities. Therefore, this study is aimed toward the evaluation of the effects of different exercise intensities (moderate and high) on cardiopulmonary function in patients with CAD, to optimize the exercise mode, and provide actionable recommendations for the improvement of cardiopulmonary function and prognosis.

Methods

Registration

The protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42022328475). And our Meta-analysis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search strategy

The following electronic databases were searched systematically: PubMed, EMBASE, the Cochrane Library,

TABLE 1 American college of sports medicine exercise intensity ratings for healthy adults.

Strength	Relative exercise intensity					Absolute exercise intensity				
	Oxygen uptake reserve (%)	Maximum oxygen uptake (%)	Heart rate reserve (%)	Maximum heart rate (%)	Degree of subjective force (points)	Young[a] (< MET)	Middle age[b] (MET)	Aging[c] (MET)	Senior citizens[d] (MET)	Static resistance training maximum load (%)
Very light	<20	<25	<20	<35	<10	<2.4	<2.0	<1.6	<1.0	<30
Light	20–39	25–44	20–39	35–54	10–11	2.4–4.7	2.0–3.9	1.6–3.1	1.1–1.9	30–49
Moderate	40–59	45–59	40–59	55–69	12–13	4.8–7.1	4.0–5.9	3.2–4.7	2.0–2.9	50–69
Heavy	60–84	60–84	60–84	70–89	14–16	7.2–10.1	6.0–8.4	4.8–6.7	3.0–4.24	70–84
Very heavy	≥85	≥85	≥85	≥90	17–19	≥10.2	≥8.5	≥6.8	≥4.25	≥85
Maximum	100	100	100	100	20	12.0	10.0	8.0	5.00	100

Oxygen uptake reserve: maximal oxygen uptake - oxygen uptake when quiet; Maximal oxygen uptake: the absorption and utilization of oxygen when each system of human body develops maximum function in the process of movement can be obtained by the cardiopulmonary endurance test of body fitness detection; Heart rate reserve = maximum heart rate - heart rate at rest; Maximum heart rate = 220 – age; Subjective force: Borg rating scale was used; MET, metabolic equivalent, expressed as oxygen metabolism per minute, 1 MET = 3.5 $m \cdot kg^{-1} \cdot min^{-1}$.
[a]20–39 years old.
[b]40–64 years old.
[c]65–79 years old.
[d]80 years of age or older.

China National Knowledge Infrastructure (CNKI), Sino-Med, Chongqing VIP, and the Wanfang database, from their inception to March 2022. Taking PubMed as an example, the search strategy was (((((coronary heart disease) OR (coronary artery bypass grafting)) OR (PCI)) OR (ischemic heart disease)) OR (coronary artery stenosis)) AND (exercise intensity) AND (randomized controlled trial). All retrieved articles are imported into EndNote (English publication) and NoteExpress (Chinese publication).

Inclusion criteria

Clinical trials that satisfy the following criteria were included in our study: (1) Population: the target population was patients with CAD (including patients who have undergone PCI and CABG), consistent with 2014 ACC/AHA/AATS/PCNA/SCAI/STS the diagnostic criteria for patients with stable ischemic heart disease (21), and there are no restrictions on gender, age or duration of disease; (2) Intervention and comparisons: studies that compared exercise training at high (intervention measures) and moderate (control measures) intensities (the classification of exercise intensity was based on the American College of Sports Medicine Exercise Intensity Classification for Healthy Adults, as shown in Table 1), the intervention time for each exercise intervention time is more than 30 mins (22), and the intervention period is more than 4 weeks (23); (3) Outcomes: Outcome indicators must include at least one of peak oxygen uptake and anaerobic threshold; (4) Study Design: Randomized controlled trials (RCTs).

Exclusion criteria

(1) Studies involving patients with other serious comorbidities; (2) Repeated studies (when there is an overlapping of patient populations overlapped in multiple studies, only the studies with the largest sample sizes were included).

Data extraction

The literature screening and data extraction were independently performed by two investigators (Zheng LY and Gu YM) independently. The basic information extracted includes: (1) first author, publication time and country; (2) study characteristics: including the patients' ages, sample size, type of exercise, exercise duration and frequency; (3) intervention and control measures; (4) outcome indicators; (5) the Jadad scale quality scoring.

If there are any discrepancies, a third author is consulted to resolve these differences.

Risk of bias assessment

The quality of the included studies was independently assessed by two researchers independently and scored using the Jadad scale for quality assessment of randomized controlled trials. The total score on the Jadad scale is 5. And a score

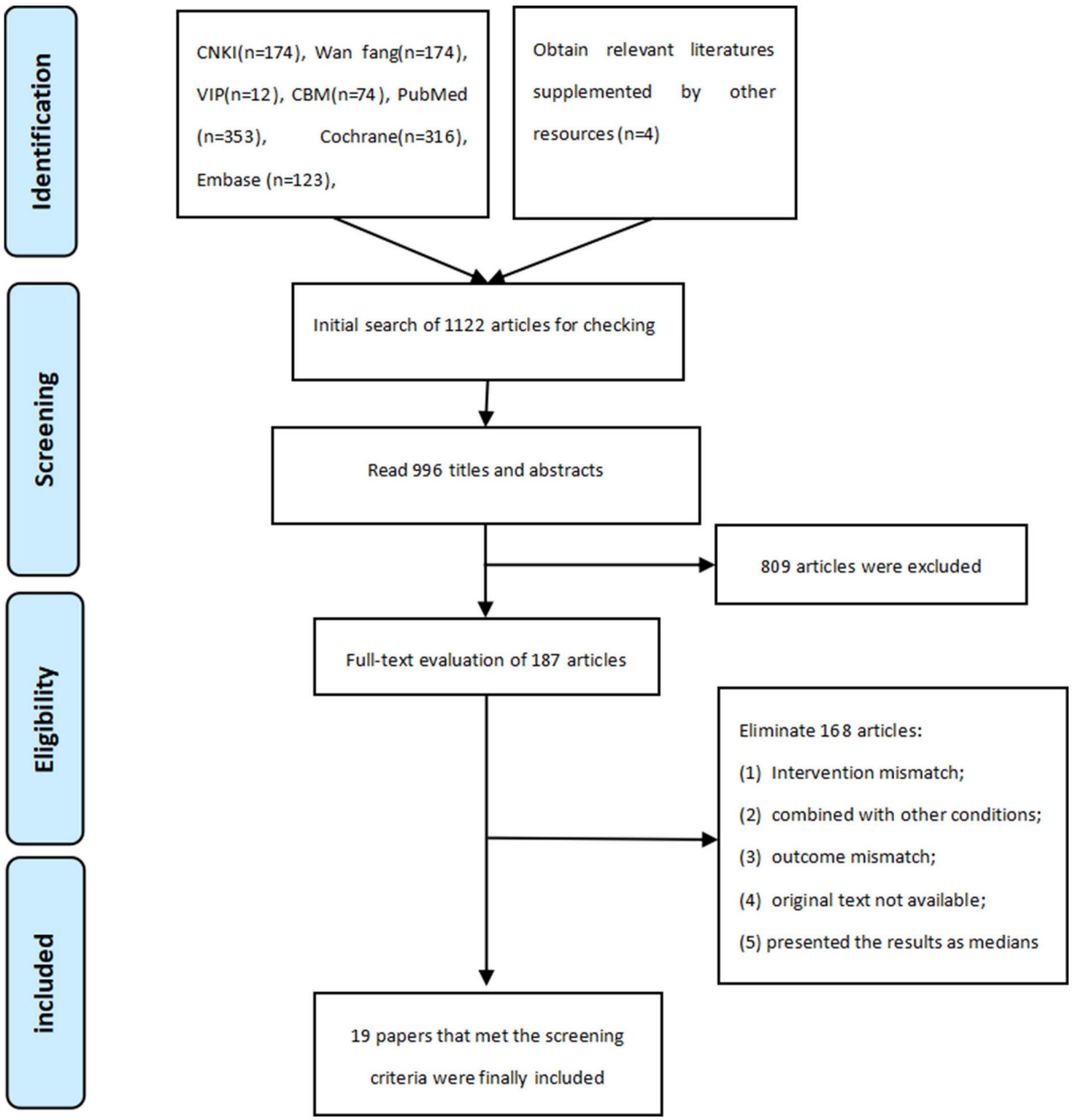

FIGURE 1
Flow diagram of literature search and selection process.

of 1–2 indicates low literature quality, while a score of 3–5 indicates high literature quality. The risk of bias for each study was assessed using the risk of bias assessment tool recommended in the Cochrane Systematic Evaluator's Manual 5.3. The evaluation indexes include seven aspects such as: the randomization method, assignment hiding, subject blindness, outcome evaluation blindness, data integrity, selective reporting and other bias. Each item was deemed to either be low risk, unknown risk and/or high risk.

Data synthesis

Data were synthesized using Review Manager Software (Version 5.3; Nordic Cochrane Center, Cochrane Collaboration). Due to the continuous nature of the extracted data, the analysis comprises mean difference (MD) and standard deviation (SD). X^2 test and I^2 statistical test were used to analyze heterogeneity. The value for high heterogeneity was set as $I^2 > 50\%$ and $P < 0.10$, and a random effects model

was used to evaluate the combined effect size of included data (24); however, if $I^2 < 50\%$ and $P > 0.10$, heterogeneity was considered small and the fixed effects model was used. In case of large heterogeneity, the sources of heterogeneity were examined through sensitivity and subgroup analyses. Publication bias was assessed using funnel plots.

Results

Study selection

Our literature search is started from the inception of the aforementioned databases to March 2022. The literature screening flow chart of literature screening is shown in Figure 1. A total of 1,225 literatures were retrieved, which comprises 434 Chinese literature and 791 English literature, and four literatures was supplemented by manual retrieval from other sources. We imported the literature into EndNote X9 and NoteExpress (both are literature management software) and eliminated 233 duplicate literature. Furthermore, 809 articles were excluded by reading the titles and abstracts, subsequently, we perused through the remaining 187 articles, and finally selected 19 articles that met the inclusion criteria (25–43).

Study characteristics

Nineteen trials were included (Table 2) involving a total of 1,036 patients which include 511 in the high-intensity exercise training group and 525 in the moderate-intensity exercise training group. 7 literatures (31, 32, 36, 38, 39, 42, 43) had an intervention period of <12 weeks, and 12 literatures (25–30, 33–35, 37, 40, 41) had an intervention period of 12 weeks or more.

Risk of bias

The quality assessment method of the 19 selected literatures is shown in Figure 2. 16 literatures (25–27, 29, 30, 32–42) described specific randomization methods, and 3 literatures (28, 31, 43) adopted a semi-randomization method combined with patients' wishes, and 5 literatures (30, 31, 37–39) implemented the allocation concealment; Considering the risk associated with exercise, patients are required to give and informed consent, so only one literature (31) implemented blind method for patients, five literatures (31, 37–39, 42) adopted the blinded outcome assessors; 13 (31–43) literatures recorded the circumstances and reasons for subjects who were lost to follow-up and dropped out of the trial. There were no selective reporting of outcome indicators, and no significant bias was identified in risk assessment.

Results of meta-analysis

Peak oxygen uptake (peak VO_2)

Peak VO_2 is reported in all the 19 included literatures (25–43) high-intensity exercise training on Peak VO_2 showed significantly better effects than that of moderate-intensity exercise training [MD = 2.67, 95% CI (2.24, 3.09), $P < 0.00001$, $I^2 = 19\%$] (Figure 3).

Anaerobic threshold

Five literatures (25–27, 29, 30) reported anaerobic threshold (AT), and the results showed that there are no statistically significant difference in the improvement effect of two types of exercise intensity on AT [MD = 0.49, 95%CI (−0.12, 1.10), $P = 0.11$, $I^2 = 18\%$] (Figure 4).

Left ventricular ejection fraction

Six articles were reported on left ventricular ejection fraction (LVEF) (25–27, 29, 30, 32), and the result shows that high-intensity exercise training has a significantly better effect on LVEF than moderate-intensity exercise training [MD = 3.60, 95% CI (2.17, 5.03), $P < 0.00001$, $I^2 = 0\%$] (Figure 5).

Exercise duration

Seven literatures (25–28, 36, 38, 39) reported exercise duration (ED). The result shows that high-intensity exercise has significant effects on ED compared to moderate-intensity exercise training [MD = 37.51, 95%CI (34.02, 41.00), $P < 0.00001$, $I^2 = 10\%$] (Figure 6).

Respiratory exchange ratio

Ten studies (31, 32, 34, 36–42) reported respiratory exchange ratio (RER) with little heterogeneity. The result shows that there is no significant difference in the improvement effect of the two exercise intensities on RER [MD = 0.00, 95%CI (−0.01, 0.02), $P = 0.56$, $I^2 = 33\%$] (Figure 7).

Resting heart rate

Nine literatures (30–32, 34–37, 39, 42) reported resting heart rate (RHR), and the results shows that there is no significant difference in the improvement effect of RHR between the two exercise intensities [MD = 1.10, 95% CI (−0.43, 2.63), $P = 0.16$, $I^2 = 0\%$] (Figure 8).

Peak heart rate

Twelve studies (28, 30, 31, 34–42) reported peak heart rate (PHR). The result showed that high-intensity exercise training

TABLE 2 Characteristics of included studies.

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
Luan et al. (25)	2018	China	41	41	T:34/7 C:32/9	59.73 ± 7.9	59.67 ± 7.93	Power bikes	12 weeks, 3 times/week	(1) The initial exercise load was 60% of the peak power in cardiopulmonary exercise test, and the adaptive training lasted for 1 week, 3 times per week; (2) 80% of the peak power was used for treadmill exercise, which was carried out in interval training mode (3-min training, 1-min rest), 10 sets per time, a total of 40 mins; 3 times per week	The exercise load was 60% of the peak power in cardiopulmonary exercise test, 40 min/time, 3 times per week	1, 2, 3, 4	0
Ju et al. (26)	2018	China	25	25	–	56.64 ± 9.86	56.64 ± 9.86	Power bikes	12 weeks, 3 times/week	80% of the peak power was used for treadmill exercise, which was carried out in interval training mode (3-minute training, 1-minute rest), 10 sets per time, a total of 40 mins; 3 times per week	60% of PP for exercise load power treadmill exercise, intermittent training mode (3-min training, 1-min rest), 10 groups/time, a total of 40 mins; 3 times per week	1, 2, 3, 4	2
Gao et al. (27)	2015	China	22	21	T:16/5 C:18/4	59.4 ± 7.9	61.2 ± 8.0	Power bikes	12 weeks, 3 times/week	80% of PP for exercise load power treadmill exercise, intermittent training mode (3-min training, 1-min rest), 10 groups/time, a total of 40 mins; 3 times per week	60% of PP for exercise load power treadmill exercise, intermittent training mode (3-min training, 1-min rest), 10 groups/time, a total of 40 mins; 3 times per week	1, 2, 3, 4	2

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
Wang et al. (28)	2010	China	22	27	T:15/7 C:18/9	65.5 ± 6.9	68.7 ± 7.0	Tai ji /Jogging	24 weeks	Jogging, exercise intensity ≥ 70% VO_{2max}, available for 4 weeks to gradually reach that exercise intensity	Tai ji practice 40 min per day, 5 d per week, exercise time about 200 min per week	1, 4, 7, 8	0
Zhang et al. (29)	2022	China	22	21	T:16/6 C:13/8	58.1 ± 13.61	62.10 ± 10.24	Power bikes	12 weeks, 3 times a week	80% of the peak power was used for treadmill exercise, which was carried out in interval training mode (3-minute training, 2-min rest), 40 mins in total; 3 times per week	60% of PP for exercise load power treadmill exercise, intermittent training mode a total of 40 mins; 3 times per week	1, 2, 3	2
Gu et al. (30)	2020	China	23	26	T:15/8 C:17/9	64.1 ± 9.2	66.5 ± 7.8	Power bikes	12 weeks, 5 times a week	(1) 70% of the peak power (PP) was used as exercise load for power treadmill training, starting with 0 W power, warming up for 3 min without power, and then increasing with a certain load range, so that patients could reach the target power within 8–10 min. During the whole process, the patient maintained a rotational speed of 55–65 r/min for treadmill exercise until reaching 70% of the maximum power assessed by the patient;	(1) 50% of the peak power is used as the exercise load for power treadmill training, resting for 5 min, starting at 0 W, warming up for 3 min without power, and then with a certain amount (specific power varies from person to person, ensuring 8–10 min to reach the target power), patients performed treadmill exercise at a rotational speed of 55–65 r/min until 50% of the maximum power assessed by the patient	1, 2, 3, 6, 7	2

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
										(2) 70% of the maximum power is used as a constant power treadmill until the 25th minute, and the last 5 mins of recovery time. A total of 30 mins, 5 times a week	power as the treadmill of constant power until the 25th minute, the last 5 mins to resume, a total of 30 mins, 5 times a week training was achieved; (2) Take 50% of the maximum		
Rognmo et al. (31)	2004	Norway	8	9	T:6/2 C:8/1	62.9 ± 11.2	61.2 ± 7.3	Treadmill	10 weeks, 3 times a week	A total of 33 min: (1) 5-min warm-up period at an intensity corresponding to 50–60% of VO_2peak (65–75% of HRpeak); (2) walking four intervals of 4 min at 80–90% of VO_2peak (85–95% of HRpeak),the intervals 3 min of walking at 50–60% of VO_2peak	41 mins continuous exercise at an intensity of 50–60% of VO_{2peak}, representing the same total training load as the high intensity aerobic exercise group	1, 5, 6, 7	5
Moholdt et al. (32)	2009	Norway	28	31	T:24/4 C:24/7	60.2 ± 6.9	62.0 ± 7.6	Treadmill	4 weeks, 5 times/week	Total time 38 mins: (1) 8 mins warm-up; (2) 4 times of 4-min intervals with HR at 90% of maximum HR, with active pauses of 3 mins of walking at the exercise session was terminated by 5 mins cool-down	Walked continuously at 70% of maximum HR for 46 mins to ensure isoenergetic training protocols	1, 3, 5, 6	4

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
Benetti et al. (33)	2010	Brazil	29	29	-	57.7 ± 6.1	57.7 ± 6.1	Treadmill	12 weeks, 5 times a week	Total time 45 mins: (1) patients exercised at around 85% of their maximum heart rate (HR) achieved in the stress test.	Total time 45 mins: patients exercised at a ∼75% of their HRmax.	1	2
Conraads et al. (34)	2015	Belgium	100	100	T:91/9 C:89/11	57.0 ± 8.8	59.9 ± 9.2	Treadmill and bicycle	12 weeks, 3 times per week	Total time 38 min: (1) 60–70% peak HR for 10 min warm-up, followed by 4 × 4 min training at 85–95% peak HR; (2) 50–70% peak active interval training was performed at an interval of 4 × 3 min performed at an interval of 4 × 3 min	Total time: 5 min warm-up at 60–70% Peak HR, followed by 37 min continuous training at 70–75% Peak HR and 5 min relaxation at 60–70% peakHR	1, 5, 6, 7, 8	2
Katharine et al. (35)	2014	United States	15	13	–	60 ± 7	58 ± 9	Treadmill	24 weeks	(1) 5-min period of active warm-up; (2) 3-min period of training at 60–70% of heart rate reserve, (3)4 higher-intensity work A 3-min recovery period set at an intensity of 60–70% of heart rate reserve followed each of the 4 higher-intensity work intervals of 4 mins each, set at an intensity corresponding to 80–90% of heart rate	A 5-min period of active warm-up, 30 mins of cardiorespiratory training, and 5 mins of active cool-down. The exercise intensity was prescribed at 60–80% of heart rate reserve throughout	1, 6, 7	2

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
										reserve. A 3-minute recovery period set at an intensity of 60–70% of heart rate reserve followed each of the four higher-intensity intervals.			
Koldobika et al. (36)	2016	Spain	36	36	–	58 ± 11	58 ± 11	Power bikes	8 weeks	Total time: 40 min: (1) 5–12 min warm up (25% PeakWR); (2) (15–30 groups) × 20 s interval (120–125% PeakWR); (3) (15–30 groups) × 40 s rest (25% PeakWR); (4) 5–13 min relaxation (25% PeakWR)	Total time of use: 40 min: (1) 5–12 min heat body; (2) 15–30 min continuous training (VT1∼VT1+10%);Relax for 5–13 mins	1, 4, 5, 6, 7	4
Lee et al. (37)	2019	Canada	17	14	Female	69.3 ± 9.9	69.6 ± 5.9	Walking/ Jogging	24 weeks, 5 times a week	(1) A warm-up period of 5–10 min of walking performed at 60–70% of Peak HR, and/or RPE of 10–12 on the 6–20 Borg Scale; (2) four 4-min intervals of walking/jogging performed at an intensity targeting 90–95% of Peak HR, and/or RPE ≥17 on the Borg Scale, interspersed with 3 min of active recovery performed at an intensity of 50–70%	Jog for about 30–40 mins at 60–80% of peak oxygen uptake, with a warm up and rest period	1, 5, 6, 7	5

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
										of Peak HR; (3) a cool-down period of 5 min of walking performed at an intensity of 50–70% of Peak HR, and/or RPE ∼10–12 on the Borg Scale.			
Koldobika et al. (38)	2019	Spain	57	53	T:50/7 C:42/11	57.6 ± 9.8	58.3 ± 9.5	Power Bikes	8 weeks, 3 times a week	Total time: 40 min: (1) 5–12 min warm up (25% PeakWR); (2) (15–30 groups) × 20 s interval (120–125% PeakWR); (3) (15–30 groups) × 40 s rest (25% PeakWR); (4) 5–13 min relaxation (25% PeakWR)	Total time of use: 40 min: (1) 5–12 min heat body; (2) 15–30 min continuous training (VT1∼VT1+10%); (3) relax for 5–13 mins	1, 4, 5, 7	4
Keteyian et al. (39)	2014	United States	15	13	T:11/4 C:12/1	60.0 ± 7.0	58.0 ± 9.0	Treadmill	4 weeks, 5 times a week	(1) 5-min period of active warm-up, (2) 3-min period of training at 60–70% of heart rate reserve and then 4 higher-intensity work a 3-min recovery period set at an intensity of 60–70% of heart rate reserve followed each of the 4 higher-intensity work intervals of 4 mins each, set at an intensity corresponding to	5-min period of active warm-up, 30 min of cardiorespiratory training, exercise intensity was prescribed at 60–80% of heart rate reserve throughout, and 5 mins of active cool-down	1, 4, 5, 6, 7, 8	5

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators	Quality score
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
										80–90% of heart rate reserve. (3) 3-min recovery period set at an intensity of 60–70% of heart rate reserve followed each of the 4 higher intensity intervals.			
Currie et al. (40)	2013	Canada	11	10	–	62 ± 11	68 ± 8	Power bikes	12 weeks, 3 times/week	Total time of use: 30 min: (1) 5 min heat body; (2) 10 × 1 min interval (89–102–110% PeakWR); (3) 10×1 min rest (10% peakWR); (4) relax in 5 mins	Total use time: 30–50 mins: (1) 5 min heat body; (2) continued training for 30–50 min (58%PeakWR); (3) relax for 5 mins	1, 5, 7	3
Cardozo et al. (41)	2015	Brazil	23	24	–	56 ± 12	62 ± 12	Treadmill	16 weeks, 3 times/week	Total time: 40 min: (1) 5 min warm-up; (2) 8 × 2 min interval (90% HRmax); (3) 7×2 min rest (60% HRmax); (4) relax in 5 mins	Total duration: 40 min (1) 5 mins heat body; (2) 30 mins continued training (70–75.0% HRmax); (3) relax for 5 mins	1, 5, 7, 8	3
Kim et al. (42)	2015	Korea	14	14	T:12/2 C:10/4	60.0 ± 13.7	57.0 ± 11.9	Treadmill	6 weeks, 3 per week times	A total of 45 mins: (1) 10-min warm-up at 50–70% of HRR, (2) four times of 4-min intervals of walking on a treadmill at 85–95% of HRR with three active pauses of 3-min walking at 50–70% of HRR, and a 10-min cooldown at 50–70% of HRR	A total of 45 mins: (1) 10-min warm-up, (2) 25-min walk on a treadmill continuously at 70–85 % of HRR and a 10-min cooldown	1, 5, 6, 7	2

(Continued)

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TABLE 2 (Continued)

References	Publish time	Country	Sample size		Gender	Age		Type of movement	Exercise time	Exercise prescription		Closing indicators score	Quality
			Test group	Control group	(Male/ Female)	Test group	Control group			Test group	Control group		
Gremeaux et al. (43)	2014	France	9	10	T:7/2 C:7/3	59.2 ± 8.1	59 ± 7.4	Treadmill and bicycle	7 weeks, 3 times a week	(1) 5 mins at 50% of the graded maximal exercise test maximal HR; (2) patients performed one set of 18 mins composed of repeated phases of 3 consecutive 6-min; (3) patients had a 3-min cool down period	(1) Patients performed a 5-min warm-up at 50% of the maximal HR measured; (2) a continuous workout at 70% of the maximal HR measured on the graded maximal exercise test for 18 mins, followed by 3 mins of active recovery	1	0

(1) Peak oxygen uptake (Peak VO_2); (2) anaerobic threshold (AT); (3) left ventricular ejection fraction (LVEF); (4) exercises duration (ED); (5) respiratory exchange ratio (RER); (6) resting heart rate (RHR); (7) peak heart rate (PHR); (8) oxygen pulse (O_2 pulse).

has a significantly effect on PHR compared with moderate-intensity exercise training [MD = 6.86, 95% CI (4.49, 9.24), $P < 0.00001$, $I^2 = 0\%$] (Figure 9A).

Oxygen pulse

O_2 pulse is reported in 4 literatures (28, 34, 39, 41). The result shows that high-intensity exercise training has a significant improvement on O_2 pulse compared to moderate-intensity exercise training [MD = 0.97, 95%CI (0.34, 1.60), $P = 0.003$, $I^2 = 17\%$] (Figure 9B).

Subgroup analysis

Subgroup analysis was performed based on the duration of the intervention. We set 12 weeks as the boundary (one group was <12 weeks, the other group was ≥12 weeks). The results of subgroup analysis showed no significant difference in the influence of intervention time on Peak VO_2, RER and PHR, and the results of subgroup analysis were consistent with the results of overall analysis (Figure 10). Interestingly, the subgroup analysis of ED showed no statistically significant difference when intervention duration was <12 weeks [MD = 5.56, 95%CI (−30.23, 41.36), $P = 0.76$]. However, when intervention time was ≥12 weeks, the difference was statistically significant [MD = 37.82, 95%CI (34.31, 41.33), $P < 0.00001$] (Figure 11A). Subgroup analysis of RHR showed that the difference was statistically significant when intervention time was <12 weeks, and high-intensity was better than moderate-intensity [MD = 3.26, 95%CI (0.73, 5.78), $P = 0.01$]. However, there was no significant difference when intervention time was ≥12 weeks [MD = −0.14, 95%CI (−2.06, 1.78), $P = 0.89$] (Figure 11B).

Publication bias

The funnel plot (Figure 12) was generated to reflect the publication bias. It can be seen from the figure that the distribution of all included studies is relatively concentrated and basically all data are located in the funnel plot. Therefore the possibility of publication bias of this study is small.

Sensitivity analysis

We performed sensitivity analysis to evaluate the influence of any individual study on the overall effect. For AT, the difference was statistically significant when Zhang et al. (29) was removed, and high-intensity was better than moderate-intensity [MD = 1.12, 95%CI (0.19, 2.06), $P = 0.02$, $I^2 = 0\%$]. For RHR, the difference was statistically significant when Conraads et al. (34) was removed, and high-intensity group showed slightly better than moderate-intensity group [MD = 2.10, 95%CI (0.13, 4.07), $P = 0.04$,

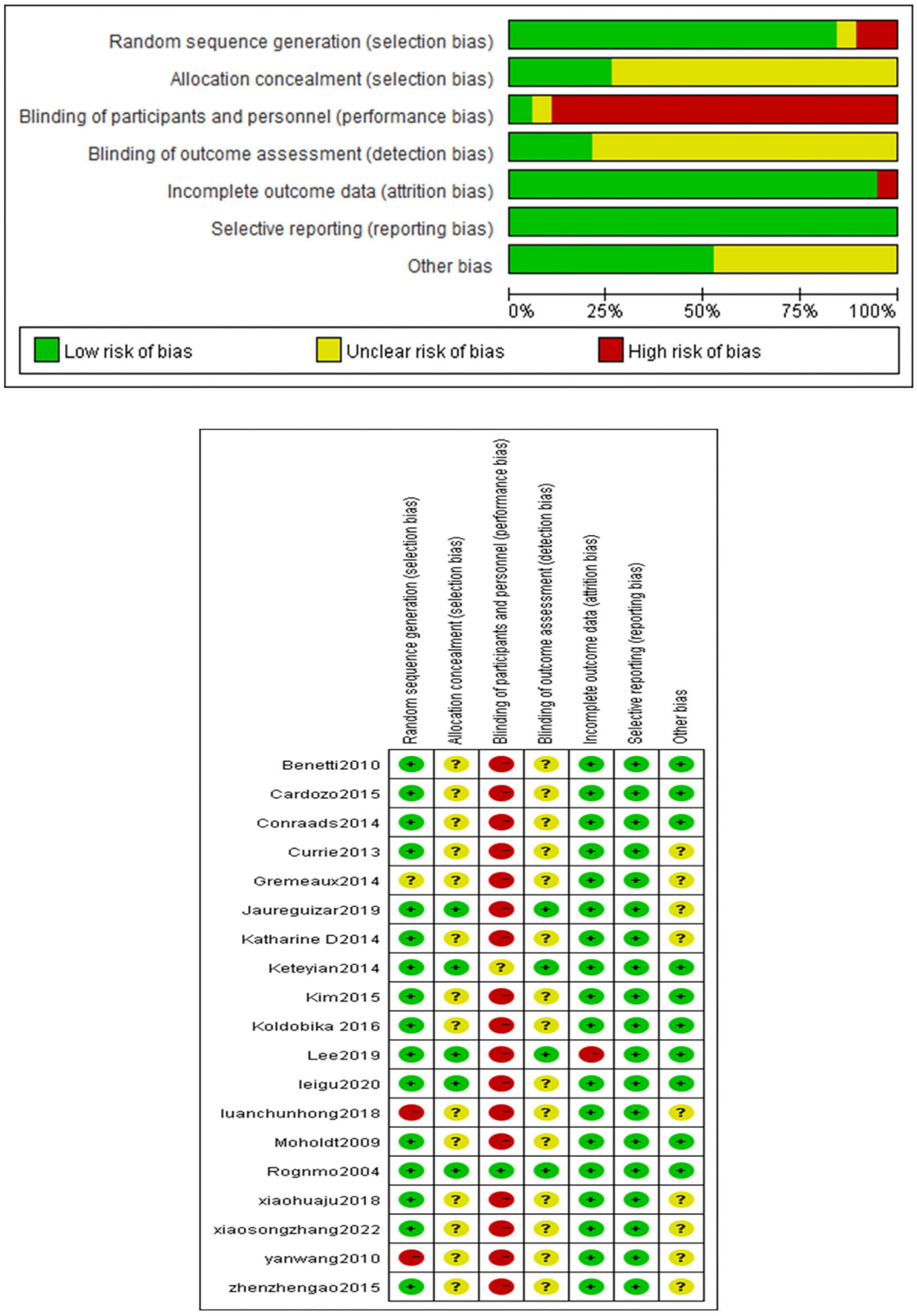

FIGURE 2
(A) Overall analysis diagram. **(B)** Single literature analysis diagram. "+" is low risk. "?" is unclear, and "−" is high risk.

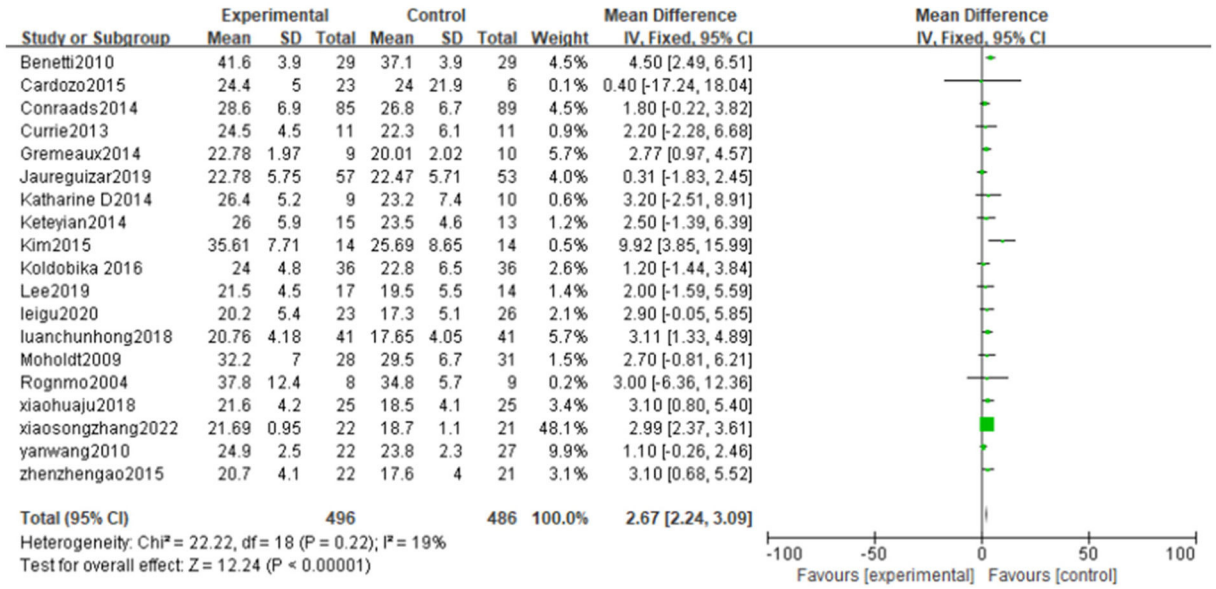

FIGURE 3
Forest plot comparing the improvement of peak VO_2 between two exercise intensity.

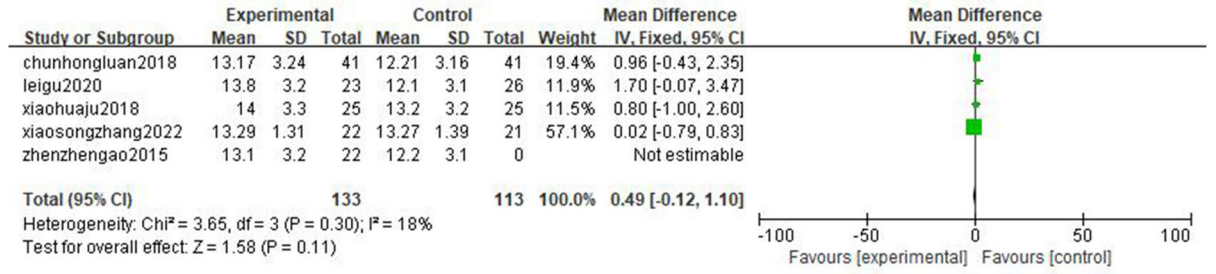

FIGURE 4
Forest plot comparing the improvement of AT between two exercise modes.

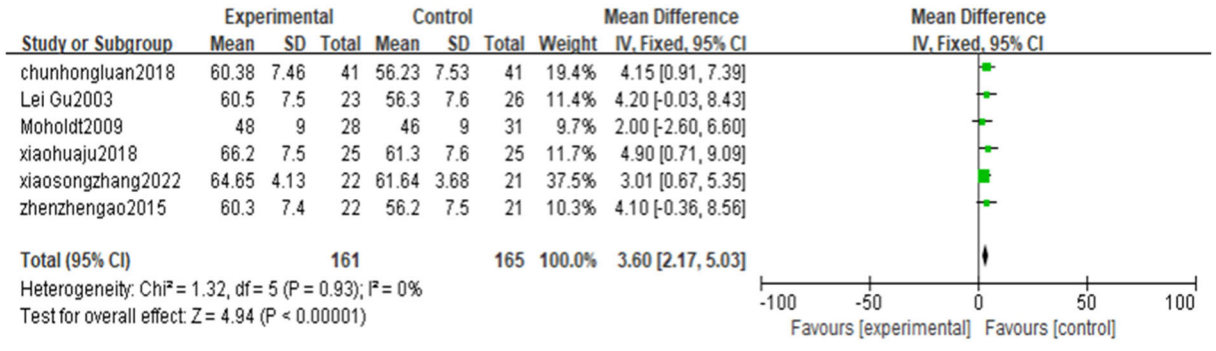

FIGURE 5
Forest plot of the effects of high-intensity and moderate-intensity exercise on LVEF in CAD patients.

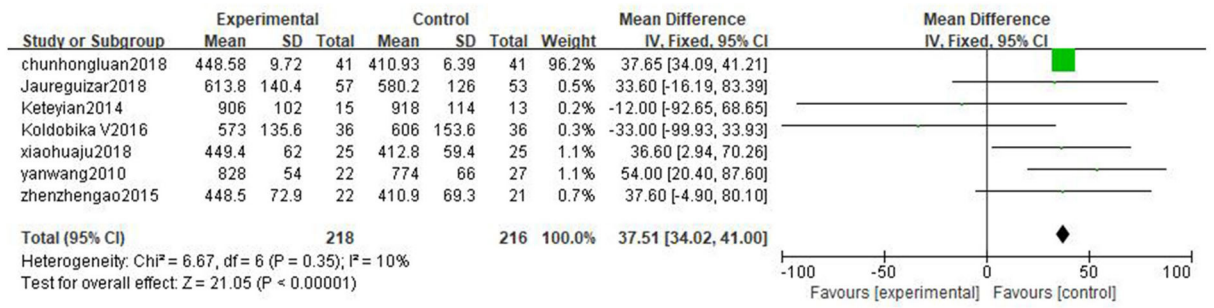

FIGURE 6
Forest plot comparing the effects of two exercise modes on ED improvement.

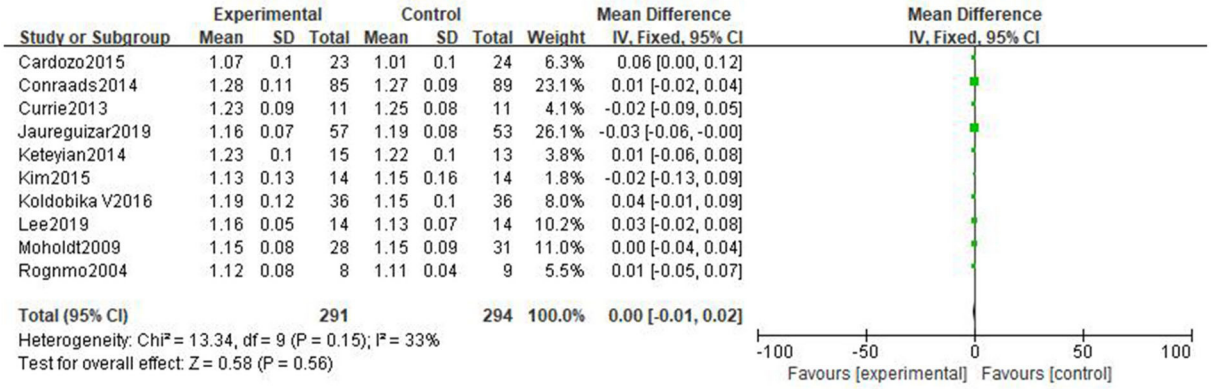

FIGURE 7
Forest plot comparing the effects of two exercise modes on RER improvement.

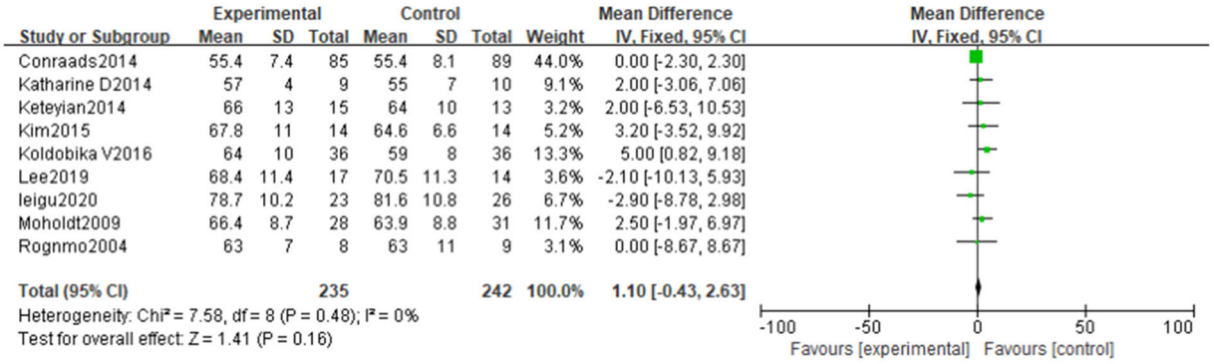

FIGURE 8
Forest plot of the effects of high-intensity and moderate-intensity exercise on RHR in CAD patients.

I^2 = 0%]. However, there is no significant difference in the improvement effect of the two exercise intensities on O_2 pulse when Wang et al. (28) was removed [MD = 0.49, 95%CI (−0.38, 1.36), $P = 0.27$, $I^2 = 0\%$].

GRADE assessment

Table 3 shows the GRADE assessment of the certainty on the effect of exercise intensity in patients with CAD. Peak

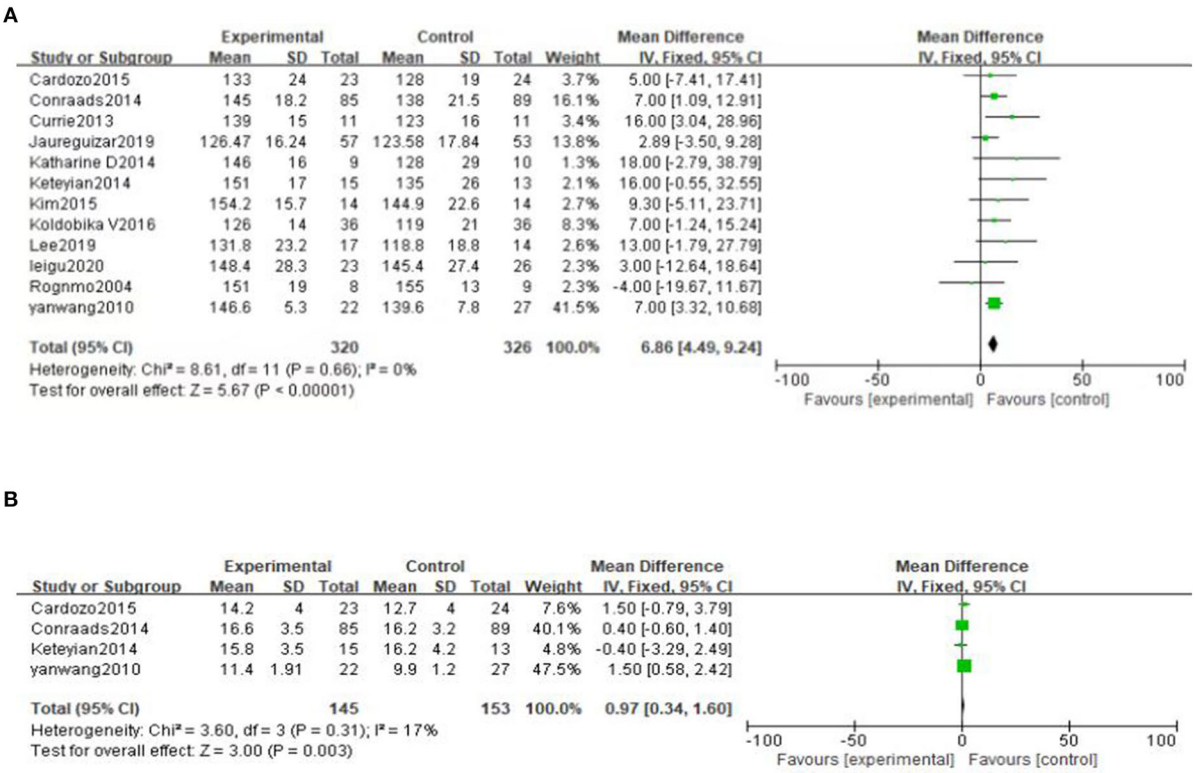

FIGURE 9
(A) Forest plot of the effects of high-intensity and moderate-intensity exercise on PHR in CAD patients. **(B)** Forest plot comparing the improvement effect of two exercise modes on O_2 pulse.

VO_2, AT, LVEF, and RER were rated as moderate, while ED, RHR, PHR, and O_2 pulse were rated as low-quality evidence. Reasons for downgrading: (1) For the risk of bias, only three literatures (28, 31, 43) refer to patients' wishes using a semi-randomized method; most studies do not specify whether allocation concealment is implemented; Considering the risk associated with exercise, patients are required to give and informed consent, so only one literature (31) implemented blind method for patients. (2) In terms of inconsistency: the results of two studies (36, 39) were inconsistent for the effect of exercise intensity on ED. For RHR, two studies (30, 37) had inconsistent results. For PHR, there was one study (31) with different results. For O_2 pulse, one study (39) showed different results from others. And these may be due to differences in study population, gender, and duration of intervention.

Discussion

Summary of the evidence

In this study, we selected 19 RCTs with a total of 1,036 patients. Our results show that compared with moderate-intensity exercise training, high-intensity exercise training has better improvement effects on Peak VO_2, LVEF, ED, PHR and O_2 pulse in patients with CAD. However, there are no significant differences in the effects of AT, RER and RHR. Furthermore, our subgroup analysis showed that there is no statistical difference in the influence of intervention time on peak VO_2, RER and PHR. The effect of exercise intensity on ED and RHR was influenced by the intervention time. For the ED outcome, high-intensity exercise was superior to moderate-intensity exercise only when the intervention time was $\geq$12 weeks. For the outcome of RHR, when the intervention time was $<$12 weeks, high-intensity exercise has better improvement effects than moderate-intensity exercise. But when the intervention time was $\geq$12 weeks, the difference was not statistically significant.

Results in relation to other studies

There were some relevant meta-analysis articles published lately, such as the recently published one by Gomes-Neto (44). However, the retrieval deadline of the study mentioned above was in November 2016. Only 12 literatures were selected in that study and the efficacy indicators of

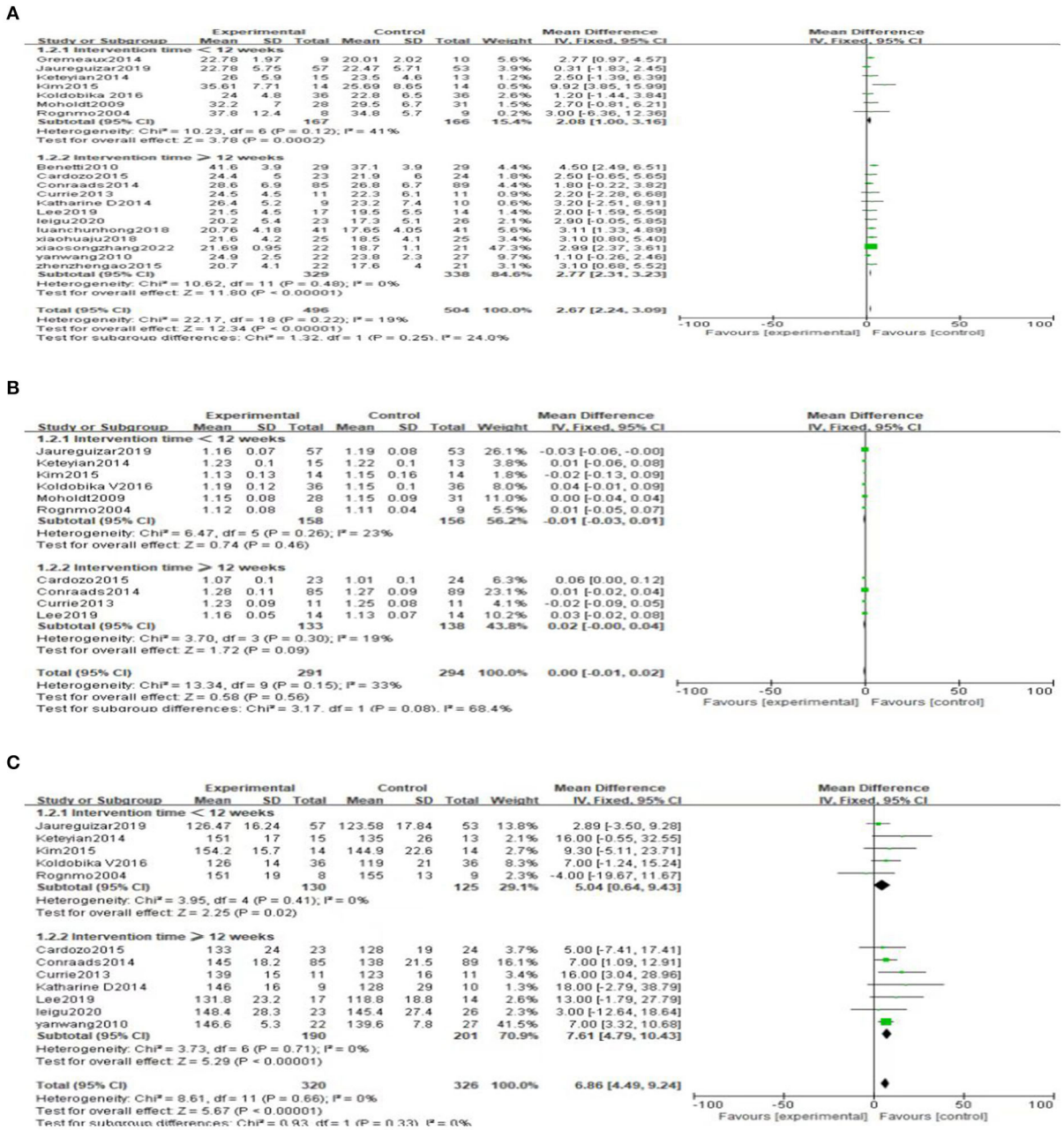

FIGURE 10
(A) Subgroup analysis of peak VO_2. **(B)** Subgroup analysis of RER. **(C)** Subgroup analysis of PHR.

cardiopulmonary function were very limited (only peak VO_2). On the contrary, our study screened all qualified literature from the establishment of the relevant databases to April 2022, and 19 RCTs were selected. We also analyzed more indicators related to cardiopulmonary function (peak VO_2, AT, LVEF, ED, RER, RHR, PHR, and O_2 pulse). Hence, our study provided more robust and comprehensive evidence for evaluating the effect of moderate and high exercise intensity on cardiopulmonary function.

Potential mechanism

The underlying mechanism of high-intensity exercise improving peak VO_2 in patients with CAD may be related to the fact that high-intensity exercise can stimulate muscle vascularization, improve blood circulation, and enhance blood oxygen-carrying capacity (45); In addition, studies (46) have shown that higher intensity exercise can stimulate the pumping capacity of the heart to a greater extent, increase blood flow,

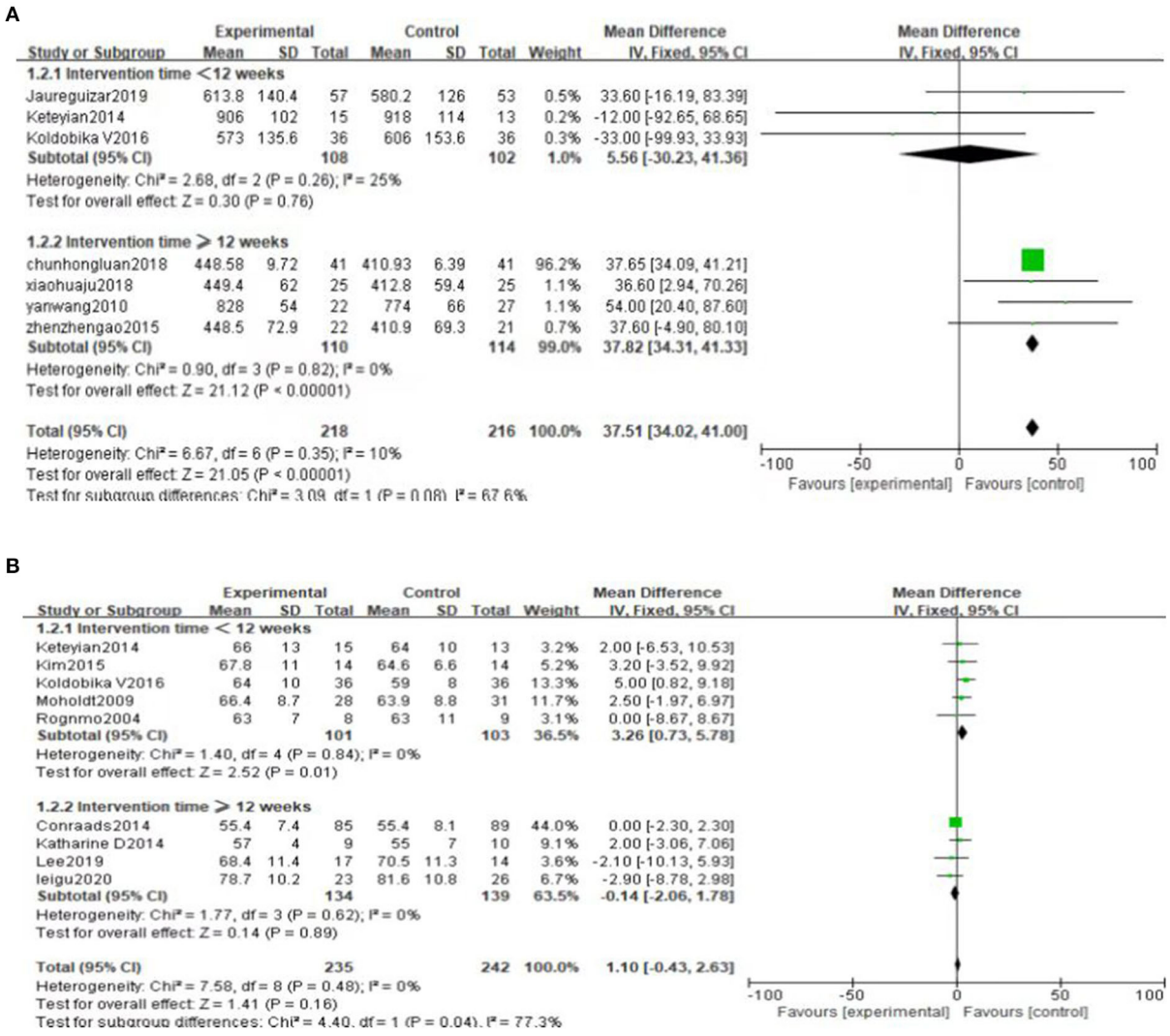

FIGURE 11
(A) Subgroup analysis of ED. **(B)** Subgroup analysis of RHR.

increase endothelial shear stress, activate endothelial nitric oxide synthase, and increase antioxidant status. Thus, nitric oxide synthesis is improved and its bioavailability is increased, which consequently improves the vascular endothelial function (47–49). High-intensity exercise can also increase oxisome proliferator-activated receptor γ coactivator 1α (a regulator of mitochondrial biogenesis) to improve mitochondrial function and enhance rapid adaptation and metabolic capacity in skeletal muscles (50). Therefore, high-intensity exercise is more effective in increasing Peak VO_2 than moderate-intensity exercise training. LVEF is a major indicator of the pumping capacity of the heart. An elevated LVEF level indicates improved cardiac function. The mechanism of which exercise intensity improves LVEF is not clear, but it may be related to the ability of higher intensity exercise reduces left ventricular end-diastolic volume (EDV) and left ventricular end-systolic volume (ESV), improves ventricular remodeling and myocardial contractility. The PHR improvement in the high-intensity exercise group is better than that in the moderate-intensity exercise group, which can be attribute to the fact that higher exercise intensity can increase stroke volume, enhance myocardial contractility, increase the ejection fraction during extreme exercise, and improve exercise tolerance (51). ED is the duration of exercise from the beginning to the end of the evaluation in the Cardiopulmonary Exercise Test (CPET). The overall analysis showed that high-intensity exercise prolongs ED better than moderate-intensity exercise. However, our subgroup analysis results shows that when ≥12 weeks, the duration of high-intensity continuous exercise is longer than that of moderate-intensity. This implies that high-intensity exercise results in a better improvement

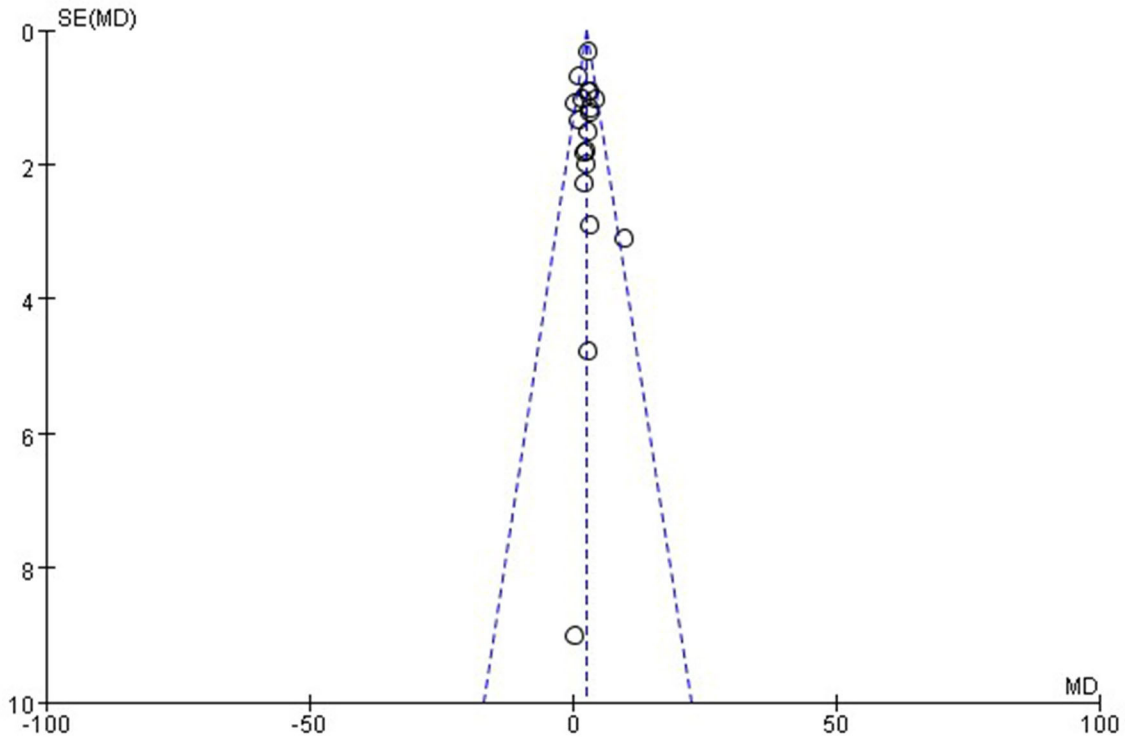

FIGURE 12
Funnel chart of publication bias.

in the exercise endurance of patients over time. Since PHR is affected by multiple factors such as age, gender, body size, muscle volume, daily activity level and exercise type (52), their specific mechanisms need to be further explored through more detailed and high-quality clinical trials. High-intensity exercise training is beneficial to anaerobic glycolysis and increases the lactic acid content in the blood. Lactate is converted back to glucose in the liver, thus AT is related to the gluconeogenic capacity of the liver. An experimental study in animal has shown that high-intensity exercise training can enhance the hepatic gluconeogenesis of lactate and increase the lactate threshold (53). However, this study shows that there is no significant difference in the effect of exercise intensity on AT, which may be due to the difference in lactic acid metabolism of human bodies. In addition, the concept of AT is still controversial, and the calculation methods, equipment used and detection personnel under various concepts will also have a significant impact on the results (54). RER expresses the relationship between carbon dioxide produced (CO_2) and oxygen consumed (O_2) which can be used to determine the rate of lipid oxidation (55). Studies showed that a reduction in the levels of RER levels after high-intensity exercise compared to moderate-intensity exercise (56, 57). However, our study did not find differences in the effect of exercise intensity on RER, which may be due to the lack of consideration for the effect of related gene expression on RER. Furthermore, some studies found that differences in exercise performance and muscle metabolic activity are associated with ACTN3 gene polymorphisms (58–60). RER decreases in subjects with only X allele after high-intensity exercise training, while there is no significant change in RER in RR homozygous subjects (61). High-intensity exercise has an advantage over moderate-intensity exercise in reducing RHR (62). And our study found that when the intervention time is $<$12 weeks, the RHR of high-intensity is slower than that of moderate-intensity, but there is no difference in intensity between the two exercises when it is $\geq$12 weeks. This may be attribute to the fact that high-intensity exercise is better at slowing PHR for a short period of time, but when patients keep exercising for longer time, the advantage of high-intensity exercise disappears.

Limitations

Due to time and funding constraints, most of the current studies only observed the impact of exercise training on cardiopulmonary function indicators in patients. But there are few reports regarding long-term follow-up and clinical endpoint events as the salient endpoint indicators, which offers us a direction worthy of in-depth research in the future; The 19 studies adopted different exercise programs, and there are

TABLE 3 Grading of Recommendations Assessment, Development, and Evaluation (GRADE) assessment.

Quality assessment							No of patients		Effect		Quality	Importance
No. of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High-intensity vs. moderate-intensity	Control	Relative (95% CI)	Absolute		
Peak oxygen uptake (better indicated by lower values)												
19	Randomized trials	Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	None[b]	496	486	–	MD 2.67 higher (2.24–3.09 higher)	⊕⊕⊕◯ Moderate	Critical
Anaerobic threshold (better indicated by lower values)												
5	Randomized trials	Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	None	133	113	–	MD 0.49 higher (0.12 lower to 1.1 higher)	⊕⊕⊕◯ Moderate	Critical
Left ventricular ejection fraction (better indicated by lower values)												
7	Randomized trials	Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	None	169	174	–	MD 3.7 higher (2.28 to 5.11 higher)	⊕⊕⊕◯ Moderate	Important
Exercises duration (better indicated by lower values)												
7	Randomized trials	Serious[a]	Serious[c]	No serious indirectness	No serious imprecision	None	218	216	–	MD 37.51 higher (34.02 to 41 higher)	⊕⊕ Low	Important

(Continued)

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TABLE 3 (Continued)

Quality assessment							No of patients		Effect		Quality	Importance
No. of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High-intensity vs. moderate-intensity	Control	Relative (95% CI)	Absolute		
RER (better indicated by lower values)												
10	Randomized trials	Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	None	291	294	–	MD 0 higher (0.01 lower to 0.02 higher)	⊕⊕⊕◯ Moderate	Important
Resting heart rate (better indicated by lower values)												
10	Randomized trials	Serious[a]	Serious[d]	No serious indirectness	No serious imprecision	None	243	251	–	MD 1.21 higher (0.28 lower to 2.71 higher)	⊕⊕ Low	Important
Peak heart rate (better indicated by lower values)												
12	Randomized trials	Serious[a]	Serious[e]	No serious indirectness	No serious imprecision	None	320	326	–	MD 6.86 higher (4.49 to 9.24 higher)	⊕⊕◯◯ Low	Important
Oxygen pulse (better indicated by lower values)												
4	Randomized trials	Serious[a]	Serious[f]	No serious indirectness	No serious imprecision	None	145	153	–	MD 0.97 higher (0.34 to 1.6 higher)	⊕⊕ Low	Important

[a]The included studies were biased in terms of allocation concealment and blinding.
[b]The results of the included studies were highly consistent.
[c]The results of the two studies were inconsistent.
[d]The results of the two studies were inconsistent.
[e]The results of the one studies were inconsistent.
[f]The results of the one studies were inconsistent.
The ⊕ symbol indicates the level of evidence quality. ⊕⊕⊕⊕ indicates the high level of evidence quality. ⊕⊕⊕ indicates the moderate level of evidence quality. ⊕⊕ indicates the low level of evidence quality. ⊕ indicates the very low level of evidence quality.

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differences in race, gender and age among the subjects. In addition, exercise time (morning/ afternoon/ evening) and the total calorie consumption during exercise was not uniform, which may be responsible for the bias in our study. And there were large differences in the quality of the literature and the sample size of each study.

Future directions

Due to the limitation of the sample size of the included studies, for patients with severe disease or patients with multi-vessel coronary artery disease, whether it is possible to continue to recommend high-intensity exercise programs is an interesting direction for future research. With the extension of exercise time, there are special changes in cardiopulmonary function indicators, which also provides an interesting direction for the design of future research duration. Given the limitations mentioned above, further high-quality studies are still needed to provide more reliable and higher-level evidence-based evidence on this subject matter in the future.

Conclusion

Compared with moderate-intensity exercise training, high-intensity exercise training is more effective in improving peak VO_2, LVEF, ED, PHR and, O_2 pulse in patients with CAD. Nonetheless, there is no statistical difference in the effects of the two exercise intensities on AT, RER, and RHR. Among them, high-intensity exercise did not show an advantage in prolonging ED until intervention time reached 12 weeks. Also, high-intensity exercise is better at slowing RHR within 12 weeks, but this advantage disappeared with increased exercise duration.

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

LZ, DP, and YG designed the study and assessed the risk of bias. LZ and DP analyzed the data and wrote the first and revised version of the manuscript. RW and YW screened and extracted the data. LZ, DP, and MX modified the final manuscript. All authors read and approved the final manuscript, contributed to the conceptualization of the research questions, interpretation of the results, and article writing.

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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.

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References

1. Members WG, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. *Circulation.* (2016) 133:e38–360. doi: 10.1161/CIR.0000000000000350

2. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol.* (2017) 70:1–25. doi: 10.1016/j.jacc.2017.04.052

3. Prabhakaran D, Jeemon P, Sharma M, Roth GA, Johnson C, Harikrishnan S, et al. The changing patterns of cardiovascular diseases and their risk factors in the states of India: the Global Burden of Disease Study 1990–2016. *Lancet Glob Health.* (2018) 6:e1339–51. doi: 10.1016/S2214-109X(18)30407-8

4. Ambrosetti M, Abreu A, Corrà U, Davos CH, Hansen D, Frederix I, et al. Secondary Prevention through Comprehensive Cardiovascular Rehabilitation: From Knowledge to Implementation. 2020 Update. A Position Paper from the Secondary Prevention and Rehabilitation Section of the European Association of Preventive Cardiology. *Eur J Prev Cardiol.* (2021) 28:460–95. doi: 10.1177/2047487320913379

5. Grace F, Herbert P, Elliott AD, Richards J, Beaumont A, Sculthorpe NF. High Intensity interval training (HIIT) improves resting blood pressure, metabolic (Met) capacity and heart rate reserve without compromising cardiac function in sedentary aging men. *Exp Gerontol.* (2018) 109:75–81. doi: 10.1016/j.exger.2017.05.010

6. Higgins TP, Baker MD, Evans SA, Adams RA, Cobbold C. Heterogeneous responses of personalised high intensity interval training on type 2 diabetes mellitus and cardiovascular disease risk in young healthy adults. *Clin Hemorheol Microcirc.* (2015) 59:365–77. doi: 10.3233/CH-141857

7. Maillard F, Pereira B, Boisseau N. Effect of high-intensity interval training on total, abdominal and visceral fat mass: a meta-analysis. *Sports Med (Auckland, NZ).* (2018) 48:269–88. doi: 10.1007/s40279-017-0807-y

8. Kessler HS, Sisson SB, Short KR. The potential for high-intensity interval training to reduce cardiometabolic disease risk. *Sports Med.* (2012) 42:489–509. doi: 10.2165/11630910-000000000-00000

9. Fernández-Rubio H, Becerro-de-Bengoa-Vallejo R, Rodríguez-Sanz D, Calvo-Lobo C, Vicente-Campos D, Chicharro JL. Exercise training and interventions for coronary artery disease. *J Cardiovasc Dev Dis.* (2022) 9:131. doi: 10.3390/jcdd9050131

10. Angadi SS, Mookadam F, Lee CD, Tucker WJ, Haykowsky MJ, Gaesser GA. High-intensity interval training vs. moderate-intensity continuous exercise training in heart failure with preserved ejection fraction: a pilot study. *J Appl Physiol.* (2015) 119:753–78. doi: 10.1152/japplphysiol.00518.2014

11. Pelliccia A, Sharma S, Gati S, Bäck M, Börjesson M, Caselli S, et al. 2020 ESC guidelines on sports cardiology and exercise in patients with cardiovascular disease. *Eur Heart J.* (2021) 42:17–96. doi: 10.1093/eurheartj/ehaa605

12. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the sixth joint task force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by Representatives of 10 Societies and by Invited Experts): developed with the special contribution of the European Association for Cardiovascular Prevention and Rehabilitation (EACPR). *Eur J Prev Cardiol.* (2016) 74:821–936. doi: 10.1177/2047487316653709

13. Price KJ, Gordon BA, Bird SR, Benson AC. A review of guidelines for cardiac rehabilitation exercise programmes: is there an international consensus? *Eur J Prev Cardiol.* (2016) 23:1715–33. doi: 10.1177/2047487316657669

14. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by Representatives of 10 Societies and by Invited Experts) developed with the special contribution of the european association for cardiovascular prevention and rehabilitation (EACPR). *Atherosclerosis.* (2016) 252:207–74. doi: 10.1016/j.atherosclerosis.2016.05.037

15. Weston KS, Wisløff U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med.* (2014) 48:1227–34. doi: 10.1136/bjsports-2013-092576

16. Marijon E, Uy-Evanado A, Reinier K, Teodorescu C, Narayanan K, Jouven X, et al. Sudden cardiac arrest during sports activity in middle age. *Circulation.* (2015) 131:1384–91. doi: 10.1161/CIRCULATIONAHA.114.011988

17. Chugh SS, Weiss JB. Sudden cardiac death in the older athlete. *J Am Coll Cardiol.* (2015) 65:493–502. doi: 10.1016/j.jacc.2014.10.064

18. Palermo P, Corrà U. Exercise prescriptions for training and rehabilitation in patients with heart and lung disease. *Ann Am Thorac Soc.* (2017) 14:59–66. doi: 10.1513/AnnalsATS.201702-160FR

19. Lee D-c, Artero EG, Sui X, Blair SN. Mortality trends in the general population: the importance of cardiorespiratory fitness. *J Psychopharmacol.* (2010) 24:27–35. doi: 10.1177/1359786810382057

20. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA.* (2009) 301:2024–35. doi: 10.1001/jama.2009.681

21. Fihn SD, Blankenship JC, Alexander KP, Bittl JA, Byrne JG, Fletcher BJ, et al. 2014 ACC/AHA/AATS/PCNA/SCAI/STS focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, and the American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Thorac Cardiovasc Surg.* (2015) 149:e5–e23. doi: 10.1016/j.jtcvs.2014.11.002

22. Li B, Tian Z. Research progress on exercise intervention in ischemic heart disease and its mechanism. *Life Sci.* (2020) 32:864–77. doi: 10.13376/j.cbls/2020108

23. Achttien RJ, Staal JB, van der Voort S, Kemps HMC, Koers H, Jongert MWA, et al. Exercise-based cardiac rehabilitation in patients with coronary heart disease: a practice guideline. *Netherlands Heart J.* (2013) 21:429–38. doi: 10.1007/s12471-013-0467-y

24. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 327:557–60. doi: 10.1136/bmj.327.7414.557

25. Luan CH, Yang XL, Luan LP. Effects of intermittent aerobic exercise of different intensities on cardiac function, exercise tolerance and quality of life in patients with coronary heart disease after PCI. *Guizhou Med.* (2019) 43:277–9. doi: 10.3969/j.issn.1000-744X.2019.02.038

26. Ju XH. Effects of intermittent aerobic exercise of different intensities on cardiac function, exercise tolerance and quality of life in patients with coronary heart disease after PCI. *Heilongjiang Med.* (2018) 31:675–6. doi: 10.14035/j.cnki.hljyy.2018.03.105

27. Gao ZZ Ji P, Xia YQ, Wang L. Effects of different intensities of aerobic exercise on cardiac function and exercise tolerance in patients after percutaneous coronary intervention. *Chin J Rehabil Med.* (2015) 30:344–8. doi: 10.3969/j.issn.1001-1242.2015.04.007

28. Wang Y, Zhu LY, Ren AH. Effects of different intensities of exercise on cardiopulmonary function in elderly patients with coronary heart disease who lack exercise. *Chin J Phys Med Rehabil.* (2010) 2010:845–8. doi: 10.3760/cma.j.issn.0254-1424.2010.11.012

29. Zhang XS, Zhong JP, Tang YX, Song BT, Deng SP, Hu SS, et al. Effects of high-intensity intermittent and moderate-intensity continuous aerobic training on stratified low-risk patients with exercise rehabilitation after percutaneous coronary intervention. *Chin J Phys Med.* (2022) 44:47–51. doi: 10.3760/cma.j.issn.0254-1424.2022.01.009

30. Gu L, Wu L, Li Y, Wan L, A. comparative study of different exercise intensities for cardiac rehabilitation in patients after percutaneous coronary intervention. *Hebei Med.* (2020) 42:853–6. doi: 10.3969/j.issn.1002-7386.2020.06.012

31. Rognmo Ø, Hetland E, Helgerud J, Hoff J, Slørdahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehab.* (2004) 11:216–22. doi: 10.1097/01.hjr.0000131677.96762.0c

32. Moholdt TT, Amundsen BH, Rustad LA, Wahba A, Løvø KT, Gullikstad LR, et al. Aerobic interval training versus continuous moderate exercise after coronary artery bypass surgery: a randomized study of cardiovascular effects and quality of life. *Am Heart J.* (2009) 158:1031–7. doi: 10.1016/j.ahj.2009.10.003

33. Benetti M, Araujo CL, Santos RZ. Cardiorespiratory fitness and quality of life at different exercise intensities after myocardial infarction. *Arquivos Bras Cardiol.* (2010) 95:399–404. doi: 10.1590/s0066-782x2010005000089

34. Conraads VM, Pattyn N, De Maeyer C, Beckers PJ, Coeckelberghs E, Cornelissen VA, et al. Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study. *Int J Cardiol.* (2015) 179:203–10. doi: 10.1016/j.ijcard.2014.10.155

35. Currie KD, Bailey KJ, Jung ME, McKelvie RS, MacDonald MJ. Effects of resistance training combined with moderate-intensity endurance or low-volume high-intensity interval exercise on cardiovascular risk factors in patients with coronary artery disease. *J Sci Med Sport.* (2015) 18:637–42. doi: 10.1016/j.jsams.2014.09.013

36. Jaureguizar KV, Vicente-Campos D, Bautista LR, de la Peña CH, Gómez MJ, Rueda MJ, et al. Effect of high-intensity interval versus continuous exercise training on functional capacity and quality of life in patients with coronary artery disease: a randomized clinical trial. *J Cardiopulm Rehabil Prev.* (2016) 36:96–105. doi: 10.1097/HCR.0000000000000156

37. Lee LS, Tsai M-C, Brooks D, Oh PI. Randomised controlled trial in women with coronary artery disease investigating the effects of aerobic interval training versus moderate intensity continuous exercise in cardiac rehabilitation: cat versus mice study. *BMJ Open Sport Exerc Med.* (2019) 5:e000589. doi: 10.1136/bmjsem-2019-000589

38. Villelabeitia-Jaureguizar K, Vicente-Campos D, Berenguel Senen A, Hernández Jiménez V, Ruiz Bautista L, Barrios Garrido-Lestache ME, et al. Mechanical efficiency of high versus moderate intensity aerobic exercise in coronary heart disease patients: a randomized clinical trial. *Cardiol J.* (2019) 26:130–7. doi: 10.5603/CJ.a2018.0052

39. Keteyian SJ, Hibner BA, Bronsteen K, Kerrigan D, Aldred HA, Reasons LM, et al. Greater improvement in cardiorespiratory fitness using higher-intensity interval training in the standard cardiac rehabilitation setting. *J Cardiopulm Rehabil Prev.* (2014) 34:98–105. doi: 10.1097/HCR.0000000000000049

40. Currie KD, Dubberley JB, McKelvie RS, MacDonald MJ. Low-volume, high-intensity interval training in patients with Cad. *Med Sci Sports Exerc.* (2013) 45:1436–42. doi: 10.1249/MSS.0b013e31828bbbd4

41. Cardozo GG, Oliveira RB, Farinatti PT. Effects of high intensity interval versus moderate continuous training on markers of ventilatory and

cardiac efficiency in coronary heart disease patients. *Sci World J.* (2015) 2015:192479. doi: 10.1155/2015/192479

42. Kim C, Choi HE, Lim MH. Effect of high interval training in acute myocardial infarction patients with drug-eluting stent. *Am J Phys Med Rehabil.* (2015) 94:879–86. doi: 10.1097/phm.0000000000000290

43. Gremeaux M, Hannequin A, Laurent Y, Laroche D, Casillas JM, Gremeaux V. Usefulness of the 6-minute walk test and the 200-metre fast walk test to individualize high intensity interval and continuous exercise training in coronary artery disease patients after acute coronary syndrome: a pilot controlled clinical study. *Clin Rehabil.* (2011) 25:844–55. doi: 10.1177/0269215511403942

44. Gomes-Neto M, Durães AR, Reis H, Neves VR, Martinez BP, Carvalho VO. High-intensity interval training versus moderate-intensity continuous training on exercise capacity and quality of life in patients with coronary artery disease: a systematic review and meta-analysis. *Eur J Prev Cardiol.* (2017) 24:1696–707. doi: 10.1177/2047487317728370

45. Wisløff U, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation.* (2007) 115:3086–94. doi: 10.1161/CIRCULATIONAHA.106.675041

46. Tinken TM, Thijssen DHJ, Hopkins N, Black MA, Dawson EA, Minson CT, et al. Impact of shear rate modulation on vascular function in humans. *Hypertension* (Dallas, Tex: 1979) (2009) 54:278–85. doi: 10.1161/HYPERTENSIONAHA.109.134361

47. Johnson BD, Mather KJ, Wallace JP. Mechanotransduction of shear in the endothelium: basic studies and clinical implications. *Vasc Med.* (2011) 16:365–77. doi: 10.1177/1358863X11422109

48. Tucker WJ, Lijauco CC, Hearon CM Jr, Angadi SS, Nelson MD, Sarma S, et al. Mechanisms of the improvement in peak VO_2 with exercise training in heart failure with reduced or preserved ejection fraction. *Heart Lung Circ.* (2018) 27:9–21. doi: 10.1016/j.hlc.2017.07.002

49. Lavier J, Beaumann M, Menétrey S, Bouzourène K, Rosenblatt-Velin N, Pialoux V, et al. High-intensity exercise in hypoxia improves endothelial function via increased nitric oxide bioavailability in C57bl/6 mice. *Acta Physiologica (Oxford, England).* (2021) 233:e13700. doi: 10.1111/apha.13700

50. Haykowsky MJ, Timmons MP, Kruger C, McNeely M, Taylor DA, Clark AM. Meta-analysis of aerobic interval training on exercise capacity and systolic function in patients with heart failure and reduced ejection fractions. *Am J Cardiol.* (2013) 111:1466–9. doi: 10.1016/j.amjcard.2013.01.303

51. Huang SC, Wong MK, Lin PJ, Tsai FC, Fu TC, Wen MS, et al. Modified high-intensity interval training increases peak cardiac power output in patients with heart failure. *Eur J Appl Physiol.* (2014) 114:1853–62. doi: 10.1007/s00421-014-2913-y

52. Oberman A, Fletcher GF, Lee J, Nanda N, Fletcher BJ, Jensen B, et al. Efficacy of high-intensity exercise training on left ventricular ejection fraction in men with coronary artery disease (the training level comparison study). *Am J Cardiol.* (1995) 76:643–7. doi: 10.1016/s0002-9149(99)80189-1

53. Itoh H, Ajisaka R, Koike A, Makita S, Omiya K, Kato Y, et al. Heart rate and blood pressure response to ramp exercise and exercise capacity in relation to age, gender, and mode of exercise in a healthy population. *J Cardiol.* (2013) 61:71–8. doi: 10.1016/j.jjcc.2012.09.010

54. Muller GY, Matos FdO, Perego Junior JE, Kurauti MA, Diaz Pedrosa MM. High-intensity interval resistance training (HIIRT) improves liver gluconeogenesis from lactate in Swiss mice. *Appl Physiol Nutr Metab.* (2022) 47:439–46. doi: 10.1139/apnm-2021-0721

55. Rogers B, Giles D, Draper N, Mourot L, Gronwald T. Detection of the anaerobic threshold in endurance sports: validation of a new method using correlation properties of heart rate variability. *J Funct Morphol Kinesiol.* (2021) 6:38. doi: 10.3390/jfmk6020038

56. Farinatti P, Castinheiras Neto AG, Amorim PR. Oxygen consumption and substrate utilization during and after resistance exercises performed with different muscle mass. *Int J Exerc Sci.* (2016) 9:77–88.

57. Islam H, Townsend LK, Hazell TJ. Modified sprint interval training protocols. Part I Physiological responses. *Appl Physiol Nutr Metab.* (2017) 42:339–46. doi: 10.1139/apnm-2016-0478

58. McGarvey W, Jones R, Petersen S. Excess post-exercise oxygen consumption following continuous and interval cycling exercise. *Int J Sport Nutr Exerc Metab.* (2005) 15:28–37. doi: 10.1123/ijsnem.15.1.28

59. MacArthur DG, North KN, A. Gene for speed? The evolution and function of alpha-actinin-3. *BioEssays.* (2004) 26:786–95. doi: 10.1002/bies.20061

60. Papadimitriou ID, Lucia A, Pitsiladis YP, Pushkarev VP, Dyatlov DA, Orekhov EF, et al. Actn3 R577x and Ace I/D gene variants influence performance in elite sprinters: a multi-cohort study. *BMC Genom.* (2016) 17:1–8. doi: 10.1186/s12864-016-2462-3

61. Quinlan KG, Seto JT, Turner N, Vandebrouck A, Floetenmeyer M, Macarthur DG, et al. Alpha-actinin-3 deficiency results in reduced glycogen phosphorylase activity and altered calcium handling in skeletal muscle. *Hum Mol Genet.* (2010) 19:1335–46. doi: 10.1093/hmg/ddq010

62. Ribeiro HS, Maya ÁTD. Neves RP, de Moraes MR, Lima RM, et al. Influence of the Actn3 genotype and the exercise intensity on the respiratory exchange ratio and excess oxygen consumption after exercise. *J Strength Condition Res.* (2021) 35:1380–8. doi: 10.1519/JSC.0000000000002911

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Prevalence and factors associated with cognitive impairment in Chinese heart failure patients: A pilot study

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Background: The prevalence of Cognitive impairment (CI) is high in patients with heart failure (HF). It leads to poor prognosis, such as self-care, hospital readmission and increased mortality. However, such information among Chinese population is unclear.

Objective: The purpose of this study was to examine the prevalence of CI in Chinese patients with HF, and explore its correlation with biomarkers and clinical factors to better manage HF patients with CI.

Methods: This study is a cross-sectional study of 200 hospitalized HF patients in China. The cognitive function of HF patients was assessed by the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Examination (MMSE).

Results: The majority are male (62.5%, $n = 125$), have primary school and below level of education (57.5%, $n = 115$), NYHA III and above (62%, $n = 124$). They have an average MoCA score of 15.10 ± 8.18, MMSE score of 19.55 ± 8.23. Age, NYHA class, and atrial fibrillation were independently associated with CI ($p < 0.05$). There was a significant association between CI and the 4th quartile of TNT ($p = 0.013$), and the 3rd and 4th quartile of NT-proBNP ($p = 0.015$, $p = 0.038$).

Conclusions: The prevalence of undiagnosed CI in Chinese HF patients is high (81%). HF patients with high levels of TNT or NT-proBNP or both values may be at risk of developing CI. Therefore, we suggest that HF patients with older age, atrial fibrillation, NYHA class II and III, as well as elevated TNT or NT-proBNP or both values to be followed up with a formal evaluation for CI. Nurses need to provide targeted health education program for cognitively impaired HF population to improve their self-care ability and nursing outcome.

KEYWORDS

heart failure, cognitive impairment, Chinese, nursing, prevalence

Introduction

Chronic heart failure (HF) is a syndrome resulting from multiple, long-standing cardiovascular abnormalities, such as coronary artery disease or hypertension (1). HF is increasing in prevalence and is a public health problem in the world. Currently, the overall prevalence of HF is 1.3–6.7% in Asian population (2). The China Hypertension

Survey (CHS) of 22,158 participants from 2012 to 2015 reported the prevalence of HF was 1.3% (3). There is increasing evidence reported that HF is associated with cognitive impairment (CI) independently (4). CI is highly prevalent in Asian HF patients (44%) (5), but such information in Chinese population is scant. Additionally, CI is closely associated with poor prognosis, such as self-care, hospital readmission and increased mortality (6, 7). The 2016 European Society of Cardiology guidelines focused on self-management of HF patients with CI (8). To better understand self-management of cognitively impaired HF patients, this study examined the prevalence of CI in Chinese HF population and explored its correlation with biomarkers and clinical factors.

Materials and methods

Study design and participants

We performed a cross-sectional study of hospitalized HF patients at Linyi People Hospital, Shandong Province, China. We recruited 200 inpatients aged 18 years old. Patients were deemed ineligible if they had significant language or physical impairment impeding their abilities in cognitive tests (e.g., aphasia, hearing and vision impairment, severe hemiplegia, etc.). Patients who had clinically significant psychiatric disorders (e.g., anxiety, depression) hyperthyroidism, hypothyroidism, substance abuse (e.g., alcoholism, drug abuse) were also excluded. This study was approved by the Shandong First Medical University (Shandong Academy of Medical Sciences) Human Research Ethic Review Committee. All participants provided written informed consents.

Demographic and clinical factors

As part of a research, demographic and clinical factors collected by the researchers, including age, sex, race, New York Heart Association (NYHA) class, troponin T (TNT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), left ventricular ejection fraction (LVEF, determined by echocardiogram performed within the past 6 months), Heart Failure with Reduced Ejection Fraction (HFrEF), Heart Failure with Preserved Ejection Fraction (HFpEF), medical history of vascular diseases [hypertension, coronary artery disease (CAD), diabetes mellitus (DM), stroke, atrial fibrillation (AF), Chronic kidney diseases (CKD)], other risk factors such as smoking and alcohol consumption.

Cognitive function

Cognitive function was assessed with the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Examination (MMSE). To correct for education effects, 1 point was added for participants with 12 years of education or less on their total MoCA score (9). Scores on both cognitive tools range from 0 to 30 points, with a lower score reflecting greater CI. A recent study in the Asian HF population suggests a cut-off of <25 on the MoCA or <28 on the MMSE for CI (4). Hence, in this study, CI was defined by either MoCA<25 or MMSE<28, or both MoCA<25 and MMSE<28. The MMSE and MoCA were administered by a trained research personnel.

Statistical analysis

SPSS22.0 software was used for statistical analysis. To examine the population characteristics of the study, we calculated proportions for categorical variables, means and standard deviations for continuous variables. We compared the characteristics of patients with and without CI, using the independent *t*-tests for continuous variables and the chi-square tests for categorical variables. After adjusted for age, gender, NYHA class, AF, prior stroke, and DM, binary logistic regression was used to analyzed the correlation between CI and other variables. Pearson correlation analysis was used to examine the correlation between MoCA, MMSE and other variables. For NT-proBNP and TNT, quartile measures were used to form 4 groups. The lowest quartile was used as a reference group. Logistic regression analysis was used to analyze the association between CI and NT-proBNP, TNT, other variables.

Results

Demographic description

Participants had an average age of 76.11 $\pm$ 13.38 years. The majority were male (62.5%, $n = 125$), primary school and below (57.5%, $n = 115$), NYHA III and above (62%, $n = 124$). Cardiovascular risk factors included largely CAD (55%, $n = 100$), followed by smoking (40.5%, $n = 81$), hypertension (38.5%, $n = 77$), AF (33.5%, $n = 67$), alcohol consumption (31%, $n = 62$), DM (29.5%, $n = 59$), prior stroke (15%, $n = 30$), and CKD (12.5%, $n = 25$). These participants had an average of 2.55 $\pm$ 1.21 total cardiovascular risk factors. Our HF population had an average LVEF of 42.36 $\pm$ 11.09, TNT of 0.09 $\pm$ 0.20, NT-proBNP of 4083.57 $\pm$ 5663.43. Patients had an average MoCA score of 15.10 $\pm$ 8.18, MMSE score of 19.55 $\pm$ 8.23. As shown in Table 1, compared with patients without CI, those with CI were older (71.14 $\pm$ 10.12 vs. 49.95 $\pm$ 11.95 years, $p \leq 0.001$), had higher proportion of female (42.7 vs. 10.5%, $p \leq 0.001$), less educated ($\leq$12 years of education: 29.6 vs. 97.4%, $p \leq 0.001$). They also had approximately twice higher rates of AF (37.7 vs. 15.8%, $p < 0.05$) and levels of NT-proBNP (4518.93 $\pm$ 6107.31 vs. 2227.54 $\pm$ 2416.37, $p \leq 0.001$).

TABLE 1 Study sample characteristics.

Variables	Whole sample ($N = 200$)	No CI ($N = 38$)	CI ($N = 162$)	p-value
Age (years)	76.11 ± 13.38	49.95 ± 11.95	71.14 ± 10.12	**≤0.001**
Sex				
Male	125(62.5%)	34(89.5%)	114(70.4%)	**≤0.001**
Female	75(37.5%)	4(10.5%)	48(29.6%)	
Education				
Primary school and below	115(57.5%)	1(2.6%)	39(24.1%)	**≤0.001**
Secondary school and above	85(42.5%)	37(97.4%)	26(16.0%)	
NYHA class				
NYHA I	12(6%)	3(7.9%)	9(5.6%)	
NYHA II	64(32%)	13(34.2%)	51(31.5%)	0.719
NYHA III	64(32%)	15(39.5%)	49(30.2%)	0.907
NYHA IV	60(30%)	7(18.4%)	53(32.7%)	0.254
Cardiovascular risk factors				
Hypertension	77(38.5%)	14(36.8%)	63(38.9%)	0.745
CAD	110(55%)	17(44.7%)	93(57.4%)	0.505
DM	59(29.5%)	10(26.3%)	49(30.2%)	0.802
Prior stroke	30(15%)	6(15.8%)	24(14.8%)	0.757
CKD	25(12.5%)	4(10.5%)	21(13.0%)	0.404
AF	67(33.5%)	6(15.8%)	61(37.7%)	**<0.05**
Smoking	81(40.5%)	21(55.3%)	60(37.0%)	0.200
Alcohol consumption	62(31%)	17(44.7%)	45(27.8%)	0.126
Total cardiovascular risk factors	2.55 ± 1.21	2.50 ± 1.18	2.56 ± 1.22	0.778
LVEF(%)	42.36 ± 11.09	42.26 ± 9.69	42.41 ± 11.39	0.298
HFrEF (EF≤40%)	104 (52%)	19 (9.5%)	85 (42.5%)	0.787
HFpEF (EF≥50%)	59 (29.5%)	9 (4.5%)	50 (25%)	0.373
TNT	0.09 ± 0.20	0.19 ± 0.39	0.07 ± 0.11	0.479
NT-proBNP	4083.57 ± 5663.43	2227.54 ± 2416.37	4518.93 ± 6107.31	**≤0.001**
MMSE	19.55 ± 8.23	28.50 ± 0.65	17.45 ± 7.77	**≤0.001**
MoCA	15.10 ± 8.18	26.63 ± 1.05	12.39 ± 6.60	**≤0.001**

Values are expressed as mean ± standard deviation, or n (%).
CI, cognitive impairment; NYHA, New York Heart Association; CAD, coronary artery disease; DM, diabetes mellitus; CKD, chronic kidney disease; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; TnT, troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment. The bold values indicate $p < 0.05$.

Prevalence of cognitive impairment

The prevalence of CI was 81% based on MoCA <25, or MMSE <28, or a combination of both in our HF patients. 80% of patients had MoCA scores<25, 74.5% had MMSE scores<28, 6.5% of patients had normal MMSE score (i.e., ≥28) but failed MoCA test (i.e., <25), 1% of patients who had normal MoCA score (i.e., ≥25) but failed MMSE test (i.e., <28).

Factors association with cognition

Multiple regressions showed that age, educational level, NYHA class II and III, history of stroke, CKD, AF and LVEF were independently associated with MMSE and MoCA scores ($p < 0.05$). Logistic regression showed that age, education level, NYHA class, AF and alcohol consumption were independently associated with CI ($p < 0.05$), as shown in Table 2. Regarding cardiac biomarkers, the association between CI and TNT was not significant, while the association between CI and 3rd and 4th quartile of NT-proBNP was significant ($p = 0.027$, $p = 0.017$), as shown in Table 3. Controlling covariates, i.e., age, gender, NYHA class II and III, AF, prior stroke and DM, there was significant association between the 4th quartile of TNT and CI (OR = 0.06, $p = 0.013$), and TNT was negatively correlated with CI. Similarly, there was a statistically significant association between CI and the 3rd and 4th quartile of NT-proBNP after adjustment ($p = 0.015$, $p = 0.038$).

TABLE 2 Association between MMSE, MoCA and CI.

Variables	Correlation with MMSE		Correlation with MoCA		CI	
	β	*p*-value	*β*	*p*-value	OR(95%CI)	*p*-value
Age(years)	−0.194	**<0.001**	−0.193	**<0.001**	**1.151**(1.054-1.258)	**<0.05**
Sex						
Male						
Female	−0.511	**<0.001**	−0.147	**<0.001**	2.065(0.134-31.79)3)	0.603
Education						
Primary school and below						
Secondary school and above	0.675	**<0.001**	0.758	**<0.001**	0.011(0.002–0.085)	**<0.001**
NYHA class						
NYHA I						
NYHA II	4.225	**<0.05**	4.035	**<0.05**	0.026(0.001–0.940)	**<0.05**
NYHA III	4.090	**<0.05**	4.928	**<0.05**	0.012(0.000–0.974)	**<0.05**
NYHA IV	5.436	**<0.05**	5.622	**≤0.001**	0.170(0.002–11.589)	0.411
Risk factors						
Hypertension	0.096	0.907	−0.168	0.808	1.257(0.229–6.908)	0.792
CAD	1.222	0.177	1.086	0.157	0.478(0.057–4.037)	0.498
DM	0.577	0.535	0.160	0.839	0.538(0.071–4.078)	0.548
Prior stroke	−3.170	**<0.05**	−2.447	**<0.05**	0.325(0.033–3.203)	0.335
CKD	3.415	**<0.05**	2.986	**<0.05**	0.095(0.003–2.824)	0.174
AF	−2.703	**≤0.001**	−1.435	**<0.05**	**13.582**(1.249–147.663)	**<0.05**
Smoking	−0.260	0.827	−0.813	0.419	3.526(0.393–31.670)	0.261
Alcohol consumption	−0.147	0.900	0.786	0.427	**0.070**(0.008–0.604)	**<0.05**
Total cardiovascular risk factors	−0.217	0.797	−0.217	0.521	1.043(0.778–1.399)	0.777
LVEF(%)	0.113	**<0.05**	0.083	**<0.05**	1.006(0.910–1.111)	0.909
HFrEF (EF≤40%)	−0.123	0.082	−0.102	0.152	2.810(0.838–9.422)	0.094
HFpEF (EF≥50%)	0.027	0.705	0.003	0.967	3.428(0.890–13.200)	0.073

CI, cognitive impairment; NYHA, New York Heart Association; CAD, coronary artery disease; DM, diabetes mellitus; CKD, chronic kidney disease; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; TnT, troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment. The bold values indicate $p < 0.05$.

Cognitive subtests performance

As shown in Table 4. Compared with patients without CI, a higher proportion of patients with CI did not achieve full scores in 4 domain subtests, i.e., naming (80.0 vs. 2.5%), attention (81.9 vs. 0%), abstraction (81.3 vs. 5.0%) and orientation (74.4 vs. 2.5%) on the MoCA. By comparison, they did not achieve full scores in 2 domain subtests, i.e., orientation (84.6 vs. 2.5%), attention and calculation (74.4 vs. 2.5%) on the MMSE. There were more patients passed MMSE yet failed MoCA than those failed MMSE yet passes MoCA (13 vs. 2, shown in Table 5). We further compared average scores of the domain subtests in these 2 groups. MMSE had no domian subtests that could differentiate these 2 groups, while MoCA had 2 domain subtests (Visuospatital and executive function, Abstraction) differentiated these 2 groups. Overall, MoCA had more domain subtests than the MMSE in differentiating those with and without CI. Additionally, there were more patients passed MMSE yet failed MoCA than those passed MoCA yet failed MMSE.

Discussion

In this pilot study, the prevalence of undiagnosed CI based on cognitive screening tests in Chinese HF patients was high (81%), which may be due to the following reasons. First, optimal cut-points of MMSE <28 and MoCA <25 in our study were based on recently published HF population specific cut-off points (5, 10), rather than previous studies that used cut-off points validated for psychiatric inpatients (MMSE<24) (11) or those with mild CI (MoCA<26) (9). Second, age was significantly related to CI, our HF patients were much older than previous studies (e.g., ∼20 years older than the sample in Dong et al. (58.7 ± 10.5 years old) (5) and Vellone et al. (66.9 ± 11.7 years old) (12).

TABLE 3 Association between cardiac markers (quartiles) and CI.

	CI ($N = 38$)	No CI($N = 162$)	*p*-value	CI (before adjustment)		CI (after adjustment*)	
				OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
NT-proBNP							
1st quartile	590.949 ± 255.610	506.760 ± 217.010	0.243	1	0.057	1	0.062
2nd quartile	1674.400 ± 441.432	1464.091 ± 393.457	0.229	1.672(0.650-4.299)	0.286	2.387(0.454-12.563)	0.304
3rd quartile	3508.558 ± 770.628	2962.000 ± 303.457	**0.01**	3.559(1.154-10.978)	**0.027**	24.211(1.846-317.480)	**0.015**
4th quartile	11216.818 ± 8475.586	7194.667 ± 1501.370	**0.007**	4.159(1.284-13.467)	**0.017**	13.175(1.158-149.887)	**0.038**
TNT							
1st quartile	0.017 ± 0.006	0.016 ± 0.006	0.724	1	0.293	1	0.105
2nd quartile	0.031 ± 0.005	0.032 ± 0.004	0.655	0.643(0.206-2.006)	0.447	0.343(0.050-2.336)	0.274
3rd quartile	0.049 ± 0.007	0.045 ± 0.005	0.051	0.451(0.159-1.277)	0.134	0.438(0.071-2.713)	0.375
4th quartile	0.191 ± 0.160	0.620 ± 0.571	**0.042**	0.356(0.110-1.148)	0.084	0.060(0.006–0.557)	**0.013**

CI, cognitive impairment; TnT, troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.
For NT-proBNP and TNT, quartile measures were used to form 4 groups.
*Adjusted for age, gender, NYHA class, AF, prior stroke, and DM. The bold values indicate $p < 0.05$.

TNT and NT-proBNP were early biomarker of cardiac dysfunction (13). Our study has shown that TNT and NT-proBNP were significantly associated with CI, consistent with previous studies by Dong et al. (5), Hilal et al. (14), and Gunstad et al. (15). Pokharel et al. (16) indicated that elevated TNT values were more common in older than in younger patients. Our findings on TNT is consistent with a previous study by Hilal et al. (14). The age of our patients with CI were older and comparable to the sample in the study by Hilal et al. (14) (71.14 ± 10.12 vs. 70.20 ± 9.60 years old). Our sample consisted of HF patients only, different from the study sample from Hilal et al. (14) which consisted of community and memory clinic older adults with few HF patients. Furthermore, our finding shows that TNT value is significantly associated with CI at its fourth quartile (Table 3), although it has a statistically non-significant and lower value in those with CI (Table 1). By comparison, NT-proBNP value is significantly associated with CI at its third and fourth quartile, showing lower threshold. This suggests that NT-proBNP might be a more sensitive cardiac biomarker than TNT in HF population.

The possible mechanisms of NT-proBNP associating with CI are as follows. First, the left ventricular dysfunction and ischemic heart disease not only release these biomarkers (NT-proBNP) but also activates several other inflammatory markers leading to ischemic damage in regions selective to cognitive function (17). Second, high levels of NT-proBNP are associated with endothelial dysfunction, possibly linked to cognitive function (18). Third, the association between NT-proBNP and cognitive dysfunction could be due to subclinical cardiovascular disease (i.e., early atherosclerosis) (19). Fourth, higher level of NT-proBNP is also linked to AF (20) which in turn is associated with reduced cognitive function (17).

Our results indicated that AF was associated independently with CI, which was consistent with the conclusion of Kalantarian et al. (21). Beyond clinically recognized shared risk factors (aging and cardiac function), one of the leading potential mechanisms was the occurrence of silent cerebral infarcts (22). AF was associated with a more than two-fold increase in the risk of developing silent cerebral infarcts (23). Although silent infarcts were not associated with clinically apparent acute neurologic deficits, there was a significant association between silent infarcts and the development of cognitive decline and dementia (24). The other possible mechanism was that AF and CI share a common link with regards to protein misfolding and amyloidgenesis (25). Misfolded atrial natriuretic peptide may lead to the formation and deposition of atrial amyloid fibers in elderly patients with AF. β-Amyloid protein and tau protein forming cerebral plaques which exert cytotoxic effects leading to cerebral atrophy, consequently cognitive decline (26, 27). Studies suggested that the occurrence of Alzheimer's disease was related to hypoperfusion, inflammation, oxidative stress, and endothelial dysfunction, and those factors resulting in an atrial cardiomyopathy which in turn, leads to AF (27).

The cognitive deficits of HF patients were shown in several subtests, especially in domains of orientation, short-term memory, attention, concentration and working memory, language (e.g., naming), and executive functions (e.g., abstraction). The domain subtests in the MoCA seems to be better than the MMSE in differentiating HF patients with and without CI. Cognitive deficits interfere with patients' self-care abilities, such as recognizing worsening symptoms, adhering to complex medication regimens, dietary restrictions and numerous lifestyle modifications. CI may contribute to suboptimal self-care in several ways. Several studies have suggested that deficits in memory, attention, and executive

TABLE 4 Comparisons of Proportions of MoCA and MMSE domain subtest below full scores.

	CI (%)	No CI (%)	*p*
MMSE domain subtests [a]			
Orientation (10) *	84.60	3.00	<0.001
Registration (3)	30.60	0.00	<0.001
Attention and calculation (5) *	74.40	3.00	<0.001
Recall (3)	99.00	73.00	<0.001
Language and praxis (9)	98.70	60.00	<0.001
MoCA domain subtests [b]			
Visuospatial and executive function (5)	84.60	60.00	<0.001
Naming (3) *	80.00	2.50	<0.001
Attention (6) *	81.90	0.00	<0.001
Language (3)	98.80	85.00	<0.001
Abstraction (2) *	81.30	5.00	<0.001
Delayed recall (5)	96.90	95.00	<0.001
Orientation (6) *	74.40	2.50	<0.001

[a]MMSE domain subtest full score/details: Orientation (10)—Orientation to place and time; Registration (3)—Repeat 3 words; Attention and Calculation (5)—Serial 7 s; Recall (3)—Recall a list of 3 words; Language and praxis (9)—Sentence repetition, comprehension, reading, writing, copy intersecting pentagons.
[b]MoCA domain subtest full score/details: Visuospatial and executive function (5)—Trail B test, Cube copy, Clock drawing; Naming (3)—Confrontation naming (lion, rhinoceros, camel). Attention (6)—Digit span, Vigilance (tapping at the number 1 in a list of numbers), Serials 7 s; Language (3)—Sentence repetition, verbal fluency; Abstraction (2)—Similarities between 2 items; Delayed recall (5)—Recall a list of 5 words; Orientation (6)—Date, month, year, day, place, city.
*The proportion of subtests below full scores of CI differs from the proportion of subtests below full scores of no CI by more than 50%.

TABLE 5 Comparisons of the average MoCA and MMSE domain subtest scores.

‘	MMSE≥28 &MoCA<25 (*n* = 13)	MMSE<28 &MoCA≥25 (*n* = 2)	*p*
MMSE domain subtest [a]			
Orientation (10)	9.92 ± 0.28	9.50 ± 0.71	0.116
Registration (3)	3.00 ± 0.00	3.00 ± 0.00	**
Attention and calculation (5)	4.92 ± 0.28	4.50 ± 0.71	0.116
Recall (3)	2.15 ± 0.69	1.50 ± 0.71	0.234
Language and praxis (9)	8.15 ± 0.38	8.50 ± 0.71	0.287
MoCA domain subtest [b]			
Visuospatial and executive function (5) *	1.77 ± 1.59	4.50 ± 0.71	**<0.05**
Naming (3)	1.92 ± 0.95	3.00 ± 0.00	0.146
Attention (6)	5.61 ± 0.77	6.00 ± 0.00	0.505
Language (3)	1.77 ± 0.73	2.00 ± 0.00	0.670
Abstraction (2) *	1.61 ± 0.51	2.00 ± 0.00	**<0.05**
Delayed recall (5)	1.38 ± 0.96	2.50 ± 0.71	0.144
Orientation (6)	5.92 ± 0.28	5.50 ± 0.71	0.116

[a]MMSE domain subtest full score/details: Orientation (10)—Orientation to place and time; Registration (3)—Repeat 3 words; Attention and Calculation (5)—Serial 7 s; Recall (3)—Recall a list of 3 words; Language and praxis (9)—Sentence repetition, comprehension, reading, writing, copy intersecting pentagons.
[b]MoCA domain subtest full score/details: Visuospatial and executive function (5)—Trail B test, Cube copy, Clock drawing; Naming (3)—Confrontation naming (lion, rhinoceros, camel). Attention (6)—Digit span, Vigilance (tapping at the number 1 in a list of numbers), Serials 7 s; Language (3)—Sentence repetition, verbal fluency; Abstraction (2)—Similarities between 2 items; Delayed recall (5)—Recall a list of 5 words; Orientation (6)—Date, month, year, day, place, city.
*Not calculate.
**p < 0.05. The bold values indicate $p < 0.05$.

functioning were associated with difficulties adhering to recommendations, because of forgetfulness and poor learning ability (28, 29). Executive functioning affected the ability of HF patients to adapt to treatment and lifestyle regimens, by affecting learning and recall efficiency (30). Decline in language function led to poor understanding of medical instructions and contributes to worse adherence, while poor memory and attention have an adverse impact on daily tasks such as attending appointments, adhering to medication and weighing. Therefore, we should identify HF patients with CI early through routine cognitive screening, so as to develop individualized health education according to their cognitive problems. For example, based on the characteristic deficits on MoCA and MMSE, health education for our HF patients should be simpler and easy to understand, kept at a shorter duration, delivered with frequent repetition to strengthen the recall, etc. If necessary, upon discharge from hospital, closer follow-up and ongoing nursing management should be carried out for HF patients with CI, in order to improve their self-care and prognosis. In the future, we can also carry out health education for HF population with CI through home- or community-based care services and remote monitoring by medical professionals (Wechat, apps and so on), so as to prevent the increase of mortality and readmission rate.

Several limitations require acknowledgment. Firstly, small sample size and single study site limit the generalizability of our findings. Larger and multicenter studies are needed to establish the prevalence of CI in Chinese HF patients. Secondly, this study did not conduct a formal neuropsychological assessment to determine CI due to time constraint. Finally, our study showed that alcohol consumption decreased the incidence of CI in patients with HF, which may be related to cardiovascular benefits of light alcohol consumption (31). However, the amount and types of alcohol consumption were not investigated in our study. Future study should examine the impact of alcohol consumption on CI in patients with HF.

In conclusion, the prevalence of undiagnosed CI in Chinese HF patients is high. HF patients with elevated TNT or NT-proBNP or both values may be at risk of developing CI. Therefore, we suggest that HF patients with older age, AF, NYHA class II and III, as well as elevated TNT or NT-proBNP or both values to be followed up with a formal evaluation for CI. Future study should develop customized health education programs for

HF patients with CI, so as to improve their self-care ability and nursing outcome.

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 Shandong First Medical University (Shandong Academy of Medical Sciences) Human Research Ethic Review Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

QN designed this study and drafted the manuscript with the help from YD. WL and FW reviewed the manuscript. YD conceptualized this study, contributed to the design and provided critical review of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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References

1. Bennett SJ, Sauvé MJ. Cognitive deficits in patients with heart failure: a review of the literature. *J Cardiovasc Nurs.* (2003) 18:219–42. doi: 10.1097/00005082-200307000-00007

2. Wang H, Chai K, Du M, Wang S, Cai JP Li Y, et al. Prevalence and incidence of heart failure among urban patients in China: a national population-based analysis. *Circ Heart Fail.* (2021) 14:e008406. doi: 10.1161/CIRCHEARTFAILURE.121.008406

3. Hao G, Wang X, Chen Z, Zhang L, Zhang Y, Wei B, et al. China hypertension survey investigators. Prevalence of heart failure and left ventricular dysfunction in china: the china hypertension survey, 2012-2015. *Eur J Heart Fail.* (2019) 21:1329–37. doi: 10.1002/ejhf.1629

4. Cacciatore F, Abete P, Ferrara N, Calabrese C, Napoli C, Maggi S, et al. Congestive heart failure and cognitive impairment in an older population. Osservatorio Geriatrico Campano Study Group. *J Am Geriatr Soc.* (1998) 46:1343–8. doi: 10.1111/j.1532-5415.1998.tb05999.x

5. Dong Y, Teo SY, Kang K, Tan M, Ling LH, Yeo PSD, et al. Cognitive impairment in Asian patients with heart failure: prevalence, biomarkers, clinical correlates, and outcomes. *Eur J Heart Fail.* (2019) 21:688–90. doi: 10.1002/ejhf.1442

6. Cameron J, Worrall-Carter L, Page K, Riegel B, Lo SK, Stewart S. Does cognitive impairment predict poor self-care in patients with heart failure? *Eur J Heart Fail.* (2010) 12:508–15. doi: 10.1093/eurjhf/hfq042

7. Lee CS, Gelow JM, Bidwell JT, Mudd JO, Green JK, Jurgens CY, et al. Blunted responses to heart failure symptoms in adults with mild cognitive dysfunction. *J Cardiovasc Nurs.* (2013) 28:534–40. doi: 10.1097/JCN.0b013e31826620fa

8. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* (2016) 18:891–975. doi: 10.1002/ejhf.592

9. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc.* (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x

10. Hawkins MA, Gathright EC, Gunstad J, Dolansky MA, Redle JD, Josephson R, et al. The MoCA and MMSE as screeners for cognitive impairment in a heart failure population: a study with comprehensive neuropsychological testing. *Heart Lung.* (2014) 43:462–8. doi: 10.1016/j.hrtlng.2014.05.011

11. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* (1975) 12:189–98. doi: 10.1016/0022-3956(75)90026-6

12. Vellone E, Chialà O, Boyne J, Klompstra L, Evangelista LS, Back M, et al. Cognitive impairment in patients with heart failure: an international study. *ESC Heart Fail.* (2020) 7:46–53. doi: 10.1002/ehf2.12542

13. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med.* (2009) 361:858–67. doi: 10.1056/NEJMoa0900428

14. Hilal S, Chai YL, Ikram MK, Elangovan S, Yeow TB, Xin X, et al. Markers of cardiac dysfunction in cognitive impairment and dementia. *Medicine (Baltimore).* (2015) 94:e297. doi: 10.1097/MD.0000000000000297

15. Gunstad J, Poppas A, Smeal S, Paul RH, Tate DF, et al. Relation of brain natriuretic peptide levels to cognitive dysfunction in adults > 55 years of age with cardiovascular disease. *Am J Cardiol.* (2006) 98:538–40. doi: 10.1016/j.amjcard.2006.02.062

16. Normann J, Mueller M, Biener M, Vafaie M, Katus HA, et al. Effect of older age on diagnostic and prognostic performance of high-sensitivity troponin T in patients presenting to an emergency department. *Am Heart J.* (2012) 164:698–705.e4. doi: 10.1016/j.ahj.2012.08.003

17. Angermann CE, Frey A, Ertl G. Cognition matters in cardiovascular disease and heart failure. *Eur Heart J.* (2012) 33:1721–3. doi: 10.1093/eurheartj/ehs128

18. Chong AY, Blann AD, Patel J, Freestone B, Hughes E, Lip GY. Endothelial dysfunction and damage in congestive heart failure: relation of flow-mediated dilation to circulating endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. *Circulation.* (2004) 110:1794–8. doi: 10.1161/01.CIR.0000143073.60937.50

19. Daniels LB, Laughlin GA, Kritz-Silverstein D, Clopton P, Chen WC, Maisel AS, et al. Elevated natriuretic peptide levels and cognitive function in community-dwelling older adults. *Am J Med.* (2011) 124:670.e1–8. doi: 10.1016/j.amjmed.2011.02.027

20. Richards M, Di Somma S, Mueller C, Nowak R, Peacock WF, Ponikowski P, et al. Atrial fibrillation impairs the diagnostic performance of cardiac natriuretic peptides in dyspneic patients: results from the BACH Study (Biomarkers in ACute Heart Failure). *JACC Heart Fail.* (2013) 1:192–9. doi: 10.1016/j.jchf.2013.02.004

21. Kalantarian S, Ruskin JN. Atrial Fibrillation and Cognitive Decline: Phenomenon or Epiphenomenon? *Cardiol Clin.* (2016) 34:279–85. doi: 10.1016/j.ccl.2015.12.011

22. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Rotterdam Scan Study. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. *Stroke.* (2003) 34:392–6. doi: 10.1161/01.STR.0000052631.98405.15

23. Kalantarian S, Ay H, Gollub RL, Lee H, Retzepi K, Mansour M, et al. Association between atrial fibrillation and silent cerebral infarctions: a systematic review and meta-analysis. *Ann Intern Med.* (2014) 161:650–8. doi: 10.7326/M14-0538

24. Dagres N, Chao TF, Fenelon G, Aguinaga L, Benhayon D, Benjamin EJ, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) expert consensus on arrhythmias and cognitive function: What is the best practice? *J Arrhythm.* (2018) 34:99–123. doi: 10.1002/joa3.12050

25. Rollo J, Knight S, May HT, Anderson JL, Muhlestein JB, Bunch TJ, et al. Incidence of dementia in relation to genetic variants at PITX2, ZFHX3, and ApoE ε4 in atrial fibrillation patients. *Pacing Clin Electrophysiol.* (2015) 38:171–7. doi: 10.1111/pace.12537

26. Röcken C, Peters B, Juenemann G, Saeger W, Klein HU, Huth C, et al. Atrial amyloidosis: an arrhythmogenic substrate for persistent atrial fibrillation. *Circulation.* (2002) 106:2091–7. doi: 10.1161/01.CIR.0000034511.06350.DF

27. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace.* (2016) 18:1455–90. doi: 10.1093/europace/euw161

28. Alosco ML, Spitznagel MB, van Dulmen M, Raz N, Cohen R, Sweet LH, et al. Cognitive function and treatment adherence in older adults with heart failure. *Psychosom Med.* (2012) 74:965–73. doi: 10.1097/PSY.0b013e318272ef2a

29. Dickson VV, Tkacs N, Riegel B. Cognitive influences on self-care decision making in persons with heart failure. *Am Heart J.* (2007) 154:424–31. doi: 10.1016/j.ahj.2007.04.058

30. Lovell J, Pham T, Noaman SQ, Davis MC, Johnson M, Ibrahim JE. Self-management of heart failure in dementia and cognitive impairment: a systematic review. *BMC Cardiovasc Disord.* (2019) 19:99. doi: 10.1186/s12872-019-1077-4

31. O'Keefe JH, Bybee KA, Lavie CJ. Alcohol and cardiovascular health: the razor-sharp double-edged sword. *J Am Coll Cardiol.* (2007) 50:1009–14. doi: 10.1016/j.jacc.2007.04.089

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Convergent cardiorespiratory neurons represent a significant portion of cardiac and respiratory neurons in the vagal ganglia

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Significant cardiorespiratory coordination is required to maintain physiological function in health and disease. Sensory neuronal "cross-talk" between the heart and the lungs is required for synchronous regulation of normal cardiopulmonary function and is most likely mediated by the convergence of sensory neural pathways present in the autonomic ganglia. Using neurotracer approaches with appropriate negative control experiments in a mouse model, presence of cardiorespiratory neurons in the vagal (nodose) ganglia are demonstrated. Furthermore, we found that convergent neurons represent nearly 50% of all cardiac neurons and approximately 35% of all respiratory neurons. The current findings demonstrate a pre-existing neuronal substrate linking cardiorespiratory neurotransmission in the vagal ganglia, and a potentially important link for cardiopulmonary cross-sensitization, which may play an important role in the observed manifestations of cardiopulmonary diseases.

KEYWORDS

autonomic nervous, vagal, neurocardiology, cardiac neurons, respiratory neurons, cardiorespiratory neurons, convergent neurons

Introduction

The autonomic nervous system innervates all visceral organs, regulating every aspect of their function and allowing them to operate in a coordinated fashion (1). A significant highway for afferent and efferent communication between the brain and the visceral organs is the vagus nerve, which carries cardiovascular and respiratory signals to the brainstem *via* sensory neurons in the vagal ganglia (nodojugular-complex, NJG).

Mechanical interactions between the heart and lungs are well-established. Pathological processes, such as myocardial injury that affect the heart cause autonomic remodeling in the peripheral and central nervous system, leading to sympathetic activation as well as parasympathetic dysfunction (1). This autonomic remodeling results in progression of heart failure and arrhythmias (1–3). On the other hand, injury to the central nervous system, such as subarachnoid hemorrhage, can affect vagal neuronal density, leading to cardiac electrophysiological abnormalities However, while axonal branching is known to be ubiquitous in the brain (4), and specific cardiopulmonary preganglionic parasympathetic neurons that coordinate cardiopulmonary function have been identified in the nucleus ambiguous of the brainstem (5), little attention has been given to potential cardiac and respiratory interactions that may exist *via* a shared peripheral autonomic network. Using retrograde fluorescent labeling techniques, Tomney and colleagues reported evidence of branching post-ganglionic sympathetic efferent cardiopulmonary neurons in the canine sympathetic ganglia (6). Given the tight physiological coordination of the cardiorespiratory system and sensory innervation of both organs *via* the vagal nerve, we hypothesized that a significant portion of cardiac and respiratory neurons in the vagal ganglia may also be convergent, allowing for the processing of afferent inputs from both organs, and tested this hypothesis using several retrograde labeling techniques.

Materials and methods

All animal experimental procedures were approved by the University of California at Los Angeles. C57BL/6J (000664) mice were purchased from the Jackson Laboratory. Benzyl ether, dichloromethane, and dispase II were purchased from Millipore Sigma. CTB-Alexa Fluor-555 was purchased from Thermofisher Scientific (Waltham, MA, USA). Collagenase I was obtained from Life Technology Corporation (Carlsbad, CA, USA). RetroAAV2-Td-tomato was procured from Boston Children's Hospital Core (Boston, MA, USA). Retro-AAV9-GFP was from Addgene, (Watertown, MA, USA). Fast blue (FB) was obtained from Polyscience, (Warrington, PA, USA).

Administration of neurotracers in the lungs and heart

Three months old mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg IP). Anesthetized mice were positioned on the surgical platform. A laryngoscope was used for direct visualization of the glottis. The trachea was intubated with a 20-gauge blunt catheter. For non-viral neuro-tracer approaches, mice (n = 6) received 30 μL of 2% FB intratracheally, following confirmation of intratracheal catheter placement *via* visualization of condensation on a hand-held mirror. After 11 days, cardiac injections of cholera toxin B Alexa Fluor-555 (CTB, n = 6) were performed in FB administrated mice, as previously described (7). Briefly, mice received carprofen (5 mg kg^{-1}, s.c.) and buprenorphine (0.05 mg/kg, s.c.) 1 hour before surgery. Animals were anesthetized with isoflurane (induction at 5%, maintenance at 1–3%, inhalation), intubated, and mechanically ventilated. Core body temperature was maintained at 37°C. The surgical incision site was cleaned using 10% povidone-iodine and 70% ethanol. A left lateral thoracotomy was performed at the fourth intercostal space and the heart was exposed, cardiac injections of the left and right ventricles were performed with 10 μL of 0.1% CTB. The surgical wounds were closed with 6–0 sutures. Buprenorphine (0.05 mg/kg, s.c.) was administered daily for 2 days after surgery. Animals were sacrificed 3 days later and bilateral NJG were dissected and collected for imaging and fluorescence-activated cell sorting. For viral neurotracer studies, retro-AAV2-td-tomato (1 × 10ˆ12 vg/mL, 1 in 5 dilutions) (n = 5) was administered intratracheally followed by cardiac retro-AAV9-GFP (1 × 10ˆ13 vg/mL1 in 10 dilutions) injections after 2 days (n = 5). Three weeks later, bilateral NJGs were isolated. In two other groups of mice (n = 3 per group), only cardiac injections of neurotracers (CTB in one group and retro-AAV2-td-tomato in the other group) were performed. In the mice receiving CTB, NJG, lungs, and lumbar dorsal root ganglia (DRG) were isolated after 3 days, while in the group receiving retro-AAV2-td-tomato only, those organs were isolated after 21 days for imaging.

Tissue clearing

Tissue clearing was performed using the iDISCO protocol (7). Briefly, fixed lungs lobes were dehydrated with graded methanol [20, 40, 60, and 80% methanol in H_2O (vol/vol) sequentially, each for 1 h at room temperature], washed twice with 100% methanol for 1 hour at room temperature, and chilled at 4°C. Lungs were then incubated in 66% dichloromethane/33% methanol overnight, washed twice in 100% methanol for 1 h at room temperature, and chilled to 4°C. Following these steps, tissue was bleached with 5% H_2O_2 in methanol (vol/vol) overnight at 4°C. After bleaching, the lungs were again rehydrated with graded methanol, in series, followed by washes with 0.01 M PBS, and then dehydrated again with graded methanol, in series and, incubated in 66% dichloromethane/33% methanol for 3 h at room temperature. Then, the lung tissue was washed using 100% dichloromethane and stored in benzyl ether for imaging.

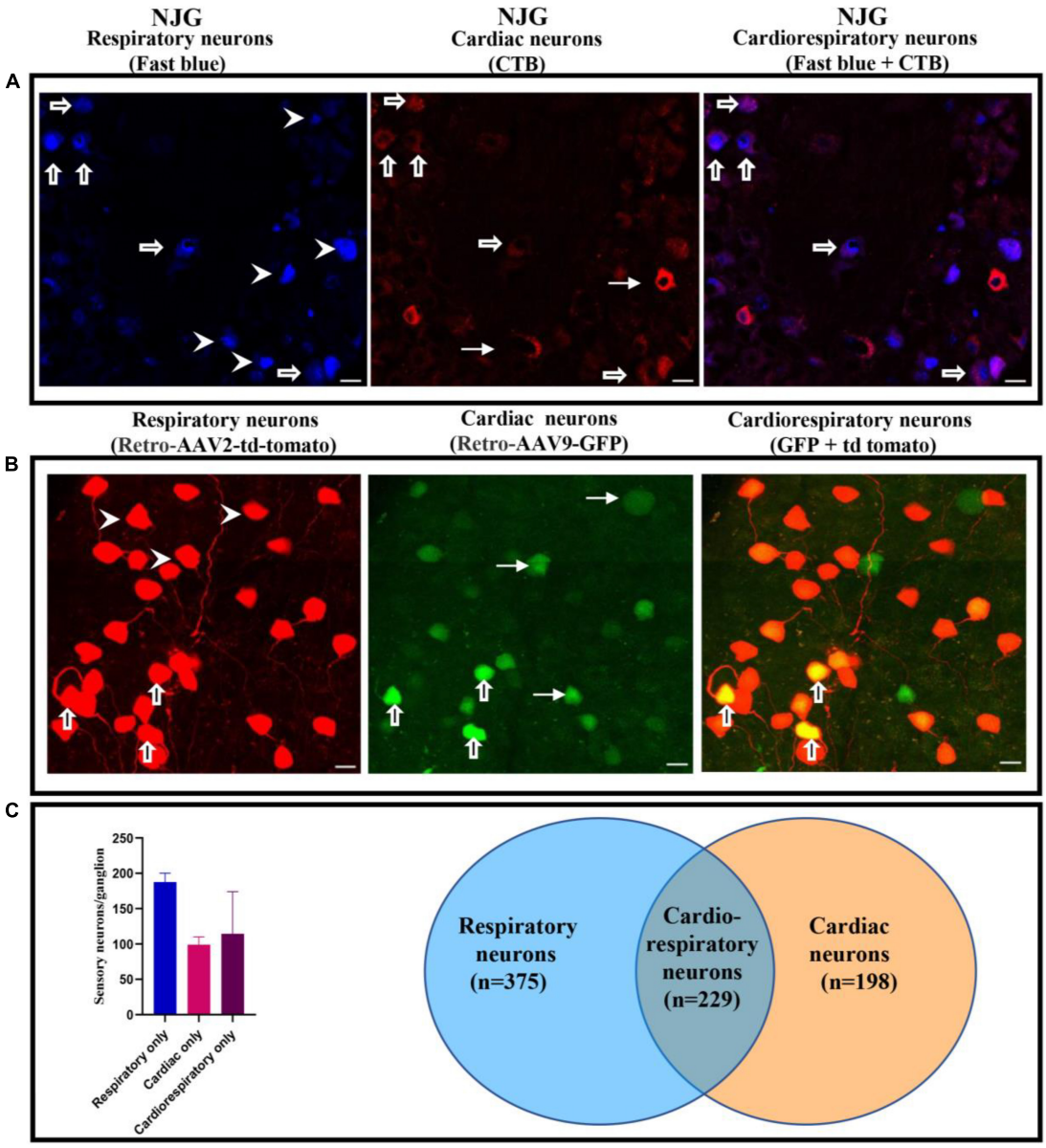

FIGURE 1
Presence of cardiorespiratory neurons in the NJG. **(A)** NJGs were isolated from FB (n = 6) and CTB administered mice (n = 6), cryosectioned (7 um slices), and imaged using confocal microscopy. Presence of respiratory, cardiac, and cardiorespiratory neurons (dual labeling of CTB and FB) was observed in all ganglia (Bars = 20 μm). Thick arrows point to cardiorespiratory neurons, arrowheads to respiratory only, and thin arrows to cardiac only neurons. **(B)** NJGs were isolated from retro-AAV2-td-tomato (n = 5) and retro-AAV9-GFP (n = 5) mice. Individual slices were imaged by confocal microscopy. Retro-AAV2-td-tomato and retro-AAV9-GFP also demonstrated evidence of neurons with dual labeling (Bars = 20 μm). Thick arrows point to examples of convergent cardiorespiratory neurons, arrowheads to examples of respiratory only, and thin arrows to examples of cardiac only neurons. **(C)** Flow cytometry data from NJG (2 separate groups of 3–4 mice) also confirmed evidence of convergent neurons.

Confocal imaging

Whole NJG or cryo-sectioned NJG (7 μm slices) and lumbar DRGs were fixed in 4% PFA. NJGs, lumbar DRGs, and, cleared lung tissue were imaged using a confocal laser scanning microscope (Zeiss, LSM 880). Individual slices were analyzed from non-viral tracer (CTB and FB) and viral tracer (retro-AAV2-td-tomato and retro-AAV9-GFP) administrated mice using Z-stack imaging with confocal microscopy. For CTB imaging, excitation wavelength of 633 nm (filter wavelength range of 562–642 nm) and for FB imaging, excitation wavelength of 561 nm (laser DPSS 561-10, GFP filter wavelength range

of 410–513 nm) was used. For GFP imaging, laser wavelength of 549 nm (filter 491–606 nm) and for td-tomato imaging, excitation wavelength of 561 (laser HENE633, filter range of 566–697 nm) was used. Images were processed with Zen 2 (Zeiss) software. Labeled neurons were manually quantified for 3 animals that received retrograde viral tracers and 5 animals that received non-viral (CTB/FB) tracers.

Fluorescence-activated cell sorting using flow cytometry in CTB/FB labeled ganglia

NJG isolated from CTB/FB mice were digested with collagenase I, and Dispase II at 37°C for 45 min, washed with L-15 medium, gently triturated with glass aspiration pipettes of decreasing diameter, and filtered with a 40 μm cell strainer. The cell suspensions were sorted on a 5 Laser SORP Aria II (488, 562,633, 405, and 350 nm) 4-way 4°C chilled sorter. The 670/14 635LP filter on the 562 nm laser was used to detect CTB-555 and the 405 nm laser with the 450/50 filter set was used for FB detection.

Statistical analysis

All values are expressed as mean ± standard mean error (SEM). One-way ANOVA with Tukey multiple comparison test was used for multiple group comparisons. PRISM (GraphPad) was used for all statistical analyses.

Results and discussion

Cardiac and respiratory neuronal labeling was observed with both CTB/FB and retro-AAV tracers using confocal imaging (**Figures 1A,B** and **Supplementary Figure 1**). Notably, colocalization of CTB and FB and colocalization of retro-AAV9-GFP and retro-AAV2-td-tomato tracers were observed in multiple NJG neurons in all animals undergoing both cardiac and respiratory tracer administration, demonstrating the existence of convergent neurons. Image quantification of neurons showed that that mice that received a non-viral neuronal tracer (CTB/FB) exhibited two to threefold higher neuronal labeling as compared to mice that received a retro-AAV tracers (**Table 1**). This is likely due to the different mechanisms of retrograde transport employed by these tracers. Non-viral neurotracers, such as CTB and FB, directly bind with host biomolecules. CTB binds with GM1 gangliosides that are present on the surface of the plasma membranes, whereas FB binds to nucleic acids. In the case of retrograde viral neurotracers (retro-AAVs), at least three physiological barriers must be crossed by the AAV to transduce host cells: (1) AAV has to bind with the plasma membrane, (2) AAV internalization has to occur *via* a dynamin-dependent mechanism (for AAV2 it has to be the endocytosed *via* clathrin-coated vehicles), and (3) the virus has to enter into the nucleoplasm through the nuclear pore and then integrate its genome into the nucleic acid (8). Finally, the AAV virus that has successfully integrated expresses the fluorescent protein allowing for detection (8). Therefore, it is not surprising that retrograde viral tracers have lower expression/labeling than non-viral tracers. Regardless of the type of neurotracer administered, the number of labeled respiratory neurons was approximately twofold higher than the number of cardiac or cardiorespiratory neurons. This is likely due to the wider distribution of FB throughout the lungs following intratracheal administration. With both viral and non-viral neurotracer techniques, cardiorespiratory neurons represented a significant portion of cardiac and respiratory neurons in the NJG complex. The presence of convergent-dual-labeled CTB/FB neurons was also confirmed by flow cytometry. Flow cytometry detected 185 ± 17 labeled respiratory neurons, 99 ± 11 labeled cardiac neurons and 114 ± 59 dual-labeled cardiorespiratory neurons present in per nodose ganglion. Similar to results obtained from imaging techniques, the number of respiratory neurons was 1.8 times greater than cardiac neurons on flow cytometry analysis (**Figure 1C**, raw data in **Supplementary Figure 1**).

To ensure that the labeling of convergent neurons was not due to leakage of dye from the heart into the lungs or systemic circulation, analysis of the NJGs, lumbar DRGs (ganglia that are not associated with cardiac innervation) and cleared lung tissue was performed in mice injected with only cardiac CTB and retro-AAV. The results revealed that in all mice, labeled NJG neurons were observed, while no labeling (**Figures 2A,B**) of nerves or neurons was seen in the cleared lung tissue or the lumbar DRGs, suggesting that

TABLE 1 Quantification of cardiac, respiratory, and dual labeled cardiorespiratory neurons on confocal imaging in the mouse NJG complex.

Non-viral neurotracers		
Respiratory neurons/Ganglion	**Cardiac neurons/Ganglion**	**Cardiorespiratory neurons/Ganglion**
112 ± 19	55.3 ± 8*	30 ± 7***
Viral neurotracers		
Respiratory neurons/Ganglion	**Cardiac neurons/Ganglion**	**Cardiorespiratory neurons/Ganglion**
34 ± 3	19 ± 3***	14 ± 2***

CTB/FB group: $n = 5$ animals; retro-AAV2-td-tomato and retro-AAV9-GFP group: $n = 5$ animals; $^{*}P < 0.05$ for comparison of cardiac to respiratory neurons using non-viral techniques, $^{***}P < 0.001$ for comparison of cardiorespiratory neurons to respiratory neurons. The number of cardiorespiratory compared to number of cardiac neurons was not statistically different.

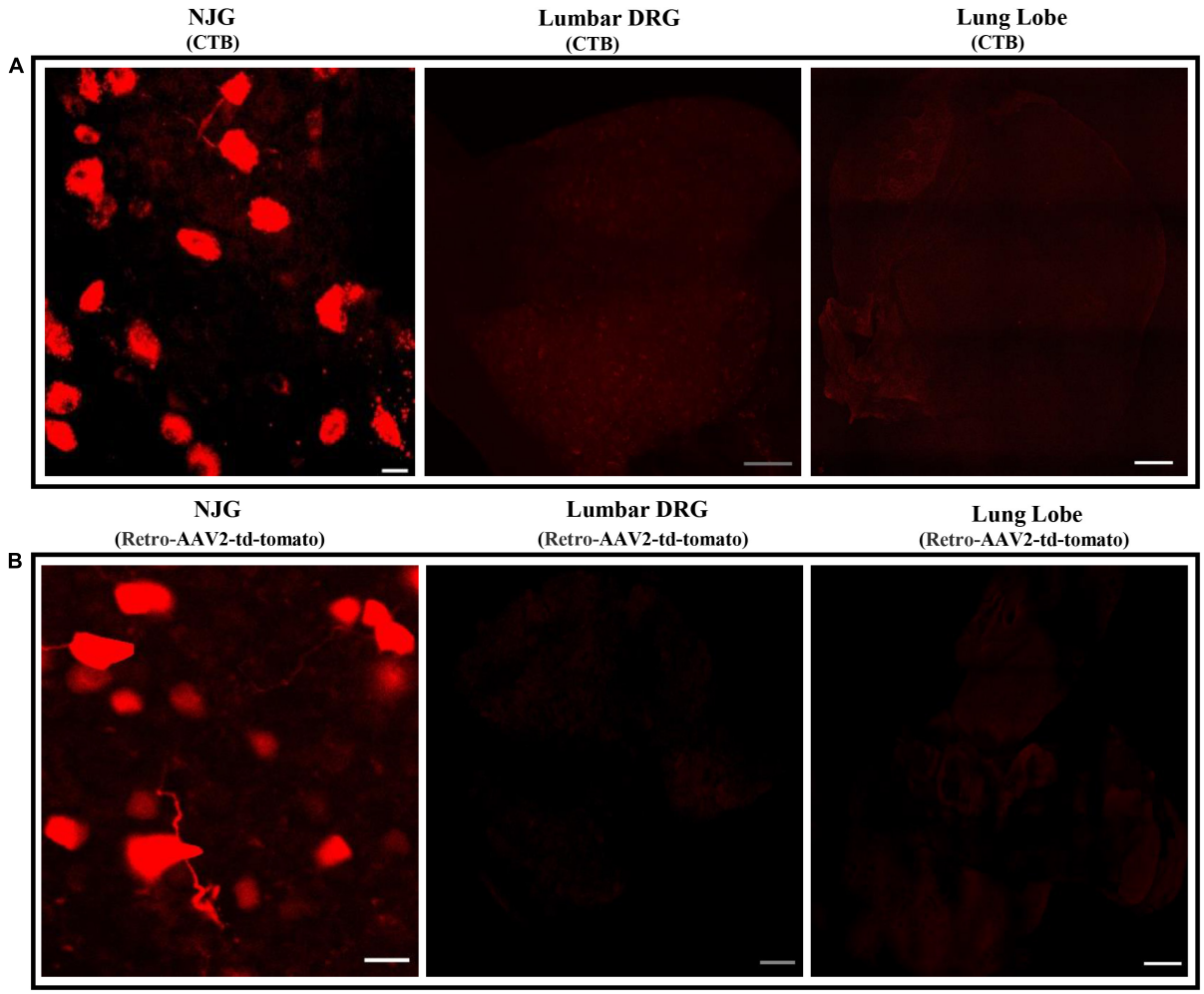

FIGURE 2
Absence of neuronal labeling in lumbar DRGs and the lungs following cardiac injections of neurotracers. **(A,B)** The lungs, lumbar DRGs, and NJG were isolated from mice after CTB (n = 3) or cardiac retro-AAV2-td-tomato (n = 3) injections and maximum intensity projections were imaged. While neuronal labeling in the NJGs was clearly observed, no labeling of neurons or nerves was seen in the lungs (confirming lack of leakage of dye) or in the lumbar DRGs (confirming lack of significant leakage into the systemic circulation). NJG: Bars = 20 μm. Lumbar DRGs: Bars = 100 μm. Cleared lung tissue: Bars = 500 μm.

localized or systemic spread of CTB/AAV was unlikely to explain convergent neuronal labeling. Our results demonstrate that convergent cardiorespiratory neurons represent a significant portion of all cardiac and respiratory sensory neurons in the peripheral vagal ganglia, a novel finding of this study. A recent study suggested that neurovisceral organs may have parallel processing within peripheral ganglia, with neurotransmission convergence occurring primarily at the level of the brainstem (9). Given the significant portion of convergent cardiorespiratory neurons noted in this study, it's possible that an important degree of cardiorespiratory coordination may occur at the vagal ganglia and other shared peripheral ganglia. These neurons appear to be a specific subset of sensory neurons that innervate both organs and may play an important role in synchronizing cardiorespiratory function.

It is established that pulmonary pathologies affect cardiac function, and conversely, cardiac disease is known to negatively impact respiratory processes. Until now, much focus has relied on mechanical interactions between these organs. Yet, many patients demonstrate evidence of multi-organ autonomic dysfunction after single organ pathology, which could be mediated *via* their shared autonomic sensory neuronal network. For example, patients with chronic obstructive pulmonary disease demonstrate abnormal heart rate recovery after exercise (10). The prevalence of cardiac arrhythmias, especially atrial fibrillation, where vagal neurotransmission plays an important role, is increased in these patients (11). Conversely, Cheyne-Stroke respiration and sleep apnea are associated with the occurrence of atrial fibrillation and increased mortality in the setting of heart failure (12). Most mechanistic studies,

however, have focused on the effects of a single organ pathology on the autonomic nervous system and vice-versa. The specific effects of cardiac pathology on the autonomic nervous system, for example, have been extensively studied. Myocardial injury is known to cause remodeling of the cardiac autonomic nervous system, leading sympathetic activation and parasympathetic dysfunction (1). In a chronic porcine infarct model, myocardial infarction was associated with significant functional and structural remodeling of nodose ganglia neurons that transmit cardiac nociceptive signals, reducing cardiac nociceptive neurotransmission (13). Nodose ganglion degeneration after subarachnoid hemorrhage is associated with coronary vasospasm, and ischemic neurodegeneration of the vagal ganglia can lead to ventricular arrhythmias during subarachnoid hemorrhage (14, 15). While these studies have clearly demonstrated a two-way relationship between the heart and the nervous system *via* the peripheral vagal ganglia, data regarding multi-organ interactions through the peripheral autonomic nervous system are sparse. A possible mechanism for dual labeling of neurons may be due to branching axons, that has also been observed in the sympathetic chain of canines and is known to exist in the brain (6, 16).

Our study suggests that many of the cardiac and respiratory sensory innervation share the same neurons at the level of the peripheral vagal ganglia, and processes that affect the heart or the lung may, in fact, affect other organs through this peripheral neural link. The current study's findings open up a potentially new and important avenue of investigation in the context of cardiopulmonary disease, linking cardiorespiratory interactions *via* their shared sensory neurons in the NJGs. Several studies are underway in our laboratory to generate molecular maps of cardiorespiratory neurons in health and disease. Identifying neural pathways and functions of these neurons may shed important light on how these organs are coordinated through the autonomic network in health and disease.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by the University of California at Los Angeles Animal Research Committee.

Author contributions

AD, KW, and KS performed the experiments and analyzed data, and drafted manuscript. YS and JV assisted with protocols creation and experiments, and edited the manuscript. XS assisted with design of studies, data interpretation, and editing of the manuscript. MV oversaw design of the study, protocols, experiments, data interpretation, and drafted and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MV has performed educational consulting for Medtronic Inc., and Biosense Webster and has shares in NeuCures, Inc. University of California, Los Angeles has patents relating to cardiac neural diagnostics.

The remaining 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.

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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.2022.959815/full#supplementary-material

References

1. Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. *Prog Cardiovasc Dis.* (2008) 50:404–19. doi: 10.1016/j.pcad.2008.01.003

2. Zipes DP, Rubart M. Neural modulation of cardiac arrhythmias and sudden cardiac death. *Heart Rhythm.* (2006) 3:108–13. doi: 10.1016/j.hrthm.2005.09.021

3. Wu P, Vaseghi M. The autonomic nervous system and ventricular arrhythmias in myocardial infarction and heart failure. *Pacing Clin Electrophysiol.* (2020) 43:172–80. doi: 10.1111/pace.13856

4. Dumoulin FL, Daniel WG, Lichtlen P, Schmidt RE. [Pulmonary thromboembolism in antiphospholipid syndrome]. *Dtsch Med Wochenschr.* (1993) 118:903–6. doi: 10.1055/s-2008-1059403

5. Veerakumar A, Yung AR, Liu Y, Krasnow MA. Molecularly defined circuits for cardiovascular and cardiopulmonary control. *Nature.* (2022) 606:739–46. doi: 10.1038/s41586-022-04760-8

6. Tomney PA, Hopkins DA, Armour JA. Axonal branching of canine sympathetic postganglionic cardiopulmonary neurons. A retrograde fluorescent labeling study. *Brain Res Bull.* (1985) 14:443–52. doi: 10.1016/0361-9230(85)90023-1

7. Rajendran PS, Challis RC, Fowlkes CC, Hanna P, Tompkins JD, Jordan MC, et al. Identification of peripheral neural circuits that regulate heart rate using optogenetic and viral vector strategies. *Nat Commun.* (2019) 10:1944. doi: 10.1038/s41467-019-09770-1

8. Bartlett JS, Wilcher R, Samulski RJ. Infectious entry pathway of adeno-associated virus and adeno-associated virus vectors. *J Virol.* (2000) 74:2777–85. doi: 10.1128/JVI.74.6.2777-2785.2000

9. Zhao Q, Yu CD, Wang R, Xu QJ, Dai Pra R, Zhang L, et al. A multidimensional coding architecture of the vagal interoceptive system. *Nature.* (2022) 603:878–84. doi: 10.1038/s41586-022-04515-5

10. Zhao D, Abbasi A, Casaburi R, Adami A, Tiller NB, Yuan W, et al. Identifying a heart rate recovery criterion after a 6-minute walk test in COPD. *Int J Chron Obstruct Pulmon Dis.* (2021) 16:2545–60. doi: 10.2147/COPD.S311572

11. Liu X, Chen Z, Li S, Xu S. Association of chronic obstructive pulmonary disease with arrhythmia risks: a systematic review and meta-analysis. *Front Cardiovasc Med.* (2021) 8:732349. doi: 10.3389/fcvm.2021.732349

12. Bradley TD, Floras JS. Sleep apnea and heart failure: part I: obstructive sleep apnea. *Circulation.* (2003) 107:1671–8. doi: 10.1161/01.CIR.0000061757.12581.15

13. Salavatian S, Hoang JD, Yamaguchi N, Lokhandwala ZA, Swid MA, Armour JA, et al. Myocardial infarction reduces cardiac nociceptive neurotransmission through the vagal ganglia. *JCI Insight.* (2022) 7:e155747. doi: 10.1172/jci.insight.155747

14. Aydin MD, Kanat A, Yilmaz A, Cakir M, Emet M, Cakir Z, et al. The role of ischemic neurodegeneration of the nodose ganglia on cardiac arrest after subarachnoid hemorrhage: an experimental study. *Exp Neurol.* (2011) 230:90–5. doi: 10.1016/j.expneurol.2010.09.018

15. Yolas C, Kanat A, Aydin MD, Altas E, Kanat IF, Kazdal H, et al. Unraveling of the effect of nodose ganglion degeneration on the coronary artery vasospasm after subarachnoid hemorrhage: an experimental study. *World Neurosurg.* (2016) 86:79–87. doi: 10.1016/j.wneu.2015.09.004

16. Dumoulin A, Ter-Avetisyan G, Schmidt H, Rathjen FG. Molecular analysis of sensory axon branching unraveled a cGMP-dependent signaling cascade. *Int J Mol Sci.* (2018) 19:1266. doi: 10.3390/ijms19051266

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Case report: Different clinical manifestations of the rare Loeffler endocarditis

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Background: Loeffler endocarditis is a rare and fatal disease, which is prone to be misdiagnosed, owing to its various clinical manifestations. Consequently, an early identification of Loeffler endocarditis and its effective treatment are crucial steps to be undertaken for good prognosis.

Case presentation: This report describes two cases of Loeffler endocarditis with different etiologies and clinical manifestations. Case 1 was caused by idiopathic eosinophilia and presented with a thrombus involving the tricuspid valve and right ventricular inflow tract (RVIT). The patient suffered from recurrent syncope following activity. After the patient underwent tricuspid valve replacement and thrombectomy, he took oral prednisone and warfarin for 2 years, consequent to which he discontinued both drugs. However, the disease recurred 6 months later, this time manifesting as edema of both legs. Echocardiography showed that a thrombus had reappeared in the RVIT. Thus, oral prednisone and warfarin therapy was readministered. Three months later, the thrombus had dissolved. Low-dose prednisone maintenance therapy was provided long term. Case 2 involved a patient who presented with recurrent fever, tightness in the chest, and asthma, and whose condition could not be confirmed, despite multiple local hospitalizations. In our hospital, echocardiography revealed biventricular apical thrombi. After comprehensive examinations, the final diagnosis was eosinophilic granulomatosis polyangiitis (EGPA) involving multiple organs, including the heart (Loeffler endocarditis), lungs, and kidneys. After administration of corticosteroid, anticoagulant, and immunosuppressive agents along with drugs to improve cardiac function, the patient's symptoms improved significantly.

Conclusion: In Loeffler endocarditis due to idiopathic eosinophilia, long-term corticosteroid use may be required. Diverse and non-specific symptoms cause Loeffler endocarditis to be easily misdiagnosed. So, when a patient shows a persistent elevation of the eosinophil count with non-specific myocardial damage, the possibility of this disease, should always be considered. Furthermore, even when an invasive clinical procedure such as endomyocardial biopsy (EMB) is not available or

acceptable, corticosteroids should be administered promptly to bring the eosinophil count back to the normal range, thereby halting the progression of disease and reducing patient mortality.

KEYWORDS

Loeffler endocarditis, eosinophilia, thrombus, corticosteroid, anticoagulant

Introduction

Loeffler endocarditis, also known as eosinophilic endocarditis, is a severe complication of eosinophilia (1). As this condition manifests as diverse clinical presentations, it is easily misdiagnosed as a cardiac emergency such as acute myocarditis or acute myocardial infarction. If left untreated, the patients may develop potentially fatal complications such as cardioembolism, arrhythmia, or acute heart failure (2). Consequently, early identification and effective treatment are crucial for improving the prognosis. However, no consensus guidelines are available for managing Loeffler endocarditis (3), because the management treatment of each patient is individualized and relates to (1) an acute treatment, (2) a symptomatic cardiac treatment based on diverse manifestations (anti-arrhythmics, pressure support, ejection fraction support (EF support), thrombus targeting, prophylaxis, etc.), and (3) finally a thorough diagnostic workup to identify the cause of eosinophilia (idiopathic, primary, or secondary). This report presents in a detailed manner how we had used these three components in the treatment of our two patients. We hope that it provides valuable information for the early recognition and appropriate treatment of this rare and fatal disease.

Case description

Case 1

A 61-year-old man was admitted to our hospital on May 20, 2016, due to syncope after activity. One month before that incident, he had experienced tightness in his chest and dizziness, with both symptoms showing reprieve following a short rest (3–5 min). A few days later, he felt his condition was progressively worsening with each passing day. He occasionally had sudden syncope during activity, but it then revived on its own without any intervention. On admission to our hospital, his blood pressure was 110/70 mmHg. An electrocardiogram showed flat T waves in leads II, III, and arteriovenous fistula (AVF). Routine blood work showed a white blood cell (WBC) count of 11.4 $\times$ 10^9/L (reference, 4–10 $\times$ 10^9/L), eosinophil count of 3.1 $\times$ 10^9/L (reference, 0.02–0.52 $\times$ 10^9/L) with a percentage of eosinophils at 38% (reference, 0.4–8.0%), a hemoglobin level of 135 g/L (reference, 120–160 g/L), and a platelet count of 146 $\times$ 10^9/L (reference, 100–300 $\times$ 10^9/L). Moreover, the level of cardiac troponin I (cTnI) was 0.05 ng/mL (nanograms/milliliter) (reference, <0.2 ng/mL), and the level of B-type natriuretic peptide (BNP) was 441 pg/mL (pictograms/milliliter) (reference, <100 pg/mL). Cardiac computed tomography (CT) showed right atrial (RA) enlargement, small right ventricle (RV), and small pericardial effusion. Echocardiography showed a mass (Figure 1A) in the right ventricle (RV) involving the right ventricular inflow tract (RVIT), tricuspid annulus, chordae tendineae, and papillary muscle, resulting in RVIT stenosis (Figure 1B) and restriction of the motion of the tricuspid leaflet and chordae. Left ventricular ejection fraction (LVEF) was 66%. Contrast echocardiography showed a mass in the right ventricle and no blood perfusion within it. The patient did not show any history of hypertension, cerebrovascular disease, coronary heart disease, heart failure, arrhythmia, food and drug allergies, infectious diseases, or parasitic infection. Parasite and leukemic fusion gene [Fip1-like 1/platelet-derived growth factor receptor-alpha (FIP1/PDGFR)] tests were all negative. Eosinophilia and Loeffler endocarditis with thrombus in the RV was considered a probable diagnosis.

Case 2

A 58-year-old man presented with chest tightness, cough, and asthma without fever in late October 2020. The patient took aminophylline and inhaled budesonide, but without obvious effect. His condition began to worsen gradually and he was admitted to a local hospital on 4 November, 2020. Electrocardiogram showed an elevated ST segment in leads II, III, and arteriovenous fistula (AVF). Echocardiography showed segmental wall motion abnormalities, and the LVEF was decreased (36%). Laboratory examinations showed elevated cardiac troponin I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels. Thus, the patient was transferred to a cardiology care unit (CCU) for acute inferior myocardial

Abbreviations: EGPA, Eosinophilic granulomatosis polyangiitis; LVEF, Left ventricular ejection fraction; RVIT, Right ventricular inflow tract; LA, Left atrial; RA, Right atrial; LV, Left ventricle; RV, Right ventricle; cTnI, Cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BNP, B-type natriuretic peptide.

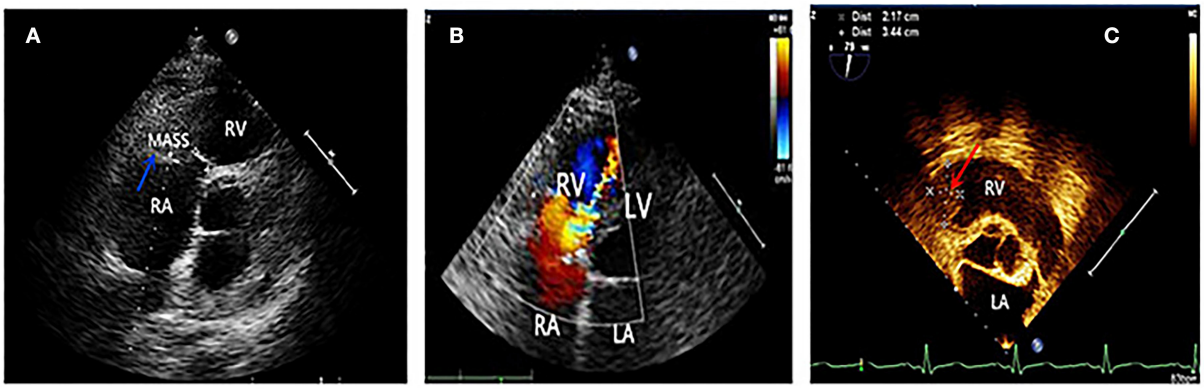

FIGURE 1
(A) Blue arrow showed a mass in the right ventricle, which was later confirmed thrombus by surgery. **(B)** Four chamber view showed the mosaic blood flowing through the stenotic right ventricular inflow tract. **(C)** Two and a half years after tricuspid valve replacement and thrombectomy, a recurrence of thrombus (indicated by red arrow) was seen in the right ventricular inflow tract by transesophageal echocardiography.

infarction and heart failure. Coronary computed tomography (CT) angiography results showed only mild stenosis in the coronary arteries. The patient's symptoms improved insignificantly after the administration of medications that improved cardiac function. During hospitalization, he had fever with temperature of 39.5°C. Blood work showed a white blood cell (WBC) count of 9.7 × 10^9/L, hemoglobin level of 117 g/L, platelet count of 186 × 10^9/L, and high levels (2.2 × 10^9/L) and percentage (22.8%) of eosinophils. As the patient presented with cough and asthma, he was administered anti-infective and antipyretic treatment. Four days later, the patient's body temperature decreased to normal, and the fever symptoms were relieved. He was discharged from the hospital on 19 November, 2020. Two weeks later, the symptoms including fever, cough, and asthma recurred. Upon revisiting the local hospital, the patient's chest CT showed bronchiectasis with infection. He was administered antibiotics for 7 days but with poor effect and had fever with a temperature of 38°C.

With no significant relief from symptoms, he revisited our hospital and was admitted to the respiratory department with bronchiectasis. Physical examinations revealed a body temperature of 37.6°C, pulse rate of 111 times/min, respiration rate of 20 breaths/min, and blood pressure of 130/80 mmHg. The laboratory examination showed the following test results: eosinophil count 3.36 × 10^9/L, percentage of eosinophils 32.10%, cTnI level of 1.032 ng/mL, and BNP level of 1,073 pg/mL. The patient had 1-year history of bronchial asthma and allergic rhinitis. He had a 30-year smoking history, smoking approximately 7 cigarettes per day and quitting smoking for 1 year. The patient did not show any history of hypertension, coronary heart disease, diabetes mellitus, food or drug allergies, infectious diseases, or parasitic infections. Echocardiography showed an enlarged heart and decreased LVEF (47%). It also showed that there were masses in both ventricular apexes with a small amount of pericardial effusion (Figure 2A). Contrast echocardiography showed no perfusion in either of the ventricular apical masses (Figure 2B). Hence, we considered the masses to be thrombi.

Diagnostic assessment and therapeutic interventions

Case 1

Eosinophilia and Loeffler endocarditis with thrombus in the RV was considered a probable diagnosis. The patient was administered low-molecular-weight heparin (subcutaneous injection, 5,000 IU (international units), twice daily) and oral prednisone (60 mg, once daily). Nine days later, a routine blood work showed that eosinophils had decreased to within the normal range (0.37 × 10^9/L); however, frequent syncope persisted. The patient then underwent thrombectomy and tricuspid valve replacement. Pathological results showed an RV thrombus, endocardial fibroplasia, and eosinophilic infiltration. The symptoms relieved significantly after the operation, and he was discharged with oral prednisone (60 mg, once daily) and warfarin (3.75 mg, once daily). Six months later, prednisone was gradually tapered down to 10 mg daily. The patient continued to feel well for 2 years following surgery when he stopped taking prednisone and warfarin on his own. However, 6 months later, the patient experienced edema in both legs and was readmitted. Blood work showed elevated eosinophils once again (5.13 × 10^9/L). Echocardiography revealed a hypoechoic mass attached to the RVIT (Figure 1C), and contrast echocardiography showed no perfusion in the mass. Thus, the patient was diagnosed to have a recurrence of thrombus . He was once again prescribed oral prednisone (60 mg, once daily) and warfarin (4.5 mg, once

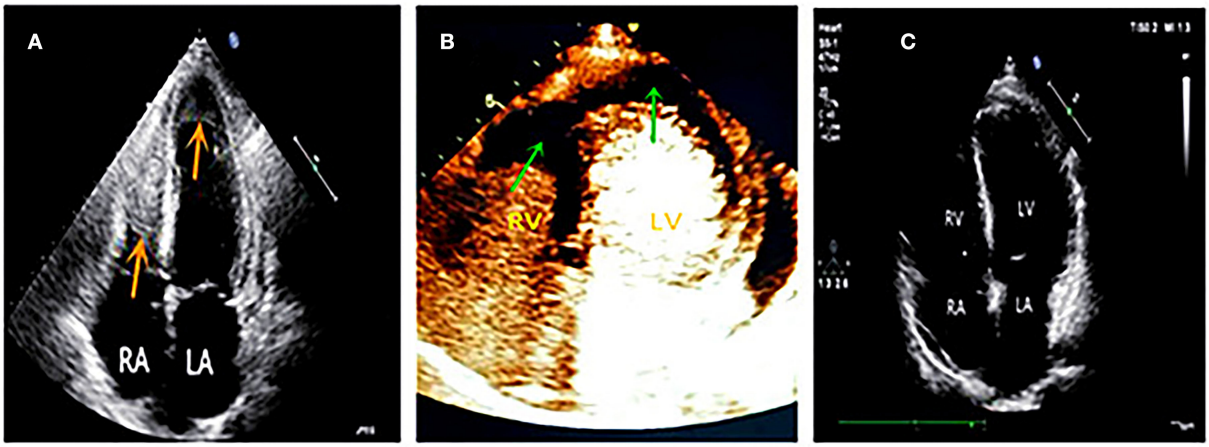

FIGURE 2
(A) Four chamber view showed masses in both of the ventricular apex (red arrows). **(B)** Contrast echocardiography showed no perfusion in both apical masses (green arrows), which suggested thrombi. **(C)** Follow-up at six months later, both ventricular apical thrombi were disappeared.

daily). At the 3-month follow-up, no edema was noticed in the patient's legs. The eosinophils had restored to normal conditions, and the RV thrombus had dissolved. Thus, the medication was changed to long-term low-dose prednisone (10 mg, once daily) maintenance therapy. The patient remained in good health at later follow-up visits.

Case 2

A probable diagnosis of Loeffler endocarditis was considered. The patient was advised to undergo an endomyocardial biopsy but he refused invasive testing. He also refused to undergo cardiac magnetic resonance (CMR) due to noise intolerance. A bone marrow puncture showed eosinophilia. Genetic FIP1L1/PDGFRA, platelet-derived growth factor receptor-beta (PDGFRB), FGFR1/d8z2 (8p11), and parasite testing were negative. Other abnormal examination results included immunoglobulin G (IgG) 24.9 g/L↑, immunoglobulin E (IgE) 1,890 IU /mL↑, and urinary microalbumin 122.74 mg/L↑. Combined with clinical symptoms and examinations, the final diagnosis was eosinophilic granulomatosis polyangiitis (EGPA) involving multiple organs, including the heart (Loeffler endocarditis), lungs, kidneys, and nasal sinuses. This diagnosis was made according to the Classification Criteria of the American College of Rheumatology for eosinophilic granulomatosis polyangiitis (EGPA) (4). The therapeutic medications included prednisone and mycophenolate mofetil for anti-immunotherapy, warfarin for anticoagulation, sacubitril valsartan, metoprolol, spironolactone, and dapagliflozin for heart failure. The patient's temperature returned to normal, and symptoms of tightness in chest, shortness of breath, and asthma alleviated substantially. During the regular outpatient follow-up visits, eosinophil count, IgG, and IgE levels returned to the normal range, electrocardiogram returned to a normal pattern, LVEF was improved to above 50%, and mural thrombi in both ventricles disappeared in echocardiography (Figure 2C). The patient's exercise tolerance improved significantly and he reported the ability to play basketball for up to an hour. Prednisone and mycophenolate mofetil were gradually tapered down to a maintenance dose. Because the patient had no uncomfortable symptoms and was in a good condition, he requested discontinuation of the medications for heart failure. A recent follow-up reported normal immunoglobulin levels, normal cardiac function, and no mural thrombus in the heart.

Discussion

Eosinophilia is defined as a persistent peripheral blood eosinophil count $\geq 1.5 \times 10^9$/L with an extensive or local eosinophilic infiltration (5), which involves multiple organs, including the heart, lungs, kidneys, central nervous system, and skin. Cardiac involvement is the most serious complication of this condition. Its incidence is over 50% and is a major cause of morbidity and mortality (6). Eosinophilia can be classified as idiopathic, primary, and secondary according to the etiology (7). The etiologies of primary eosinophilia include stem cells, myeloid or eosinophilic neoplasm (PDGFRA, PDGFRB, FGFR1), and T-cell lymphoma. The etiologies of secondary eosinophilia include parasitic infections, allergic diseases (bronchial asthma, drug allergies), certain infectious diseases (tuberculosis, AIDS), and hematologic diseases (8). However, there is no apparent etiology in idiopathic eosinophilia.

Loeffler endocarditis refers to eosinophilia that involves the endocardium, with the first case having been reported by Loeffler in 1936 (9). According to its pathological characteristics, Loeffler endocarditis can be divided into three phases: (1) the necrotic phase: eosinophils infiltrate the myocardium, and inflammation leads to myocardial damage and necrosis; (2) the thrombotic phase: as myocardial inflammation subsides, the heart cavity subsequently forms a mural thrombus; (3) the fibrotic phase: eosinophils disappear, and the primary characteristic is extensive endocardial fibrous proliferation. In the third phase, the ventricular diastolic function is the main pathophysiological change, often manifested as recalcitrant chronic heart failure (10). Once it progresses to the fibrotic stage, the survival rate is only 35%−50%, even after 2 years of active treatment (11). As the disease progresses, serious outcomes, such as heart failure, arrhythmia, and embolic events, manifest (12). In the early stages, it is easy to misdiagnose this condition due to diverse clinical manifestations (13).

Endomyocardial biopsy is the gold standard for diagnosing Loeffler endocarditis (14); however, no clinical guidelines for managing Loeffler endocarditis are currently in vogue, and treatment expertise is mostly derived from a handful of previous case reports. The management of Loeffler endocarditis requires consideration of the etiology of eosinophilia, the stage of progress, and the involvement of organs (3). The treatment goals should include the following: (1) eosinophils being reduced to the normal range to avoid cardiac damage (eosinophils can release toxic substances), and (2) treatment of the underlying etiology and complications (heart failure, arrhythmia, thrombus). Primary eosinophilia, especially in patients with FIP1L1-PDGFRA-positive, is highly sensitive to imatinib (15, 16). Imatinib is the first choice for treatment. The Loeffler endocarditis originates from idiopathic and secondary eosinophilia, corticosteroids are usually the primary medication (17). In addition, it is important to save samples for clonality (genetic, flow cytometry) before corticosteroids' initiation, if possible, because corticosteroids often have a very rapid effect, blurring the diagnostic clues.

In our report, Case 1, with idiopathic eosinophilia, had only cardiac involvement. A thrombus caused stenosis of the tricuspid valve and RVIT, leading to decreased cardiac output and frequent syncope. After thrombectomy and tricuspid valve replacement and administration of a corticosteroid and an anticoagulant, the patient felt remarkably better. The current literature does not contain exact guidelines for when to cease corticosteroid administration in idiopathic eosinophilia; however, from Case 1 in this study, we observed that when the patient stopped taking medications after several months, the eosinophil level increased and the thrombus reappeared. This indicated that if the etiology of eosinophilia was unknown, discontinuation of corticosteroids may lead to recurrence of the disease. Thus, corticosteroids may need to be taken long term in idiopathic eosinophilia.

Case 2 was of secondary eosinophilia resulting from an autoimmune disease involving multiple organs and leading to diverse clinical manifestations. At the initial visit, the patient from Case 2 was in the first stage of Loeffler endocarditis, showing non-specific symptoms such as myocardial injury and heart failure. In fact, eosinophils were already high at this stage. However, because of the rarity of Loeffler endocarditis and the lack of awareness of this disease by many physicians, it is prone to be misdiagnosed. In our hospital, echocardiography showed disease progression to the thrombotic phase, with contrast echocardiography playing an important role in the differential diagnosis of the thrombus.

Although the gold standard of diagnosis for Loeffler endocarditis is an endomyocardial biopsy, it can sometimes turn out to be a difficult diagnostic procedure. Because it is an invasive and risky examination, it may also be rejected outright by the patient, as it occurred in Case 2. Moreover, not all hospitals possess the necessary infrastructure, conditions, or technology to perform an endomyocardial biopsy. However, as Loeffler endocarditis is fatal, and where endomyocardial biopsy is not available, corticosteroids should be administered early to patients who present with persistent elevation of eosinophils and non-specific heart damage. Timely treatment alone can reduce the damage of eosinophils to the heart, prevent disease progression, and reduce patient mortality.

Prolonged use of corticosteroids gives rise to an increased risk of osteoporosis and other long-term complications. If the disease is effectively alleviated without recurrence, the corticosteroids should then be tapered to a minimum maintenance dose only. Calcium and vitamin D supplementation can be administered, according to guidelines. However, it is pertinent to conduct a careful diagnostic workup, at diagnosis or at recurrence after terminating steroids, to decide the glucocorticoid-sparing treatments for idiopathic, primary, or secondary eosinophilia with alternative immunosuppressive or cytoreductive agents (6, 7, 15, 16).

In addition, multidisciplinary collaboration is essential for patient management especially because of the different etiologies of Loeffler endocarditis, the diverse clinical presentations, and the possibility of involving multiple organs. All these different spheres of activity have to work in close unison toward effective patient care. This is exactly what happened in Case 2. Through a multidisciplinary collaboration including those of Departments of Cardiology, Respiratory, Hematology, and Rheumatology, the patient was correctly diagnosed as having EGPA and was given an effective therapy with good clinical outcomes.

Despite the prognoses of the two patients being good, our study still has certain limitations. First, patient of Case 2 was in poor health condition when he first visited our hospital. He refused to follow the doctors' suggestions to undergo endomyocardial biopsy and CMR. Therefore, it was unfortunate that we could not obtain the endocardial pathology results of his endocardium. CMR is yet another important non-invasive

imaging tool, besides echocardiography, for evaluating tissue properties and detecting thrombus in Loeffler endocarditis (18). Hence, the use of comprehensive multimodality imaging, inclusive of echocardiography, contrast echocardiography, and CMR, should be considered in the future for the evaluation of Loeffler endocarditis, if available. Overall, the two patients are presently in good condition and are satisfied with our diagnosis and treatment.

Conclusion

When the etiology of eosinophilia remains unknown, eosinophils continue to reelevate if corticosteroids are discontinued. This suggests that corticosteroids should be used long term to prevent the recurrence of idiopathic eosinophilia. In its initial stage, Loeffler endocarditis is prone to be misdiagnosed due to its diverse and unspecific symptoms. When the patients show persistent eosinophil elevation with non-specific myocardial damage, the possibility of this disease, must always be considered. Even if an endomyocardial biopsy is unavailable, corticosteroids should be administered promptly to reduce eosinophil levels to normal, thereby halting the progression of disease and reducing patient mortality.

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 Medical Ethics Committee of Qilu Hospital of Shandong University (Qingdao). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YZ initially wrote the manuscript. PJ and XC collected the data. GY reviewed and revised the manuscript. All authors contributed to the diagnosis and treatment of the patients, read the manuscript, and agreed to publication.

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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.

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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.2022.970446/full#supplementary-material

References

1. Hernandez CM, Arisha MJ, Ahmad A, Oates E, Nanda NC, Nanda A, et al. Usefulness of three-dimensional echocardiography in the assessment of valvular involvement in Loeffler endocarditis. *Echocardiography.* (2017) 34:1050–6. doi: 10.1111/echo.13575

2. Gao M, Zhang W, Zhao W, Qin L, Pei F, Zheng Y. Loeffler endocarditis as a rare cause of heart failure with preserved ejection fraction: a case report and review of literature. *Medicine.* (2018) 97:e0079. doi: 10.1097/MD.0000000000010079

3. Salih M, Ibrahim R, Tirunagiri D, Al-Ani H, Ananthasubramaniam K. Loeffler's endocarditis and hypereosinophilic syndrome. *Cardiol Rev.* (2021) 29:150–5. doi: 10.1097/CRD.0000000000000324

4. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum.* (1990) 33:1094–100. doi: 10.1002/art.1780330806

5. Crane MM, Chang CM, Kobayashi MG, Weller PF. Incidence of myeloproliferative hypereosinophilic syndrome in the United States and an estimate of all hypereosinophilic syndrome incidence. *J Allergy Clin Immunol.* (2010) 126:179–81. doi: 10.1016/j.jaci.2010.03.035

6. Ogbogu PU, Bochner BS, Butterfield JH, Gleich GJ, Huss-Marp J, Kahn JE, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. *J Allergy Clin Immunol.* (2009) 124:1319-25.e3. doi: 10.1016/j.jaci.2009.09.022

7. Roufosse F, Weller PF. Practical approach to the patient with hypereosinophilia. *J Allergy Clin Immunol.* (2010) 126:39–44. doi: 10.1016/j.jaci.2010.04.011

8. Mankad R, Bonnichsen C, Mankad S. Hypereosinophilic syndrome: cardiac diagnosis and management. *Heart.* (2016) 102:100–6. doi: 10.1136/heartjnl-2015-307959

9. Metze M, Davierwala PM, Andreas H, Klingel K, Laufs U, Klöter T. Progression of left ventricular thrombus in Loeffler's endocarditis without eosinophilia-case report and review of the literature. *Clin Res Cardiol.* (2019) 108:1163–70. doi: 10.1007/s00392-019-01483-1

10. Kim NK, Kim CY, Kim JH, Jang SY, Bae MH, Lee JH, et al. A hypereosinophilic syndrome with cardiac involvement from thrombotic stage to fibrotic stage. *J Cardiovasc Ultrasound.* (2015) 23:100–2. doi: 10.4250/jcu.2015.23.2.100

11. Alam A, Thampi S, Saba SG, Jermyn R. Loeffler endocarditis: a unique presentation of right-sided heart failure due to eosinophil-induced endomyocardial fibrosis. *Clin Med Insights Case Rep.* (2017) 10:1–4. doi: 10.1177/1179547617723643

12. Beedupalli J, Modi K. Early-stage Loeffler's endocarditis with isolated right ventricular involvement: management, long-term follow-up, and review of literature. *Echocardiography.* (2016) 33:1422–7. doi: 10.1111/echo.13264

13. Kleinfeldt T, Nienaber CA, Kische S, Akin I, Turan RG, Körber T, et al. Cardiac manifestation of the hypereosinophilic syndrome: new insights. *Clin Res Cardiol.* (2010) 99:419–27. doi: 10.1007/s00392-010-0144-8

14. Hsieh FH. Hypereosinophilic syndrome. *Ann Allergy Asthma Immunol.* (2014) 112:484–8. doi: 10.1016/j.anai.2014.03.004

15. Qu SQ, Qin TJ, Xu ZF, Zhang Y, Ai XF, Li B, et al. Long-term outcomes of imatinib in patients with FIP1L1/ PDGFRA associated chronic eosinophilic leukemia: experience of a single center in China. *Oncotarget.* (2016) 7:33229–36. doi: 10.18632/oncotarget.8906

16. Metzgeroth G, Schwaab J, Naumann N, Jawhar M, Haferlach T, Fabarius A, et al. Treatment-free remission in FIP1L1-PDGFRA-positive myeloid/lymphoid neoplasms with eosinophilia after imatinib discontinuation. *Blood Adv.* (2020) 4:440–3. doi: 10.1182/bloodadvances.2019001111

17. Baltazares-Lipp ME, Soto-González JI, Aboitiz-Rivera CM, Carmona-Ruíz HA, Ortega BS, Blachman-Braun R. Hypereosinophilic syndrome: a case of fatal Löffler endocarditis. *Case Rep Cardiol.* (2016) 2016:2359532. doi: 10.1155/2016/2359532

18. Koyama T, Yamamoto H, Matsumoto M, Isogai J, Isomura T, Tanaka S. Late-stage Löffler's endocarditis mimicking cardiac tumor: a case report. *Front Cardiovasc Med.* (2020) 7:589212. doi: 10.3389/fcvm.2020.589212

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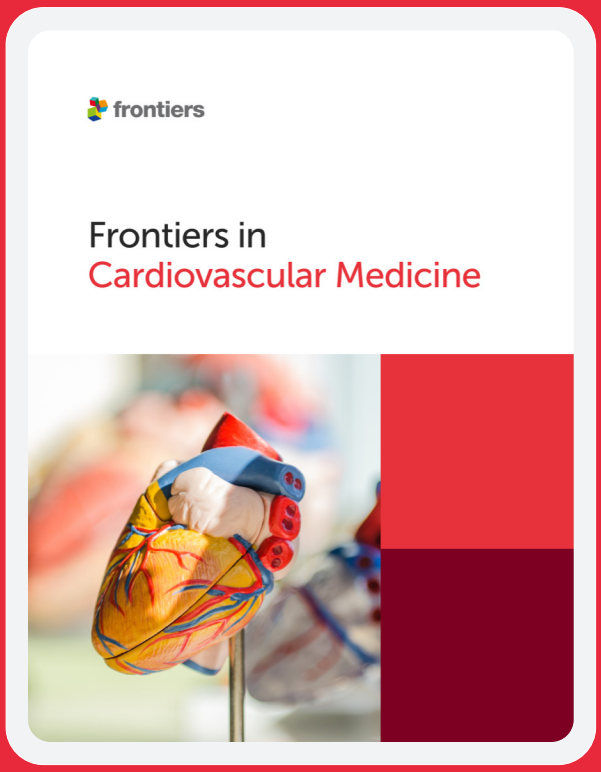

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