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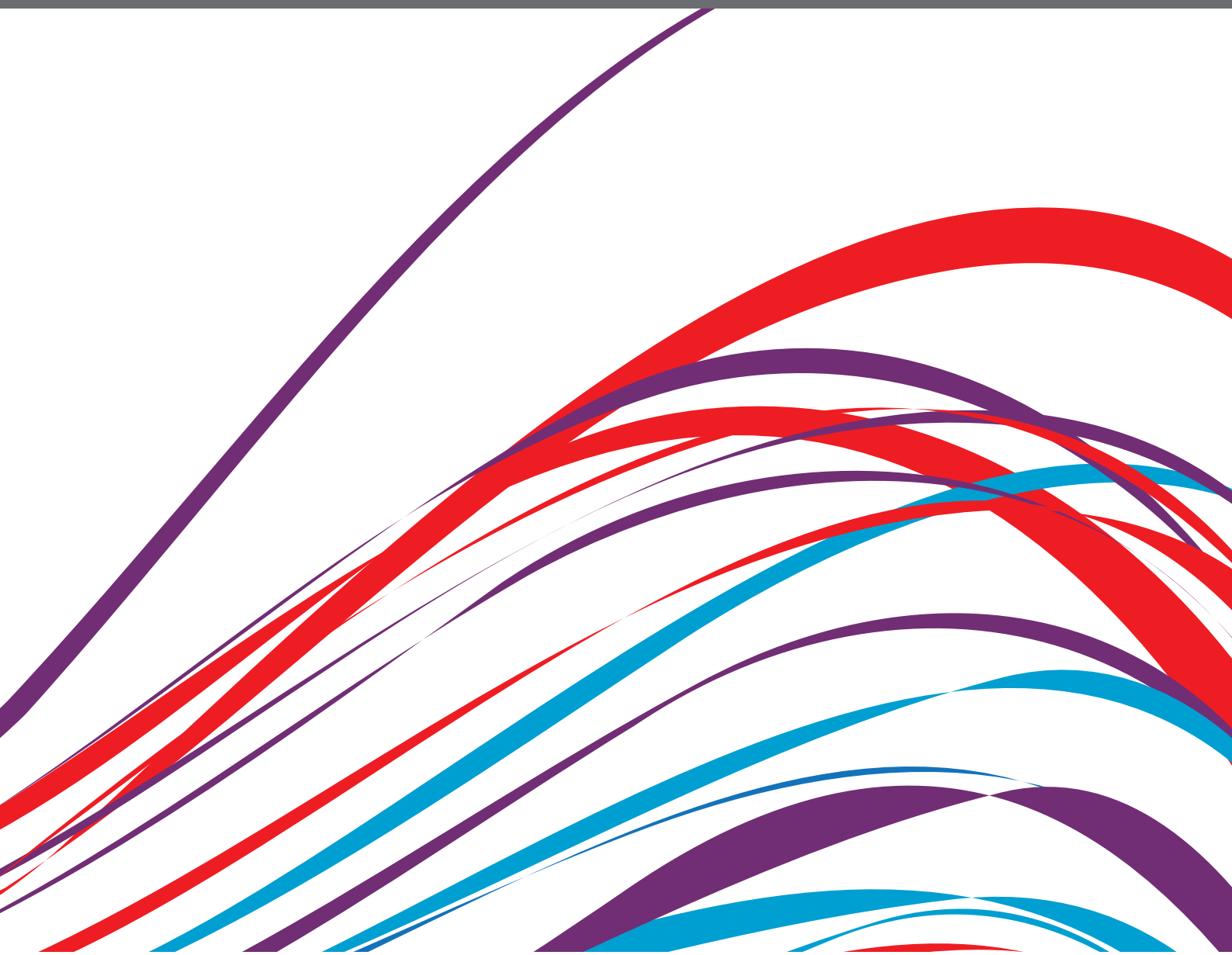

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INSIGHTS IN CARDIOVASCULAR EPIDEMIOLOGY AND PREVENTION: 2021

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Develop and Apply Electrocardiography-Based Risk Score to Identify Community-Based Elderly Individuals at High-Risk of Mortality

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With an aging world population, risk stratification of community-based, elderly population is required for primary prevention. This study proposes a combined score developed using electrocardiographic (ECG) parameters and determines its long-term prognostic value for predicting risk of cardiovascular mortality. A cohort-study, conducted from December 2008 to April 2019, enrolled 5,380 subjects in Taiwan, who were examined, using three-serial-12-lead ECGs, and their health/demographic information were recorded. To understand the predictive effects of ECG parameters on overall-survival, Cox hazard regression analysis were performed. The mean age at enrollment was 69.04 ± 8.14 years, and 47.4% were males. ECG abnormalities, LVH [hazard ratio (HR) = 1.39, 95% confidence intervals (CI) = (1.16–1.67), $P = 0.0003$], QTc [HR = 1.31, CI = (1.07–1.61), $P = 0.007$] and PR interval [HR = 1.40, CI = (1.01–1.95), $P = 0.04$], were significantly associated with primary outcome all-cause death. Furthermore, LVH [HR = 2.37, CI = (1.48–3.79), $P = 0.0003$] was significantly associated with cardiovascular death, while PR interval [HR = 2.63, CI = (1.24– 5.57), $P = 0.01$] with unexplained death. ECG abnormality (EA) score was defined based on the number of abnormal ECG parameters for each patient, which was used to divide all patients into sub-groups. Competing risk survival analysis using EA score were performed by using the Gray's test, which reported that high-risk EA groups showed significantly higher cumulative incidence for all three outcomes. Prognostic models using the EA score as predictor were developed and a 10-fold cross validation design was adopted to conduct calibration and discrimination analysis, to establish the efficacy of the proposed models.

Overall, ECG model could successfully predict people, susceptible to all three death outcomes ($P < 0.05$), with high efficacy. Statistically significant ($P < 0.001$) improvement of the c-indices further demonstrated the robustness of the prediction model with ECG parameters, as opposed to a traditional model with no EA predictor. The EA score is highly associated with increased risk of mortality in elderly population and may be successfully used in clinical practice.

Keywords: prevalence, long-term prognosis, electrocardiographic abnormality score, Han Chinese population, community-based

INTRODUCTION

The world's population is gradually aging as every country in the world is facing an increase in the number and proportion of older people in its population. Based on data from the United Nations' World Population Prospects, 1 in 11 people (9%) was over the age of 65 years in 2019, and this proportion will increase to nearly 1 in 6 people (17%) by 2050. Therefore, it is to be expected that the prevalence of diseases associated with old age, such as cardiovascular disease, will also rise. Thus, for the patients, health providers, and families, risk stratification for elderly people who have or are susceptible to chronic diseases such as hypertension, diabetes mellitus or chronic kidney diseases, is important, for primary prevention. Therefore, finding an effective, low-cost, non-invasive and readily available risk stratification tool for clinical practice is an important issue that needs to be addressed.

Although traditional cardiovascular risk factors (e.g., hypertension, diabetes or hyperlipidemia) have been identified and are widely used in clinical practice for risk stratification of cardiovascular disease development and cardiovascular events (1–3), the predictive value is less accurate in older adults than in middle-aged adults (4). An increased risk of sudden cardiac deaths has also been reported to be associated with several electrocardiographic (ECG) parameters (5–7), however, most of these parameters are used as a single predictor (e.g., QT interval) to predict clinical outcomes, resulting in limited predictive value. Combining several ECG parameters into integrated traditional risk models may improve risk stratification of cardiovascular events or mortality. This approach has not been investigated, until now, in elderly people.

Mostly, prior studies have been conducted in relatively young or middle-aged general populations (<65 years old) (5, 6, 8, 9) or in disease cohorts (10, 11) and the use of ECG parameters as predictive values of cardiovascular events for the general elderly population is scarce. The Healthy Aging Longitudinal Study (HALST) is a large, multi-site cohort study of community-dwelling older-middle-aged and elderly adults (>55 years old), with at least a 10-year follow-up, from the Han Chinese general population in Taiwan. Here, we sought to develop a combined ECG score using several ECG parameters and determine the score's long-term prognostic value for all-cause mortality, cardiovascular mortality and unexplained mortality in the HALST cohort.

METHODS

Study Subjects: Taiwan Geriatric Health Survey population

We performed a prospective community-based cohort study in Taiwan, the HALST study (**Supplementary Material**), a community-based cohort study, that enrolled 5,380 study participants (12–14). Participants were enrolled from December 2008 through March 2013, and were followed up until April 2019. We collected three serial 12-lead ECGs and other relevant clinical and demographic information prospectively at the time of enrollment according to the study protocol. All analysis were conducted between May 2019 and September 2020.

We excluded individuals with a history of any cancer, significant heart diseases (e.g., documented coronary artery disease, severe heart failure (New York Heart Association class III/IV), myocardial infarction, severe valvular diseases, any kinds of cardiomyopathies and infectious cardiac diseases), ventricular conduction delay (2nd or 3rd degree atrioventricular block), atrial fibrillation or flutter, congenital short or long QT syndrome (15, 16), Brugada-type ECG (17), catecholaminergic polymorphic ventricular tachycardia (18), ventricular pre-excitation, and implanted pacemakers. The final study cohort population consisted of 4,615 individuals, constituting a random sample representative of the entire source population. All those who died of cardiac or other causes ($n = 87$ and $n = 683$, respectively) during the follow-up (mean follow-up time 95.1 ± 21.9 months) were identified.

Long-Term Prognosis and Follow-Up

The clinical outcomes that are considered in this study are all-cause death, cardiovascular death, and unexplained death. Death from cardiovascular causes was defined by the ICD codes I01-I02.0, I05-I09, I20-I25, I27, and I30-I52. The definition of unexplained deaths are sudden deaths with unexplained causes, occurring in an individual older than 1 year, according to 2020 AHPRS/HRS expert consensus statement (19). Taiwan maintains extensive administrative registers that record every death in the country. Two clinicians blinded to the ECG results determined the causes of death by examining death certificates from the National Taiwan Institute of Health and Welfare. The causes of death were adjudicated by a committee of experienced cardiologists, who were blinded to the data from the electrocardiographic analyses.

ECG Measurement and Definition of Abnormality Score

The 12-lead ECGs (Hewlett Packard, USA) were recorded at the standard settings of 10 mm/mV and 25 mm/s. PR and QRS intervals were computed automatically, while QTc was computed using Bazett's formula. We selected ECG variables, that are associated with mortality and relatively easily obtained in clinical practice, for risk analysis. The criteria for abnormality for each of the ECG parameters were defined as follows: PR interval >220 ms, QRS duration >110 ms, spatial QRS-T angle >90°, corrected QT interval (QTc) >450 ms in men or >460 ms in women, heart rate > 80/min, ST segment elevation in lead aVR, early repolarization pattern (ERP) in inferior or lateral leads (lead II, III, aVf; V4–6), fragmented QRS (fQRS) (20, 21) and left ventricular hypertrophy (LVH) by Sokolow-Lyon criteria or modified Cornell Criteria (22, 23). Each abnormal ECG variable counted for 1 point.

ECGs were displayed on a 24-in computer screen in multiple formats, enabling careful classification of slurring on the downslope of the R and J waves. All ECGs were analyzed and interpreted independently in random order by two trained cardiologists, who were blinded to clinical data and follow-up status. In case of divergent results, a third blinded cardiologist re-interpreted the ECG, and a preliminary decision on each ECG parameters was achieved by majority vote. After the preliminary decision on each ECG parameters, two trained cardiologists jointly re-assessed, and a final decision was reached by consensus. The inter-observer agreement was $k = 0.97$, and agreement proportion = 0.98.

Statistical Analysis

Cumulative ECG Abnormality Score

All-cause death, cardiovascular (CV) death, and unexplained death were considered as the study endpoints (**Supplementary Materials**). All-cause death, is considered as the primary study endpoint as it is comprised of CV death, unexplained death and deaths due to other causes. A Cox proportional hazards model was used to estimate the hazard ratio and 95% confidence intervals (CI) of the nine ECG risk parameters (**Table 1**) (24) using all study samples. A univariate analysis, age- and sex-adjusted, were conducted with each ECG parameter as predictor for the three clinical outcomes, followed by a joint analysis of all ECG parameters, with an age-adjusted and sex-adjusted model. As each of the ECG parameters would generally have a low predictive power, explaining only a small percentage of the variance, we proceeded to include all nine parameters to calculate a primary, cumulative ECG based score, named as ECG abnormality (EA) score to increase the explanatory power by looking at the joint effect of multiple ECG parameters. Subjects were stratified into four groups: (a) subjects in Group 0 showed no abnormal ECG readings, (b) Group 1's subjects exhibited one ECG abnormality, (c) Group 2's exhibited two ECG abnormalities, and (d) subjects in Group 3 showed three or more abnormalities. Additionally, a secondary EA score was developed, as a comparison, by stratifying the subjects into (i) a low-EA group (ECG abnormalities $\leq$1) and (ii) a high-EA group (ECG abnormalities $\geq$2).

Competing Risk Analysis

This study consists of three events where the occurrence of one may preclude or significantly alter the probability of the other two events. Therefore, competing risk analysis, using the complete data ($N = 4{,}530$), were performed for each of the three events, all-cause death, CV death and unexplained death, to estimate the hazard of failing from each of the given causes (cause-specific hazard function) in the presence of other two competing risk events, respectively (25). The cumulated incidence function (CIF) (probability of failing from a specific cause C before time) was employed using the "survminer" (26) and "cmprsk" (27) packages in R and CIF plots were used to compare the cumulative incidence of subjects with EA scores. Gray's test was performed to elucidate whether significant differences exist in the different groups: (A) all-cause death, (B) CV death, and (C) unexplained death.

Development of Prognostic Models With ECG Risk Scores

The EA scores were used to propose prognostic models using all patients ($N = 4{,}530$) in this study, including (i) an age and sex adjusted EA score prognostic model and (ii) a multivariate-adjusted EA score prognostic model, for all three endpoints in this study. The variables included in the multivariate adjustment consisted of traditional variables from the baseline model, such as age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), current smoking status, hypertension, diabetes mellitus, hyperlipidemia, stroke, and chronic kidney disease. The Cox proportional hazards model was employed using the "Survival" package in R (24) to estimate the hazard ratio and the corresponding 95% CIs. Statistical tests and graphical diagnostics based on the scaled Schoenfeld residuals testing, were used to check the proportional hazards (PH) assumption for all outcomes and for all models (age and sex adjusted ECG model and Multivariate adjusted ECG model) that have been proposed in this study (28).

Model Evaluation Using Internal Validation Cohort

To evaluate whether EA scores can help to identify elderly people's susceptibility to death outcomes, a 10-fold cross-validation (10CV) was performed to conduct model calibration and discrimination as indicators of performance (29). The study subjects from the HALST cohort were randomly split into training and testing sets in the ratio of 9:1, and repeated 10 times, to ensure all of the 10 data folds were used as the testing data. The training data were used on the (i) a multivariate-adjusted EA score prognostic model (ECG model), and (ii) a baseline model consisting of only traditional variables adjusted by age and sex (traditional model), to estimate the coefficients of the predictor variables, and the testing data were used to validate the fitted models, for all three outcomes.

Harrell's c-index from the "Survival" package in R (24) was utilized for the discrimination analysis to evaluate the concordance of predicted and observed survival. For a prognostic model to have a robust prediction performance for a pair of patients, the patient with shorter time to event (T) should have higher risk scores (R), as assigned by the model. In this study c-indices from all 10 cross-validation runs were averaged

TABLE 1 | Association between ECG parameters and all-cause death. [#](*N* = 4,530).

ECG Parameter	*N (%)*	Univariate model		Multivariate model	
		HR (95%CI)	*P*-value	HR(95%CI)	*P*-value
Heart rate >80/min	452 (9.97)	1.14 (0.91−1.45)	0.27	1.07 (0.84−1.36)	0.56
Early repolarization pattern (ERP)	880 (19.42)	1.03 (0.84−1.26)	0.79	1.01 (0.82−1.24)	0.94
Fragmented QRS complex (fQRS)	1,869 (41.26)	1.01 (0.86−1.18)	0.91	1.01 (0.86−1.18)	0.97
ST elevation in lead aVR	0 (0)	NA	NA	NA	NA
Left ventricular hypertrophy (LVH)*	735 (16.22)	1.39 (1.17−1.67)	0.0002	1.39 (1.16−1.67)	0.0003
QRS duration >110 ms	311 (6.86)	0.97 (0.74−1.28)	0.85	0.89 (0.67−1.17)	0.42
Corrected QT interval (QTc) (ms)*	1,001 (22.09)	1.32 (1.09−1.59)	0.004	1.31 (1.07−1.61)	0.007
PR interval (ms)*	115 (2.54)	1.43 (1.03−1.98)	0.03	1.40 (1.01−1.95)	0.04
QRS-T angle > 90°	88 (1.94)	1.10 (0.62−1.96)	0.73	1.12 (0.63−1.99)	0.7

[#]*P-value (age-adjusted and sex-adjusted) is calculated from a Cox hazard regression model. HR: hazard ratio; CI: confidence interval; *P < 0.05.*

to evaluate the models' performances for all three outcomes. Furthermore, the c-indices of the two prediction models, with and without adding ECG parameters, were compared using a bootstrapping strategy (30) to check if adding the EA score into the prediction model leads to significant improvement in the discrimination ability, as opposed to only traditional variables.

For the calibration analysis, difference between the observed and predicted mortality for both the ECG model and the traditional model, were computed as percentages, over all study subjects, by a given follow up time. Furthermore, a proportion test was conducted to check if the differences attained statistical significance ($P < 0.01$).

RESULTS

Baseline Characteristics

From a total of 5,380 relatively healthy and ambulatory individuals, enrolled in this study (**Supplementary Figure 1**), 755 individuals were excluded due to cancer, underlying severe cardiovascular diseases (e.g., myocardial infarction or pacemaker implantation), or missing follow-up information. Finally, 4,530 individuals with complete data were used for analysis (**Supplementary Table 1**).

Prognostic Value of Individual ECG Parameters

To evaluate whether ECG parameters were associated with the death outcomes, we performed a log-rank test using both univariate and multivariate approaches. As shown in **Table 1**, LVH, QTc and the PR interval were significantly associated with the primary outcome, all-cause death ($P < 0.05$). The hazard ratios (HRs) of LVH, QTc and the PR interval were 1.39 [95% CI = (1.16–1.67)], 1.31 [95% CI = (1.07–1.61)] and 1.40 [95% CI = (1.01–1.95)], respectively, in the multivariate model. Similarly, the LVH was a significant predictor of CV death [HR = 2.37, 95% CI = (1.48–3.79), $P < 0.05$] (**Supplementary Table 2**), and PR interval was the risk predictor of unexplained death [HR = 2.63, CI= (1.24– 5.57), $P < 0.05$] (**Supplementary Table 3**).

ECG Abnormality Risk Groups' Association With All-Cause Mortality, Cardiovascular Mortality and Unexplained Mortality

To further evaluate whether ECG parameters can be used to stratify people into different risk groups, we divided subjects into subgroups based on how many ECG abnormal parameters they displayed. The baseline characteristics of each ECG group are summarized in **Table 2**. Comparison among all groups were conducted to report *p*-values (**Table 2** and **Supplementary Table 4**).Some clinical factors showed significant differences among the 4 groups, including age, systolic and diastolic blood pressures, smoking and hypertension ($P < 0.05$).

To explore whether the EA score was associated with the survival outcomes, we examined the proportion of death events for each EA level. The results are illustrated in **Supplementary Figure 2**; the proportion of events for EA subgroups increased with each additional ECG abnormal parameter. Group with 3 ECG abnormalities exhibited the highest proportion of events for outcomes, all-cause death (10.25, 13.04, 18.39, and 19.82%), CV death (0.78, 129, 2.16, and 2.80%) and unexplained death (0.78, 1.29, 2.16, and 2.80%), respectively. We further performed a secondary analysis, using only two EA groups: low and high. The samples with no or one ECG abnormal parameter (0 or 1) were classified as low EA, whereas samples displaying two or more ECG abnormal parameters were classified as high EA. As shown in **Figure 1**, the high EA group showed a higher proportion of events for all-cause deaths (18.83%), CV deaths (2.82%) and unexplained deaths (2.36%) compared to that of the low EA group (11.97%, 1.13%, and 1.10%, respectively).

Furthermore, a competing risk survival analysis of the two-level EA groups were performed by using the Gray's tests to elucidate their associations with each of the death outcomes studied, while taking into consideration the possibility of the event to have an affect due to deaths by other causes (**Figure 2**). Notably, the high EA group showed significantly higher cumulative incidence in all three outcomes: all-cause death ($P = 0.004$, **Figure 2A**), CV death ($P < 0.001$, **Figure 2B**), and unexplained death ($P = 0.006$, **Figure 2C**). The detailed classification (using four EA categories) of each group and

TABLE 2 | Demographic characteristics of study subjects ($N = 4,530$) divided into subgroups based on number of ECG abnormal parameters (0, 1, 2, ≥3).

ECG Parameters	0 *N* = 1,151	1 *N* = 1,855	2 *N* = 1,060	> = 3 *N* = 464	*P*-value
Gender					0.09603
Male (%)	609 (52.91)	944 (50.89)	567 (53.49)	265 (57.11)	
Female (%)	542 (47.09)	911 (49.11)	493 (46.51)	199 (42.89)	
Age (years)	68.31 ± 8.06	68.73 ± 7.88	69.81 ± 8.287	70.32 ± 8.74	3.46E−07
Systolic blood pressure (mmHg)	126.28 ± 17.76	126.33 ± 17.84	131.39 ± 19.88	134.63 ± 19.44	<2e−16
Diastolic blood pressure (mmHg)	69.87 ± 10.22	69.59 ± 10.31	71.34 ± 10.89	72.91 ± 11.79	2.30e−10
Body mass index	24.39 ± 3.295	24.48 ± 3.47	24.51 ± 3.49	24.58 ± 3.75	0.735
Current smoker (%)	123 (10.68)	275 (14.82)	134 (12.64)	59 (12.71)	0.02678
Hypertension (%)	445 (38.67)	731 (39.40)	527 (49.72)	269 (57.97)	< 2.2e−16
Diabetes mellitus (%)	185 (16.07)	330 (17.79)	193 (18.20)	96 (20.69)	0.1633
Stroke (%)	47 (4.08)	85 (4.58)	60 (5.66)	28 (6.03)	0.1979
Hyperlipidemia (%)	353 (30.67)	590 (31.81)	354 (33.40)	145 (31.25)	0.5754
Chronic respiratory disease (%)	26 (2.26)	59 (3.18)	35 (3.30)	16 (3.45)	0.387
Chronic kidney disease (%)	170 (14.77)	285 (15.36)	140 (13.21)	63 (13.58)	0.4034

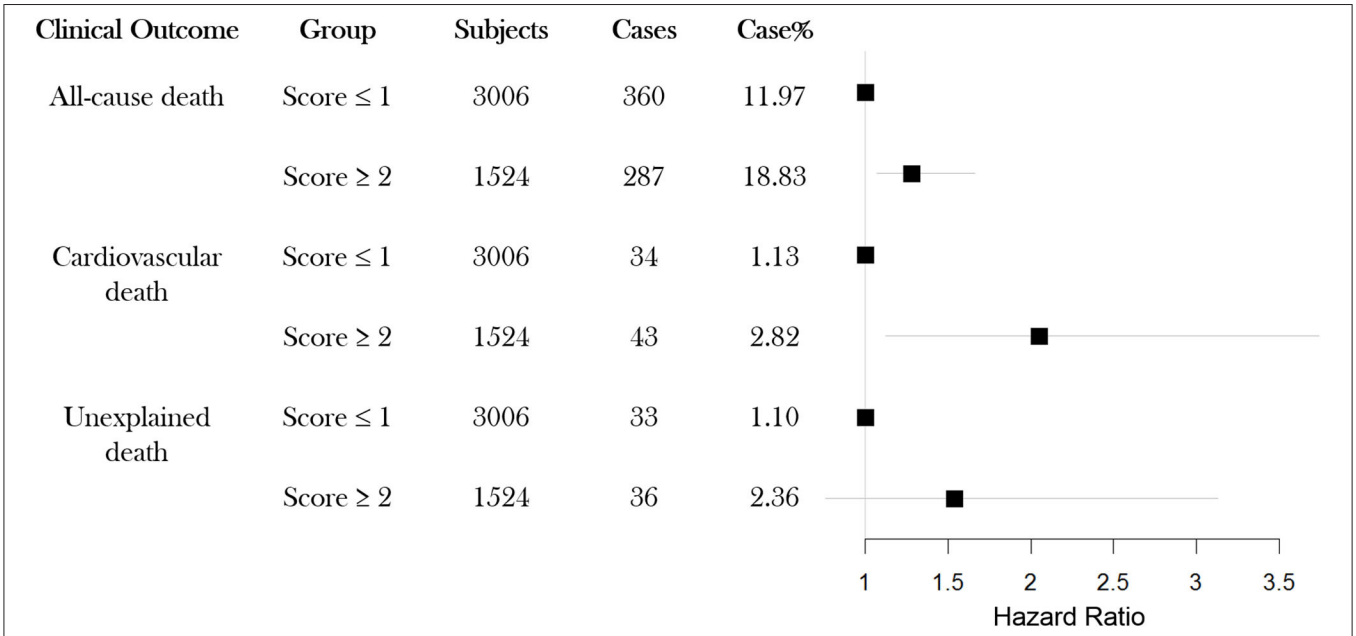

FIGURE 1 | Event rates for each of three possible outcomes All-cause death, CV death and Unexpected death, for study subjects classified into two groups (high-EA or low-EA) based on ECG abnormal parameters ($N = 4,530$). EA: ECG abnormality; CV: cardiovascular.

its association with the different death outcomes is shown in **Supplementary Figure 3**, which showed similar pattern as that of the two-level EA groups for all three events. In conclusion, these results indicate that ECG parameters can help in identifying people at a higher risk for all-cause-death, cardiovascular death, or unexplained death.

Development of Prognostic Model

Supplementary Tables 5–13 displays the results for the scaled Schoenfeld residuals testing that were conducted for all three study outcomes to check for the validity of the proportional hazard assumption for the covariates under consideration, in each of the proposed prognostic models in this study. The findings along with the global tests were not found to be statistically significant, implying that the proportional hazard assumption is valid for all models. Cox - proportion hazards regression was performed on an age and sex adjusted univariate model and a multivariate adjusted prognostic model, to check if the EA score is a significant predictor for all three endpoints all-cause death, CV death and unexpected death. The results for the primary outcome, all-cause death are summarized in **Table 3**. Notably, the EA (high, low) was a significant predictor in the age- and sex-adjusted model (HR: 1.31; 95% CI= (1.12–1.53), $P = 0.0008$) and the multivariate model (HR:

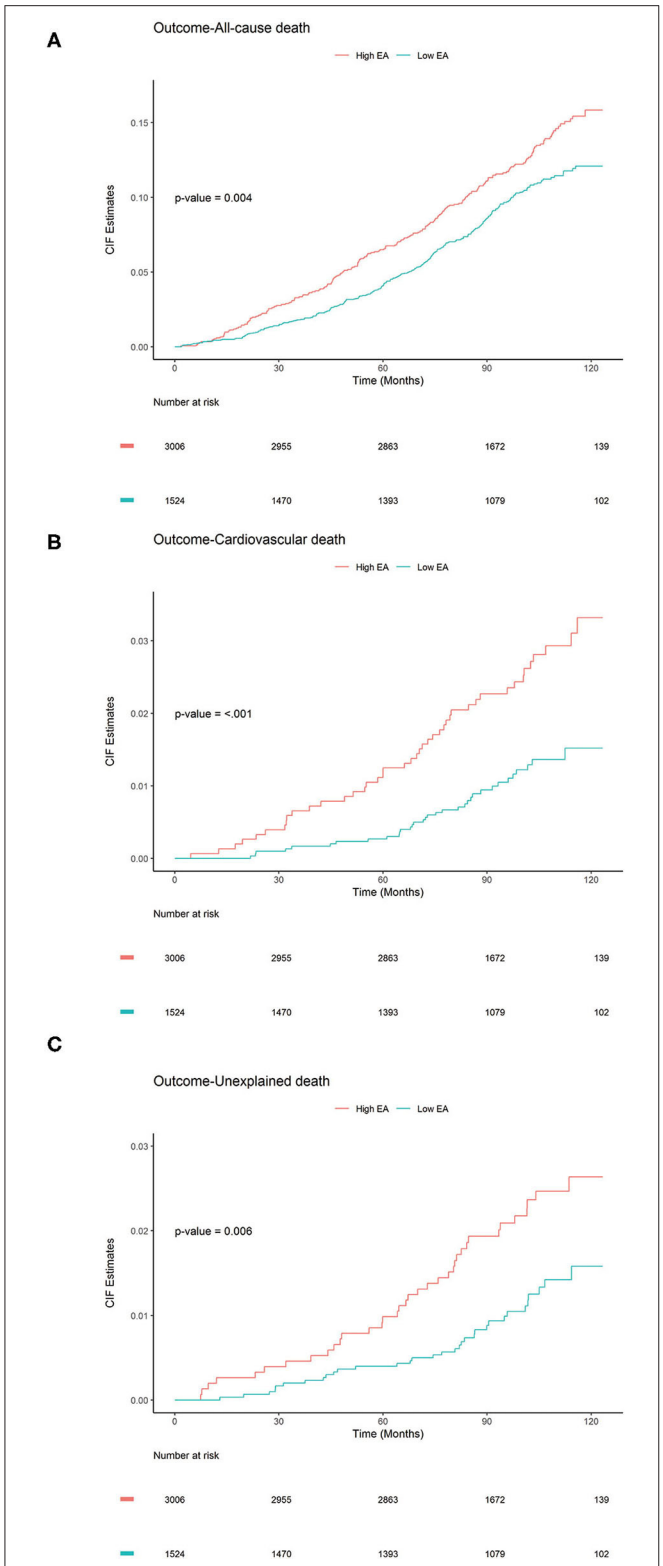

FIGURE 2 | Cumulative Incidence Function plots to compare the cumulative incidence of subjects ($N = 4,530$) with ECG abnormality (EA) score (high, low). *P*-values were calculated using Gray's test to elucidate whether significant differences exist in the different groups: **(A)** All-cause death, **(B)** CV death, and **(C)** Unexplained death.

TABLE 3 | Prediction performance of ECG abnormality score (low EA and high EA) associated with all-cause death ($N = 4,530$).

Event	Low	High
	***N* = 3,006**	***N* = 1,524**
All-cause death		
[#]All-cause deaths (%)	360 (11.97)	287 (18.83)
Age-Adjusted and Sex-Adjusted HR (95%CI)	1	1.31 (1.12–1.53)
P-Value		0.0008
Multivariate-Adjusted HR (95% CI)	1	1.28 (1.09–1.50)
P-Value		0.002

[#]The ECG abnormality score is a sum of ECG abnormality for each of 9 ECG parameters. The age-adjusted and sex-adjusted and the multivariate-adjusted HRs and 95% CIs. Deaths in the 10-year follow-up period were calculated using Cox-proportional Hazards Model. Variables included in the multivariate analysis consisted of age, sex, systolic blood pressure, diastolic blood pressure, BMI, smoking status, hypertension, diabetes mellitus, hyperlipidemia, and chronic kidney disease. Low EA is defined as <= 1 ECG abnormalities, and high EA is defined as > = 2 ECG abnormalities.

1.28; 95% CI = (1.09–1.50); $P = 0.002$) after considering all traditional risk factors. The c-index was 0.786 in the multivariate model, suggesting a good-fit of the prediction model and the real death events in all-cause death. As shown in **Supplementary Tables 14**, **15**, the EA score was a significant predictor for both CV death, and unexplained death, respectively.

ECG Abnormality Score Validation and Model Improvement

To evaluate the performance of the multivariate adjusted prediction model in comparison with the traditional model, discrimination analysis was conducted using a 10CV design. **Table 4**, **Supplementary Table 16** lists the average and the standard deviations of the C- indices over all 10CVs for both the models and for all three study outcomes. Harrel's c-index values for all three outcomes were reported to be 0.786, 0.798, and 0.851, implying that the ECG model is an effective predictor of the study events and can correctly discriminate patient's survival for a given patient pair. Furthermore, comparison of the c-indices between ECG model and traditional model, using a bootstrapping strategy, revealed that adding ECG parameters can significantly improve the predictive capability ($P < 0.001$), based on our data.

Finally, a calibration analysis was conducted to examine if the predicted events were significantly different from the observed events, for all three study outcomes. **Figure 3** displays the results of the calibration analysis that was performed using a 10CV for each of the 10 years where the average difference of the proportions between the predicted and observed events were plotted for both ECG model (high, low) and traditional model. For all-cause death the differences in the proportions were $\leq$5% for the first 5 years, while for both CV death and unexpected death the differences in the proportions were negligible (<3%) for all 10 years. The higher differences that were observed for all-cause death after 5 years may be attributed to the fact that these elder patients may die of diseases other than cardiovascular ones, such as cancers. Similar patterns

TABLE 4 | Average and standard deviation of c-indices from 10-fold cross-validation.

Models	All-cause death		CV death		Unexplained death	
	Avg_C-index	Stdev_C-index	Avg_C-index	Stdev_C-index	Avg_C-index	Stdev_C-index
Multivariate adjusted ECG model	0.786	0.001	0.798	0.015	0.851	0.007
Traditional Model	0.775	0.001	0.782	0.016	0.846	0.007

Multivariate adjusted model: using ECG abnormality (high, low) adjusted by age, sex, systolic blood pressure, diastolic blood pressure, BMI, current smoker, Dx_HTN, Dx_DM, Dx_HypLipid, Dx_CRD, Dx_CKD, and Dx_Stroke. Avg_C-index: average of c-indices over all 10 cross-validations for the training data. Stdev_C-index: standard deviation of c-indices over all 10 cross-validation for the training data.

were observed for the other EA score model (0, 1, 2, and 3) (**Supplementary Figure 4**) and traditional models (**Figure 3** and **Supplementary Figure 4**). **Supplementary Tables 17–22** further lists the detailed calibration analysis results for both ECG model and traditional model, for all three outcomes. All results suggest that our prediction model can help to identify subjects with a high risk of a death event among the elderly.

DISCUSSION

Due to the increasing elderly population, world-wide, risk assessment and stratification of elderly people becomes an important issue in patient care. To our knowledge, this is the first study to specifically investigate the usefulness of an EA score as a predictive tool for an elderly population (mean age ≥65 years).

This study identified LVH, QTc and PR interval to be independently associated with increased risk of the primary study outcome, all-cause mortality, while LVH and PR as the only risk factor of cardiovascular mortality and unexplained mortality, respectively. Previous studies, including the Framingham experience, have demonstrated that LVH, manifested by repolarization abnormality and increased voltage, is one of the less common but ominous risk factors for coronary artery disease, stroke and heart failure. It was associated with a 3–15-fold increase of cardiovascular events (31–33). QTc and PR prolongation has also been reported as cardiac risk factors of sudden death (34, 35) in coronary patients or risk equivalent for middle age and older adults (36). Our findings were not only consistent with previous studies, but also extended the utility of the three ECG parameters to community-based elder individuals.

Several ECG parameters have been tested in many previous studies, both in general populations (37, 38) and in cohorts with cardiac specific diseases (39, 40). These studies showed that the predictive value of a single ECG parameter for sudden cardiac death was generally lower than combining multiple ECG parameters (6, 41, 42). This study uses a specific set of 9 ECG parameters to define the EA score, used to construct the prognostic models. This is because, these ECG parameters have more clinical meanings after having been used in medical practice for 100 years and their potential in predicting the outcomes of cardiovascular events have already been verified through numerous prior studies (6, 20, 21, 43–48) One of the more representative studies is one where the authors analyzed 6,830 participants and reported important ECG abnormalities associated with sudden cardiac death risks (6). To make the results in this study comparable to the previous study, the same ECG parameters were used for defining the EA score in this study. After adjusting for multiple traditional cardiovascular risk factors, our combined and validated EA scores successfully revealed that elderly subjects with ≥3 ECG abnormalities had a 29% higher risk of all-cause mortality compared to elderly subjects with ≤3 ECG abnormalities. Combined ECG risk parameters and traditional cardiovascular risk factors have a cumulative effect on the risk of all-cause mortality. In summary, these results suggest that the combined EA score has the potential to serve as a predictive tool of death events in the elderly.

ECG is a globally used, essential, inexpensive, and non-invasive technique to detect electric abnormalities of the heart and more and more elderly individuals receive annual health examinations, including ECG. We expect that ECG abnormalities would be an incidental finding during a routine health examination. Our study provides an important reference for clinicians or health professionals when they encounter asymptomatic elderly individuals susceptible to chronic diseases or with comorbidities.

Although we performed internal validation, a limitation of this study is that the findings have not been validated in a prospective, external cohort. However, as our cohort takes into consideration the complexity associated with diverse outcomes of cardiac related deaths in general elder population, we believe that our results are generalizable to other similar elder cohorts. Future studies will be conducted to validate the findings on independent cohorts. Secondly, as some of the covariates in our proposed model for the primary outcome, all-cause death (diabetes mellitus and stroke) could possibly have a have a non-proportional hazard effect (**Supplementary Tables 5–7**), further studies are needed for better modeling by conducting the possible interaction of these variables with time. Lastly, detailed clinical information such as echocardiographic assessment (e.g., left ventricular ejection fraction), coronary angiography, or medications are not available in the HALST database. For screening purposes in a large cohort, these information are seldom available, thus this study mimics the setting in which screening would normally take place.

CONCLUSION

The findings in this study show that combined EA score is highly associated with an increased risk of all-cause mortality, CV mortality and unexplained mortality in an

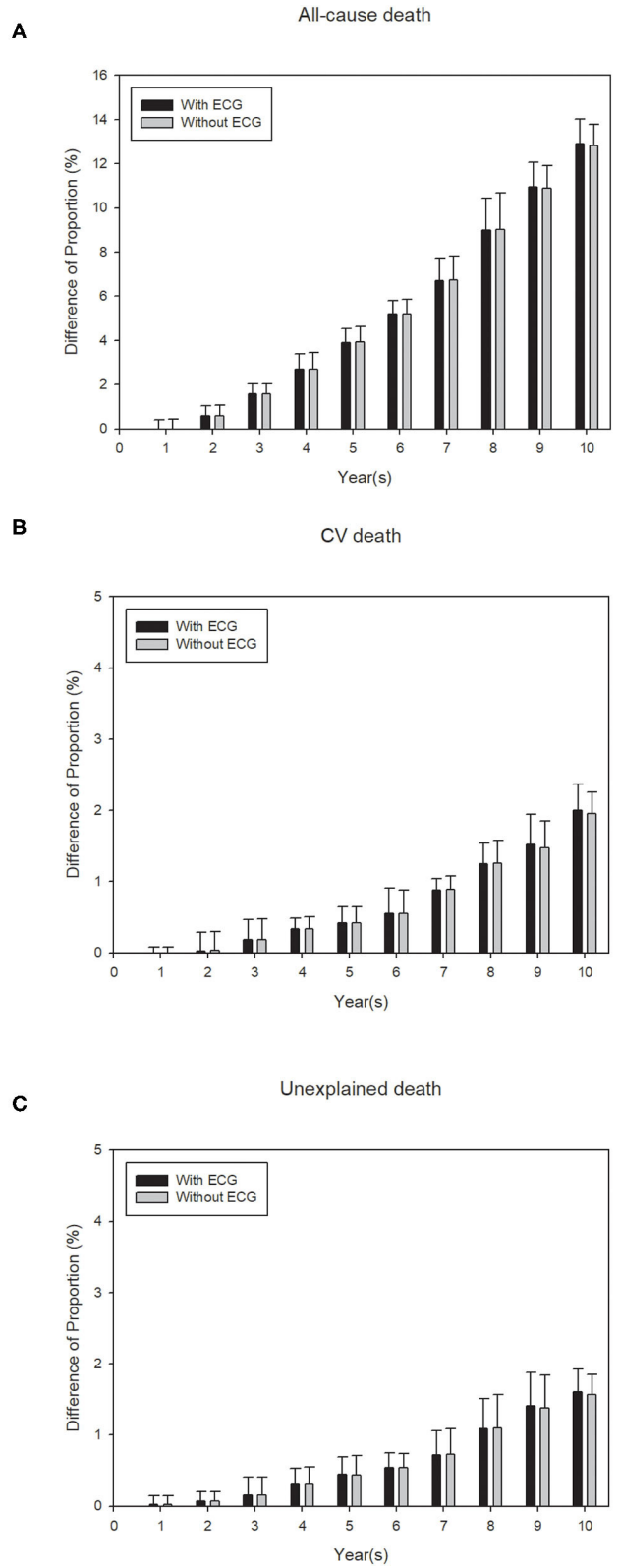

FIGURE 3 | Calibration plots for the events **(A)** all-cause death, **(B)** cardiovascular death and, **(C)** unexplained death, to show the difference between observed and predicted survival probability for proposed ECG model (with EA score (high, low) and traditional variables) and traditional model (only traditional variables without EA score). Calibration for each of the models is conducted using 10-fold cross-validation (CV) and each bar shows an average of the probability difference over 10 models for each CV. Black bars: model with EA score + traditional variables (with ECG). Gray bars: model with only traditional variables (without ECG).

elderly population, which will be confirmed through future validation in independent cohorts. Use of this abnormality score may improve the risk stratification for elderly population 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 studies involving human participants were reviewed and approved by this study was approved by the Ethics Committee of the National Health Research Institutes and conducted according to the principles of the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was conceptualization and designed by T-PL, S-FY, J-MJ, C-YC, and J-YC. Enrolled individuals, collected data, and laboratory work were done by CH, I-SC, I-CW, C-CH, T-YC, and W-TT. Data analysis and interpretation were done by T-PL, AC, K-CL, S-FY, J-MJ, C-YC, and J-YC. Resources and supervision was done by CH and J-MJ. Writing, reviewing, and editing the manuscript was done by T-PL, AC, K-CL, S-FY, J-YC, and J-MJ. All authors have read and approved the final version of the submitted 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.2021.738061/full#supplementary-material

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Comparison of the Association Between Arterial Stiffness Indices and Heart Failure in Patients With High Cardiovascular Risk: A Retrospective Study

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Objective: Study findings of the relationship of each arterial stiffness index with incident heart failure (HF) are conflicting. We aimed to compare the association between the indices of arterial stiffness and the risk of HF.

Methods: We analysed 3,034 patients from a prospective cohort that enrolled patients with high cardiovascular risk. They underwent brachial-ankle pulse wave velocity (baPWV), brachial pulse pressure (PP), carotid-femoral pulse wave velocity (cfPWV), and central PP measurements.

Results: Over a median follow-up of 4.7 years (interquartile range, 3.4–5.8 years), 65 HF events occurred. The incidence rate of HF was 4.7 per 1,000 person-years [95% confidence interval (CI), 3.7–6.0]. There was no difference in baPWV in those with and without HF events (1,561 ± 401 and 1,520 ± 321 cm/s, respectively, $P = 0.415$); however, there was a significant difference in brachial PP (63.2 ± 16.9 vs. 52.3 ± 11.5 mmHg, $P < 0.001$), cfPWV (11.0 ± 3.1 vs. 9.4 ± 2.4 m/s, $P < 0.001$) and central PP (56.6 ± 19.9 vs. 42.9 ± 13.8 mmHg, $P < 0.001$). In the multivariable-adjusted model, brachial PP [hazards ratio (HR) per standard deviation unit (SDU), 1.48; 95% CI, 1.19–1.84, $P < 0.001$], cfPWV (HR per SDU, 1.29; 95% CI, 1.02–1.63, $P = 0.032$) and central PP (HR per SDU, 1.44; 95% CI, 1.17–1.78; $P < 0.001$) were associated with incident HF, but baPWV was not (HR per SDU, 0.83; 95% CI, 0.63–1.10; $P = 0.198$). In the receiver operating characteristic analysis, the area under the curve (AUC) of brachial PP ($P < 0.001$), cfPWV ($P = 0.003$) or central PP ($P = 0.001$) was larger than that of baPWV, and there was no difference in the AUCs of brachial PP, cfPWV and central PP.

Conclusion: Among arterial stiffness indices, brachial PWV was less associated with the risk of heart failure, and brachial PP and measures representing central hemodynamics were highly associated with incident HF.

Keywords: heart failure, arterial stiffness, central blood pressure, pulse wave velocity, retrospective study, prospective cohort, brachial pulse pressure

INTRODUCTION

It is estimated that there are 65.3 million people with heart failure (HF) worldwide, and the prevalence of HF is increasing (1). The prognosis of HF is very poor, and the survival rate at 5 years after HF diagnosis is only 45.5% (2). Despite the development of medications and devices for HF, the risk of death owing to HF remains high. Therefore, there is a need for a pre-emptive prevention strategy that appropriately selects patients at high risk of incident HF and controls risk factors.

The hemodynamic burden on the heart contributes to HF development (3). The heart is an organ that produces blood flow; however, it is affected by the pressure within the aorta, which is called ventricular–arterial coupling (4). A rise in afterload increases myocardial oxygen demand and causes myocardial wall thickening, and this long-term change has a detrimental effect on the left ventricle (LV), leading to symptomatic HF (5). Afterload is affected by complex factors such as pulsatile blood flow and the properties of blood vessels. In particular, an aorta with reduced elasticity has a low capacity as a reservoir for the pulsatile pressure of ventricular ejection and increases afterload (4). Therefore, arterial stiffness, which is a general term for reduced arterial elasticity, showed an association with the development of HF in previous studies (6, 7).

Arterial stiffness can be expressed in several ways. Carotid-femoral pulse wave velocity (cfPWV) and brachial-ankle pulse wave velocity (baPWV) are representative non-invasive measures of arterial stiffness; they are related to the occurrence of cardiovascular disease (CVD) and can be utilised for cardiovascular risk assessment (8, 9). Another index of arterial stiffness is pulse pressure (PP). Like brachial PP, central PP is closely related to CVD (10). Among these measures, brachial PP and cfPWV have been investigated as a predictor for incident HF (6, 11) but studies on other measures are lacking. Therefore, this study aimed to compare the association between the indices of arterial stiffness and the risk of HF.

MATERIALS AND METHODS

Ethics Statements

The Institutional Review Board of the Yonsei University Health System Clinical Trial Center approved the study protocol (4-2013-0581), and written informed consent was obtained from all participants.

Study Design and Population

This retrospective study analysed participants of the Cardiovascular and Metabolic Disease Etiology Research Center-High Risk Cohort (CMERC-HI). Briefly, CMERC-HI is a prospective cohort study aimed at developing more specific preventive strategies for patients with a high risk of CVD (ClinicalTrials.gov ID: NCT02003781). The following patients were included in the cohort: high-risk patients with hypertension [estimated glomerular filtration rate (eGFR) $>$ 60 mL/min/1.73 m^2 with target organ damage or eGFR $\leq$ 60 mL/min/1.73 m^2]; patients with diabetes mellitus with albuminuria; anuric patients with end-stage renal disease who were undergoing dialysis; relatives of patients with acute myocardial infarction (men aged $<$ 55 years; women aged $<$ 65 years); patients with asymptomatic atherosclerotic CVD [abdominal aorta diameter $\geq$ 3 cm or ankle-brachial index (ABI) $<$ 0.9, carotid plaque or carotid intima-media thickness $\geq$ 0.9 mm, asymptomatic old cerebrovascular accident, or $>$30% stenosis in at least one major coronary artery]; patients with rheumatoid arthritis aged $>$ 40 years and taking methotrexate and a steroid; patients with atrial fibrillation with a CHA2DS2-VASc score $\geq$ 1; and kidney transplant recipients at $>$3 months after transplantation. Persons aged $>$ 20 years who met at least one of the inclusion criteria were enrolled. The exclusion criteria were as follows: a history of acute coronary syndrome, symptomatic coronary artery disease or symptomatic peripheral artery disease, or HF; a life expectancy of $<$6 months; pregnancy; or a history of contrast allergy and related adverse effects.

Central Hemodynamic Measurements, baPWV and Brachial Pulse Pressure

The central hemodynamic were evaluated using the SphygmoCor system (AtCor Medical, Sydney, Australia) with patients in the sitting position after 10 min of rest. A high-fidelity micromanometer (Millar Instruments, Houston, TX, USA) was used to record peripheral pressure waveforms from the radial arteries, as reported previously (12, 13). Radial artery waveforms were obtained from the patient's arm without an arteriovenous fistula. The SphygmoCor system obtains the ascending aortic pressure waveform from the radial artery waveform using its validated mathematical transfer function. The central systolic blood pressure (BP), diastolic BP, PP, augmentation pressure, forward wave amplitude, and augmentation index were acquired from the aortic pressure waveform analyses. Central PP was calculated as the difference between the systolic and diastolic BPs. cfPWV was measured as specified previously (14). Briefly, electrocardiogram and carotid/femoral pulse waves were obtained simultaneously to calculate the transit time using the foot-to-foot method. The distance travelled by the pulse wave was calculated by subtracting the sternal notch-right carotid site from the right femoral site-sternal notch distances (14).

baPWV was measured using a volume-plethysmography device (OMRON, Tokyo, Japan). The patients were examined while resting in the supine position. Electrocardiographic electrodes were placed on both wrists, and cuffs were wrapped on both arms and ankles. Pulse volume waveforms at both brachial and posterior tibial arteries were recorded using a semiconductor pressure sensor after patients rested for at least 5 min. The heart rate was continuously recorded with flow and pressure tracings gated to the electrocardiogram, and baPWV was calculated automatically using time-phase analysis. The distance between the upper arm and ankle was estimated based on height. We used the average baPWV from right and left measurements in the analysis. Brachial pulse pressure was calculated using the systolic and diastolic blood pressures derived during baPWV measurement.

TABLE 1 | The clinical characteristics of study participants according to incident heart failure.

	Total (*N* = 3,034)	No incident HF (*N* = 2,969)	Incident HF (*N* = 65)	*P*-value
Age, years	59.2 ± 11.6	59.1 ± 11.6	61.4 ± 12.3	0.110
Men, *N* (%)	1,656 (54.6)	1,618 (54.5)	38 (58.5)	0.611
BMI, kg/m^2	25.1 ± 3.6	25.1 ± 3.6	24.7 ± 3.2	0.390
Hypertension, *N* (%)	2,534 (83.5)	2,474 (83.3)	60 (92.3)	0.155
Type 2 diabetes, *N* (%)	1,381 (45.5)	1,346 (45.4)	35 (53.8)	0.217
SBP, mmHg	128.3 ± 18.0	128.0 ± 17.7	141.3 ± 25.6	<0.001
DBP, mmHg	76.7 ± 10.7	76.8 ± 10.6	74.8 ± 14.2	0.285
Antihypertensive medications, *N* (%)	2,508 (82.7)	2,446 (82.4)	62 (95.4)	0.010
RASB, *N* (%)	1,935 (63.8)	1,891 (63.7)	44 (67.7)	0.594
CCB, *N* (%)	1,507 (49.7)	1,462 (49.3)	45 (69.2)	0.002
BB, *N* (%)	807 (26.6)	770 (25.9)	37 (56.9)	<0.001
Diuretics, *N* (%)	755 (24.9)	718 (24.2)	37 (56.9)	<0.001
Laboratory				
Hemoglobin, g/dL	13.3 ± 2.0	13.4 ± 2.0	11.5 ± 2.1	<0.001
TC, mg/dL	173.4 ± 40.2	173.3 ± 38.6	179.6 ± 85.4	0.558
HDL-C, mg/dL	49.5 ± 13.7	49.6 ± 13.6	42.2 ± 15.1	<0.001
LDL-C, mg/dL	95.2 ± 33.1	95.0 ± 32.0	103.8 ± 66.5	0.314
TG, mg/dL	141.0 ± 91.0	141.2 ± 91.4	131.6 ± 72.1	0.315
eGFR, mL/min/1.73 m^2	68.5 ± 34.3	69.4 ± 33.8	28.8 ± 30.8	<0.001

HF, heart failure; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RASB, renin angiotensin system blockers; CCB, calcium channel blockers; BB, beta blockers; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

Assessment of Heart Failure Outcomes

An HF event was identified by hospitalisations related to HF symptoms. HF diagnoses were adjudicated by a three-physician committee after extensive review of inpatient medical records using the following clinical criteria modified from the European Society of Cardiology definition (15): (1) HF with reduced ejection fraction (HFrEF) was defined as left ventricular ejection fraction (LVEF) $\leq$ 40% on imaging study (echocardiography, technetium-99m sestamibi myocardial imaging, or cardiac magnetic resonance imaging) and plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) level $\geq$ 600 pg/mL and (2) HF with preserved ejection fraction (HFpEF) was defined as LVEF $>$ 40% and relaxation abnormality of the LV filling pattern on echocardiography and NT-proBNP level $\geq$ 300 pg/mL. The date of onset was noted as the first date of hospitalisation owing to HFrEF or HFpEF.

Statistical Analysis

We divided study participants into two groups according to incident HF status. We used the *t*-test and chi-square test to compare continuous and categorical variables, respectively, between the two groups. Continuous variables are expressed as mean ± standard deviation, and categorical variables are expressed as *n* (%).

Receiver operating characteristic (ROC) analysis was performed to identify the best cut-off values and assess the performance of baPWV, brachial PP, cfPWV, and central PP for predicting incident HF. We calculated the areas under the ROC curve (AUCs) for baPWV, brachial PP, cfPWV, and central PP and used the method of DeLong et al. to test the statistical significance of the differences between them (16). We defined the high-risk category as a value higher than the cut-off values of baPWV, brachial PP, cfPWV, and central PP. Kaplan-Meier survival curve analysis was used to assess the cumulative rate of incident HF according to high-risk categories based on baPWV, brachial PP, cfPWV, and central PP values. We used Cox proportional hazards regression analysis to assess the associations between categorical (high-risk categories) and continuous measures of arterial stiffness and incident HF using the univariable model, age- and sex-adjusted model, and multivariable model adjusted for age, sex, body mass index, antihypertensive medication usage, hemoglobin level, and eGFR. Statistical analyses were conducted using R software, version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria), assuming a threshold of significance at $P < 0.05$.

RESULTS

In total, 3,270 participants were enrolled from November 2013 to June 2018 at the Severance Hospital in Seoul, Republic of Korea. Among the participants, 236 did not undergo central hemodynamic measurements or baPWV measurement. Finally, 3,034 participants were included in the final analyses.

Over a median follow-up of 4.7 years (interquartile range, 3.4–5.8 years), 65 HF events occurred, of which 11 were HFrEF and 54 were HFpEF. The incidence rate of HF was 4.7 [95% confidence interval (CI), 3.7–6.0] per 1,000 person-years. **Table 1** shows the baseline characteristics of all study participants. Among 3,034

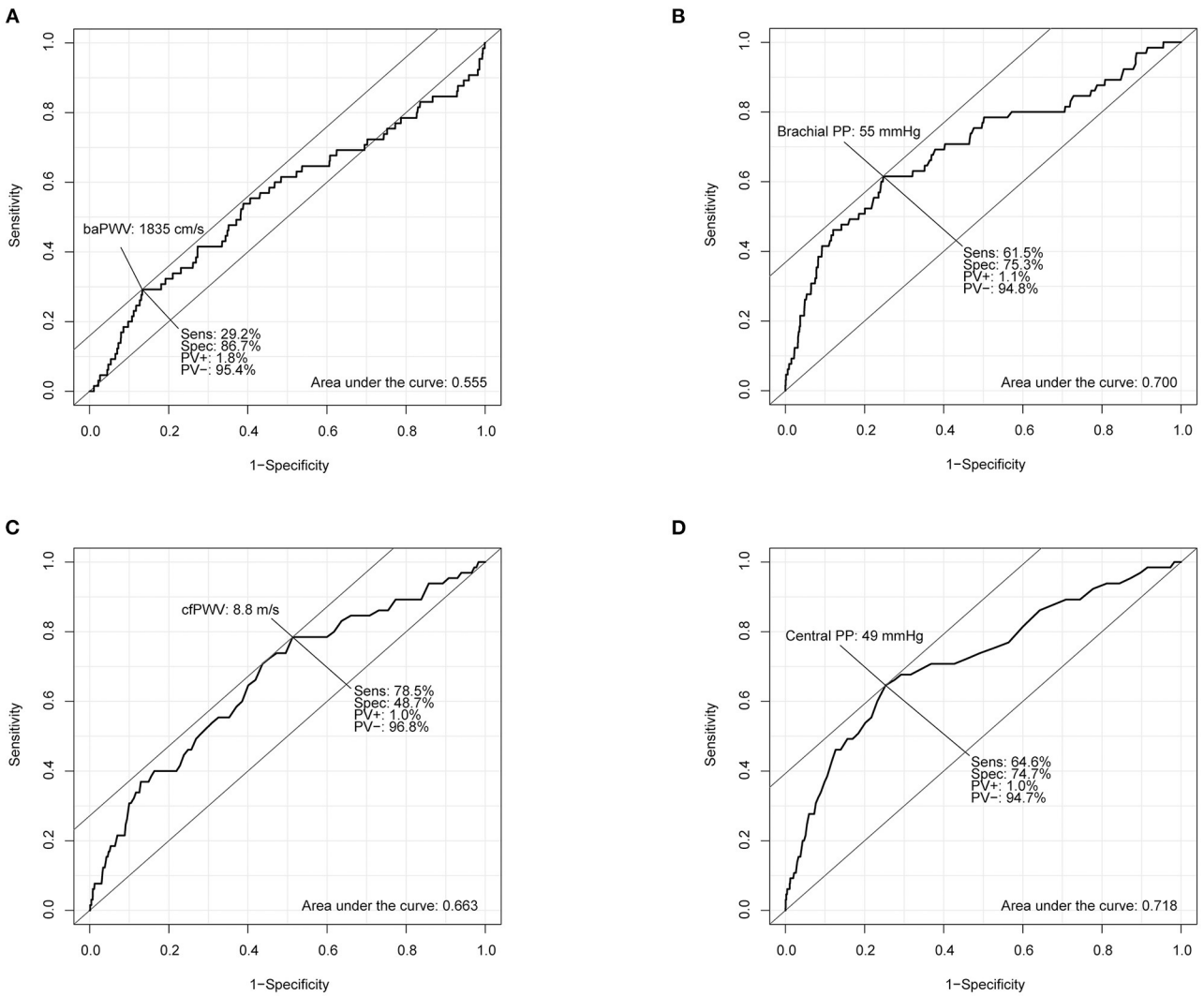

FIGURE 1 | Receiver operating characteristic curve analyses of brachial-ankle pulse wave velocity (baPWV; **A**), brachial pulse pressure (brachial PP; **B**), carotid-femoral pulse wave velocity (cfPWV; **C**), and central pulse pressure (central PP; **D**) for predicting incident heart failure.

participants, the average age was 59.2 ± 11.6 years, 54.6% of the participants were men, and 83.5 and 45.5% had hypertension and diabetes, respectively. Participants with incident HF had higher systolic BP than those without incident HF, despite a higher rate of antihypertensive drug use. Based on the laboratory findings, we found that participants with incident HF had lower levels of hemoglobin and high-density lipoprotein cholesterol and lower eGFR than those without.

Results of the ROC curve analysis of the association between arterial stiffness measures and incident HF are shown in **Figure 1**. According to the ROC curve of baPWV, the AUC was 0.555 (95% CI, 0.474–0.636) and best cut-off value was 1,835 cm/s for prediction of incident HF. However, the AUCs of brachial PP, cfPWV and central PP were 0.700 (95% CI, 0.625–0.774), 0.663 (95% CI, 0.593–0.733) and 0.718 (95% CI, 0.650–0.787), respectively. The AUC of baPWV ($P < 0.001$), cfPWV ($P = 0.003$) or central PP ($P < 0.001$) was larger than that of baPWV. There was no difference in the AUCs between brachial PP and cfPWV ($P = 0.292$), the AUCs between brachial PP and central PP ($P = 0.445$), and the AUCs between brachial PP and central PP ($P = 0.145$). The ROC curve analysis revealed that brachial PP of 55 mmHg, cfPWV of 8.8 m/s and central PP of 49 mmHg were cut-off values with the highest sensitivity and specificity for predicting incident HF.

Table 2 presents the arterial stiffness measures and central hemodynamic parameters according to incident HF. Although there was no difference in baPWV between the two groups, the proportion of participants who had baPWV ≥ 1,835 cm/s was higher among those with incident HF than among those without incident HF. In contrast, brachial PP was significantly different between two groups. There a higher proportion of participants

TABLE 2 | The arterial stiffness measures and central hemodynamic parameters according to incident heart failure.

	Total (*N* = 3,034)	No incident HF (*N* = 2,969)	Incident HF (*N* = 65)	*P*-value
baPWV, cm/s	1,521.1 ± 322.9	1,520.2 ± 321.0	1,561.3 ± 401.0	0.415
baPWV ≥ 1,835 cm/s, *N* (%)	480 (15.8)	396 (13.3)	19 (29.2)	<0.001
Brachial PP, mmHg	52.6 ± 11.7	52.3 ± 11.5	63.2 ± 16.9	<0.001
Brachial PP ≥ 55 mmHg, N (%)	1,126 (37.1)	1,083 (36.5)	43 (66.2)	<0.001
cfPWV, m/s	9.5 ± 2.4	9.4 ± 2.4	11.0 ± 3.1	<0.001
cfPWV ≥ 8.8 m/s, *N* (%)	1,002 (33.0)	1,581 (53.3)	51 (78.5)	<0.001
Central SBP, mmHg	118.8 ± 18.9	118.5 ± 18.6	132.8 ± 25.5	<0.001
Central DBP, mmHg	75.6 ± 10.5	75.6 ± 10.5	76.2 ± 12.7	0.704
Augmentation index	27.1 ± 12.6	27.0 ± 12.6	29.8 ± 11.7	0.079
Central PP, mmHg	43.2 ± 14.1	42.9 ± 13.8	56.6 ± 19.9	<0.001
Central PP ≥ 49 mmHg, N (%)	793 (26.1)	818 (27.6)	43 (66.2)	<0.001

HF, heart failure; baPWV, brachial ankle pulse wave velocity; cfPWV, carotid femoral pulse wave velocity; PP, pulse pressure.

with brachial PP ≥ 55 mmHg among participants with incident HF than among those without HF.

In terms of central hemodynamic parameters, cfPWV was significantly higher in participants with incident HF than in those without incident HF. The proportion of participants with cfPWV ≥ 8.8 m/s was also higher among those with incident HF than among those without incident HF. Additionally, participants with incident HF had higher central PP than those without incident HF. There was a higher proportion of participants with central PP ≥ 49 mmHg among participants with incident HF than among those without HF.

Participants with high-risk category of arterial stiffness were older, had higher rates of diabetes, higher blood pressure, and more reduced renal function than participant with low-risk category (**Supplementary Tables 1**–**4**). We analysed the associations of high-risk categories of arterial stiffness with incident HF. In the unadjusted analyses, baPWV ≥ 1,835 cm/s, brachial PP ≥ 55 mmHg, cfPWV ≥ 8.8 m/s, and central PP ≥ 49 mmHg were associated with the risk of incident HF (all, $P < 0.05$; **Figure 2**; **Table 3**). In the age- and sex-adjustment model, baPWV ≥ 1,835 cm/s, brachial PP ≥ 55 mmHg, cfPWV ≥ 8.8 m/s, and higher central PP ≥ 49 mmHg were associated with incident HF (all, $P < 0.05$; **Table 3**). There was significant association of cfPWV ≥ 8.8 m/s and central PP ≥ 49 mmHg with incident HF even after adjusting for additional covariates, except baPWV ≥ 1,835 cm/s and brachial PP ≥ 55 mmHg (**Table 3**).

We analysed the association between arterial stiffness measures as continuous variables and incident HF. In the unadjusted models, brachial PP, cfPWV and central PP were significantly associated with the risk of incident HF, but baPWV was not. These associations were slightly attenuated but remained in the age- and sex-adjustment model and in the additional adjustment model (**Table 3**). Additionally, we performed a sensitivity analysis in participants with ABI > 0.9 because baPWV may not be reliable in participants with significant peripheral artery stenosis. In this analysis, baPWV was not associated with the risk of HF, and cfPWV showed a weak association (**Supplementary Table 5**).

DISCUSSION

In this study, brachial PP, cfPWV and central PP were higher in those with incident HF than in those without incident HF, but baPWV was not different between the two groups. However, there were more participants with high-risk category of each arterial stiffness measure among those with incident HF than among those without incident HF. When several covariates were adjusted, cfPWV ≥ 8.8 m/s and central PP ≥ 49 mmHg were significantly associated with incident HF. In previous studies, baPWV > 1,800 cm/s, brachial PP ≥ 55 mmHg, cfPWV > 10 m/s, and central PP > 50 mmHg were defined as high risk for cardiovascular events (10, 17–19), and our results were not markedly different from them. As a continuous variable for each index, the risk of incident HF increased as brachial PP, cfPWV and central PP increased, except for baPWV. Therefore, through this study, we revealed that arterial stiffness is an independent risk factor for HF, and among the indices of arterial stiffness, measures representing central hemodynamics and brachial PP were more relevant for incident HF than baPWV.

cfPWV has been studied considerably in Europe and the United States, and a significant amount of clinical data have been accumulated; thus, it is considered a gold-standard measurement of arterial stiffness (20). In contrast, baPWV has been mainly studied in Asia, especially Japan (21). Although baPWV lacks large-scale and long-term study data compared to cfPWV, it has been extensively studied recently. The two measures differ methodologically. In the case of cfPWV, the carotid and femoral artery pulses are measured using tonometry and calculated using the time difference between the two pulses. In contrast, for baPWV, the pulses of the brachial artery and the ankle artery are measured, and the time difference between the two pulses is used to calculate pulse wave velocity (PWV). Therefore, cfPWV is considered to represent aortic stiffness, whereas baPWV represents both aortic stiffness and peripheral arterial stiffness (22). In a community-based cohort study of 2,287 patients, cfPWV and baPWV showed a strong positive association, and the two indices were nearly equivalent for predicting the presence of coronary artery disease and stroke (22). Nevertheless, the

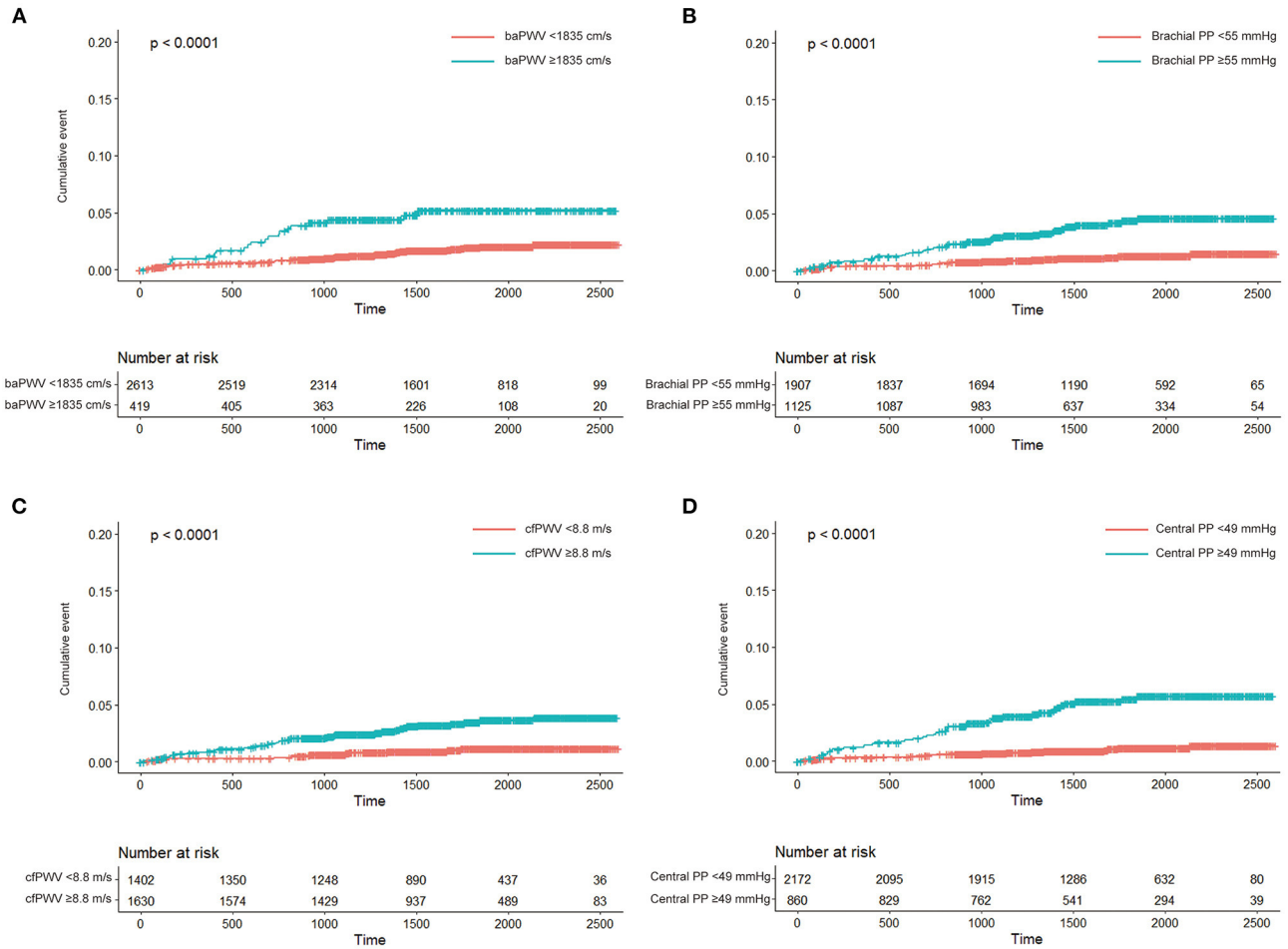

FIGURE 2 | Kaplan-Meier curve analyses of cumulative incidence of heart failure according to high-risk category of brachial-ankle pulse wave velocity (baPWV; **A**), brachial pulse pressure (brachial PP; **B**), carotid-femoral pulse wave velocity (cfPWV; **C**), and central pulse pressure (central PP; **D**).

TABLE 3 | The association between arterial stiffness measures and incident heart failure.

	Univariable model		Age- and sex-adjusted		Multivariable model	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Categorical variables						
baPWV ≥ 1,835 cm/s	2.69 (1.57–4.59)	<0.001	2.54 (1.44–4.48)	0.001	1.48 (0.82–2.67)	0.197
Brachial PP ≥ 55 mmHg	3.39 (2.03–5.67)	<0.001	3.43 (2.02–5.82)	<0.001	1.73 (0.95–3.14)	0.073
cfPWV ≥ 8.8 m/s	3.21 (1.78–5.80)	<0.001	3.18 (1.70–5.93)	<0.001	2.01 (1.02–3.95)	0.043
Central PP ≥ 49 mmHg	4.86 (2.91–8.13)	<0.001	5.15 (2.30–8.85)	<0.001	3.07 (1.68–5.64)	<0.001
Continuous variables*						
baPWV, cm/s	1.13 (0.90–1.43)	0.293	1.06 (0.82–1.37)	0.644	0.83 (0.63–1.10)	0.198
Brachial PP, mmHg	1.92 (1.62–2.28)	<0.001	1.93 (1.62–2.29)	<0.001	1.48 (1.19–1.84)	<0.001
cfPWV, m/s	1.62 (1.36–1.93)	<0.001	1.63 (1.35–1.97)	<0.001	1.29 (1.02–1.63)	0.032
Central PP, mmHg	1.85 (1.57–2.17)	<0.001	1.88 (1.59–2.22)	<0.001	1.44 (1.17–1.78)	<0.001

**HR for incident HF expressed per standard deviation increment in each measure. SD for each measure were as follows: baPWV SD = 322.9 cm/s, brachial PP SD = 11.7 mmHg, cfPWV SD = 2.4 m/s, central PP SD = 14.1 mmHg.*

Multivariable model adjusted for age, sex, body mass index, diabetes, mean blood pressure, hypertensive medication usage, hemoglobin level, and estimated glomerular filtration rate. HR, hazard ratio; CI, confidence interval; baPWV, brachial ankle pulse wave velocity; cfPWV, carotid femoral pulse wave velocity; PP, pulse pressure; SD, standard deviation.

relationship between HF and arterial stiffness has been studied for only cfPWV.

However, a recent study on the relationship of baPWV with LV remodelling and diastolic dysfunction was published. In 202 untreated hypertensive patients, baPWV was significantly associated with parameters of LV remodelling and diastolic function, and it predicted diastolic dysfunction well (23). Although this study did not examine the association of baPWV with HF, it suggests that baPWV contributes to the development of HF, as LV remodelling and diastolic dysfunction in hypertensive patients are considered to be the first steps leading to HF.

Our study showed that indices such as cfPWV and central PP related to central hemodynamics were more closely related to HF than baPWV. This is probably because cfPWV or central PP is a representative index that better represents the hemodynamic burden on the LV in the pathophysiologic aspect of the development of HF. In a small study, central systolic BP and central PP were more strongly associated with LV diastolic dysfunction than baPWV (24).

Study findings on the relationship between central hemodynamic parameters and incident HF are conflicting (6, 7, 25). In Connie et al.'s study, both cfPWV and central PP were associated with incident HF in the age- and sex-adjusted model, but there was no statistical significance in the association between central PP and incident HF in a multivariable model that included several cardiovascular risk factors (6). In contrast, in our study, the association between central PP and incident HF was clear even in the model adjusted for multiple covariates. Connie et al. analysed the Framingham cohort study sample, whereas the cohort used in our study was hospital-based and had a relatively higher risk of CVD than the Framingham cohort. This can be supported by the fact that our study cohort had a higher rate of antihypertensive drug use and a higher prevalence of diabetes, although their age was younger than that of the Framingham cohort.

It is unclear whether central PP or cfPWV is more predictive of the occurrence of CVD. Although PP is considered a surrogate marker of arterial stiffness, aortic PWV and peripheral pressure wave reflection are the two main determinants of central PP. Although one study showed that both central PP and cfPWV were associated with renal microvascular damage, in a model that considered both, only central PP was an independent factor for determining changes in renal hemodynamics (26). There is often a mismatch between PP and PWV, and the Framingham offspring cohort analysed the relative contribution of PP and PWV to CVD. Patients with high central PP and high cfPWV had the highest cardiovascular risk. However, cfPWV was more likely to be involved in left ventricular hypertrophy and incident CVD than central PP (27). In our study, central PP showed a significant association with the risk of HF in both the categorical variable analysis model and the continuous variable analysis model. In contrast, cfPWV showed an association in the continuous variable model. Since there was no difference in the AUC of the ROC curves for incident HF in cfPWV and central PP, it is difficult to conclude which of the two indices is superior for predicting HF even in our study.

Brachial PP is the simplest measure of arterial stiffness. The association between brachial PP and the risk of HF has been known in the elderly population (11). In our study, brachial PP showed a close relationship with incident HF, and the AUC value for incident HF in brachial PP was comparable to that of cfPWV or central PP. Although brachial PP and baPWV are highly correlated indices (28), it was confirmed through this study that brachial PP is a superior index for predicting heart failure compared to baPWV. It may be because PP well reflects the afterload along with arterial stiffness. Since central aorta is located close to the target organ, it is thought that central PP has a stronger effect on the target organ damage and the development of cardiovascular disease than brachial PP (29). However, in our study, brachial PP was associated with the risk of HF to a degree very similar to that of central PP. This may be due to the characteristics of our study participants. Pressure amplification (peripheral/central pulse pressure ratio) is high in younger people, but pressure amplification is reduced in older people or people with advanced arterial stiffness (30).

This study had some limitations. First, since this cohort mainly included hypertensive or diabetic patients with target organ damage, and asymptomatic atherosclerotic CVD, the risk of cardiovascular disease was higher than that of general population (31, 32). The 10-year atherosclerotic CVD risk calculated by the pooled cohort equation was 15% on average. Therefore, the results of our study must be cautiously applied to the general population. In particular, most of those who progressed to HF had poor renal function at baseline. It seems to be natural because chronic kidney disease is an independent risk factor for HF (33). Nevertheless, this study showed that cfPWV and central PP were associated with the development of HF in the multivariable model including renal function, which is similar to previous findings (7). Second, despite the follow-up period of ∼4 years, the number of individuals who developed HF was smaller than expected. This may be because we limited incident HF events to only HF hospitalisation to clarify the adjudication through the retrospective chart review. Heart failure is a clinical syndrome and its definition is ambiguous and not standardised. Therefore, we used modified criteria with an increased cut-off for NT-proBNP levels in the European Society of Cardiology definition (15). Because the NT-proBNP level supporting hospitalisation for heart failure is presented as 300 pg/mL in the European Society of Cardiology position paper (34). Third, as this hospital-based cohort study recruited patients from a single tertiary centre, most of the study individuals regularly visited outpatient clinics and were being managed for underlying cardiovascular risk factors. Although this study could not reflect the natural relationship between arterial stiffness measures and incident HF, its results are valuable in real-world clinical practise and applicable to patients with high cardiovascular risk.

In conclusion, among arterial stiffness indices, brachial PP, cfPWV, and central PP were better predictors of HF than baPWV. Brachial PP or central hemodynamic measures may help in risk stratification for the development of HF in patients at high cardiovascular risk.

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 Yonsei University Health System Clinical Trial Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CJL: conceptualisation, funding acquisition, and writing—original draught. MY, JH, and SP: data curation. SP, S-HL, and S-MK: supervision. JO and S-MK: validation. S-MK: writing—review and editing. 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.2021.782849/full#supplementary-material

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Anticoagulation Control in Different Ethnic Groups Receiving Vitamin K Antagonist for Stroke Prevention in Atrial Fibrillation

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Vitamin K antagonist such as warfarin reduces the risk of stroke in atrial fibrillation (AF) patients. Since warfarin has a narrow therapeutic index, its administration needs to be regularly monitored to avoid any adverse clinical outcomes such as stroke and bleeding. The quality of anticoagulation control with warfarin therapy can be measured by using time in therapeutic range (TTR). This review focuses on the prevalence of AF, quality of anticoagulation control (TTR) and adverse clinical outcome in AF patients within different ethnic groups receiving warfarin therapy for stroke prevention. A literature search was conducted in Embase and PubMed using keywords of "prevalence," "atrial fibrillation," "stroke prevention," "oral anticoagulants," "warfarin," "ethnicities," "race" "time in therapeutic range," "adverse clinical outcome," "stroke, bleeding." Articles published by 1st February 2020 were included. Forty-one studies were included in the final review consisting of AF prevalence ($n = 14$ studies), time in therapeutic range ($n = 18$ studies), adverse clinical outcome ($n = 9$ studies) within different ethnic groups. Findings indicate that higher prevalence of AF but better anticoagulation control among the Whites as compared to other ethnicities. Of note, non-whites had higher risk of strokes and bleeding outcomes while on warfarin therapy. Addressing disparities in prevention and healthcare resource allocation could potentially improve AF-related outcomes in minorities.

Keywords: atrial fibrillation, ethnicity, time in therapeutic range (TTR), adverse clinical outcomes, anticoagulant

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INTRODUCTION

Atrial fibrillation (AF) is an atrial tachyarrhythmia that has an uncoordinated atrial activation, with consequent atrial mechanical function deterioration (1). Based on the latest European Society of Cardiology Guidelines, AF had remained one of the world's main causes of stroke, heart failure, sudden death and cardiovascular disease (2). The prevalence of AF has been increasing throughout the world affecting 8 million people in Europe and this is expected to increase 2-3-fold by 2030 (3). Older age, male sex, diabetes, and ischemic heart disease are factors associated with AF diagnosis (4). The use of Vitamin K antagonist (VKA) in AF patients is associated with a reduction in the risk of thromboembolic complications when time in therapeutic range (TTR) >70% is achieved (5). TTR is the time spent within the therapeutic range of INR (2.0–3.0) (6). Patients with TTR ≥

70% are considered to have a well-controlled warfarin therapy while those with TTR $\leq$ 70% are considered as poorly controlled warfarin therapy (6). Current research suggests that high TTR in patients treated with warfarin for AF correlates with better patient outcomes (6). However, challenges arise in identifying patients who are likely to achieve and maintain therapeutic INR as well as good anticoagulation control. The quality of anticoagulation control can be influenced by many factors. Ethnicity has been established as one of the factors that might impact anticoagulation control (7). A clinical scoring system, the SAMe-TT_2R_2 score was developed in 2013 that presents the most common clinical and demographic factors that might influence anticoagulation control in AF patients (7) and non-white ethnicity is included in this scoring system. The score can be used to aid decision making by identifying those patients who would probably do well when treated with VKA (achieving a high TTR, >65%) or, conversely, those who would need additional interventions to achieve good INR control or

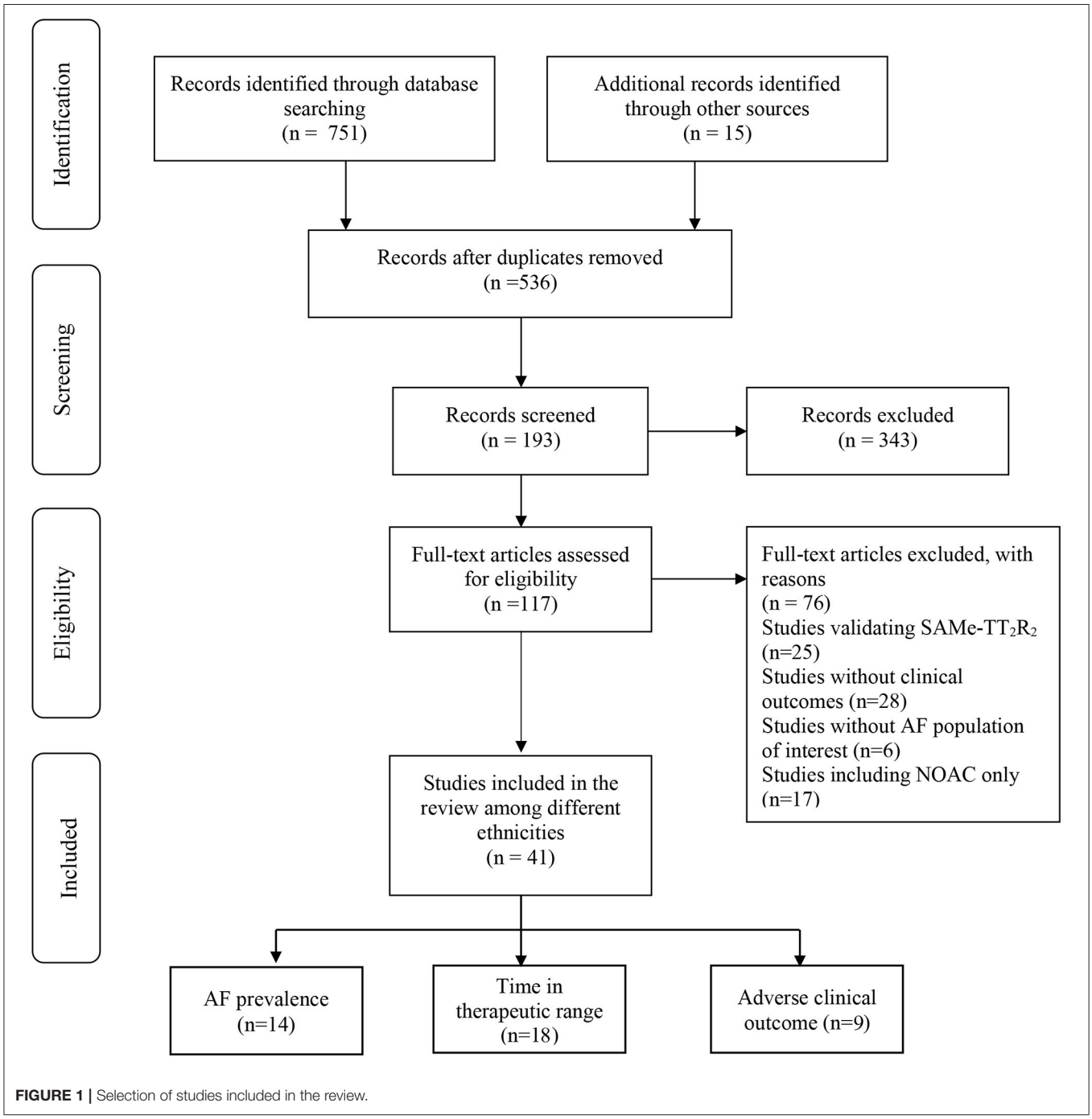

FIGURE 1 | Selection of studies included in the review.

to be initiated on/switched to a non-VKA oral anticoagulant (NOAC) (7).

Stroke is a long-term complication related to AF when effective measures of stroke prevention is not taken (2). Stroke tends to be more severe with a higher recurrence in AF patients reaching 6.9% compared to 4.7% in patients with stroke without atrial fibrillation (2). The CHA_2DS_2-VASc score, a well-validated score has been used worldwide to identify AF patients' risk factors of stroke and the need for oral anticoagulation therapy. Patients with a low risk factor of stroke from the CHA_2DS_2-VASc (CHA_2DS_2-VASc 0 in male and 1 in female) do not require antithrombotic treatment, while patients with risk factors for strokes (i.e., CHA_2DS_2-VASc of 1 or more for males and 2 or more for females) require oral anticoagulation therapy to prevent the risk of stroke (2). Vitamin K antagonist (e.g., warfarin) is one of the effective oral anticoagulants (OAC) that can be used to reduce the risk of stroke from AF by 64% (8). Nonetheless, bleeding risk is a major concern for AF patients taking warfarin therapy. Uncontrolled monitoring of warfarin therapy is associated with major bleeding in AF patients (9, 10). The risk of bleeding can be measured by using the HAS-BLED scoring system where a risk of bleeding $\geq$ 3 indicates high-risk bleeding and a score of <3 indicates low risk of bleeding (2).

The prevalence of AF, quality of anticoagulation and adverse clinical outcome among different ethnic groups varies and was seen to be poor among non-white ethnicities. Hence, this is the first review that reports on the prevalence of AF, the quality of anticoagulation control (TTR) and adverse clinical outcome among different ethnic groups receiving warfarin therapy for stroke prevention.

METHOD

A literature search was conducted on Embase and PubMed using keywords of "prevalence," "atrial fibrillation," "stroke prevention," "oral anticoagulants," "warfarin," "ethnicities," "race" "time in therapeutic range," "adverse clinical outcome," "stroke, bleeding." Articles published by 1st February 2020 were included. Given the lack of large scale RCTs in this population, evidence from prospective and retrospective studies were incorporated. Besides, they serve as "real-world" evidence on oral anticoagulant (OAC) use in AF patients with different ethnicities. Forty-one studies were included in the final review consisting of AF prevalence ($n = 14$ studies), time in therapeutic range ($n = 18$ studies), adverse clinical outcome ($n = 9$ studies) within different ethnic groups (**Figure 1**).

AF AMONG DIFFERENT ETHNIC GROUPS

The prevalence of AF differs between each ethnics group. **Table 1** represents 14 studies (11–24) on the prevalence of AF based on different ethnic groups. According to all studies, White people have the highest prevalence of AF compared to Afro-Caribbean, Asian, Hispanic and others ranging from 1.2% (12) to 29% (19). Only 3 studies (11, 22, 24) investigated AF diagnosis within the Hispanics ranging from 2.6 to 7.8%. While eight other studies (11–13, 15, 18, 20, 22, 24) reported on AF prevalence within Asians ranging from 0.05 to 10.1%. There was only one study (24) reported prevalence of AF among Native American (4.4%) and Pacific Islandar/Hawaiian (4.6%) and another study (15) which reported the prevalence of AF in Malaysia according to its ethnic groups with Malay (0.77%), Chinese (0.05%), others (0.06%). Besides, a study by Shavadia et al. (25) also reported that Asian ethnicity has been associated with considerably lower AF rates compared to White ethnicity. Differences in clinically detected AF among different ethnic group might be evident. It could reflect the variations in clinical recognition of AF, perception of AF symptoms or access to health care, the limited participation of minorities in trials and clinical studies for AF; or also due to difference in the completeness of clinical assessment when patients are presented with AF symptoms (11).

TIME IN THERAPEUTIC RANGE AMONG WARFARIN USERS WITHIN DIFFERENT ETHNIC GROUPS

Time within the therapeutic range (TTR) is used to evaluate anticoagulation control in patients on warfarin therapy for stroke prevention in AF. TTR has a significant impact on patient outcomes such as stroke and mortality (6, 26). As shown in **Table 2**, most of the studies used TTR $\geq$ 70 as their cut-off point indicating good anticoagulant control in AF patients. Moreover, there were also other studies (30, 32) that used TTR $\geq$ 60 and TTR $\geq$ 65 as their cut-off point to indicate good anticoagulant control among their AF patients.

Among the 18 studies (9, 26–42) from the findings, eight studies (27, 28, 32, 33, 37–39, 42) stated TTR among each specific ethnic groups in their population and the other 10 studies (9, 29–31, 34–37, 40, 41) only mentioned the TTR in their overall population (whites, Asians). So far, in Malaysia, there is no study being conducted that focused on TTR among different ethnic groups. However, a study by Yap et al. (35) at Malaysia's National Heart Institute reported only 53.2% of their overall patients had a TTR $\geq$ 70. Furthermore, in one Singaporean study (33), Chinese patients were reported to have higher mean TTR than Malays with 58.7 and 55.2%, respectively. One study in China (36) showed the percentage of people with TTR$\geq$70 was only 10.7% while TTR $\leq$ 70% was 89.3% which indicated poor anticoagulation control of warfarin therapy in both countries. Interestingly, Nguyen et al. (28) also reported lower TTR among their 512 Indigenous vs. non-indigenous Australian patients with AF [40 (29) vs. 50 (31); $p = 0.006$]. Based on the previous study by Golwala et al. (37) black individuals had lower median TTRs (59%) than Hispanic (62%) and white (68%) participants; consistent with findings from the sub study of IMPACT trial (38). In relation to a previous study by Zulkifly et al. (27) the researchers also found that the quality of anticoagulant control differs based on ethnicity whereby South Asians and Afro-Caribbeans had poor

TABLE 1 | Prevalence of AF by ethnicity.

Country	References	a) Study design b) Follow-up c) Sample size	Prevalence (%)				
			White	Black	Asian	Hispanic	Others
US	Heckbert et al. (11)	a) Cross sectional b) 14.4 years c) 1,556	11.3	6.6	9.9 - Chinese	7.8	-
London	Mathur et al. (12)	a) Cross sectional b) 3 years c) 6,292	1.2	0.4	0.2 - South Asian	-	-
England	Gillot et al. (13)	a) Observational b) N/A c) 277,218	2.4	-	0.4 - South Asian	-	-
US	Magnani et al. (14)	a) Prospective b) 6.2 years c) 15,080	8.1	5.8	-	-	-
Malaysia	Lim et al. (15)	a) Prospective b) 3 years c) 10,805	-	-	0.77 - Malay 0.05- Chinese 0.06 - Other	-	
California, Florida, New York	Kamel et al. (16)	a) Retrospective b) 4 years c) 101,773	25.5	21.4	-	-	-
Washington	Jensen et al. (17)	a) Prospective b) 11.2 years c) 1,585	19	17	-	-	-
North America, Europe, Asia	Lau et al. (18)	a) Prospective b) 2.5 years c) 2,580	18	8.3	10.1- Chinese 9.5- Japanese	-	-
US	Lahiri et al. (19)	a) Retrospective b) 4 years c) 1,001	29	19	-	-	-
US	Winkelmayer et al. (20)	a) Cross sectional b) 15 years c) 2,483,199	14	6.5	9.0- South Asian	-	-
US, Washington	Marcus et al. (21)	a) Combination CHS and ARIC study b) N/A c) 19,784	23	15	-	-	-
California	Shen et al. (22)	a) Cross sectional b) 1 year c) 430,317	8	3.8	3.9 - East Asian	3.6	-
California	Go et al. (23)	a) Cross sectional b) 1 year c) 17,974	2.2	1.5	-	-	-
US	Borzecki et al. (24)	a) Cross sectional b) 1 year c) 664,654	6.1	2.6	3.4	2.6	4.4- Native American 4.6- Pacific Islandar/Hawaiian

TABLE 2 | Mean TTR of patients among different ethnic group.

Country	References	a. b.	Study design length of follow up	a. b.	Sample size race/ethnicity	Method INR monitoring	Mean TTR
United Kingdom	Zulkifly et al. (27)	a. b.	Retrospective 11 months	a. b.	1,070 Whites, Afro Caribbean, South Asian	Anticoagulant clinic	White = 67.9 Afro- Caribbean = 61.3 South Asian = 60.5
Australia	Nguyen et al. (28)	a. b.	Retrospective n/a	a. b.	512 Indigenous, non-Indigenous	Hospital based	Indigenous = 40 (29) non-Indigenous = 50 (31)
China	Li et al. (29)	a. b.	Prospective 3 months	a. b.	379 Chinese	Hospital based	Overall mean TTR 58.35 (26.3)
Lithuania	Urbonas et al. (30)	a. b.	Retrospective 12 months	a. b.	406 -	Primary health care centre	TTR > 65 = 20.4 TTR < 65 = 79.6
Spain	Roldán Rabadán et al. (31)	a. b.	Prospective 3 years	a. b.	1,584 Whites	Hospital based	TTR > 70 = 40 TTR < 70 = 60
Australia	Bernaitis et al. (9)	a. b.	Retrospective 6 months	a. b.	3,199 Whites	Hospital based	TTR > 70 = 82 TTR < 70 = 20
Qatar	Mohammed et al. (32)	a. b.	Retrospective >6 months	a. b.	241 Asian	Anticoagulant clinic	TTR > 65 = 65.1 TTR < 65 = 34.9 Mean TTR: Arab = 71 Asian= 67
Singapore	Bernaitis et al. (33)	a. b.	Retrospective 6 months	a. b.	1,137 Malay Chinese Indian	Hospital based	Mean TTR: Malay = 55.2 Chinese = 58.7 Indian = 49.7
Iran	Abbasinazari et al. (34)	a. b.	Cross sectional 6 months	a.	470	Anticoagulant clinic	TTR > 70 = 37.3 TTR < 70 = 62.7
Malaysia	Yap et al. (35)	a. b.	Retrospective >12 months	a. b.	500 Asian	Hospital based	TTR > 70 = 53.2 TTR < 70 = 46.8
China	Chan et al. (36)	a. b.	Retrospective 14 years	a. b.	1,428 Chinese	Hospital based	TTR > 70 = 10.7 TTR < 70 = 89.3
California	Golwala et al. (37)	a. b.	Prospective 15 months	a. b.	9,542 White Black Hispanic	Outpatient	Mean TTR: White = 68 Black = 59 Hispanic = 62
North America, Europe, Australia	Lip et al. (38)	a. b.	Randomised controlled trial (RCT) 2 years	a. b.	2,718 White, Black, Asian, Non-Hispanic, Hispanic	RCT	Mean TTR: White; 55.2 Black: 44.0 Asian: 67.0 Non-Hispanic: 53.7 Hispanic: 47.8
US	Yong et al. (39)	a. b.	Retrospective 1 year	a. b.	184, 161 Blacks, whites	Outpatient setting	Mean TTR White: 57% Black: 49%; $p < 0.001$

(Continued)

TABLE 2 | Continued

Country	References	a. b.	Study design length of follow up	a. b.	Sample size race/ethnicity	Method INR monitoring	Mean TTR
South Dakota, US	Barta et al. (40)	a. b.	Retrospective 16 months	a. b.	837 Native American Asian Caucasian/white	Tertiary care clinic	TTR > 70 = 43.9 TTR < 70 = 56.1
United Kingdom	MacEdo et al. (41)	a. b.	Population based 12 months	a. b.	29,717 White Black Asian	Hospital based	TTR > 70 = 44 TTR < 70 = 56
Portugal	Calderia et al. (26)	a. b.	Retrospective >12 months	a. b.	274 Whites	Anticoagulant clinic	TTR > 60 = 53.3 TTR < 60 = 46.7
Iran	Singer et al. (42)	a.	Prospective/ Clinical trial	a. b.	1,178 White African/American Asian American Indian/Alaskan Hawaiian	Clinical trial center	Mean TTR White = 56.3 African/American = 51.9 Asian = 48.3 American Indian/Alaskan = 51.2 Hawaiian = 52.6

anticoagulant control with their mean TTR of 60.5 and 61.3%, respectively as opposed to White people with 67.9% (27). Hence, these studies indicate that white people have better anticoagulant control compared to other ethnic groups. Birman-Deych et al. (43) claimed that warfarin did not offer advantages in blacks and Hispanics, partially due to less effective warfarin care and anticoagulation monitoring.

These observations may be due to various reasons, for example differences comorbid disease, socioeconomic status, poor understanding of therapy, adherence issue and genetic background. Ethnic differences in anticoagulation control were evident in a cohort of 9,542 patients (37) receiving warfarin therapy for various indications (AF, VTE, and other mixed conditions), with lower mean TTR among the Blacks compared to Whites. Blacks were younger and lived in areas of highest quartile of poverty, had higher illness burden including more comorbid disease, requiring more medications and hospitalisations to manage those conditions compared to White patients (37). After accounting for all these factors, which are mostly non-modifiable, Black patients still had a recorded TTR 2.3% lower than White patients (37). Meanwhile, poor TTR among Asians might be affected by their dietary intake and extensive use of herbal medications (44). Furthermore, it is not common to have a structured anticoagulant clinic in many parts of the Asian countries causing more challenges in optimising INR control (44).

In terms of pharmacogenetics, warfarin metabolism and dose requirements might differ between ethnic groups. Studies have shown that warfarin dosage requirements are higher in Blacks compared to Whites partly due to racial differences in genotype frequencies (45). Blacks have been found to have additional CYP2C9 alleles which are associated with reduced function of the CYP2C9 activity and thus might contribute to dose variability (45). In addition, issues like variability of health literacy, adherence to medication might also contribute to the differences in quality of anticoagulation therapy among different ethnic groups (37). Perhaps these issues could "flag" the physicians to have a closer and more frequent follow up among ethnic minority patients who are having difficulties in achieving therapeutic INR with warfarin therapy. Otherwise, if without budget constraint, NOACs are in preference to Vitamin K antagonist (VKA) in these patients based on the latest ESC guideline on management of AF (evidence grade 1C) (2).

ADVERSE CLINICAL OUTCOME AMONG AF PATIENTS WITHIN DIFFERENT ETHNIC GROUPS

Warfarin, apart from having a narrow therapeutic index which requires frequent INR monitoring, multiple major drug-drug interaction and drug-food interaction have been documented. Its usage is also associated with adverse event such as thromboembolic and bleeding complications if the quality of anticoagulation control is not optimized. According to Pastori et al. (4) good TTR (>70%) is associated with a low risk of stroke and bleeding. **Table 3** outlines adverse clinical outcomes among different ethnic groups based on 9 studies (10, 27, 36, 38, 46–50). Five studies (27, 36, 46–48) focused on the adverse clinical outcomes in each ethnic group while the other four studies (10, 38, 49, 50) focused on the overall population. Referring to study Shen et al. (47). African American or black people has a high risk of stroke compared to other ethnic groups. Similarly, Zulkifly et al. (27) reported that black people had the highest proportion of stroke and bleeding complications (9.8 and 6.5%) compared to White (4.5 and

TABLE 3 | Percentage of adverse clinical outcome by ethnicity.

References	Adverse outcome	Adverse outcome (%)					
		White	Black	Asian	Hispanic	Others	Overall population
Kabra et. al (46)	Stroke	23	38 - African American	-	27.8	28.7 – Native American 22.8– Pacific islander	-
Shen et. al (47)	ICH	0.34	0.77	1.75	0.73	-	-
Chan et. al (36)	IS	-	-	23.7 – Chinese	-	-	-
Wang et. al (48)	ICH	-	-	11	-	0.97 – non-Asian	-
Zulkifly et. al (27)	Stroke / TIA	4.5	6.5	4.9	-	-	-
	Bleeding	4.5	9.8	5.9	-	-	-
	CVS hospitalization	21.3	25.6	32.3	-	-	-
	Death	2.5	1.2	2.0	-	-	-
Graham et al. (49)	Bleeding	-	-	-	-	-	1.3
Lip et al. (38)	Bleeding	-	-	-	-	-	1.3–7.4
	ICH	-	-	-	-	-	0.3–2.5
Guo et al. (10)	Bleeding	-	-	-	-	-	1–1.5
	ICH	-	-	-	-	-	0.4
Guo et al. (50)	Major bleeding	-	-	-	-	-	1.14
	Major bleeding + ICH	-	-	-	-	-	0.52

ICH, intracranial haemorrhage; TIA, transient ischemic attack; IS, ischemic stroke.

4.5%) and Asian (4.9 and 5.9%) population, respectively. A meta-analysis of 10 studies comparing the prevalence of AF among African Americans to Whites in the United States concluded that being African American was associated with a "protective effect" from AF [OR 0.51 (95% CI 0.44–0.59); $p < 0.001$]. Despite that, African Americans have twice the risk of first ever stroke compared to Whites and this might be due to higher risk factor burden of stroke, for example, hypertension (51).

Besides stroke and TIA, patients on warfarin therapy are also at risk of getting intracranial haemorrhage (ICH) with double the risk in Asians relative to the Whites (43). Clinical trials (52, 53) have shown low TTR among Asians compared to non-Asians; however, the rates of major bleeding were significantly higher in Asian patients (53, 54). The reasons behind this are not completely understood but one small Chinese study ($n = 290$) (54) reported the presence of cerebral microbleeds (CMBs) which was associated with numerically higher incidence of ICH among their AF patients on warfarin therapy compared to those without CMBs (3.6 vs. 0.7%, $p = 0.129$).

STRENGTHS AND LIMITATIONS OF THE REVIEW

This is the first review summarising the prevalence, quality of anticoagulation control (TTR), and adverse clinical outcome among AF patients on warfarin therapy within different ethnic groups. Clinicians could understand the underlying factors that influence the treatment outcome among these ethnic groups. Rational prescribing of NOAC and warfarin could be improved by having the snapshot view of AF disease burden and their clinical outcomes in terms of stroke prevention. Nevertheless, the findings derived from this review are limited with caveats such as more than half of the included studies are retrospective in nature, the number of patients included were small and not represented in some parts of the world such as the Middle East and areas other than mainland China and Western Europe.

CONCLUSION

In conclusion, this review represents the differences in the prevalence of AF, anticoagulation control with warfarin therapy and adverse clinical outcomes among different ethnic groups across the globe. Findings suggest higher prevalence of AF but better anticoagulation control among the Whites as compared to other ethnicities. Unfortunately, non-whites had higher risk of strokes and bleeding outcomes while on warfarin therapy. Addressing disparities in prevention and healthcare resource allocation will likely improve AF-related outcomes in minorities.

AUTHOR CONTRIBUTIONS

HZ: conceptualization and supervision. NZ, IA, and HZ: methodology, formal analysis, investigation, data curation, writing—original draft preparation, and project administration.

NZ, IA, LM, and HZ: software and validation. LM, HPG, and HZ: resources, writing—review and editing, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL RESEARCH
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Obstructive Sleep Apnoea in Stanford Type B Aortic Dissection Is Associated With Multiple Imaging Signs Related to Late Aortic Events

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Background: Obstructive sleep apnoea (OSA) is highly prevalent in patients with Stanford type B aortic dissection (TBAD). Few studies have evaluated the effects of OSA on vascular changes in TBAD patients. This study aimed to explore the effect of OSA on aortic morphological changes in TBAD patients and its relation to late aortic events (LAEs).

Methods: This case-control study included 143 TBAD patients. The diameters of different parts of the aorta were measured based on computed tomography angiography (CTA). According to the apnoea-hypopnoea index (AHI), OSA was classified as mild ($5 \leq AHI \leq 15$), moderate ($15 < AHI \leq 30$), or severe ($AHI > 30$). The false lumen (FL) status was evaluated and classified as partially thrombosed, patent, or completely thrombosed.

Results: The OSA prevalence in TBAD patients was 64.3%, and image differences related to LAEs between TBAD patients with and without OSA included the maximum aortic diameter at onset (37.3 ± 3.9 vs. 40.3 ± 4.5 mm, $p < 0.001$), the FL diameter of the proximal descending thoracic aorta (16.0 ± 6.8 vs. 20.3 ± 4.7 mm, $p < 0.001$), and the proportion of the FL that was partially thrombosed (39.2 vs. 64.1%, $p = 0.004$). Additionally, in the multivariable analysis of patients with OSA, the risks of an aortic diameter $\geq$40 mm, a proximal descending aorta FL $\geq$ 22 mm and a partially thrombosed FL were 4.611 (95% CI: 1.796–11.838, $p = 0.001$), 2.544 (95% CI: 1.050–6.165, $p = 0.039$), and 2.565 (95% CI: 1.167–5.637, $p = 0.019$), respectively, after adjustment for confounding factors. Trend tests showed that the risks of an aortic diameter $\geq$40 mm and a partially thrombosed FL increased with increasing OSA severity.

Conclusions: TBAD patients with moderate to severe OSA have aortic dilatation in different parts of the aorta. OSA is an independent risk factor for multiple imaging signs related to LAEs, suggesting that OSA is an important factor affecting the prognosis of TBAD patients.

Keywords: Stanford type B aortic dissection, obstructive sleep apnoea (OSA), late aortic events, aortic morphological changes, aortic dilatation

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INTRODUCTION

Stanford type B aortic dissection (TBAD) is a catastrophic vascular disease with high mortality and poor prognosis (1, 2) that is characterized by the blood passing through the first one-third of the external media in the aortic wall (3). The annual incidence of AD is estimated to be 2.6–3.6 per 100,000 adults worldwide (4–6). Most patients die within the first week, and ∼25–50% of patients who survive the acute phase require surgery during the chronic phase (7, 8). The overall in-hospital mortality of TBAD was reported to be 13%. Despite the increase in the use of thoracic endovascular aortic repair (TEVAR) and the application of hybrid surgery, in-hospital mortality has not changed significantly in the past 20 years (9). Compared with medical therapy, TEVAR has proven to be more effective in improving aortic remodeling and long-term survival (10). However, neither TEVAR nor medical therapy can fully prevent aortic wall degeneration or disease progression. Approximately 35% of patients treated with TEVAR and 65% of patients receiving medical therapy develop late aortic events (LAEs) within 5 years (11, 12).

The occurrence of LAEs severely affects the prognosis of patients with TBAD (13, 14). In most studies, LAEs were defined as the development of aortic expansion (>55 mm), rapid dilatation of the dissected aorta (>10 mm per 10–12 mo), new dissection, malperfusion, rupture, or impending rupture and aortic-related death after patients were discharged following surgery or medical treatment (10, 15–19). Previous studies have demonstrated that several predictors could be used to identify TBAD patients at high risk for aortic expansion during follow-up and predict the individual risk of LAEs. Radiologic predictors included an initial maximum diameter of the aorta ≥40 mm (10, 15, 16, 20–23), a proximal descending thoracic aortic false lumen (FL) diameter ≥22 mm (24), a partially thrombosed FL (17, 25–28), a large entry tear >10 mm (10), a fusiform index ≥0.64 (29), and ulcer-like projections (30, 31). Clinical predictors included younger age (<60 years) (32, 33), heart rate ≥60/min (34), and Marfan syndrome (35). Laboratory findings involved fibrinogen-fibrin degradation product levels ≥20 mg/ml at admission (36) and peak C-reactive protein levels ≥9.61 mg/dl (37). Identification of these predictors might benefit patients through early TEVAR or closer follow-up and contribute to the development of individualized treatment strategies (14, 19, 38).

Obstructive sleep apnoea (OSA) is characterized by recurrent upper airway obstruction with interruption of airflow and persistence of inspiratory effort during sleep (39) and is one of the most common risk factors for cardiovascular diseases (40). The prevalence of OSA in TBAD is 66.2–81.7% (41–43), which is dramatically higher than the reported incidence of 9–38% in the general population (44, 45). The potential correlation between OSA and TBAD was first reported by Sampol et al. (46). An increasing number of studies have shown that OSA is an important cause of AD (42, 47–49).

However, few studies have evaluated the effects of OSA on vascular changes in TBAD patients. The aim of this study was to explore the effect of OSA on aortic morphological changes in TBAD patients and its relation to LAEs.

MATERIALS AND METHODS

Study Population

From August 1, 2019, to July 1, 2021, a total of 167 TBAD patients were admitted to the vascular surgery department of the First Hospital of China Medical University. All patients underwent computed tomography angiography (CTA) examinations and arterial blood gas analysis on admission. We routinely performed sleep monitoring on the patients. We asked the patients' medical history and queried their electronic medical records to determine whether the patients had undergone polysomnography (PSG) for the first time or had been previously treated with continuous positive airway pressure (CPAP) for OSA or other respiratory diseases. Smoking history was determined as a history of smoking (current or former) or no history of smoking. **Figure 1** illustrates the exclusion criteria for this study, including (1) type A AD; (2) patients with Marfan syndrome; (3) patients with traumatic dissection; (4) patients who refused to undergo sleep monitoring; (5) poor-quality CTA data or a lack of imaging data before surgery; and (6) aortic dissection rupture, shock, unconsciousness or other life-threatening situations. A total of 143 TBAD patients were ultimately enrolled in our study.

Imaging Data

CTA images were produced by a SIEMENS SOMATOM Definition Flash CT (Siemens Healthcare, Erlangen, Germany). The thickness of the CTA imaging slice was 1 mm. The aortic morphology was measured using image processing software (IMPAX Client, Agfa HealthCare N.V., Version 6.5.3.1509). The imaging data we measured included (1) the maximum aortic diameter at onset (the first CTA scan was performed during the acute event on initial imaging of the aorta); (2) the maximum diameter of the descending aorta; (3) the FL diameter of the proximal descending thoracic aorta (the FL at the upper descending thoracic aorta on initial CTA); (4) the diameter of the distal aortic arch (at the level of the left subclavian artery, including the retrograde dissection extending to the proximal aortic arch); (5) the diameter of the descending aorta (at the level of origin, the main pulmonary artery); (6) the maximum diameter of the FL; (7) the FL status (completely thrombosed, partially thrombosed, patent); (8) the fusiform index (defined as A/(B+C), where A = maximum diameter of the descending aorta, B = diameter of the distal aortic arch, C = diameter of the descending aorta at the level of origin, the main pulmonary artery) (29); and (9) the number of dissection process-involved zones according to the Society for Vascular Surgery/Society of Thoracic Surgeons (SVS/STS) classification system reporting standards for TBAD (50).

Sleep Study

A sleep study was performed with a portable sleep-respiration monitor [PSM, PSM100A; Sealand Technology (Chengdu) Co., Ltd]. Sleep monitoring was performed before surgery and after all the patients were transferred from the intensive care unit to the general ward. The initial symptoms of each patient were relieved, which did not affect sleep monitoring due to pain or dyspnoea. Neither sedatives nor alcohol was used within

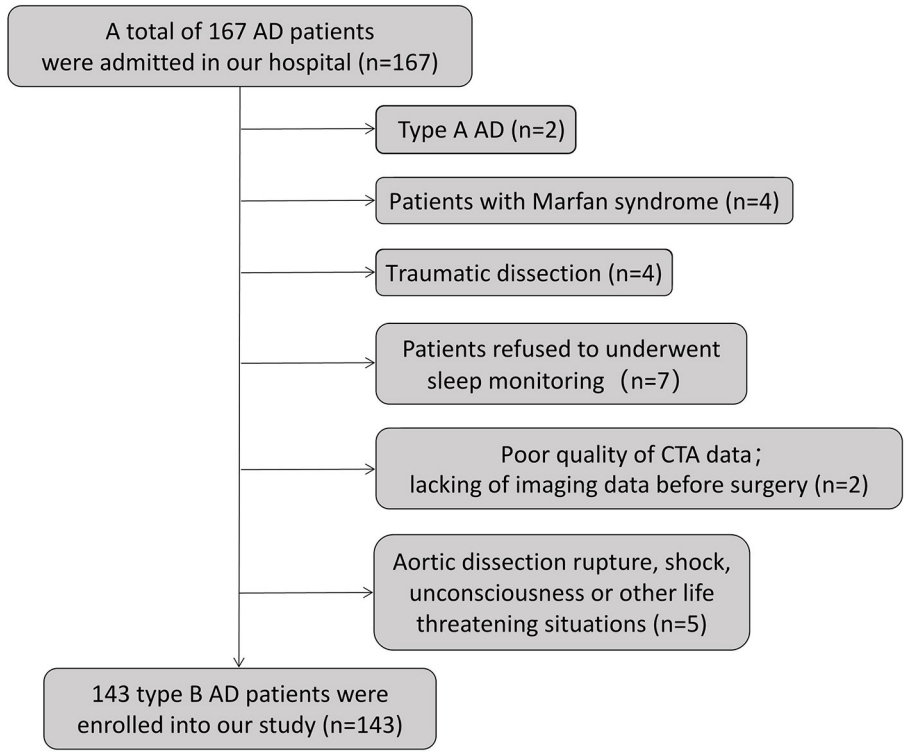

FIGURE 1 | Flow chart of the exclusion criteria.

48 h before the study. The recording duration was between 10:00 P.M. to 7:00 A.M. and lasted at least 7 h. The content of the records included the apnoea-hypopnoea index (AHI), nasal airflow pressure, chest movement, body position, snoring and fingertip oximetry. The diagnostic criteria for OSA refer to the American Academy of Sleep Medicine (AASM) Guidelines (2017) (51) and were defined as AHI $\geq$ 5 events/h. OSA severity was categorized according to the AHI as follows: mild OSA (5 $\leq$ AHI $\leq$ 15), moderate OSA (15 $<$ AHI $\leq$ 30), and severe OSA (AHI $>$ 30).

Statistics

All data are expressed as the mean $\pm$ standard deviation (SD) or number of participants (percentages). Normality was assessed using the Shapiro-Wilk normality test. Student's *t*-test was used for comparisons between TBAD patients with or without OSA if the data fit the normal distribution, and the Mann–Whitney *U*-test otherwise. Pearson's chi-square test or Fisher's exact test was used to compare the percentages in different groups. Univariate and multivariate logistical regressions were performed to analyse the risk factors and risk magnitude for a maximum aortic diameter at onset $\geq$40 mm, a diameter of the FL of the proximal descending thoracic aorta $\geq$22 mm and a partially thrombosed FL. Variables with a $p \leq 0.05$ on univariate analysis and clinically important factors [age, hypertension, and body mass index (BMI)] were included in the multivariate analysis. The results are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). $P \leq 0.05$ were considered significant. Statistical analyses were performed using SPSS version 26 (IBM Corp.). The minimal sample size was estimated by PASS 11 (NCSS, Kaysville, Utah) and met the requirements of our study (for a maximum aortic diameter at onset $\geq$40 mm, a FL diameter of the proximal descending aorta $\geq$22 mm, and a partially thrombosed FL, the minimum sample size of TBAD patients with and without OSA was 58 and 32, 74 and 41, 87 and 48, respectively).

RESULTS

Baseline Characteristics and Prevalence of OSA Among TBAD Patients

Of all 143 patients, the mean age was 52.0 $\pm$ 11.7 (years), and the average AHI was 15.1 $\pm$ 15.5 (times/h). Most of the patients underwent sleep monitoring for the first time, except for two patients who were previously diagnosed with OSA. One of the two patients received surgical treatment for OSA, and none of them had received CPAP. However, the AHI of the two patients was still as high as 28.5 and 22.2 times/h, respectively. Therefore, we still included the two patients in the trial group. A total of 71 patients (49.7%) had a $pO_2 < 80$ mmHg, of which seven patients (4.9%) had a $pO_2 < 60$ mmHg. In addition, the pCO_2 and lactate levels of all the patients were within the normal range at admission. According to the latest SVS/STS classification system, we also counted the number of dissection process-involved zones.

TABLE 1 | Baseline characteristics of the patients.

Variable	No. or mean (%)
Demographic characteristics	
Male	115 (80.4)
Age, years	52.0 ± 11.7
Young adults (>18, ≤45)	37 (25.9)
Middle-aged adults (>45, <60)	68 (47.6)
Older adults (≥60)	38 (26.6)
Patients with OSA	92 (64.3)
Mild OSA	38(26.6)
Moderate OSA	27(18.9)
Severe OSA	27(18.9)
BMI (kg/m^2)	26.8 ± 4.1
Hypertension	107 (74.8)
Diabetes	5 (3.5)
History of cardiovascular disease	8 (5.6)
Smoking	71 (49.7)
AHI (times/h)	15.1 ± 15.5
Arterial blood gas analysis	
PH	7.42 ± 0.03
pCO_2 (mmHg)	38.7 ± 3.4
pO_2 (mmHg)	82.0 ± 15.3
Lactate (mmol/L)	1.37 ± 0.56
Contrast-enhanced CT data	
Maximum aortic diameter at onset (mm)	39.2 ± 4.5
≥40 mm	63 (44.1)
<40 mm	80 (55.9)
Maximum diameter of the descending aorta (mm)	39.7 ± 4.8
FL diameter of the proximal descending aorta (mm)	18.8 ± 5.9
≥22 mm	50 (35.5)
<22 mm	91 (64.5)
Diameter of the distal aortic arch (mm)	31.5 ± 3.9
Diameter of the descending aorta at the level of the main pulmonary artery (mm)	34.6 ± 4.7
Maximum diameter of FL (mm)	20.6 ± 6.0
False lumen status	
Partially thrombosed	79 (55.2)
Patent	37 (25.9)
Completely thrombosed	27 (18.9)
Fusiform index	0.60 ± 0.04
≥0.64	22 (15.4)
<0.64	121 (85.6)
Numbers of dissection process-involved zones	7.8 ± 2.0

TABLE 2 | Characteristics of the TBAD patients with and without OSA.

Variable	Patients without OSA (n = 51)	Patients with OSA (n = 92)	p-value
Male, n (%)	39 (76.5)	76 (82.6)	0.38
Age, years	51.4 ± 11.2	52.3 ± 12.1	0.67
BMI (kg/m^2)	24.7 ± 3.2	28.0 ± 4.1	<0.001
Hypertension, n (%)	37 (72.5)	70 (76.1)	0.64
Smoking, n (%)	26 (51.0)	45 (48.9)	0.81
AHI (times/h)	2.8 ± 1.4	22.0 ± 15.5	<0.001
Maximum aortic diameter at onset (mm)	37.3 ± 3.9	40.3 ± 4.5	<0.001
Maximum aortic diameter at onset ≥40 mm, n (%)	13 (25.5)	50 (54.3)	<0.001
Maximum diameter of the descending aorta (mm)	38.2 ± 4.9	40.5 ± 4.5	0.029*
FL diameter of the proximal descending thoracic aorta (mm)	16.0 ± 6.8	20.3 ± 4.7	<0.001
FL diameter of the proximal descending thoracic aorta ≥22 mm, n (%)	11 (21.6)	39 (42.4)	0.012
Diameter of the distal aortic arch (mm)	30.4 ± 3.4	31.9 ± 3.3	0.008
Diameter of the descending aorta at the level of the main pulmonary artery (mm)	33.7 ± 5.3	35.0 ± 4.3	0.045*
Maximum diameter of the FL (mm)	18.8 ± 7.5	21.6 ± 4.7	0.018
Fusiform index	0.597 ± 0.04	0.600 ± 0.04	0.287
Fusiform index ≥0.64, n (%)	6 (11.8)	16 (17.4)	0.372
Partially thrombosed, n (%)	20 (39.2)	59 (64.1)	0.004
Numbers of dissection process-involved zones	7.4 ± 2.2	8.1 ± 1.8	0.065*

**This variable was analyzed with the Mann–Whitney U-test.*

The clinical characteristics and imaging data of all the patients are shown in **Table 1**.

The prevalence of OSA in TBAD patients was 64.3%: 38 patients (26.6%) had mild OSA, 27 (18.9%) had moderate OSA, and 27 (18.9%) had severe OSA. TBAD patients with OSA showed a higher BMI of 28.0 ± 4.1 kg/m^2 (p<0.001) and a series of imaging differences. The main manifestation was aortic dilatation, which included the maximum aortic diameter at onset (37.3 ± 3.9 vs. 40.3 ± 4.5 mm, $p < 0.001$), the proximal descending thoracic aorta FL diameter (16.0 ± 6.8 vs. 20.3 ± 4.7 mm, $p < 0.001$), and the proportion of FL that was partially thrombosed (39.2 vs. 64.1%, $p = 0.004$). Other imaging differences included the maximum diameter of the descending aorta (38.2 ± 4.9 vs. 40.5 ± 4.5 mm, $p = 0.029$), the diameter of the distal aortic arch (30.4 ± 3.4 vs. 31.9 ± 3.3 mm, $p = 0.008$), and the maximum diameter of the FL (18.8 ± 7.5 vs. 21.6 ± 4.7 mm, $p = 0.018$). Two of the patients' FL were found in the abdominal aorta; therefore, we excluded these patients when analyzing the association between OSA status and the diameter of the FL of the proximal descending aorta. The characteristics of the TBAD patients with and without OSA are shown in **Table 2**.

Association Between OSA Status and the Maximum Aortic Diameter at Onset

As shown in **Figure 2A**, compared with patients without OSA, patients with OSA showed larger aortic diameters. **Table 3**

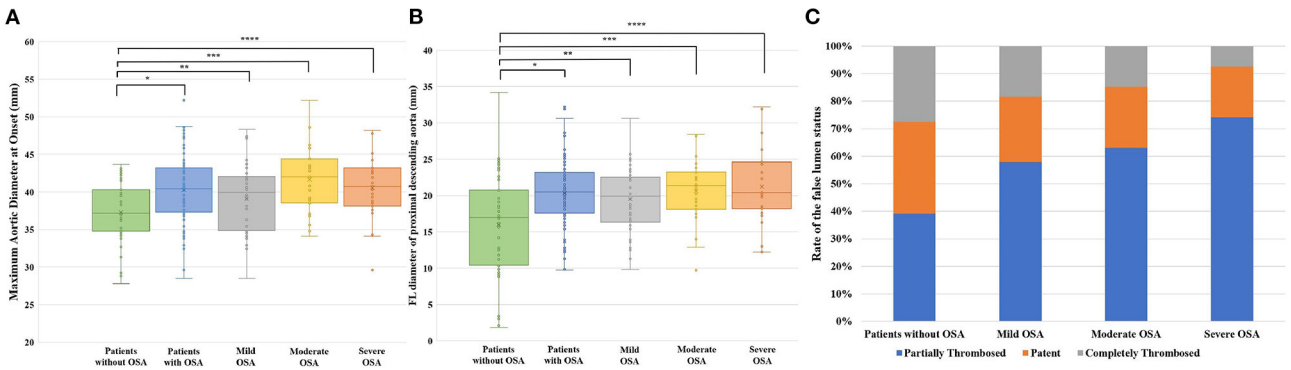

FIGURE 2 | Imaging signs related to LAEs in different OSA severities. **(A)** Aortic diameter at onset for different degrees of OSA severity. *$p < 0.001$; **$p = 0.044$; ***$p < 0.001$; ****$p = 0.001$. **(B)** FL diameter of the proximal descending thoracic aorta for different OSA severities. *$p < 0.001$; **$p = 0.004$; ***$p < 0.001$; ****$p < 0.001$. **(C)** Rate of the FL status in different OSA severities.

TABLE 3 | Univariate and multivariate logistic analyses of imaging signs related to LAEs.

Variables	Univariate analysis		Multivariate (Model 1)		Multivariate (Model 2)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Maximum aortic diameter at onset ≥40 mm						
Age	1.081 (1.044–1.120)	<0.001	1.084 (1.041–1.128)	<0.001	1.097 (1.047–1.149)	<0.001
Sex	0.643 (0.281–1.475)	0.298	–	–	0.894 (0.306–2.612)	0.838
BMI	1.030 (0.951–1.116)	0.469	–	–	1.074 (0.956–1.206)	0.228
OSA	4.225 (1.962–9.099)	<0.001	5.689 (2.361–13.704)	<0.001	4.611 (1.796–11.838)	0.001
Hypertension	4.723 (1.904–11.716)	0.001	4.078 (1.457–11.420)	0.007	3.646 (1.278–10.401)	0.016
FL diameter of the proximal descending thoracic aorta ≥22 mm						
Age	1.008 (0.978–1.038)	0.605	–	–	1.007 (0.970–1.044)	0.730
Sex	1.113 (0.460–2.690)	0.813	–	–	1.160 (0.448–3.003)	0.760
BMI	1.071 (0.983–1.167)	0.118	1.024 (0.932–1.125)	0.617	1.032 (0.926–1.151)	0.568
OSA	2.868 (1.278–6.437)	0.011	2.626 (1.102–6.257)	0.029	2.544 (1.050–6.165)	0.039
Hypertension	2.523 (1.010–6.305)	0.048	2.440 (0.958–6.219)	0.062	2.381 (0.899–6.311)	0.081
Partially thrombosed FL						
Age	1.005 (0.977–1.034)	0.724	–	–	1.002 (0.969–1.037)	0.886
Sex	0.909 (0.395–2.092)	0.822	–	–	0.826 (0.341–2.002)	0.672
BMI	1.067 (0.982–1.159)	0.126	1.021 (0.932–1.117)	0.660	1.027 (0.927–1.137)	0.616
OSA	2.771 (1.369–5.610)	0.005	2.583 (1.201–5.557)	0.015	2.565 (1.167–5.637)	0.019
Hypertension	1.790 (0.835–3.834)	0.134	1.762 (0.803–3.865)	0.158	1.723 (0.767–3.870)	0.188

TABLE 4 | Logistic regression and trend analyses of imaging signs related to LAEs for different OSA severities.

OSA severity classification	OR (95% CI) *p*-value		
	Non-adjusted	**Model 1***	**Model 2****
Maximum aortic diameter at onset ≥40 mm			
Non-OSA	Reference	Reference	Reference
Mild OSA	5.381 (2.083–13.902) 0.001	4.665 (1.650–13.191) 0.004	4.189 (1.454–12.071) 0.008
Moderate OSA	7.380 (2.616–20.817) <0.001	8.157 (2.469–26.941) 0.001	6.684 (1.933–23.107) 0.003
Severe OSA	5.125 (1.836–14.308) 0.002	6.489 (2.056–20.481) 0.001	4.830 (1.387–16.824) 0.013
P for trend	<0.001	<0.001	0.006
FL diameter of the proximal descending thoracic aorta ≥22 mm			
Non-OSA	Reference	Reference	Reference
Mild OSA	3.106 (1.171–8.237) 0.023	2.810 (1.042–7.574) 0.041	2.792 (1.019–7.646) 0.046
Moderate OSA	3.644 (1.279–10.386) 0.016	3.301 (1.121–9.719) 0.030	3.207 (1.050–9.793) 0.041
Severe OSA	3.644 (1.279–10.386) 0.016	2.733 (0.867–8.620) 0.086	2.669 (0.835–8.530) 0.098
P for trend	0.011	0.060	0.079
Partially thrombosed FL			
Non-OSA	Reference	Reference	Reference
Mild OSA	2.131 (0.907–5.010) 0.083	2.111 (0.877–5.081) 0.096	2.067 (0.846–5.052) 0.111
Moderate OSA	2.635 (1.007–6.898) 0.048	2.763 (1.006–7.591) 0.049	2.775 (0.971–7.933) 0.057
Severe OSA	4.429 (1.584–12.380) 0.005	4.207 (1.286–13.766) 0.018	4.228 (1.282–13.945) 0.018
P for trend	0.003	0.010	0.011

**For the maximum aortic diameter at onset ≥40 mm, the model was adjusted for age and hypertension. For the FL diameter of the proximal descending thoracic aorta ≥22 mm and partially thrombosed FL, the model was adjusted for BMI and hypertension; **Adjusted for age, hypertension, sex, and BMI.*

displays the results for the univariate and multivariate logistic regression analyses on a maximum aortic diameter at onset ≥40 mm. The risk of an aortic diameter larger than 40 mm in TBAD patients with OSA was ∼4 times higher (OR: 4.225, 95% CI: 1.962–9.099, $p < 0.001$) than that in patients without OSA. After adjusting for age and hypertension (model 1) and for age, hypertension, sex, and BMI (model 2), the ORs were 5.689 (95% CI: 2.361–13.704, $p < 0.001$) and 4.611 (95% CI: 1.796–11.838, $p = 0.001$), respectively. We also analyzed the effect of different OSA severities on the aortic diameter, as shown in **Table 4**. After adjusting for all the relevant factors (model 2), the ORs for patients with mild, moderate and severe OSA were 4.189 (95% CI: 1.454–12.071, $p = 0.008$), 6.684 (95% CI: 1.933–23.107, $p = 0.003$), and 4.830 (95% CI: 1.387–16.824, $p = 0.013$), respectively. Trend analysis showed that the risk of an aortic diameter ≥40 mm increased with increasing OSA severity ($p = 0.006$; **Table 4**).

Association Between OSA Status and the FL Diameter of the Proximal Descending Aorta

TBAD patients with OSA showed a larger proximal descending aorta FL diameter (**Table 2**). As OSA severity increased, the FL diameter also increased gradually (**Figure 2B**), but for a proximal descending aorta FL ≥ 22 mm in different OSA severities, as shown in **Table 4**, a trend analysis did not show a significant increase in OSA severity ($p = 0.079$). Univariate logistic regression showed that age, sex, and BMI were not risk factors for dilatation of the proximal descending aorta FL (≥22 mm). However, OSA was a significant risk factor (OR: 2.868, 95% CI: 1.278–6.437, $p = 0.011$) for FL dilatation. After adjusting for all the relevant factors (age, hypertension, sex, and BMI), the OR was 2.544 (95% CI: 1.050–6.165, $p = 0.039$; **Table 3**).

Association Between OSA Status and Thrombosed FL

Most of the patients had partially thrombosed FLs. However, the proportion of partially thrombosed FLs in severe OSA patients was as high as 74.1%, which was much higher than that in patients without OSA (39.2%) ($p = 0.004$, $\chi^2 = 8.238$). The proportions of patients with mild and moderate OSA were 57.9 and 63.0%, respectively (**Table 5**). Univariate and multivariate logistic regression analyses on partially thrombosed FL are shown in **Table 3**. After adjusting for confounding factors, the ORs of OSA for model 1 and model 2 were 2.583 (95% CI: 1.201–5.557, $p = 0.015$) and 2.565 (95% CI: 1.167–5.637, $p = 0.019$), respectively. **Figure 2C** shows the rate of the FL status in different OSA severities. Trend analysis showed that the risk of a partially thrombosed FL increased with increasing OSA severity ($p = 0.011$; **Table 4**).

Other Imaging Differences Between TBAD Patients With and Without OSA

As shown in **Figure 3**, other significant imaging differences between the two groups included the maximum diameter of the descending aorta (38.2 ± 4.9 vs. 40.5 ± 4.5 mm, $p = 0.029$), the diameter of the distal aortic arch (30.4 ± 3.4 vs. 31.9 ± 3.3 mm, $p = 0.008$), and the maximum diameter of the FL (18.8 ± 7.5 vs. 21.6 ± 4.7 mm, $p = 0.018$). We further analyzed these imaging

TABLE 5 | Thrombosed FL with different OSA severity levels.

Variable	Patients without OSA ($n = 51$)	Patients with OSA			p-value	χ^2
		Mild OSA ($n = 38$)	Moderate OSA ($n = 27$)	Severe OSA ($n = 27$)		
Partially thrombosed	20 (39.2%)	22 (57.9%)	17 (63.0%)	20 (74.1%)	0.004	8.238
Patent	17 (33.3%)	9 (23.7%)	6 (22.2%)	5 (18.5%)	0.129	2.300
Completely thrombosed	14 (27.5%)	7 (18.4%)	4 (14.8%)	2 (7.4%)	0.051	3.801

differences according to OSA severity and found that the most significant difference was between non-OSA patients and patients with moderate and severe OSA.

DISCUSSION

To our knowledge, this is the first study to find that TBAD patients with OSA have a higher risk of aortic dilatation and that some of the imaging differences are related to LAEs.

This case-control study included 143 TBAD patients, and the prevalence of OSA was as high as 64.3%. In 2003, Sampol et al. (46) first found a correlation between OSA and aortic dissection, and an increasing number of studies have shown that OSA is an independent risk factor for AD, especially for TBAD patients with moderate to severe OSA (47–49). Previous studies have indicated that OSA may lead to dilatation of the aorta, including the aortic root (52–55), thoracic aorta (56) and abdominal aorta (57); other studies have come to the opposite conclusion (58), and most of the OSA patients in these studies had no history of cardiovascular disease. However, the effect of OSA on vascular morphology changes in TBAD has rarely been reported. As seen in our results, the main change in the aorta for TBAD patients with OSA is aortic dilatation, which also includes enlargement of the FL. The mechanism of aortic dilatation and the development of TBAD induced by OSA has not been fully elucidated. The possible mechanism includes changes in intrathoracic pressure, meaning that the increase in pleural negative pressure can lead to mechanical stretch of the aorta (54, 55, 59–61), which further leads to the expansion of the aortic root, thoracic aorta and thoracic FL; patients with frequent intermittent hypoxia leading to oxidative stress and increases in sympathetic activity exhibit an increase in blood pressure due to the activation of the renin–angiotensin–aldosterone system (59, 60, 62). The state of chronic intermittent hypoxemia and the altered microenvironment in OSA patients, such as the increase in IL-6 levels and reduction in TGF-β levels, could damage the function of endothelial cells and promote a Th17/Treg imbalance, which leads to a reduction in the production of endothelium-dependent vasodilator substances and contributes to vascular dysfunction and stimulation of systemic inflammation (63–67).

Another important finding in our study showed that vascular changes in TBAD patients with OSA were related to the predictors of LAEs, which included an initial aortic diameter ≥40 mm, a FL diameter of the proximal descending thoracic aortic ≥22 mm, and a partially thrombosed FL. Previous studies have proven that the postdischarge mortality of patients with these factors will be substantially increased (10, 15, 20, 21, 23–25). An aortic diameter >40 mm and a FL diameter >22 mm have been suggested as high-risk features by SVS/STS research (50). Our results indicate that for patients with OSA, the risk of these imaging signs was several times higher than that of non-OSA patients. Univariate analysis revealed that the risk of aortic diameter dilation larger than 40 mm in OSA patients was ∼4.6 times higher than that in non-OSA patients when we adjusted for age, sex, BMI, and hypertension in the multivariate analysis. In addition, age is an independent risk factor for aortic enlargement in TBAD patients, which suggests that the degree of aortic dilatation is more obvious in elderly TBAD patients with OSA.

In addition, we observed the effect of OSA on the size and thrombosis of the FL. Song et al. (24) revealed that patients with an FL diameter of the initial upper descending thoracic aorta ≥22 mm showed a higher rate of aortic adverse events such as aneurysmal dilation or aortic-related death. From the results, the risk of a proximal descending aortic FL larger than 22 mm was ∼2.5 times higher in OSA patients than in non-OSA patients after adjusting for other relevant factors (model 2). This suggests that OSA is an important factor related to the prognosis of TBAD patients. Our study shows that OSA is an important factor leading to an increased risk of partial FL thrombosis. Wang et al. (41) came to the same conclusion. The results showed that the risk of FL thrombosis in patients with OSA is increased by ∼2.5-fold and that the proportion of FL thrombosis increases with the severity of OSA. Patients with OSA are in a state of chronic intermittent hypoxemia, which may lead to endothelial cell damage and the release of inflammatory factors, causing hypercoagulability of blood. This process increases the chance of FL thrombosis and is accompanied by intrathoracic pressure fluctuations, which leads to changes in aortic transmural pressure and mechanical stretching of the aorta. Furthermore, it may interfere with thrombosis of the FL, and both processes eventually cause a higher probability of partial thrombosis.

We classified OSA according to its severity as mild, moderate and severe. As seen in our results, moderate OSA patients showed a higher OR than severe and mild OSA patients in the logistic regression analyses on an aortic diameter

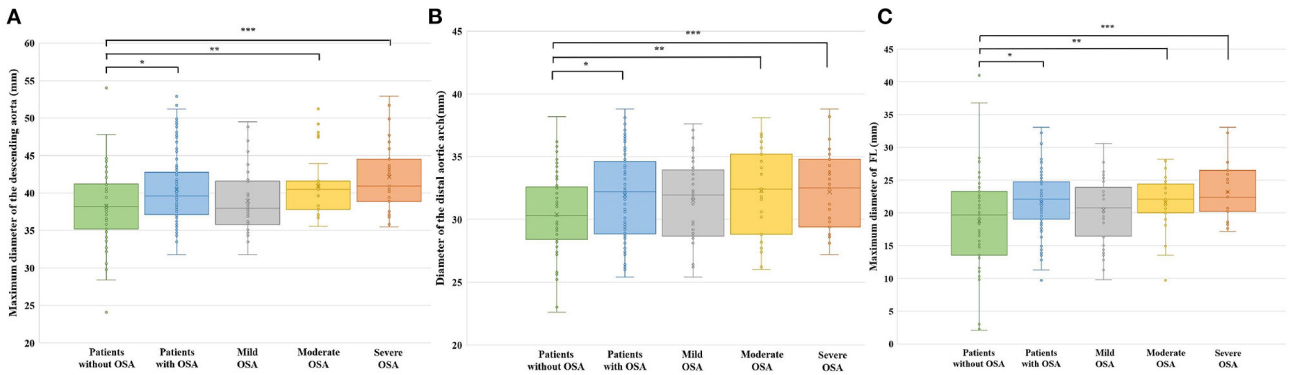

FIGURE 3 | Other imaging differences in different OSA severities. **(A)** Maximum diameter of the descending aorta for different OSA severities. $^{*}p = 0.029$; $^{**}p = 0.037$; $^{***}p = 0.001$. **(B)** Diameter of the distal aortic arch for different OSA severity levels. $^{*}p = 0.008$; $^{**}p = 0.022$; $^{***}p = 0.026$. **(C)** Maximum diameter of the FL for different OSA severity levels. $^{*}p = 0.018$; $^{**}p = 0.038$; $^{***}p = 0.006$.

≥40 mm. This may be due to the sampling error caused by the relatively small sample size after grouping or other unknown factors in moderate OSA. Most of the vascular morphology differences were reflected in the patients with moderate and severe OSA, including the aortic diameter at onset, the maximum diameter of the descending aorta, the diameter of the distal aortic arch, and the maximum diameter of the FL. Moderate to severe OSA has a significant effect on morphological changes in the aorta. Furthermore, TBAD patients with moderate to severe OSA may have a worse prognosis.

We also measured other vascular morphological parameters correlated with prognosis that were reported by previous studies, including the fusiform index (29), but there was no significant difference between OSA and non-OSA in TBAD patients. This is because the fusiform index was defined to express the dilatation degree of the descending aorta, which is a method used to describe the morphological changes in the aorta by local dilation. However, our study found that patients with OSA tend to present dilation of the whole aorta, which may explain why there was no significant difference in the degree of local expansion between OSA and non-OSA patients. We used the SVS/STS classification system to describe the extent of FL involvement. There was no significant difference between OSA and non-OSA, which shows that although OSA can lead to enlargement of the FL, it cannot lead to extension of the FL. The SVS/STS classification system also provides a way for us to further study the area of dissection involved.

From the blood gas analysis results, nearly half of the patients had a decrease in pO_2, of which 7 patients reached type I respiratory failure. We considered that this is an acute manifestation rather than a chronic progressive condition because when taking the patients' medical history, we found that almost all patients with decreased pO_2 complained of dyspnoea and sweating caused by pain, and when we rechecked the blood gas before discharge, it had returned to the normal range.

The strengths of our study include that this is the first study to explore the effect of OSA on vascular morphological changes in TBAD patients and the first to identify OSA as an independent risk factor related to LAEs. To comprehensively and systematically assess changes in the aorta, we included up to nine indicators, including not only aortic dilatation, FL status, and imaging signs with LAEs but also the dissection process-involved zones according to the SVS/STS classification system. We first found that the effect of OSA on the aorta was mainly manifested as aortic dilation rather than an increase in the involved zones. There are still some limitations to our study, which include the need for further investigations in animal experiments to reveal the molecular mechanism of the occurrence and development of TBAD caused by OSA. Further studies on the prognosis of TBAD patients with OSA are also needed.

CONCLUSION

TBAD patients with OSA, especially moderate to severe OSA, have vascular morphological changes in the different regions of the aorta that mainly manifest as aortic dilation. OSA was an independent risk factor for a maximum aortic diameter at onset ≥40 mm, an FL diameter in the proximal descending aorta ≥22 mm, and a partially thrombosed FL. The imaging differences related to LAEs suggest that OSA is an important factor that affects the prognosis of TBAD patients.

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 Medical Research Ethics Committee of the First

Hospital of China Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JiawZ and SX first conceived this article, and designed the research method of this study. JiawZ was responsible for data analysis and writing the manuscript, collected the medical records, and imaging data as well as drafted the manuscript. ZZ and YY also collected the medical records and performed sleep monitoring. LW and HW drafted the discussion. LF and BZ gave the help of statistical analysis. WW and JianZ participated in the design of the study and analyzed sleep monitoring data. SX carried out critical revision and finalization of the manuscript. All authors approved the final manuscript.

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Healthy Sleep Associated With Lower Risk of Hypertension Regardless of Genetic Risk: A Population-Based Cohort Study

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Background: Hypertension is a leading contributor to the global burden of disease and to mortality. The combined effects of sleep factors on the risk of hypertension are unclear. We aimed to evaluate the effect of combined sleep factors on the risk of hypertension and to explore whether this association is independent of genetic risk.

Methods: This population-based prospective cohort study included 170,378 participants from the UK Biobank study. We conducted a healthy sleep score based on a combination of major five sleep factors and a genetic risk score based on 118 risk variants. Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: A total of 170,378 participants were included. Compared to participants with a healthy sleep score of 0–1, those with healthy sleep scores of 2 (HR, 0.90; 95% CI, 0.83–0.98), 3 (HR, 0.81; 95% CI, 0.75–0.88), 4 (HR, 0.74; 95% CI, 0.68–0.81), or 5 (HR, 0.67; 95% CI, 0.59–0.77) had increasingly lower risks of hypertension (*P* for trend <0.001). Participants with high genetic risk and an unfavorable sleep pattern had a 1.80-fold greater risk of hypertension than participants with low genetic risk and a favorable sleep pattern. The association between sleep patterns and hypertension persisted in subgroup analysis, stratified by the genetic risk. Nearly 18.2% of hypertension events in this cohort could be attributed to unfavorable sleep pattern.

Conclusions: Favorable sleep pattern was associated with a low risk of hypertension, regardless of genetic risk. These findings highlight the potential of sleep interventions to reduce risk of hypertension across entire populations.

Keywords: sleep, genetic risk, hypertension, cohort study, epidemiology

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INTRODUCTION

Hypertension is a leading contributor to the global burden of disease and to mortality (1). It is a complex disease driven by both environmental and genetic factors (2–6). Sleep factor is an important modifiable risk factor for hypertension (7, 8). A rich body of evidence shows that healthy sleep factors, including no excessive daytime sleepiness (9), adequate sleep duration (10, 11), no insomnia (12, 13), no snoring (14, 15), and early chronotype (16), were associated with low risk of hypertension. Although those sleep factors have been independently correlated to increased risk of hypertension, a combination of sleep factors may have synergistic effects as these sleep factors are highly associated with each other (17–20). Bathgate et al. (21) have assessed sleep factors jointly and indicated the highest risk of hypertension in association with a joint effect of short sleep duration (<6 h) with insomnia. Therefore, a composite variable may better aid investigation of how sleep factors act synergistically to affect hypertension risk. However, few studies have investigated the combined impact of sleep factors, let alone all the aforementioned sleep factors jointly, on hypertension risk.

Early evidence supporting a role for genetics in the risk of hypertension came from twin studies and the Framingham Study (5, 22–24). Further evidence has emerged from genome-wide association studies (GWASs), which have identified genetic variants associated with the risk of hypertension (2, 25). These risk alleles, when aggregated into a polygenic risk score, are predictive of incident hypertension and provide a quantitative measure of the genetic risk for hypertension. It might be hypothesized that adhering to a healthy sleep pattern could attenuate the effect of genetics on the risk of hypertension. A previous study on cerebrovascular disease, a condition closely related to hypertension, found a statistically significant role of the interplay between sleep factors and genetics in the risk of cerebrovascular disease (17). However, whether a healthy sleep pattern, which integrates several modifiable sleep factors, can modify the effect of genetic predisposition on hypertension remains less certain; moreover, no study to date has investigated the risk of hypertension with regard to sleep patterns and genetics.

Therefore, in a large population-based cohort study, we prospectively investigated the association of a healthy sleep score based on a combination of major sleep factors with the risk of incident hypertension. We further explored whether the association between sleep pattern and the risk of incident hypertension was independent of genetic risk.

METHODS

Study Population

The UK Biobank study is a large, population-based prospective cohort study that recruited >500,000 individuals aged between 40 and 70 years from across the UK (Scotland, England, and Wales) between 2006 and 2010. The population and design of the UK Biobank study have been described in detail in previous reports (26–29). The study collected extensive data, including demographic, health, and lifestyle (e.g., sleep factors) data, from questionnaires, interviews, physical measurements, and health records. Blood samples were also obtained and used for genotyping (30). Approval for this research was obtained from the North West Multicenter Research Ethics Committee (11/NW/0382), and all participants provided informed consent.

In this study, after excluding participants with hypertension or cardiovascular diseases at baseline, those with missing data for any of the five sleep factors, or those without genetic data, 170,378 participants were finally included in the present analyses. A flowchart depicting the selection of the study participants is presented in **Supplementary Figure 1**.

Measure

Healthy Sleep Score and Sleep Pattern

The UK Biobank participants completed a self-reported touchscreen questionnaire on their usual sleep factors. To investigate the association between the combination of sleep factors and hypertension, we constructed a healthy sleep score based on the recommendations regarding five potentially modifiable sleep factors, including daytime sleepiness, sleep duration, insomnia, snoring, and chronotype, on the basis of previous studies (13, 17, 18). The methods of assessment of the sleep factors is described in **Appendix**. We dichotomized each sleep factor based on previous knowledge (17). Participants were assigned one point for each of five low-risk sleep factors defined as follows: sleep 7–9 h per day, no insomnia ("never/rarely" having insomnia symptoms), no snoring, early chronotype ("morning" or "morning more than evening" person), and no frequent daytime sleepiness ("never/rarely" or "sometimes"). The points for the five sleep factors were summed to obtain the healthy sleep score, which ranged from 0 (least healthy) to 5 (most healthy), and was subsequently categorized as a favorable (score of 4 or 5), intermediate (score of 2 or 3), or unfavorable (score of 0 or 1) sleep pattern, as described previously (17).

A weighted standardized healthy sleep score was then derived based on the five sleep factors with the following equation: weighted sleep score $= (\beta_1 \times$sleep factor1 $+ \beta_2 \times$sleep factor2 $+ \ldots + \beta_5 \times$sleep factor5$) \times$ (5/sum of the β coefficients). This weighted sleep score also ranged from 0 to 5 points but took into account the magnitudes of the adjusted risk for each behavior in each sleep pattern and in the combination of the five sleep factors (17).

Genotyping and Polygenic Risk Score

The genotyping process in the UK Biobank study has been reported in detail elsewhere (30). The polygenic risk score for hypertension was based on a recent GWAS of individuals of European ancestry (31). Therefore, the study considered only individuals who self-reported as British or other white backgrounds. We excluded SNPs that were missing from the UK Biobank study. Independent SNPs were selected based on the *P*-value by using the linkage disequilibrium (LD) clumping procedure (at $R^2 < 0.01$) conducted in PLINK version 2.0 (https://www.cog-genomics.org/plink2). The polygenic risk score was calculated across all selected SNPs associated with hypertension totaling 118 (**Supplementary Table 1**) (31). For each individual in the UK Biobank sample, we calculated

polygenic risk scores, defined as the sum of the number of risk alleles (0, 1, or 2) present at each locus weighted by the natural logarithm of the estimated odds ratio for that locus. Polygenic risk scores were then *z*-standardized based on values for all individuals and categorized into low (lowest quintile), intermediate (quintiles 2–4), or high (highest quintile) risk (32).

Incident Hypertension

Data on incident hypertension in the UK Biobank were based on medical history and linked to data on hospital admissions and mortality. The linkage procedure can be found on the website (http://content.digital.nhs.uk/services) in detail. We defined participants with hypertension according to the International Classification of Diseases edition 10 (ICD-10): I10 for hypertension.

Covariates

The covariates included in the present study were as follows: age, sex (male or female), education (degree [college/university degree] or no degree), Townsend deprivation index (TDI) (33), race (white or other), physical activity, smoking status (current, previous, or never), alcohol consumption (current, previous, or never), body mass index [BMI: was calculated by dividing an individual's weight (kg) by the square of height in meter (m)], family history of hypertension, and medical history (physician diagnosis of diabetes, depression, and cancer), obtained from the self-completed baseline questionnaire. Details of these measurements can be found on the website of the UK Biobank (www.ukbiobank.ac.uk).

Statistical Analysis

We imputed the missing covariate values (all covariates had <3% of values missing) through multiple imputation by chained equations (34). The mean and standard deviation (SD) (continuous variables) or number and percentage (categorical variables) were used to describe the participants' baseline characteristics.

We used Cox proportional hazard regression models to test the association of sleep factors with the incident hypertension risk. The duration of follow-up was calculated as the time between the date of attendance and the date of first diagnosis, date of death, or February 28, 2017, for Scotland, and February 25, 2018, for Wales and England, whichever occurred first. Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated. The multivariable-adjusted models were adjusted for age, sex, education, TDI, race, physical activity, smoking status, alcohol consumption, BMI, and family history of hypertension, depression, cancer, and diabetes. The proportionality of hazards assumption was assessed using the Schoenfeld residuals and was satisfied (35). We included an interaction term in the regression model to test for the statistical interaction between the sleep pattern and genetic risk categories. In addition, adjusted population attributable fractions (PAFs) and 95% CIs were calculated to estimate the proportion of hypertension cases that theoretically would not have occurred if all participants had healthy sleep factor.

We performed subgroup analyses stratified by age (<60 or ≥60 years), sex (male or female), current smoking status (yes or no), current alcohol consumption status (yes or no), physical activity (inactive [<400 MET-h/week] or active[≥400 MET-h/week]), BMI (non-obese [<30 kg/m^2] or obese [≥30 kg/m^2]), and family history of hypertension (yes or no). In addition, we conducted several sensitivity analyses. To minimize the influence of reverse causation, we performed a sensitivity analysis by excluding participants who experienced hypertension events within the first 2 years of follow-up. Moreover, the risk of incident hypertension was investigated in sensitivity analyses using non-imputed data. All analyses were performed using R software, version 4.0.0 (R Development Core Team, Vienna, Austria). *P*-values were two-sided, with statistical significance set at <0.05.

RESULTS

Baseline Characteristics

This analysis included 170,378 (mean [SD] age: 53.6 [8.0] years) participants, of whom 107,499 (63.1%) were female and 156,398 (91.8%) were white (**Table 2**). Participants with hypertension were more likely to be older, to have a lower level of education, to have a higher BMI, to smoke, to have higher prevalence rates of cancer, diabetes, and depression, and to have a lower healthy sleep score compared with those without incident hypertension (**Table 1**).

Associations of Sleep Factors With Incident Hypertension

During a median (interquartile range) follow-up of 9.0 (8.3–9.7) years, 6,581 incident hypertension cases were recorded. In the age- and sex-adjusted model, evening chronotype, long (≥9 h) or short (<7 h) sleep duration, insomnia, snoring, and often/always daytime sleep were significantly associated with an increased incident hypertension risk (**Supplementary Table 2**). In the multivariable-adjusted model, these associations remained statistically significant except for evening chronotype (**Supplementary Table 2**).

When these five sleep factors were reclassified as either a high (reference) or a low risk, sleep 7–9 h per day (HR, 0.86; 95% CI, 0.82–0.91), no insomnia (HR, 0.85; 95% CI, 0.80–0.91), no snoring (HR, 0.92; 95% CI, 0.88–0.97), and no frequent daytime sleepiness (HR, 0.94; 95% CI, 0.89–0.99) were independently associated with a decreased incident hypertension risk in the multivariable-adjusted model (**Table 2**).

We also calculated the PAF for each sleep factor separately and the combination of the five sleep factors (**Table 2**). The estimated PAFs attributable to pre-existing the low-risk sleep factors ranged from 1.6% (for chronotype) to 11.9% (for insomnia). For participants who were adherent to all five of the low-risk sleep factors, the PAF was 18.2% (95% CI: 7.3–25.1%), suggesting that 18.2% of hypertension cases in this cohort would not have occurred if all participants had been in the low-risk group for all five sleep factors.

When these five sleep factors were considered jointly by using the healthy sleep score, the risk of hypertension decreased

TABLE 1 | Baseline characteristics of participants.

	Overall (n = 170,378)	No incident hypertension (n = 163,797)	Incident hypertension (n = 6,581)	P-value
Age, mean (SD), y	53.6 (8.0)	53.4 (8.0)	57.8 (7.7)	< 0.001
Female	107,499 (63.1)	103,886 (63.4)	3,613 (54.9)	< 0.001
Education				< 0.001
Degree	65,682 (38.6)	63,875 (39.0)	1,807 (27.5)	
No degree	104,696 (61.4)	99,922 (61.0)	4,774 (72.5)	
TDI	−1.4 (3.0)	−1.4 (3.0)	−0.9 (3.3)	< 0.001
BMI, mean (SD), kg/m^2	25.99 (4.15)	25.91 (4.09)	28.04 (4.92)	< 0.001
Race				< 0.001
White	156,398 (91.8)	150,435 (91.8)	5,963 (90.6)	
Other	13,980 (8.2)	13,362 (8.2)	618 (9.4)	
Smoking status				< 0.001
Never	97,933 (57.5)	94,808 (57.9)	3,125 (47.5)	
Previous	52,692 (30.9)	50,311 (30.7)	2,381 (36.2)	
Current	19,753 (11.6)	18,678 (11.4)	1,075 (16.3)	
Alcohol consumption				< 0.001
Never	6,850 (4.0)	6,465 (3.9)	385 (5.9)	
Previous	5,568 (3.3)	5,228 (3.2)	340 (5.2)	
Current	157,960 (92.7)	152,104 (92.9)	5,856 (89.0)	
Physical activity (MET-h/week)	2,649.3 (2660.7)	2,649.5 (2654.4)	2,645. 7 (2812.8)	0.910
Family history of hypertension	66,940 (39.3)	64,416 (39.3)	2,524 (38.4)	0.116
Cancer	11,770 (6.9)	11,117 (6.8)	653 (9.9)	< 0.001
Diabetes	3,108 (1.8)	2,612 (1.6)	496 (7.5)	< 0.001
Depression	14,030 (8.2)	13,258 (8.1)	772 (11.7)	< 0.001
Healthy sleep score				< 0.001
0–1	13,072 (7.7)	12,243 (7.5)	829 (12.6)	
2	36,270 (21.3)	34,512 (21.1)	1,758 (26.7)	
3	60,895 (35.7)	58,620 (35.8)	2,275 (34.6)	
4	47,690 (28.0)	46,265 (28.2)	1,425 (21.7)	
5	12,451 (7.3)	12,157 (7.4)	294 (4.5)	
Genetic risk category				< 0.001
Low genetic risk	34,073 (20.0)	32,939 (20.1)	1,134 (17.2)	
Intermediate genetic risk	102,229 (60.0)	98,277 (60.0)	3,952 (60.1)	
High genetic risk	34,076 (20.0)	32,581 (19.9)	1,495 (22.7)	

BMI, body mass index; SD, standard deviation; TDI, Townsend deprivation index. Data are presented as n (percent) unless otherwise indicated.

significantly with an increasing healthy sleep score (**Table 3**; P for trend <0.001). In the multivariable-adjusted model, compared to participants with a healthy sleep score of 0–1, participants with a healthy sleep score of 2 (HR, 0.90; 95% CI, 0.83–0.98), 3 (HR, 0.81; 95% CI, 0.75–0.88), 4 (HR, 0.74; 95% CI, 0.68–0.81), or 5 (HR, 0.67; 95% CI, 0.59–0.77) had increasingly lower risks of hypertension. Each additional healthy sleep factor (per 1-point increase in score) was associated with a 9% (HR, 0.91; 95% CI, 0.89–0.93) lower risk of hypertension (**Table 3**). The association per 1-point higher score was similar in subgroups that were classified by sex, current smoking status, alcohol consumption status, total physical activity, BMI, or family history of hypertension (all P for interaction >0.05) (**Supplementary Table 3**). In the sensitivity analyses, the results did not markedly change after using non-imputed data (**Supplementary Table 4**), or excluding participants who experienced hypertension events within the first 2 years of follow-up (**Supplementary Table 5**). In addition, the results were not materially different for the weighted healthy sleep score (**Supplementary Table 6**).

Joint Association of Sleep Pattern and Genetic Risk With Incident Hypertension

Supplementary Figure 2 shows the cumulative incidence of hypertension according to sleep pattern and genetic risk. We further assessed the joint association of the healthy sleep score and polygenic risk score with the risk of hypertension. We found that participants with an unfavorable sleep pattern and high genetic risk had the highest risk of hypertension, even though there was no statistically significant interaction between

TABLE 2 | Risk of incident hypertension according to low-risk sleep factors.

Low-risk sleep factors[b]	*n/N*	Age- and sex-adjusted		Multivariable-adjusted[a]		PAF (%)
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	
Sleep 7–9 h per day	4,152/120,006	0.75 (0.71–0.79)	<0.001	0.86 (0.82–0.91)	< 0.001	5.2 (3.6–6.9)
No insomnia	1,363/44,400	0.82 (0.77–0.87)	<0.001	0.85 (0.80–0.91)	< 0.001	11.9 (7.7–16.3)
No snoring	3,968/116,426	0.78 (0.74–0.82)	<0.001	0.92 (0.88–0.97)	0.018	3.2 (1.5–5.1)
Early chronotype	4,003/104,585	0.89 (0.85–0.94)	<0.001	0.96 (0.91–1.01)	0.092	1.6 (0.5–3.4)
No frequent daytime sleepiness	4,716/134,132	0.82 (0.77–0.87)	<0.001	0.94 (0.89–0.99)	0.027	1.7 (0.2–3.0)
All five low-risk sleep factors	294/12,451	0.68 (0.60–0.76)	<0.001	0.81 (0.71–0.89)	< 0.001	18.2 (7.3–25.1)

CI, confidence interval; HR, hazard ratio; PAF, population attributable fraction.
[a]Model was adjusted for age, sex, education, TDI, race, physical activity level, smoking status, alcohol consumption, family history of hypertension, body mass index, depression, cancer, and diabetes. All sleep factors were included simultaneously in the same model.
[b]Long (≥9 h) or short (<7 h) sleep duration, frequent daytime sleepiness, insomnia, evening chronotype, and snoring were the reference groups.

TABLE 3 | Association between healthy sleep score and incident hypertension.

Healthy sleep score	*n/N*	Age- and sex-adjusted		Multivariable-adjusted[a]	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
0–1	860/13,378	1.00 (reference)	–	1.00 (reference)	–
2	1,802/37,145	0.77 (0.71–0.83)	<0.001	0.90 (0.83–0.98)	0.013
3	2,329/62,262	0.62 (0.57–0.67)	<0.001	0.81 (0.75–0.88)	<0.001
4	1,452/48,697	0.51 (0.47–0.56)	<0.001	0.74 (0.68–0.81)	<0.001
5	299/12,722	0.44 (0.39–0.51)	<0.001	0.67 (0.59–0.77)	<0.001
Per 1-point increase in score	–	0.81 (0.80–0.83)	<0.001	0.91 (0.89–0.93)	<0.001
P-value for trend	–	<0.001		<0.001	

CI, confidence interval; HR, hazard ratio.
[a]Model was adjusted for age, sex, education, TDI, race, physical activity level, smoking status, alcohol consumption, family history of hypertension, body mass index, depression, cancer, and diabetes. All sleep factors were included simultaneously in the same model.

the healthy sleep score and genetic susceptibility to hypertension (P for interaction = 0.693) (**Figure 1**). Participants with an unfavorable sleep pattern and high genetic risk had a 1.80-fold greater risk of hypertension (HR, 1.80; 95% CI, 1.49–2.17) than participants with a favorable sleep pattern and low genetic risk. The results were not materially different for the weighted healthy sleep score (**Supplementary Table 7**). Moreover, within each genetic risk stratum there was an increase in the strength of the association with a decreasing number of favorable sleep factors (P-value for trend <0.05) (**Table 4**). In the low genetic risk group, an unfavorable sleep pattern was associated with an increased risk of hypertension (HR, 1.29; 95% CI, 1.06–1.58); the association of hypertension risk with sleep pattern was similar in both the intermediate and high genetic risk groups.

DISCUSSION

Using data from a large, population-based cohort study, we found that a healthy sleep score based on five potentially modifiable sleep factors, including daytime sleepiness, sleep duration, insomnia, snoring, and chronotype, was inversely associated with the future hypertension risk. Interestingly, this inverse association persisted for all subgroup analyses, including after stratification by genetic risk. Approximately 18.2% of hypertension cases could potentially be prevented if all participants had all five healthy sleep factors. These results provide evidence for the importance of healthy sleep in preventing hypertension and reinforce the tremendous potential of primary prevention.

Existing evidence, together with our result of single sleep factors revealed that healthy sleep factors, such as an early chronotype, no frequent daytime sleepiness, 7–9 h per day sleep duration, no insomnia, and no snoring were independently associated with a decreased incident hypertension risk (9, 13, 14, 36, 37). In our study, out of the five sleep factors, insomnia was component of the healthy sleep score that showed substantially the highest PAF for hypertension. It is reasonable that insomnia is more modifiable and precisely targetable through behavioral therapies (38). Therefore, future clinical trials or community-based intervention studies should be conducted to test whether sleep interventions for insomnia can reduce subsequent incident hypertension risk.

It is important to evaluate the combination of these sleep factors because they are often interconnected. In agreement with our findings, previous studies evaluating other combinations of sleep factors indicated that the combination of insomnia and short sleep duration was strongly associated with the risk of incident hypertension (13, 21). However, despite this, when

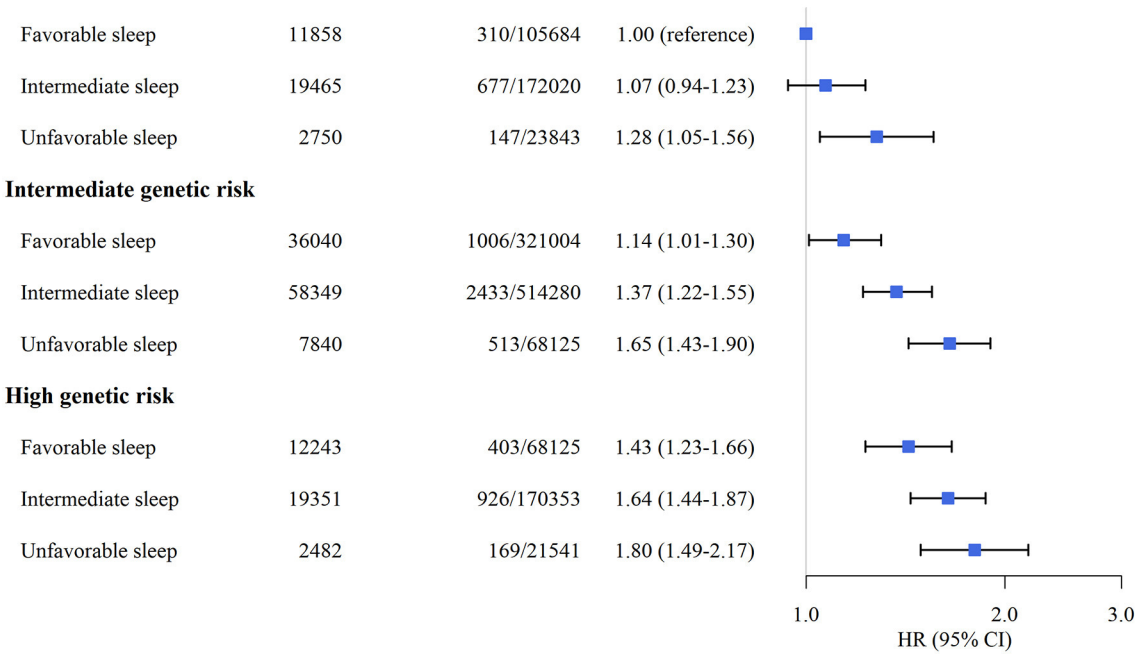

FIGURE 1 | Risk of incident hypertension according to sleep pattern and genetic risk. CI, confidence interval; HR, hazard ratio. The results were obtained after adjusting for age, sex, education, TDI, race, physical activity level, smoking status, alcohol consumption, family history of hypertension, body mass index, depression, cancer, and diabetes.

examining the different combinations of sleep factors, none of the combinations were as protective as the combination of all five sleep factors. More than 18% of hypertension cases could be prevented though modification of the combined five sleep factors. Our study, the largest to date, considered the joint effect of five major sleep factors on the risk of hypertension by constructing a healthy sleep score that reflects a more comprehensive sleep pattern. The reduction in the risk of hypertension was associated with healthy sleep factors in the present study, which highlights the importance of considering sleep factors in the management of blood pressure. The healthy sleep score defined in the present study provides a significant reference for sleep management and the identification of high-risk populations.

The potential mechanism underlying the association between combined sleep factors and the risk of hypertension is not well-understood. However, these sleep factors may individually act through several mechanisms that could operate synergistically to affect the risk of hypertension. For instance, a shortened sleep duration, insomnia symptoms, or excessive daytime sleepiness possibly relates to pathways influencing sympathetic nervous system activity, which lead to blood vessel constriction, increasing blood pressure (39–41). Habitual snoring is thought to be closely related to sleep apnea; repeated apneic episodes cause oxidative stress, accelerate atherosclerosis in the coronary and intracranial arteries, and activate hemodynamics, elevating sympathetic activity and pulmonary artery pressure (42, 43). The circadian shift toward evening causes a longer-term circadian misalignment, which strengthens the association with arterial hypertension (44).

To our knowledge, the present study is the first prospective cohort study to investigate the joint association of sleep pattern and genetic risk with incident hypertension risk. We found that no statistically significant interaction between sleep pattern and genetic risk with regard to hypertension, which suggests that the effect of sleep pattern on the risk of hypertension might be independent of the genetic risk. Interestingly, we found a strong positive association between an unfavorable sleep pattern and the risk of hypertension irrespective of the prevalent genetic risk variants. Although a genetic risk for hypertension among participants from the UK Biobank has been reported previously (31), our results provide evidence that healthy sleep factors may be associated with low risk regardless of the individuals' genetic risk profile. Therefore, our study highlights the fact that adhering to a favorable sleep pattern may be greatly beneficial in the primary prevention of hypertension among the entire population. These results should be used to strengthen the importance of modifiable risk factors in the management of hypertension, as well as to convince individuals of the importance of following healthy sleep recommendations.

TABLE 4 | Association of sleep pattern with incident hypertension in genetic risk strata.

Sleep pattern category	Low genetic risk			Intermediate genetic risk			High genetic risk		
	Favorable	Intermediate	Unfavorable	Favorable	Intermediate	Unfavorable	Favorable	Intermediate	Unfavorable
n/N	310/11,858	677/19,465	147/2,750	1,006/36,040	2,433/58,349	513/7,840	403/12,243	923/19,351	169/2,482
HR (95% CI)[a]	1.00 (reference)	1.08 (0.94–1.24)	1.29 (1.06–1.58)	1.00 (reference)	1.19 (1.11–1.29)	1.43 (1.28–1.59)	1.00 (reference)	1.15 (1.02–1.3)	1.26 (1.05–1.52)
P-value	–	0.276	0.013	–	<0.001	<0.001	–	0.019	0.015
P-value for trend		0.021			<0.001			0.006	

CI, confidence interval; HR, hazard ratio.

[a]*Cox proportional hazards regression adjusted for adjusted for age, sex, education, TDI, race, physical activity level, smoking status, alcohol consumption, family history of hypertension, body mass index, depression, cancer, and diabetes.*

Strengths and Limitations

To our knowledge, this is the first prospective cohort study to investigate the associations of five joint sleep factors with risk of incident hypertension. Another strength of this study was the large sample size, which enabled a detailed investigation of the combination of sleep factors and genetic risk. Other strengths include the comprehensive collection of sociodemographic status, medical data, and lifestyle information by self-report, which enabled us to incorporate the most prevalent lifestyle factors convincingly linked to hypertension. However, the present study also has several potential limitations. First, this is an observational study, and the associations between sleep pattern and the risk of hypertension cannot be interpreted as causal. Second, this analysis focused on only five sleep factors. Expanding the range of sleep factors (i.e., rapid eye movement sleep factor and restless legs syndrome) would be of interest in future studies. Third, although a series of confounding factors were adjusted for in the analyses, the possibility of unmeasured or unknown confounding factors may remain. Fourth, the incidence of hypertension might be underestimated by potential underdiagnosed hypertension (45). Finally, the data regarding the sleep factors in the UK Biobank were self-reported, which may have led to some misclassification.

Conclusion

In conclusion, healthy sleep score combining daytime sleepiness, sleep duration, insomnia, snoring, and chronotype are predictive of incident hypertension. Meanwhile, unfavorable sleep pattern was associated with a higher risk of incident hypertension, regardless of genetic risk. These findings could have important implications for understanding the mechanisms underlying hypertension and provide future opportunities for early intervention.

DATA AVAILABILITY STATEMENT

Data are available in a public, open access repository. The UK Biobank data are available from the UK Biobank on request (www.ukbiobank.ac.uk/).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by North West Multicenter Research Ethics Committee (11/NW/0382). 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

CM, Z-HL, and Q-MH designed the research and developed the analytical plan. CM directed the study. Z-HL and Q-MH performed the statistical analyses and had primary responsibility for writing the manuscript. P-DZ, DL, DS, X-RZ, W-FZ, QC,

P-LC, and W-QS contributed to data cleaning. CM, VB, XG, X-BW, and VC contributed to the analysis or interpretation of the data. All authors critically reviewed the manuscript for important intellectual content.

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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.2021.769130/full#supplementary-material

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Lower Masticatory Performance Is a Risk for the Development of the Metabolic Syndrome: The Suita Study

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Objectives: Declined masticatory function has recently been receiving attention as a risk factor for poor general health. The present longitudinal analysis was conducted to clarify the relationship between decreased masticatory performance and the development of the metabolic syndrome (MetS) in a general urban cohort in Japan.

Methods: We surveyed 599 participants (254 men, 345 women; mean age at baseline, 65.8 ± 7.8 years) who underwent physical health checkups in the Suita study. We evaluated masticatory performance at baseline using test gummy jelly and divided participants into two groups: a "Lower group," comprising participants in the lower 25% of the masticatory performance at baseline; and a "Normal group," comprising all others. We estimated hazard ratios (HRs) for the Lower group by using Cox proportional hazard regression analysis to develop the MetS and the components of the MetS at follow-up, adjusting for age, smoking status, and periodontal status.

Results: On Cox proportional hazard regression analysis, the multivariable adjusted hazard ratio for the development of the MetS in the Lower group was 2.24 (95% confidence interval, 1.12–4.50) in men. The multivariable adjusted hazard ratio for the development of high blood pressure was 3.12 (1.42–6.87), for high triglycerides was 2.82 (1.18–6.76), and for high fasting plasma glucose was 2.65 (1.00–7.00) in men.

Conclusions: Lower masticatory performance suggested to be a risk factor for the development of the MetS as well as MetS components such as high blood pressure, high triglycerides, and high fasting plasma glucose in Japanese men.

Keywords: geriatric dentistry, prosthodontics, mastication, epidemiology, preventive dentistry, cardiovascular diseases

INTRODUCTION

Metabolic syndrome (MetS) is a complex condition of overlapping hypertension, abdominal obesity, dyslipidemia, and hyperglycemia in the same individual, and it has been reported as a risk factor for the development of cardiovascular diseases (1). Since the World Health Organization (WHO) published the concept of the MetS in 1999 (2), its prevention has been emphasized, and various measures have been taken around the world (3, 4). Nevertheless, the prevalence of the MetS is still increasing worldwide, affecting an estimated one-quarter of the global population (5). Previously, sex (6), educational level (6), smoking habits (7), eating habits (8), and exercise habits (9) were reported as factors related to the MetS. However, the MetS is a complex pathological condition caused by the various effects of these factors, and there may be factors that have not yet been clarified. Therefore, identifying the risk factors affecting the MetS is thus a major goal for preventing cardiovascular diseases.

Many studies have reported that chronic inflammation from periodontal diseases affects the MetS (10). On the other hand, some reports have shown that the changes in dietary habits and nutritional intake resulting from the declined masticatory function accompanying tooth loss affect the MetS (11). It has been reported that factors related to masticatory function, such as tooth loss (11) and decreased occlusal support (12), are associated with the MetS. However, few studies have shown the relationship between objective oral function and the MetS (13).

Evaluating masticatory performance is one method that reflects oral function objectively (14). Previous studies have reported that tooth loss, lower occlusal force (15), and progression of periodontal disease (16) lead to decreased masticatory performance. Furthermore, some studies have reported that lower masticatory performance is related to diabetes mellitus (17) and obesity (18) through changes in nutritional intake. Lower masticatory performance may thus be related to the development of the MetS. Kikui et al. have already reported a relationship between masticatory performance as evaluated using test gummy jelly and the incidence of the MetS in a general urban Japanese population (13). However, they used a cross-sectional study design and could not make any definitive comment on the longitudinal relationship between masticatory performance and the development of the MetS.

The present study therefore aimed to evaluate the hypothesis that lower masticatory performance at baseline represented a risk factor for the future development of the MetS in a general urban Japanese population in a longitudinal study.

MATERIALS AND METHODS

Study Participants

Participants in this study were the general urban population of Suita city, Osaka prefecture, Metropolitan prefecture, Japan, who underwent physical health checkups in the Suita study, a cardiovascular disease cohort study conducted by the Department of Preventive Cardiology of the National Cerebral and Cardiovascular Center (19). We recruited 937 individuals who underwent a health check at both baseline (from June 2008 to June 2013) and follow-up (from June 2010 to February 2017). Of these, 338 individuals were excluded due to incomplete data ($n = 103$), an edentulous state at baseline ($n = 35$), or diagnosis of the MetS at baseline ($n = 200$). Finally, 599 participants (254 men, 345 women) were included in the study (**Figure 1**).

The ethical committee approved the study protocol of the National Cerebral and Cardiovascular Center (approval number M19-062-4, M25-032-2), and only individuals who had given consent after receiving full written and oral explanations of the study were evaluated.

Medical Examinations

All participants were instructed to fast for 12 h before each health check. Routine blood tests were performed, including triglycerides, high-density lipoprotein (HDL), and fasting blood glucose. Waist circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured.

The MetS was defined using National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATPIII) criteria (20). The five components of the MetS were defined as follows: high blood pressure, SBP ≥130 mmHg and/or DBP ≥85 mmHg and/or taking antihypertensive medicines; high blood glucose level, fasting blood glucose ≥110 mg/dL and/or taking diabetic medicines; hypertriglyceridemia, triglycerides ≥150 mg/dL and/or taking antilipidemic medicines; low HDL-cholesterol, HDL-cholesterol <40 mg/dL in men or <50 mg/dL in women; and abdominal obesity, waist circumference (in the Asian diagnostic criteria) ≥90 cm in men or ≥80 cm in women. The MetS was diagnosed based on the presence of ≥3 of these 5 components.

Participants were surveyed regarding lifestyle using questionnaires. Smoking status was divided into "never smoking," "quit smoking," and "current smoking." Participants who responded "current smoking" were defined as smokers.

Oral Examinations

Periodontal Tissue Examination

Periodontal tissue examination was carried out with participants in a supine position on a bed under artificial lighting of sufficient brightness. Periodontal status was evaluated on the basis of the Community Periodontal Index (CPI) (21). The teeth examined were the maxillary and mandibular left and right first and second molars, the maxillary right central incisor, and the mandibular left central incisor, for a total of 10 teeth. When this examination was not possible because the incisor to be examined was missing, the same tooth on the opposite side was examined. No evaluation was carried out when all the teeth to be surveyed were lost. Periodontal status was examined using a CPI probe (Periodontal Probe; YDM, Tokyo, Japan) at six points in the periodontal pocket of each tooth according to the following CPI code criteria, with the highest code value recorded: Code 0, no finding of gingival inflammation; Code 1, bleeding found after probing; Code 2, dental calculus deposits (including those detected by probing up to 4 mm beneath the gingival margin); Code 3, periodontal pocket depth ≥4 mm but <6 mm; and Code 4, periodontal pocket depth ≥6 mm. In this study, CPI Code ≥3 was

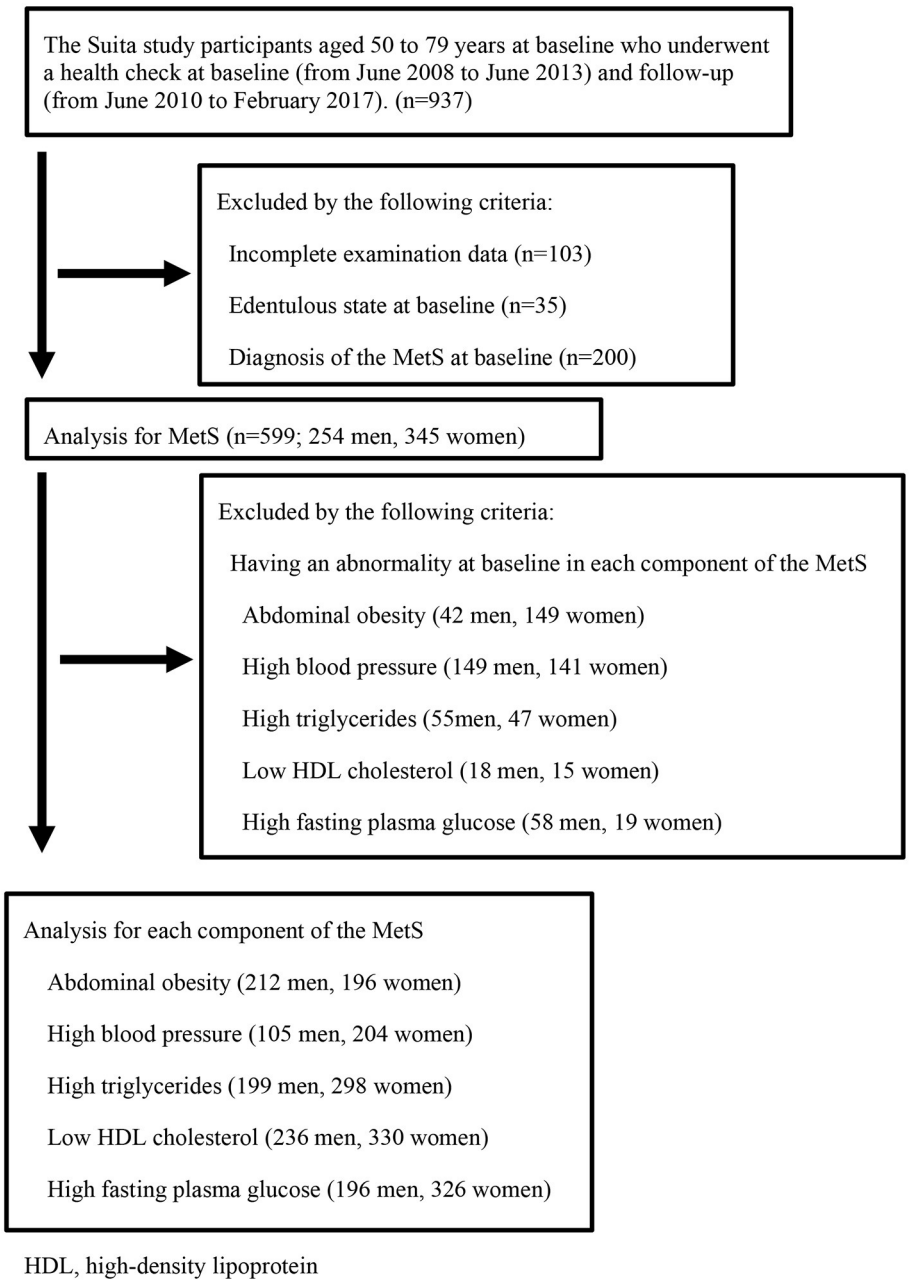

FIGURE 1 | Flow-chart of study participants. HDL, high-density lipoprotein.

defined as "with periodontitis," and CPI Code 0–2 was defined as "without periodontitis."

Masticatory Performance Examination

Participants were instructed to chew a piece of test gummy jelly (Soshaku-noryoku sokuteiyou gummy jelly; UHA Mikakuto, Osaka, Japan) using 30 chewing strokes on the preferred side, and to expectorate the comminuted jelly onto a piece of cotton gauze spread over the top of a paper cup, without leaving any gummy particles in the mouth. The cotton gauze and comminuted pieces were then rinsed under running water for 30 s to remove as much saliva and glucose adhering to the surfaces as possible. The comminuted jelly alone was subsequently placed in a plastic container, and water (35°C, 15 mL) was injected into the container. The contents were agitated for 10 s at 400 rpm with a magnetic stirrer (PC-410D Digital Stirrer; Corning Incorporated, New York, USA). Immediately after agitation, a small amount of the supernatant was collected using a set of forceps and placed in contact with the tip of a sensor fitted to a commercially available instrument for self-monitoring

of blood glucose (Glutest Every; Sanwa Kagaku Kenkyusho, Nagoya, Japan), and the glucose concentration (mg/dL) displayed after 15 s was recorded. The increase in surface area of the comminuted jelly (mm^2) (y) was calculated from the glucose concentration (x) using the regression formula y = 15x-250, and this was regarded as masticatory performance. For participants who wore removable dentures, masticatory performance was measured with the dentures in place. Participants were surveyed the presence or absence of pain in the temporomandibular joint when masticatory performance examinations were performed. We excluded the participants who answered they had pain.

In this study, participants in the lower 25% of the masticatory performance at baseline by sex were defined as the "Lower group," and all others were defined as the "Normal group" (22).

Statistical Analysis

We conducted analyses in this study by sex (1), because of the differences between the sexes in the risk factors for the MetS (6). First, we compared each variable between groups of masticatory performance using the Student's *t*-test for continuous variables and the chi-squared test for categorical variables. We used the Shapiro-Wilk test for masticatory performance (men: $p = 0.057$, women: $p = 0.274$) and Q-Q plot, and confirmed that the data had a normal distribution.

In this study, as the first-stage analysis, the participants were limited to those who did not have MetS at baseline ($n = 599$) to assess the relationship between lower masticatory performance and MetS (**Figure 1**). We estimated hazard ratios (HRs) to develop the MetS at follow-up in the Lower group using Cox proportional hazard regression, adjusting for age, smoking status, and periodontal status. Additionally, as a second-stage analysis, we assessed lower masticatory performance had a large effect which of the components of the MetS (abdominal obesity, high blood pressure, high triglycerides, low HDL cholesterol, high fasting plasma glucose). This second-stage analysis was performed by excluding those who had each component at baseline from the participants who were analyzed in the first stage. The number of participants analyzed was as follows: abdominal obesity, $n = 408$; high blood pressure, $n = 309$; high triglycerides, $n = 497$; low HDL cholesterol, $n = 566$; and high fasting plasma glucose, $n = 522$. We estimated HRs to develop each component of the MetS at follow-up in the Lower group using Cox proportional hazard regression, adjusting for age, smoking status, and periodontal status. Values of $p < 0.05$ were considered significant for all analyses. All statistical analyses were performed using IBM SPSS Statistics version 25 (SPSS Japan, Tokyo, Japan).

RESULTS

The mean age at baseline was 65.8 ± 7.8 years, and the mean follow-up was 4.4 ± 1.3 years. During follow-up, 88 participants (50 men, 38 women) developed the MetS.

Participants' characteristics at baseline according to the two groups of masticatory performance are shown in **Table 1**. In men, the Lower group was significantly older and showed higher fasting blood glucose levels than the Normal group. In women, the Lower group had significantly poor periodontal status than the Normal group.

The results of Cox proportional hazard regression analysis showed that the HRs to develop the MetS in the Lower group in men were 2.03 [95% confidence interval (CI), 1.01–4.05] in the age-adjusted model and 2.24 (1.12–4.50) in the multivariatble-adjusted model (**Table 2**). No significant association between masticatory performance and development of the MetS was seen in either the age-adjusted model or the multivariable-adjusted model in women.

During follow-up, 81 participants (39 men, 42 women) developed high blood pressure, 69 (29 men, 40 women) developed high triglycerides, 25 (11 men, 14 women) developed low HDL cholesterol, 42 (26 men, 16 women) developed high fasting plasma glucose, and 69 (31 men, 38 women) developed abdominal obesity.

The results of Cox proportional hazard regression analysis showed that HRs for the development of high blood pressure in the Lower group in men were 2.62 (1.25–5.52) in the age-adjusted model and 3.12 (1.42–6.87) in the multivariable-adjusted model (**Table 3**). The results of Cox proportional hazard regression analysis showed that HRs for the development of high triglycerides in the Lower group in men were 2.74 (1.13–6.68) in the age-adjusted model and 2.82 (1.18–6.76) in the multivariable-adjusted model. The results of Cox proportional hazard regression analysis showed that HRs for the development of high fasting plasma glucose in the Lower group in men were 2.49 (0.97–6.37) in the age-adjusted model and 2.65 (1.00–7.00) in the multivariable-adjusted model.

No significant association between masticatory performance and the development of MetS components was seen in either the age-adjusted model or the multivariable-adjusted model in women (**Table 4**).

DISCUSSION

To the best of our knowledge, this study is the first to show relationships between objective masticatory performance and the development of the MetS through a longitudinal study of a general urban population. The findings showed that lower masticatory performance might be a risk factor for the development of the MetS in men.

Mean masticatory performance in the Lower group was 2,170 ± 830 mm^2 in men and 2,185 ± 816 mm^2 in women. Kosaka et al. reported that the mean masticatory performance in a lost occlusal support group (Eichner B4 and C1-3) was 2,439 ± 1,671 mm^2 (23). Furthermore, the participants in the lost occlusal support group reportedly showed lower intakes of foods that were difficult to chew, such as vegetables and fruit (24). Participants in the present Lower group may thus have had similar masticatory performance to individuals who had lost occlusal support, and so they may have been exposed to similar adverse effects in terms of nutritional intake.

The results of Cox proportional hazard regression analysis showed a significant association between masticatory performance and development of the MetS in men. This

TABLE 1 | Characteristics of the study population by masticatory performance and sex.

	Men		Women	
	Normal group	**Lower group**	**Normal group**	**Lower group**
n	191	63	259	86
Masticatory performance[a], mm^2	5615 ± 1523	2170 ± 880	5267 ± 1310	2185 ± 816
Age[a], y	65.5 ± 7.7	70.1 ± 6.3*	64.7 ± 7.7	66.5 ± 8.3
Waist circumference[a], cm	84.6 ± 7.0	83.9 ± 6.4	78.9 ± 8.2	79.9 ± 9.0
SBP[a], mmHg	127.2 ± 17.1	129.0 ± 16.5	122.5 ± 19.5	124.1 ± 18.9
DBP[a], mmHg	79.7 ± 10.5	79.6 ± 8.9	74.3 ± 11.0	75.6 ± 9.2
Triglycerides[a], mg/dL	101.7 ± 63.2	100.5 ± 54.5	84.9 ± 33.4	80.8 ± 34.3
HDL-cholesterol[a], mg/dL	57.5 ± 14.6	55.8 ± 15.0	70.6 ± 13.9	69.1 ± 15.9
Fasting blood glucose[a], mg/dL	104.0 ± 12.8	109.0 ± 23.5*	97.8 ± 7.9	96.6 ± 6.7
Current smoking[b], %	19.4	25.4	3.9	3.5
Periodontitis[b], %	53.4	58.7	37.8	54.7*

Values are given as means ± SD or frequencies (%).
The Lower group was defined as ≤25% of masticatory performance.
SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.
Periodontitis was defined as CPI ≥ code 3.
[a]*Student's t-test.*
[b]*Chi-squared test.*
**Difference between Normal and Lower groups, $p < 0.05$.*

TABLE 2 | Multivariable-adjusted hazard ratios (95% CI) for the MetS by masticatory performance.

	Masticatory performance	
	Normal group	**Lower group**
Men (*n* = 254)		
Age-adjusted	1 (Ref)	2.03 (1.01–4.05)
Multivariable-adjusted	1 (Ref)	2.24 (1.12–4.50)
Women (*n* = 345)		
Age-adjusted	1 (Ref)	1.20 (0.54–2.69)
Multivariable-adjusted	1 (Ref)	1.14 (0.51–2.57)

CI, confidence interval; Ref, reference.
The Lower group was defined as ≤25% of masticatory performance.
Multivariable hazard ratios were estimated adjusting for age, smoking status, and periodontitis.

TABLE 3 | Multivariable-adjusted hazard ratios (95% CI) for the MetS components by masticatory performance in men.

	Masticatory performance	
	Normal group	**Lower group**
Abdominal obesity (*n* = 212)		
Age-adjusted	1 (Ref)	1.42 (0.60–3.36)
Multivariable-adjusted	1 (Ref)	1.96 (0.84–4.59)
High blood pressure (*n* = 105)		
Age-adjusted	1 (Ref)	2.62 (1.25–5.52)
Multivariable-adjusted	1 (Ref)	3.12 (1.42–6.87)
High triglycerides (*n* = 199)		
Age-adjusted	1 (Ref)	2.74 (1.13–6.68)
Multivariable-adjusted	1 (Ref)	2.82 (1.18–6.76)
Low HDL cholesterol (*n* = 236)		
Age-adjusted	1 (Ref)	2.06 (0.48–8.79)
Multivariable-adjusted	1 (Ref)	1.83 (0.30–11.36)
High fasting plasma glucose (*n* = 196)		
Age-adjusted	1 (Ref)	2.49 (0.97–6.37)
Multivariable-adjusted	1 (Ref)	2.65 (1.00–7.00)

CI, confidence interval; Ref, reference.
The Lower group was defined as ≤25% of masticatory performance.
Multivariable hazard ratios were estimated adjusting for age, smoking status, and periodontitis.

association was identified after adjusting for risk factors of the MetS such as smoking (7) and periodontitis (10), all of which have been reported as significantly related to the MetS in many prior studies. Furthermore, according to similar Cox proportional hazard regression analyses, masticatory performance was significantly associated with the development of high blood pressure, high triglycerides, and high fasting plasma glucose in men. As a background to these results, lower masticatory performance may lead to changes in dietary habits and nutritional intake, and it may cause adverse effects on general health. Inomata et al. reported that masticatory function was associated with intakes of vitamins A, C, and B4, folate, and dietary fiber, and they considered that lower masticatory performance might restrict the choice of foods (25). Furthermore, Iwasaki et al. reported that intakes of minerals, vitamins A and C, and dietary fiber were decreased in cases of reduced occlusal support in a 5-year follow-up study (26). Vitamins A and C have effects in preventing diabetes mellitus and cardiovascular disease due to their antioxidant effects, and these intakes are thus associated with the MetS (27). Increased intakes of dietary fiber cause reductions in blood pressure and

TABLE 4 | Multivariable-adjusted hazard ratios (95% CI) for MetS components by masticatory performance in women.

	Masticatory performance	
	Normal group	**Lower group**
Abdominal obesity ($n = 196$)		
Age-adjusted	1 (Ref)	1.84 (0.84–4.04)
Multivariable-adjusted	1 (Ref)	1.39 (0.63–3.08)
High blood pressure ($n = 204$)		
Age-adjusted	1 (Ref)	1.11 (0.49–2.55)
Multivariable-adjusted	1 (Ref)	1.28 (0.55–2.97)
High triglycerides ($n = 298$)		
Age-adjusted	1 (Ref)	1.55 (0.74–3.23)
Multivariable-adjusted	1 (Ref)	1.85 (0.88–3.92)
Low HDL cholesterol ($n = 330$)		
Age-adjusted	1 (Ref)	2.60 (0.85–7.96)
Multivariable-adjusted	1 (Ref)	1.42 (0.43–4.73)
High fasting plasma glucose ($n = 326$)		
Age-adjusted	1 (Ref)	0.73 (0.20–2.59)
Multivariable-adjusted	1 (Ref)	0.78 (0.22–2.79)

CI, confidence interval; Ref, reference.
The Lower group was defined as ≤25% of masticatory performance.
Multivariable hazard ratios were estimated adjusting for age, smoking status, and periodontitis.

blood glucose (28). With higher intakes of vegetables, fruit, fish, and natto (fermented soy beans), the prevalence of the MetS decreases (29). On the other hand, tooth loss can reportedly cause increased carbohydrate intake and elevated blood glucose levels (24). Lower masticatory performance may therefore restrict foods that can be chewed and cause decreases in intakes of dietary fiber and vitamins and increased carbohydrate intake. Based on these reports and the results of the present study, imbalances in nutrition may exert complex effects on blood pressure, blood glucose levels, and serum lipid levels, and the subsequent development of the MetS.

No significant associations were found between masticatory performance and the development of low HDL cholesterol and abdominal obesity. HDL cholesterol has the role of transporting increased triglycerides to the liver and promoting triglyceride metabolism. Therefore, a decrease in HDL cholesterol is a phenomenon that occurs after the increase in triglycerides, and the change during the follow-up period of 4.4 ± 1.3 years on average might be small. On the other hand, it has been reported that lack of exercise and excessive energy intake significantly affect obesity (18). Exercise habits or energy intake were not examined in this study, and their effects, which could thus not be considered in this study, might have affected the results.

In the present study, no significant association was found between masticatory performance and the development of the MetS in women. Geer and Shen reported that women after menopause showed increased insulin resistance and decreased lipid metabolism (30). Furthermore, women after menopause have higher blood pressures, triglyceride levels, and blood glucose levels and lower HDL-cholesterol levels than before menopause (31). The changing hormone balance after menopause may thus affect the development of the MetS. In a study of 4,683 Japanese women, Amagai et al. reported that menopause in Japanese women occurs from around 45 to 54 years of age (32). Because most female participants in the present study were post-menopausal, the changing hormone balance may have affected the results. Furthermore, women may have more careful dietary habits (33) and cook more frequently than men in Japan (34). Women may thus maintain intakes of foods that are difficult to chew by cooking with ingenuity and may show more well-balanced dietary habits. Because of these reports, we found no association between lower masticatory performance and the development of the MetS in women.

The results of the present study suggested that lower masticatory performance is an independent risk factor for the development of the MetS. A previous study reported that masticatory performance decreases with aging (35). On the other hand, prosthodontic treatment can improve the masticatory performance (36, 37) and use of dental services can prevent decreased masticatory performance (38). From the results of the present study, preventing the lower in masticatory performance by prosthodontic treatment and use of dental services may contribute to preventing the development of the MetS. Furthermore, preventing the MetS can contribute to preventing the onset of cardiovascular disease, because the MetS is a risk factor for cardiovascular disease (1). The present findings may offer a new approach for preventing the development of the MetS and cardiovascular disease.

This study had several limitations. The first was that the status of nutrient intakes was not be examined in spite of that lower masticatory performance affected the development of the MetS via adverse effects on nutrient intake remains hypothetical. Second, it was not possible to adjust for other risk factors for the development of the MetS such as level of education, economic status (39), exercise habits, and dietary habits (34). These factors could not be examined in this study, and they may have affected the results. We selected confounding factors based on a previous article assessing the risk factor the development of cardiovascular disease in Japanese (40), because the MetS is a risk factor for the development of cardiovascular disease. Age, sex, and smoking habit were common risk factors for the development of cardiovascular disease in Japanese. Furthermore, in this study, since the analysis was performed by sex, confounding factors were limited due to the sample size. Therefore, we selected age and smoking habit as confounding factors in this study.

CONCLUSION

In this study, lower masticatory performance was associated with the development of the MetS in men after adjusting for confounding factors. Furthermore, lower masticatory performance was associated with the development of

high blood pressure, high triglycerides, and high fasting plasma glucose in men. Improving and maintaining masticatory performance may offer a new approach to preventing the development of the MetS. The findings of this study will provide a basis for new preventive strategies against the development of the MetS and subsequent cardiovascular diseases.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because request for date disclosure will be granted at the discretion of the Facility Ethics Committee. Requests to access the datasets should be directed to Takayuki Kosaka, kosaka@dent.osaka-u.ac.jp.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Cerebral and Cardiovascular Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SF contributed to conception, design, data interpretation, performed statistical analyses, drafted, and critically revised the manuscript. TK contributed to conception, design, data acquisition and interpretation, drafted, and critically revised the manuscript. MN contributed to data interpretation, performed statistical analyses, and critically revised the manuscript. MK and YK contributed to data acquisition and interpretation and critically revised the manuscript. TN, TO, and KI contributed to conception, design, and critically revised the manuscript. MW contributed to data acquisition. YM contributed to conception, design, data acquisition, and critically revised the manuscript and supervision. All authors contributed to the article and approved the submitted version.

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The Value of Admission Serological Indicators for Predicting 28-Day Mortality in Intensive Care Patients With Acute Heart Failure: Construction and Validation of a Nomogram

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Background: Acute heart failure (AHF) is a severe clinical syndrome characterized as rapid onset or worsening of symptoms of chronic heart failure (CHF). Risk stratification for patients with AHF in the intensive care unit (ICU) may help clinicians to predict the 28-day mortality risk in this subpopulation and further raise the quality of care.

Methods: We retrospectively reviewed and analyzed the demographic characteristics and serological indicators of patients with AHF in the Medical Information Mart for Intensive Care III (MIMIC III) (version 1.4) between June 2001 and October 2012 and our medical center between January 2019 and April 2021. The chi-squared test and the Fisher's exact test were used for comparison of qualitative variables among the AHF death group and non-death group. The clinical variables were selected by using the least absolute shrinkage and selection operator (LASSO) regression. A clinical nomogram for predicting the 28-day mortality was constructed based on the multivariate Cox proportional hazard regression analysis and further validated by the internal and external cohorts.

Results: Age > 65 years [hazard ratio (HR) = 2.47], the high Sequential Organ Failure Assessment (SOFA) score (≥3 and ≤8, HR = 2.21; ≥9 and ≤20, HR = 3.29), lactic acid (Lac) (>2 mmol/l, HR = 1.40), bicarbonate (HCO_3^-) (>28 mmol/l, HR = 1.59), blood urea nitrogen (BUN) (>21 mg/dl, HR = 1.75), albumin (<3.5 g/dl, HR = 2.02), troponin T (TnT) (>0.04 ng/ml, HR = 4.02), and creatine kinase-MB (CK-MB) (>5 ng/ml, HR = 1.64) were the independent risk factors for predicting 28-day mortality of intensive care patients with AHF ($p < 0.05$). The novel nomogram was developed and validated with a promising C-index of 0.814 (95% CI: 0.754–0.882), 0.820 (95% CI: 0.721–0.897), and 0.828 (95% CI: 0.743–0.917), respectively.

Conclusion: This study provides a new insight in early predicting the risk of 28-day mortality in intensive care patients with AHF. The age, the SOFA score, and serum TnT level are the leading three predictors in evaluating the short-term outcome of intensive care patients with AHF. Based on the nomogram, clinicians could better stratify patients with AHF at high risk and make adequate treatment plans.

Keywords: acute heart failure, mortality, serological examination, nomogram, MIMIC III, intensive care unit

INTRODUCTION

Heart failure (HF) is one of the most frequent cardiovascular-related diseases in modern society, which influences the growing number of populations around the world (1–3). Moreover, HF is fundamentally recognized as one of the leading causes of hospitalization among patients aged > 65 years of age in the United States. For hospitalizations with primary HF, the estimated average cost was nearly $11,552 in 2014, totaling an estimated $11 billion (1, 3, 4). In contrast to the great improvements in the treatment of chronic heart failure (CHF), acute heart failure (AHF) is still associated with a worse prognosis, with 90-day readmission rates and 1 year mortality reaching 10–30% in the United States (2, 5). In the rest of the world, the mortality in Africa and India was determined with the highest rate of 34 and 23%, respectively, about mean mortality in Southeast Asia (15%) and the lowest mortality in China (7%), South America (9%), and the Middle East (9%) (6).

Thus, the risk stratification of AHF based on different clinical characteristics and biomarkers has been proposed. The ideal risk stratification system would identify a subpopulation of patients with similar pathophysiology and clinical presentation at admission, so that treatment may be tailored for each patient. During the past years, researchers from the different regions have made some attempts to discover the prognostic factors in predicting the short-term (28–30-days) or long-term (1–5 years) mortality and readmission in patients with AHF (7–17). Notably, reviewing the recently published literature, elderly age, multimorbidity, high blood urea nitrogen (BUN), serum creatinine, and hypoalbuminemia were significantly associated with the increased risk of AHF-related mortality (2, 3). Additionally, HIV positive was also identified as a meaningful evaluation indicator for patients with AHF from Africa (18). On the other hand, readmission (10) and lower health literacy (19) were also identified as indicators of long-term mortality after discharge. Compelling evidence demonstrated that delayed treatment delivery is associated with poor outcomes in AHF (2). Thus, following the concept of "time-to-treatment," early identifying patients with AHF at high risk of mortality at initial admission to the intensive care unit (ICU) could significantly help clinicians to achieve a timely diagnosis and individualized treatment modality.

In this study, we aim to determine the prognostic factors for predicting the 28-day mortality in intensive care patients with AHF at admission. Based on the admission serological indicators, we further aim to establish an individualized nomogram for routine clinical use.

MATERIALS AND METHODS

Data Source

The data of this study was from one public database and our medical center. Specifically, the training data with respect to clinical characteristics of patients with AHF were obtained from the Medical Information Mart for Intensive Care III (MIMIC III) (version 1.4), derived from a large, freely accessible critical care database comprising deidentified health records (58,976 hospitalization records) of 46,520 patients who were admitted to the ICU of Beth Israel Deaconess Medical Center between June 2001 and October 2012 (https://mimic.physionet.org/). The cases of the internal validating cohort were produced by 1,000 resampling bootstrap analyses from the training data. Besides, the external validation cohort was collected from hospitalizations of the Second Affiliated Hospital of Chongqing Medical University during January 2019 and April 2021.

Ethics Approval

The protocol for this study was approved by Chongqing Medical University. Ethical approval was waived by the local Ethics Committee of the Chongqing Medical University in view of the retrospective nature of this study and all the procedures being performed were part of the routine care.

Patient Selection

In the MIMIC III program, we retrospectively screened patients diagnosed with AHF before admitting them to the ICU. In our medical record system, we identified patients with AHF at first admission between January 2019 and April 2021 in the ICU, emergency ICU (EICU), and coronary care unit (CCU) (**Figure 1**).

Diagnosis of AHF

Acute heart failure is often accompanied by other morbidities and the diagnosis of AHF is frequently made clinically based on history and clinical signs (1, 3, 6). On one hand, in the MIMIC III database, the diagnosis of AHF was following the codes: icd9_code: 42821, 42823, 42831, 42833, 42841, and 42843. On the other hand, in our department, the diagnosis of AHF was based on the detailed medical history of heart disease or multimorbidity combined with some of the symptom and clinical signs including dyspnea on exertion, lower extremity edema, orthopnea, paroxysmal nocturnal dyspnea, reduced exercise tolerance, jugular vein distension, pulmonary rale, cold and clammy skin, and the presence of a third heart sound. Besides, suspicious patients with AHF are further evaluated by

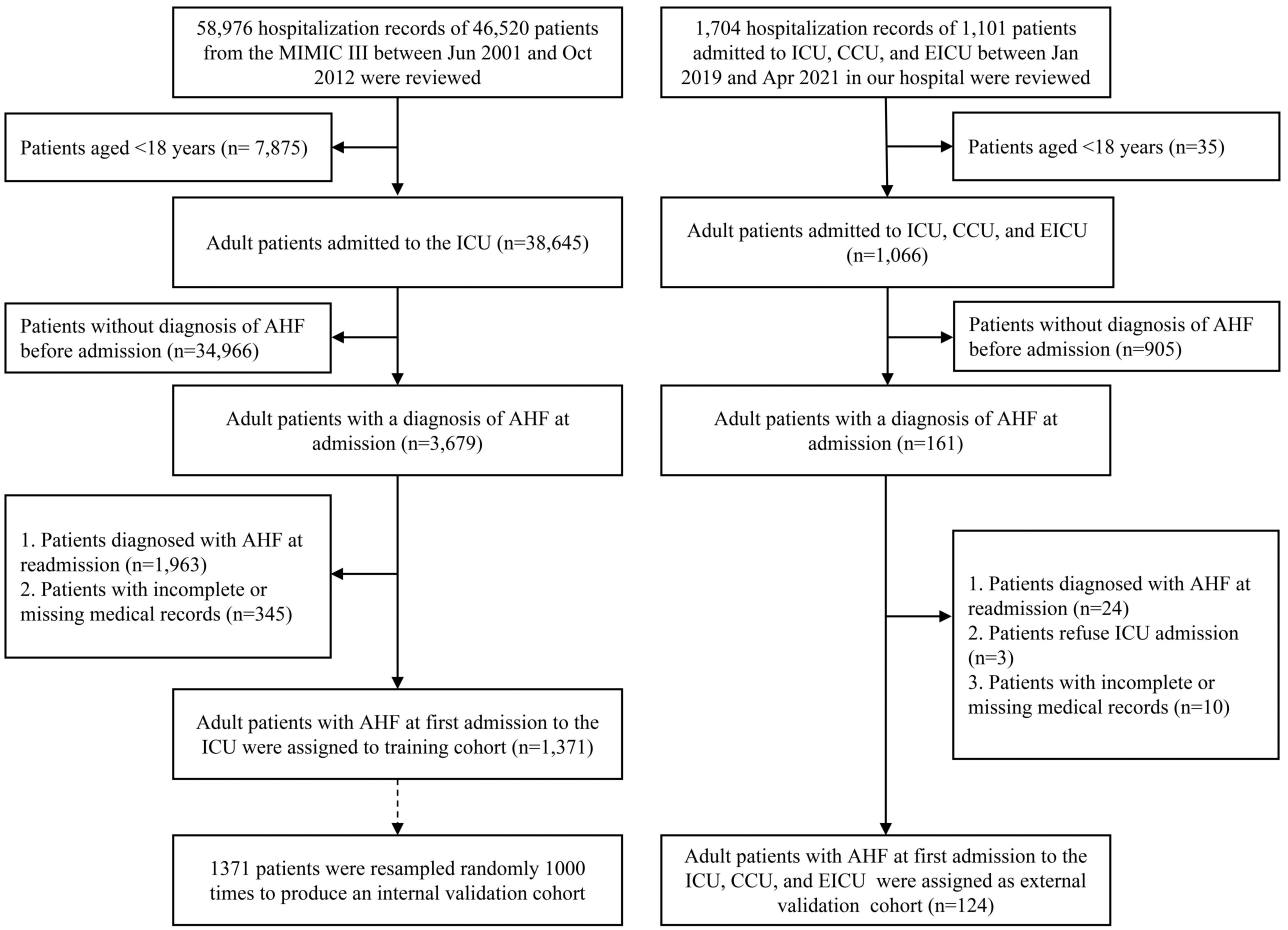

FIGURE 1 | The patient selection process for this study. AHF, acute heart failure; ICU, intensive care unit; CCU, coronary care unit; EICU, emergency ICU.

examinations including but not limited to natriuretic peptides, ECGs, and echocardiography.

Criteria to Intensive Care

The common criteria for admission to an ICU or a CCU include: (i) hemodynamic instability (heart rate < 40 beats/min or >130 beats/min); (ii) systolic blood pressure < 90 mm Hg or evidence of hypoperfusion and respiratory distress (respiratory rate $>$ 25 breaths/min, peripheral oxygen saturation $< 90\%$ despite supplemental oxygen); and (iii) use of accessory muscles for breathing or need for mechanical ventilatory support (20).

Variable Evaluation

The proportional hazards assumption was assessed by plotting Schoenfeld residuals vs. time and examining their correlation (**Supplementary Figure 1**). The continuous variables in this study did not satisfy the proportional hazards assumption ($p < 0.05$). Thus, according to the normal reference values of indicators in the MIMIC III database, all the continuous variables were analyzed as categorized.

Clinical Baseline Information

The sex (female and male), age (>18 and ≤ 65 years and >65 years), race (white, black, and other), hypertension (yes and no), chronic obstructive pulmonary disease (COPD) (yes and no), diabetes (yes and no), acute myocardial infarction (AMI) (yes and no), and body mass index (BMI) (normal: ≥ 18 and <24 kg/m^2) were reviewed.

Severity Scores

The Sequential Organ Failure Assessment (SOFA) score was analyzed individually according to the severity of system impairment (including neurologic, renal, cardiovascular, respiratory, coagulation, and hepatic) and each organ system got a score that ranges between 0 and 4. They were divided into three groups: total scores: ≥ 0 and <2, ≥ 3 and ≤ 8, and ≥ 9 and ≤ 20.

The Glasgow Coma Scale (GCS) score was calculated according to the documented motor, verbal, and eye responses at the admission medical records. We divided into three groups: total score: ≥ 13 and ≤ 15, ≥ 9 and ≤ 12, and ≥ 4 and ≤ 8.

Serological Indicators

The white blood cell (WBC) (normal: ≥4 and ≤10 k/μL), hemoglobin (Hb) (normal: male > 120 g/l, female > 110 g/l), platelet (PLT) (normal: ≥100 and ≤3,001 k/μl), sodium (Na) (normal: 135–145 mmol/l), potassium (K) (normal: ≥3.5 and ≤5.5 mmol/l), chlorine (Cl) (normal: ≥96 and ≤106 mmol/l), total calcium (tCa) (normal: ≥9 and ≤11 mg/dl), base excess (BE) (normal: ≥-3 and ≤3 mmol/l), bicarbonate (HCO_3^-) (normal: ≥22 and ≤28 mmol/l), anion gap (AG) (normal: ≥8 and ≤16 mmol/l), lactic acid (Lac) (normal: ≤2 mmol/l), blood urea nitrogen (BUN) (normal: ≤21 mmol/l), serum creatinine (Scr) (normal: male: <1.5 mg/dl, female: <1.0 mg/dl), albumin (ALB) (normal: ≥3.5 g/dl), total bilirubin (Tbil) (normal: ≤1.2 mg/dl), aspartate aminotransferase (AST) (normal: ≤35 U/l), alanine aminotransferase (ALT) (normal: ≤40 U/l), troponin T (TnT) (normal: ≤0.04 ng/ml), and creatine kinase-MB (CK-MB) (normal: ≤5 ng/ml) were screened out for constructing the database of this study (**Table 1**). The measurement unit for each variable derived from the MIMIC III database and our medical center was unified (18 × 1 mg/dl = 1 mmol/l; 1 g/dl = 10 g/l; 1 k/μl = 1 × 10^9/L). Note: the tCa was corrected by the formula = measured total calcium (mg/dl) + 0.8 [4.0 – serum albumin (g/dl)].

Variable Selection and Nomogram Construction

The following basic information and serological indicators and the two severity score scales from the MIMIC III program were screened out for investigating the risk factors associated with 28-day mortality in patients with AHF: sex, age, race, the SOFA, the GCS, WBC, Hb, PLT, Na, K, Cl, tCa, BE, HCO_3^-, AG, Lac, BUN, Scr, ALB, TBil, ALT, AST, TnT, and CK-MB. The developed nomogram of risk factors associated with 28-day mortality in patients with AHF is based on two aspects. One was based on the statistically significant different results calculated *via* the multivariate Cox proportional hazard regression analysis ($p < 0.05$). The other was based on the variables, which have been demonstrated significantly associated with cardiovascular-related death, regardless of the two-tailed *p*-value.

Statistical Analysis

The MIMIC III software was applied to identify the patients who met the inclusion criteria in the MIMIC III program. Baseline characteristics among the AHF death group and non-death group were compared using the Pearson's chi-squared test (minimal expected value > 5) and the Fisher's exact test (minimal expected value ≤5). The least absolute shrinkage and selection operator (LASSO) Cox regression algorithm with 10-fold cross-validation was used to select the optimal variables that were most relevant to the 28-day mortality of patients with AHF. A two-tailed *p*-value of < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection. The LASSO regression and the multivariate Cox proportional hazard regression analysis were performed by using the "rms" package derived from the "R" software (http://www.r-project.org, R Foundation, Vienna, Austria, version 3.5.3). The Harrell concordance indexes (C-index), which are equivalent to the area under the receiver

TABLE 1 | The demographic characteristics of patients with acute heart failure (AHF) during the first intensive care unit admission.

Variables	Subgroup	No. (%) of patients		
		Training cohort (*n* = 1,371)	Internal cohort (*n* = 685)	External cohort (*n* = 124)
Sex	Male	776 (56.6)	396 (57.8)	68 (54.8)
	Female	595 (43.3)	289 (42.2)	56 (45.1)
Age (years)	≤65	340 (24.7)	187 (27.3)	34 (27.4)
	>65	1,031 (75.3)	498 (72.7)	90 (72.6)
Race	White	1,019 (74.3)	516 (75.3)	/
	Black	128 (9.3)	53 (7.7)	/
	Other	224 (16.3)	116 (17.0)	124 (100.0)
Hypertension	No	827 (60.3)	423 (61.8)	64 (51.6)
	Yes	544 (39.7)	262 (38.2)	60 (48.4)
COPD	No	1,300 (94.8)	655 (95.6)	104 (83.9)
	Yes	71 (5.2)	30 (4.4)	20 (16.1)
Diabetes	No	807 (58.8)	405 (59.1)	83 (66.9)
	Yes	564 (41.2)	280 (40.9)	41 (33.1)
AMI	No	1,306 (95.2)	655 (95.6)	73 (58.9)
	Yes	65 (4.7)	30 (4.4)	51 (41.1)
SOFA (score)	≥0 and <2	305 (22.2)	162 (23.7)	21 (16.9)
	≥3 and ≤8	869 (63.4)	444 (64.8)	78 (62.9)
	≥9 and ≤20	197 (14.4)	79 (11.5)	25 (20.16)
GCS (score)	≥13 and ≤15	1,186 (86.5)	594 (86.7)	102 (82.2)
	≥9 and ≤12	106 (7.7)	57 (8.3)	18 (14.5)
	≥4 and ≤8	79 (5.8)	34 (5.0)	4 (3.2)
WBC (k/uL)	<4	32 (2.3)	17 (2.4)	5 (4.0)
	≥4 and ≤10	582 (42.4)	286 (41.8)	62 (50.0)
	>10	757 (55.2)	382 (55.8)	57 (46.0)
Hb (g/L)	Normal	424 (30.9)	205 (29.9)	50 (40.3)
	Low	947 (69.1)	480 (70.1)	74 (59.7)
PLT (k/uL)	<100	98 (7.1)	19 (2.8)	10 (8.1)
	≥100 and ≤300	1,005 (73.3)	498 (72.7)	96 (77.4)
	>300	268 (19.5)	348 (50.8)	18 (14.5)
Na (mmol/L)	Normal	1,074 (78.3)	551 (80.4)	72 (58.1)
	Abnormal	297 (21.7)	134 (19.6)	52 (41.9)
K (mmol/L)	<3.5	157 (11.5)	69 (10.1)	15 (12.1)
	≥3.5 and ≤5.5	1,125 (82.0)	567 (82.8)	102 (82.3)
	>5.5	89 (6.5)	49 (7.1)	7 (5.6)
Cl (mmol/L)	<96	146 (10.6)	80 (11.7)	32 (25.8)
	≥96 and ≤106	824 (60.1)	434 (63.4)	79 (63.7)
	>106	401 (29.2)	171 (24.9)	13 (10.5)
tCa (mg/dl)	Normal/ Hypercalcemia	282 (20.6)	145 (21.2)	61 (49.2)
	Hypocalcemia	1,089 (79.4)	540 (78.8)	63 (50.8)
^{adjust}tCa (mg/dl)	Normal/ Hypercalcemia	605 (44.1)	357 (52.1)	82 (66.1)
	Hypocalcemia	766 (55.9)	328 (47.9)	42 (33.9)
BE (mmol/L)	<-3	259 (18.9)	118 (17.2)	41 (33.1)
	≥-3 and ≤3	877 (64.0)	429 (62.6)	68 (54.8)
	>3	235 (17.1)	138 (20.1)	15 (12.1)

(Continued)

TABLE 1 | Continued

Variables	Subgroup	No. (%) of patients		
		Training cohort (n = 1,371)	Internal cohort (n = 685)	External cohort (n = 124)
HCO_3^- (mmol/L)	<22	418 (30.5)	183 (26.7)	44 (35.5)
	≥22 and ≤28	697 (50.8)	369 (53.9)	63 (50.8)
	>28	256 (18.7)	133 (19.4)	17 (13.7)
AG (mmol/L)	≥8 and ≤16	967 (70.5)	479 (69.9)	89 (71.8)
	>16	404 (29.5)	206 (30.1)	35 (28.2)
Lac (mmol/L)	≤2	1,030 (75.1)	526 (76.8)	90 (72.6)
	>2	341 (24.9)	159 (23.2)	34 (27.4)
BUN (mg/dl)	≤21	338 (24.7)	181 (26.4)	45 (36.3)
	>21	1,033 (75.3)	504 (73.6)	79 (63.7)
Scr (mg/dl)	Normal	684 (49.9)	357 (52.1)	59 (47.6)
	High	687 (50.1)	328 (47.9)	65 (52.4)
ALB (g/dl)	<3.5	1,112 (81.1)	567 (82.8)	48 (38.7)
	≥3.5	259 (18.9)	118 (17.2)	76 (61.3)
TBil (mg/dl)	≤1.2	1,074 (78.3)	538 (78.5)	104 (83.9)
	>1.2	297 (21.7)	147 (21.5)	20 (16.1)
ALT (U/L)	≤40	939 (68.5)	476 (69.5)	90 (72.6)
	>40	432 (31.5)	209 (30.5)	34 (27.4)
AST (U/L)	≤35	690 (50.3)	343 (50.1)	72 (58.1)
	>35	681 (49.7)	342 (49.9)	52 (41.9)
TnT (ng/ml)	≤0.04	691 (50.4)	335 (48.9)	75 (60.5)
	>0.04	680 (49.6)	350 (51.1)	49 (39.5)
CKMB (ng/ml)	≤5	792 (57.8)	386 (56.4)	70 (56.5)
	>5	579 (42.2)	299 (43.6)	54 (43.5)
BMI (kg/m^2)	<24	153 (11.2)	69 (10.1)	98 (79.0)
	≥24	602 (43.9)	285 (41.6)	26 (21.0)
	NM	616 (44.9)	331 (48.3)	/
Death	No	1,145 (83.5)	575 (83.9)	94 (75.8)
	Yes	226 (16.5)	110 (16.1)	30 (24.2)

COPD, chronic obstructive pulmonary disease; AMI, acute myocardial infarction; SOFA, Sequential Organ Failure Assessment; GCS, Glasgow Coma Scale; WBC, white blood cell; Hb, hemoglobin (normal: male > 120 g/l and female > 110 g/l); PLT, platelet; Na, sodium (normal: 135–145 mmol/l); K potassium; Cl, chlorine; tCa, total calcium (normal: ≥9 and ≤11 mg/dl); HCO_3^-, bicarbonate; BE, base excess; AG, anion gap; Lac, lactic acid; BUN, blood urea nitrogen; Scr, serum creatinine (normal: male: <1.5 mg/dl, female: <1.0 mg/dl), ALB; albumin; Tbil, total bilirubin; ALT, aspartate aminotransferase; AST, alanine aminotransferase; TnT, troponin T; CK-MB, creatine kinase-MB; BMI, body mass index; NM, not mentioned.

^{adjust}tCa: the tCa was corrected by the formula = measured total calcium (mg/dl) + 0.8 × [4.0 – serum albumin (g/dl)].

(AUC) operating characteristic (ROC) curve, were calculated for evaluating the discrimination of the model and the calibration curves were performed to assess the accuracy of the nomogram.

RESULTS

Demographic Characteristics of Patients With AHF in the Training Cohort

After excluding, there were 1,371 patients with AHF in the MIMIC III program between 2001 and 2012 and 124 patients with AHF from the Department of Cardiology Internal Medicine in the Second Affiliated Hospital of Chongqing Medical University between January 2019 and April 2021 enrolled in this study. In the training cohort, short-term mortality was observed in 16.5% (226/1,371 cases) of patients. The male patients accounted for a relatively higher rate of suffering AHF compared with the female subpopulations. The elderly patients (age > 65 years, 1,031/1,371 cases, 75.3%) and white race (1,019/1,371 cases, 74.3%) played a predominately part of the AHF population. With respect to the electrolyte indicators, approximately 20% of patients with AHF were identified with electrolyte imbalance. The specific clinical features of the patients in the training and validation cohorts are shown in **Table 1**.

Clinical Characteristics Between AHF Death Group and Non-death Group

There were significant differences between the AHF death group and non-death group in terms of age ($p < 0.001$), the SOFA score ($p < 0.001$), and the GCS score ($p < 0.001$) compositions, while no significant difference was identified in terms of race and sex. With respect to the serological indicators, there were significantly differences between the AHF death group and non-death group in terms of serum Cl ($p = 0.033$), BE ($p < 0.001$), HCO_3^- ($p < 0.001$), AG ($p < 0.001$), Lac ($p < 0.001$), BUN ($p < 0.001$), Scr ($p < 0.001$), ALB ($p = 0.004$), ALT ($p = 0.013$), AST ($p = 0.006$), TnT ($p < 0.001$), and CK-MB ($p < 0.001$) compositions (**Table 2**).

Least Absolute Shrinkage and Selection Operator Regression Analysis

A total of 24 indicators including sex, age, race, the SOFA score, the GCS score, WBC, Hb, PLT, Na, K, Cl, tCa, BE, HCO_3^-, AG, Lac, BUN, Scr, ALB, TBil, ALT, AST, TnT, and CK-MB were initially elected to the LASSO regression algorithm with 10-fold cross-validation (**Figure 2**). There were 12 predictor variables including age, the SOFA score, the GCS score, Hb, BUN, Lac, HCO_3^-, AG, TBil, ALB, TnT, and CK-MB were selected for inclusion in the multivariate Cox proportional hazard regression analysis model.

Multivariate Cox Proportional Hazard Regression Analysis

The results indicated that elderly age > 65 years [hazard ratio (HR) = 2.47, 95% CI: 1.61–3.80, $p < 0.001$), the higher SOFA score (≥3 and ≤8: HR = 2.21, 95% CI: 1.33–3.68; ≥9 and ≤20: HR = 3.29, 95% CI: 1.83–5.89, $p < 0.001$), Lac > 2 mmol/l (HR = 1.40, 95% CI: 1.05–1.89, $p = 0.022$), BUN > 21 mg/dl (HR = 1.75, 95% CI: 1.09–2.80, $p = 0.019$), HCO_3^- > 28 mmol/l (HR = 1.59, 95% CI: 1.09–2.32, $p = 0.025$), ALB < 3.5 g/dl (HR = 2.02, 95% CI: 1.34–3.05, $p = 0.001$), TnT > 0.04 ng/ml (HR = 4.02, 95% CI: 2.74–5.90, $p < 0.001$), and CK-MB > 5 ng/ml (HR = 1.64, 95% CI: 1.21–2.23, $p = 0.001$) were the independent risk factors in predicting the 28-day mortality of intensive care patients with AHF (**Table 3**).

TABLE 2 | Clinical characteristics among the AHF death group and non-death group in the training cohort.

Variables	Subgroup	No. (%) of patients		
		Death group (n = 226)	Non-death group (n = 1,145)	*P
Sex	Female	89 (39.4)	506 (44.2)	0.182
	Male	137 (60.4)	639 (55.8)	
Age (years)	≤65	24 (10.6)	316 (27.6)	**<0.001**
	>65	202 (89.4)	829 (72.4)	
Race	White	172 (76.1)	847 (74.0)	0.112
	Black	13 (5.8)	115 (10.0)	
	Other	41 (18.1)	183 (16.0)	
SOFA (score)	≥0 and <2	18 (8.0)	287 (25.1)	**<0.001**
	≥3 and ≤8	140 (61.9)	729 (63.7)	
	≥9 and ≤20	68 (30.1)	129 (11.3)	
GCS (score)	≥13 and ≤15	174 (77.0)	1,012 (88.4)	**<0.001**
	≥9 and ≤12	27 (11.9)	79 (6.9)	
	≥4 and ≤8	25 (11.1)	54 (4.7)	
WBC (k/uL)	<4	5 (2.2)	27 (2.3)	0.149
	≥4 and ≤10	83 (36.7)	499 (43.6)	
	>10	138 (61.1)	619 (54.1)	
Hb (g/L)	Normal	58 (25.7)	366 (32.0)	0.061
	Low	168 (74.3)	779 (68.0)	
PLT (k/uL)	<100	18 (8.0)	80 (7.0)	0.550
	≥100 and ≤300	159 (70.3)	846 (74)	
	>300	49 (21.7)	219 (19.0)	
Na (mmol/L)	Normal	169 (74.8)	905 (79.0)	0.155
	Abnormal	57 (25.2)	240 (21.0)	
K (mmol/L)	<3.5	29 (12.8)	128 (11.2)	0.062
	≥3.5 and ≤5.5	175 (77.4)	950 (83.0)	
	>5.5	22 (9.7)	67 (5.8)	
Cl (mmol/L)	<96	35 (15.5)	111 (9.7)	**0.033**
	≥96 and ≤106	126 (55.7)	698 (61.0)	
	>106	65 (28.8)	336 (29.3)	
^{adjust}tCa (mg/dl)	Normal/hypercalcemia	99 (43.8)	506 (44.2)	0.573
	Hypocalcemia	127 (56.2)	639 (55.8)	
BE (mmol/L)	<-3	68 (30.1)	191 (16.7)	**<0.001**
	≥-3 and ≤3	124 (54.9)	753 (65.8)	
	>3	34 (15.0)	201 (17.6)	
HCO_3^- (mmol/L)	<22	98 (43.4)	330 (28.8)	**<0.001**
	≥22 and ≤28	88 (38.9)	599 (52.3)	
	>28	40 (17.7)	216 (18.9)	
AG (mmol/L)	≤16	122 (54.0)	845 (73.8)	**<0.001**
	>16	104 (46.0)	300 (26.2)	
Lac (mmol/L)	≤2	133 (58.8)	897 (78.3)	**<0.001**
	>2	93 (41.1)	248 (21.7)	
BUN (mg/dl)	≤21	21 (9.3)	317 (27.7)	**<0.001**
	>21	205 (90.7)	828 (72.3)	
Scr (mg/dl)	Normal	84 (37.2)	600 (52.4)	**<0.001**
	High	142 (62.8)	545 (47.6)	
ALB (g/dl)	<3.5	199 (88.1)	913 (79.7)	**0.004**
	≥3.5	27 (11.9)	232 (20.3)	
Tbil (mg/dl)	≤1.2	166 (73.4)	908 (79.3)	0.051
	>1.2	60 (26.5)	237 (20.7)	
ALT (U/L)	≤40	139 (61.5)	800 (69.9)	**0.013**
	>40	87 (38.5)	345 (30.1)	
AST (U/L)	≤35	95 (42.0)	595 (52.0)	**0.006**
	>35	131 (58.0)	550 (48.0)	
TnT (ng/ml)	≤0.04	37 (16.4)	654 (57.1)	**<0.001**
	>0.04	189 (83.6)	491 (42.9)	
CKMB (ng/ml)	≤5	77 (34.1)	715 (62.4)	**<0.001**
	>5	149 (65.9)	430 (37.6)	

SOFA, Sequential Organ Failure Assessment; GCS, Glasgow Coma Scale; WBC, white blood cell; Hb, hemoglobin (normal: male > 120 g/l, female > 110 g/l); PLT, platelet; Na, sodium (normal: 135–145 mmol/l); K, potassium; Cl, chlorine; tCa, total calcium (normal: ≥9 and ≤11 mg/dl); HCO_3^-, bicarbonate; BE, base excess; AG, anion gap; Lac, lactic acid; BUN, blood urea nitrogen; Scr, serum creatinine (normal: male: <1.5 mg/dl, female: <1.0 mg/dl); ALB, albumin; Tbil, total bilirubin; ALT, aspartate aminotransferase; AST, alanine aminotransferase; TnT, troponin T; CK-MB, creatine kinase-MB.

**Two-tail Pearson's chi-squared test.*

^{adjust}tCa: the tCa was corrected by the formula = measured total calcium (mg/dl) + 0.8 × [4.0 – serum albumin (g/dl)].

Bold values indicate statistical significance ($p < 0.05$).

Nomogram Construction and Validation

Based on the multivariate results, nine variables including age, the SOFA score, and serum levels of BUN, TnT, CK-MB, Lac, HCO_3^-, AG, and ALB were used to construct an intuitive nomogram for predicting the 28-day mortality in intensive care patients with AHF (**Figure 3**). Every variable was given a score from 0 to 100 and the specific score of each variable is shown in **Table 4**. Reflecting the discrimination of the nomogram, the C-index, which was in accordance with the AUC of the time-dependent ROC, was above 0.7 and reached 0.814 (95% CI: 0.754–0.882) (**Figure 4A**). Furthermore, the AUC of the internal validation cohort, which derived from 1,000 resampling bootstrap analysis, also achieved 0.820 (95% CI: 0.721–0.897) (**Figure 4B**). To evaluate the feasibility of the nomogram in other populations, an external validation cohort from our medical center was further analyzed. The AUC of the external validation cohort also achieved a promising result of 0.828 (95% CI: 0.743–0.917) (**Figure 4C**). Moreover, to evaluate the utility of the nomogram, three calibration curves of 28-day mortality risk in patients with AHF were displayed. The curves suggested a favorable agreement in the training cohort (**Figure 5A**) and internal cohort (**Figure 5B**) and an external cohort (**Figure 5C**), respectively.

DISCUSSION

Acute heart failure is a severe clinical syndrome characterized as rapid onset or worsening of symptoms of chronic HF, which is the leading cause of unplanned hospital admission and

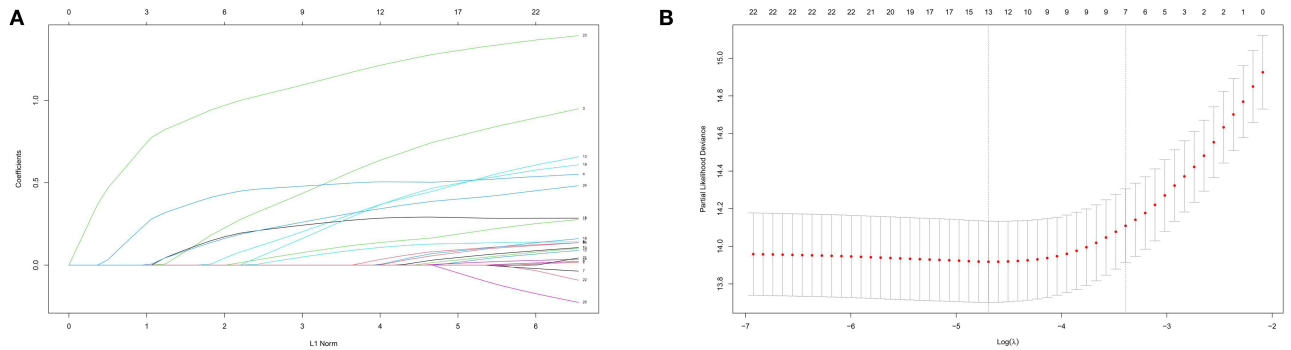

FIGURE 2 | Clinical feature selection using the least absolute shrinkage and selection operator (LASSO) Cox proportional hazard regression analysis with 10-fold cross-validation. **(A)** Tuning parameter selection in the LASSO Cox regression model. **(B)** The LASSO coefficient analysis of the clinical features.

TABLE 3 | The multivariate Cox proportional hazard regression analysis of risk factors associated with 28-day mortality in patients with AHF during the first intensive care unit admission.

Variables	Subgroup	Hazard ratio	*P*
Age (years)	≤65	1	**<0.001**
	>65	2.47 (1.61–3.80)	
SOFA (score)	≥0 and ≤2	1	**<0.001**
	≥3 and ≤8	2.21 (1.33–3.68)	
	≥9 and ≤20	3.29 (1.83–5.89)	
GCS (score)	≥13 and ≤15	1	0.260
	≥9 and ≤12	1.20 (0.79–1.82)	
	≥4 and ≤8	1.42 (0.90–2.26)	
Hb (g/L)	Normal	1	0.281
	Low	1.18 (0.87–1.60)	
HCO_3^- (mmol/L)	<22	0.91 (0.67–1.25)	**0.025**
	≥22 and ≤28	1	
	>28	1.59 (1.09–2.32)	
AG (mmol/L)	≤16	1	0.051
	>16	1.35 (0.99–1.84)	
Lac (mmol/L)	≤2	1	**0.022**
	>2	1.40 (1.05–1.89)	
BUN (mg/dl)	≤21	1	**0.019**
	>21	1.75 (1.09–2.80)	
ALB (g/dl)	<3.5	2.02 (1.34–3.05)	**0.001**
	≥3.5	1	
TBil (mg/dl)	≤1.2	1	0.205
	>1.2	1.22 (0.89–1.67)	
TnT (ng/ml)	≤0.04	1	**<0.001**
	>0.04	4.02 (2.74–5.90)	
CKMB (ng/ml)	≤5	1	**0.001**
	>5	1.64 (1.21–2.23)	

SOFA, Sequential Organ Failure Assessment; GCS, Glasgow Coma Scale; Hb, hemoglobin (normal: male > 120 g/l, female > 110 g/l); HCO_3^-, bicarbonate; AG, anion gap; Lac, lactic acid; BUN, blood urea nitrogen; ALB, albumin; TBil, total bilirubin; TnT, troponin T; CK-MB, creatine kinase-MB.
Bold values indicate statistical significance ($p < 0.05$).

even readmission in patients aged > 65 years (2, 3, 21–23). Compared with the promising improvements in the treatment of chronic HF, AHF is still associated with poor prognosis regardless of therapeutic advances (13, 24). According to the recent comprehensive review reports (2, 3), the overall in-hospital mortality of AHF ranged from 4 to 7%, whereas the highest mortality rate (reaching 17.8%) was identified in patients with ICU (25). Besides, the risk of mortality rises after hospital discharge with ∼10% mortality at 30-days and 22–27% mortality at 1 year (2). Thus, there is an urgent need for early clinical assessment, risk stratification, and increased individualization and continuation of treatment after hospital discharge to improve long-term outcomes in patients with AHF.

In this study, we ultimately included 1,371 patients with the diagnosis of AHF at initial admission to ICU from the MIMIC III program. The short-term (28-day) mortality rate was 16.5% (226/1,371 cases), which was higher than the result of Miró et al. (11) from a prospective cohort study (10.3%), but slightly lower than Follath et al. (25) results from the Acute Heart Failure Global Registry of Standard Treatment (ALARM-HF) trial (17.8%). Among the AHF death group and non-death group, there were significant differences between the groups in terms of age ($p < 0.001$), the SOFA score ($p < 0.001$), and the GCS score ($p < 0.001$) compositions, while no significant difference was identified in terms of race and sex. Interestingly, we did not determine the significant difference in sex or race composition of the two groups. However, in one large-scale population-based study with a 1 year follow-up, Sun et al. (26) demonstrated the different patterns of ethnicity in patient outcomes with AHF. They concluded a lower risk of 1 year mortality after AHF hospitalization among South Asians compared to Chinese and the general population and similar benefits of medical therapy in all the three groups. On the other hand, with respect to the serological examination, our results partially confirmed the findings derived from the previous studies and took it a step further. Notably, significantly differences were identified in serological indicators in terms of serum Cl ($p = 0.033$), BE ($p < 0.001$), HCO_3^- ($p < 0.001$), AG ($p < 0.001$), Lac ($p < 0.001$), BUN ($p < 0.001$), Scr ($p < 0.001$), ALB ($p = 0.004$), ALT ($p = 0.013$), AST ($p = 0.006$), TnT ($p < 0.001$), and CK-MB ($p < 0.001$) among AHF death group and non-death group in this study.

Furthermore, based on the multivariate Cox proportional hazard regression analysis results, we confirmed eight variables that presented a significant association with the risk of short-term

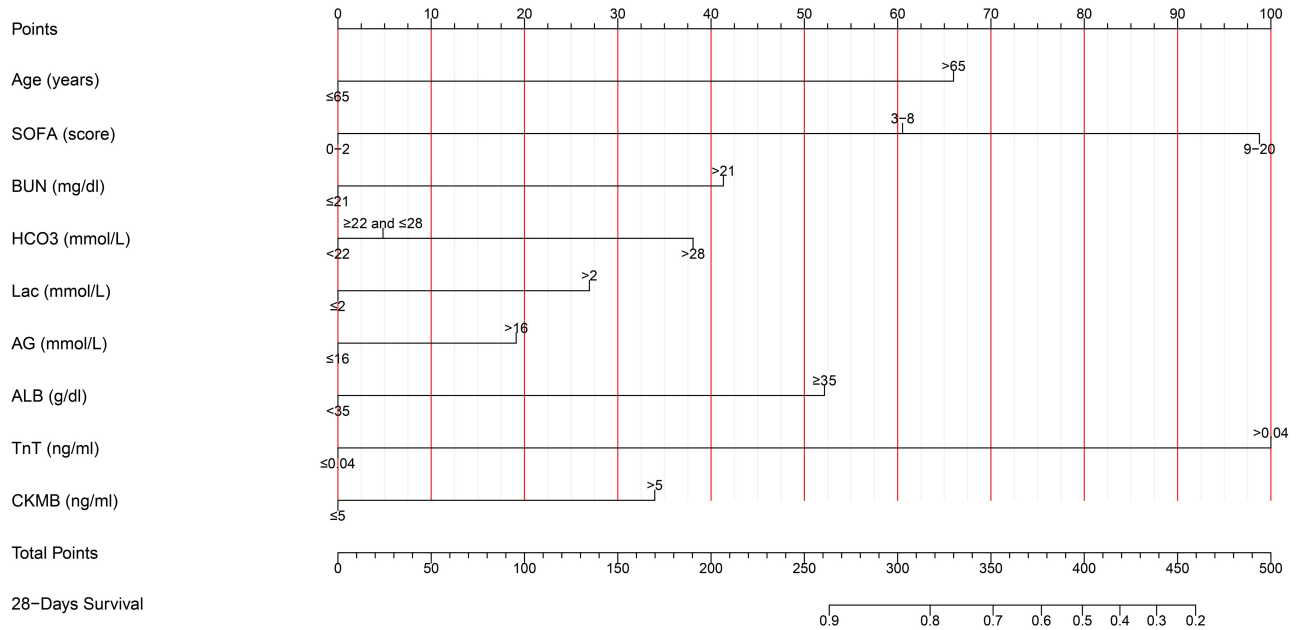

FIGURE 3 | The nomogram used for predicting the short-term mortality in intensive care patients with acute heart failure. SOFA, Sequential Organ Failure Assessment; BUN, blood urea nitrogen; TnT, troponin T; CK-MB, creatine kinase-MB; HCO_3^-, bicarbonate; Lac, lactic acid; AG, anion gap; ALB, albumin.

in-hospital mortality in patients with AHF. The age, the SOFA score, and TnT level were the three leading predictors of the short-term mortality of patients with AHF in ICU. Especially, the SOFA score was a composite and utility clinical–biological score, which could help clinicians to access the potential risk of patients. Most recently, Elias et al. (27) determined the feasibility of the SOFA score (AUC: 0.765) in predicting the short-term mortality in patients with AHF. Our results also yielded that the SOFA score could be used as a complementary risk score to early identify high-risk patients who need strict management. Although the GCS score and serum levels of AG and Scr showed statistically significant differences during the univariate analysis, these differences disappeared after adjustment for the other factors. Nonetheless, there was a tendency toward an increased risk of mortality in patients with high levels of AG [odds ratio (OR) = 1.42, $p = 0.092$]. Moreover, according to the evidence derived from the Epidemiology of Acute Heart Failure in Emergency departments (EAHFE) registry (17), the high-sensitive (hs)-TnT was recently confirmed to be an optimal biomarker in predicting the 30-days all-come mortality in patients with AHF (with the best cutoff point of 35 ng/l). A similar result was also displayed in one recent multicenter-based study, while the cutoff point was hs-TnT $\geq$ 43 ng/l (28). Although the indicator in this study was not hs-TnT but TnT (>0.04 ng/ml, HR = 4.02), it supported the promising predicting value of this serum biomarker.

One recent study concluded that it was pivotal to correct the calcium level in patients with AHF, which could help to reduce the misdiagnosis of hypocalcemia (29). In this study, concerning the impact of albumin on the calcium level, especially in patients with hypoalbuminemia, the calcium level reported was corrected for albumin [measured total calcium (mg/dl) + 0.8 × 4.0 – serum albumin (g/dl)]. After adjusting, a significant decrease in proportion of patients with hypocalcemia was observed. A similar result was also determined in our medical center (hypocalcemia rate decreased from 50.8 to 33.9%). Previous reports, especially case reports, mentioned the association between hypocalcemia and hypercalcemia and AHF (29–32). Thus, future studies are needed to evaluate its predicting value in mortality of AHF and the potential mechanisms behind it. Besides, we also confirmed the correlation between albumin level and survival of patients with HF. There were several potential explanations for the relationship between hypoalbuminemia and the survival of AHF. Hypoalbuminemia was frequently occurred in advanced age, malnutrition, and inflammation, which were known to predict a worse prognosis of AHF (33, 34). Moreover, decreased colloid osmotic pressure caused by hypoalbuminemia could lead to the development of pulmonary edema and exacerbation of AHF. Furthermore, patients with hypoalbuminemia usually present a worse prognosis in several multimorbidity conditions such as late-stage renal disease, infection, and cancer, which were highly prevalent in elderly patients with AHF and could contribute to their increased mortality risk. As for BUN, compelling evidence has demonstrated a positive correlation between BUN and increased mortality of AHF (2, 3). The high level of BUN predicted a worse renal function, which could further impair the circulation and metabolism of the body and aggravate the symptoms of AHF.

Nowadays, individual biomarkers can be utilized to predict clinical outcomes in patients with AHF, including but not limited

TABLE 4 | The specific value of clinicopathological factors in the nomogram of the training cohort.

	Characteristics	Score
SOFA		
	≥0 and <2	0
	≥3 and ≤8	61
	≥9 and ≤20	99
BUN		
	≤21	0
	>21	41
HCO_3^-		
	<22	0
	≥22 and ≤28	5
	>28	38
TnT		
	≤0.04	0
	>0.04	100
CKMB		
	≤5	0
	>5	34
Lac		
	≤2	0
	>2	27
AG		
	≤16	0
>16	19	
Age		
	≤65	0
	>65	66
ALB		
	<3.5	0
	≥3.5	52
Total point for 28-day survival		
	0.2	460
	0.3	439
	0.4	419
	0.5	399
	0.6	377
	0.7	351
	0.8	317
	0.9	263

SOFA, Sequential Organ Failure Assessment; BUN, blood urea nitrogen; HCO_3^-, bicarbonate; TnT, troponin T; CK-MB, creatine kinase-MB; AG, anion gap; ALB, albumin.

to the risk of mortality and readmission (5, 9–11, 34–36). In one earlier study, based on classification and regression tree analysis, Fonarow et al. (37) applied a model within only three factors to the clinical practice including BUN (>43 mg/dl), low admission systolic blood pressure (<115 mm Hg), and high levels of Scr (>2.75 mg/dl). With more clinical variables [using the American Heart Association Get With the Guidelines-Heart Failure (GWTG-HF) program data] involvement, they constructed another new model within seven indicators such as age, systolic blood pressure, BUN, heart rate, Na, COPD, and non-black race for predicting in-hospital mortality (7). Also, in one Spanish trial (11), the Multiple Estimation of Risk Based on the Emergency Department Spanish Score in Patients with AHF (MEESSI-AHF) scores included 13 independent risk factors to estimate the 30-day mortality in patients with AHF and achieved a C-index of 0.836. However, serological indicators combined with severity scores could be sufficient to predict the short-term risk of in-hospital mortality was rarely explored. Additionally, in China, contemporary data on the epidemiology of HF including AHF in China are scarce with only a few studies that could be reached (38, 39).

Additionally, to vividly display the results from multivariate analysis, we further constructed a prediction model for clinical use. Some prior studies mentioned above have constructed risk score models for predicting the in-hospital mortality or postdischarge prognosis in patients hospitalized with AHF (11, 34, 37). However, few researchers, to the best of our knowledge, have ever attempted to establish a nomogram that was frequently used to predict metastasis and survival in the oncology field (40, 41) to visualize the prognostic factors with different scores. For this reason, we filled this gap and developed a nomogram with nine predictors of involvement for predicting the short-term in-hospital mortality in patients with AHF. Optimistically, the C-index of the model, which was in accordance with the AUC, was above 0.70 and reached 0.795 (95% CI: 0.711–0.898). It indicated a favorable discrimination ability of our model to identify patients with AHF at high risk. Besides, an internal cohort *via* 1,000 resampling bootstrap and an external validation cohort from our medical center also proved the utility of the nomogram. Our risk model had a higher C-index than models established by Elias et al. (27) (C-index: 0.765) and Peterson et al. (7) (C-index: 0.750). Although the AUC of the model made by Kinugasa et al. (34) was 0.860, the sample size was only 349 cases and all of them were aged over 65 years. Thus, we suggest that this novel nomogram could help clinicians to identify the patients who are at high risk for death once they were admitted to the ICU and CCU.

Nevertheless, this study has some limitations, which need to be mentioned. First, the data of brain natriuretic peptide (BNP), N-terminal pro-BNP (NT-proBNP) (42) were missing in the MIMIC III program, which could be added combined with other novel biomarkers including but not limited to soluble ST2 (sST2), growth-differentiation factor-15 (GDF-15), cystatin C, galectin-3, serum uric acid, microRNAs, and low serum chloride in the further update (9, 43). Second, the distinguishment of HF with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF) left ventricular ejection fraction (LVEF) was not recorded in the MIMIC III program. Thus, this model could be applied to both the two conditions that need further evaluation. Third, although the training data was derived from a multipopulational program, the retrospective nature of this study introduced the possibility of inherent observational and selection bias. Moreover, the nomogram was established by dichotomizing continuous variables. Thus, the individualized score should be cautious to interpret during clinical practice. Last, while an external cohort from a single medical center has confirmed the utility of the nomogram, the cases of the validation cohort

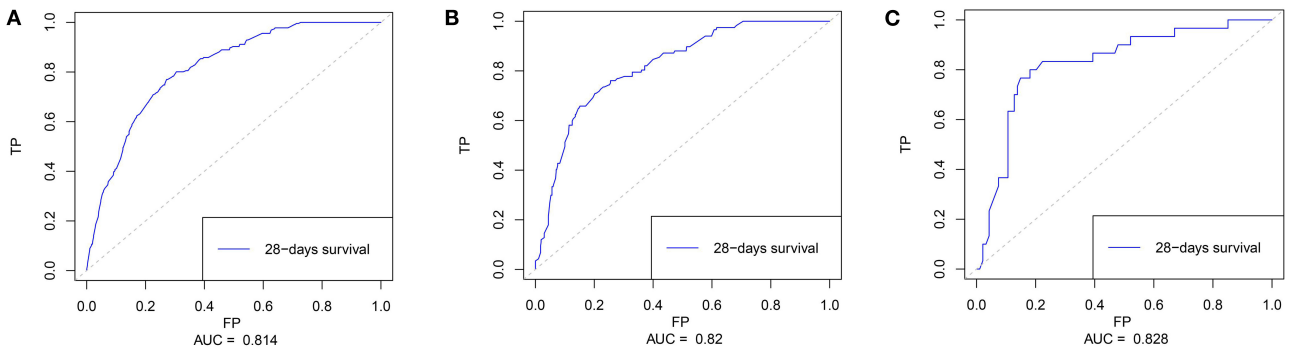

FIGURE 4 | The time-dependent receiver operating characteristics (ROC) curve and area under the ROC curve (AUC). **(A)** The ROC in the training cohort; **(B)** The ROC in the internal validating cohort; and **(C)** The ROC in the external validating cohort.

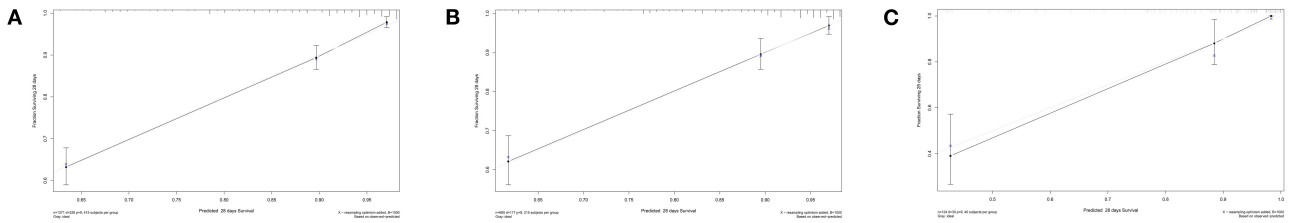

FIGURE 5 | The calibration curves for evaluating the accuracy of the nomogram. **(A)** The calibration curve in the training cohort; **(B)** The calibration curve in the internal validation cohort derived from the 1,000 resampling bootstrap analysis; and **(C)** The calibration curve in the external validation cohort.

were relatively small. Therefore, the nomogram established needs further robust prospective validation with larger sample size.

CONCLUSION

This study indicates that elderly age (>65 years), the high SOFA score (>3), HCO_3^- > 28 mmol/l, Lac (>2 mmol/l), BUN (>21 mg/dl), albumin (<3.5 g/dl), TnT (>0.04 ng/ml), and CK-MB (>5 ng/ml) are the independent risk factors in 28-day mortality of patients with AHF. Among these indicators, age > 65 years (HR = 2.47), the SOFA score ≥ 9 (HR = 3.29), and TnT (HR = 4.02) are the leading three predictors of 28-day mortality of patients with AHF. Additionally, we develop and further validate a nomogram for individualized predicting the short-term mortality once patients with AHF are admitted to the ICU. Patients at high risk of mortality are supposed to assign a higher level of active monitoring and earlier and a more intensive treatment. Meanwhile, patients estimated to have a relatively good prognosis may be suitable candidates for routine treatment, although individual factors and preferences of the patient would still require careful consideration.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

YM, XW, QW, HW, and SL organized the database. YM, XW, and JY performed the statistical analysis. All authors contributed to the conception and design of the study, wrote the first draft of the manuscript, wrote sections of the manuscript, contributed to manuscript revision, read, and 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.2021.741351/full#supplementary-material

Supplementary Figure 1 | The proportional hazards assumption was assessed by plotting Schoenfeld residuals vs. time and examining their correlation.

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Investigation of the Effects of Home-Based Exercise and Cognitive Training on Cognitive and Physical Functions in Cardiac Patients: The COVEPICARDIO Study Protocol of a Randomized Clinical Trial

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Introduction: During the COVID-19 pandemic, confinement measures are likely to produce collateral damage to health (stress, confusion, anxiety), especially in frail individuals and those living with cardiovascular disease (CVD). In cardiac patients in particular, these measures dramatically increase the level of physical inactivity and sedentary lifestyle, which can decrease cardiorespiratory capacity and increase the risk of acute events, rehospitalization, and depressive syndromes. Maintaining a minimum level of physical activity and cognitive stimulation during the COVID-19 crisis is essential for cardiac patients. This study is designed to document the effects of 6 months of home-based physical exercise alone or combined with cognitive training on cognitive and physical functions in patients with CVD over 50 years old.

Methods and Analysis: 122 patients (>50 years old) with stable CVD and no contraindication to perform physical exercise training will be recruited and randomly assigned to one of the 2 following arms: (1) Home-based physical exercise alone, (2) Home-based physical exercise combined with cognitive training. The intervention lasts 6 months, with remote assessments performed prior to, mid and post-training. A follow-up 6 months after the end of the intervention (12 month) is also proposed. The primary outcome is cognition, including general functioning (Montreal Cognitive Assessment (MoCA) score), as well as performances on measures of executive functions, processing speed, and episodic memory. The secondary outcome is physical performance, including balance, gait and mobility, leg muscle strength and estimated cardiorespiratory fitness. Tertiary outcomes include mood, anxiety, and health-related quality of life as assessed by self-reported online questionnaires.

Discussion: With the COVID-19 crisis, there is a critical need for remote exercise and cognitive training, and to further investigate this topic, in particular for cardiac patients. The present context can be viewed as an opportunity to perform a major shift from center-based programs to home-based physical exercise. This is especially important to reach out to older adults living in remote areas, where access to such interventions is limited.

ClinicalTrials.gov: [https://clinicaltrials.gov/ct2/show/NCT04661189], NCT04661189.

Keywords: cardiovascular diseases, COVID, cognition, exercise, rehabilitation, physical activity

INTRODUCTION

Because of the COVID-19 pandemic, the population has been forced to home confinement since spring 2020, drastically reducing social interactions and altering physical activity and eating behaviors (1). This sudden lockdown radically changed the population's lifestyle (gym services are also closed), with activities mainly limited to essential needs (e.g., grocery, pharmacy, or physician visits). This confinement may also have collateral effects on cognitive and physical health (2), especially in individuals at high risk of cognitive decline like older adults (3) and patients with cardiovascular diseases (CVD) (4). Hospitals and clinics must reorganize their care services, while limiting contact between individuals. Ambulatory visits and non-essential services have been reduced, like center-based cardiac rehabilitation programs (CR) stopped or delayed, despite the fact that they are a class I level A recommendation with clinical benefits that are now well documented (5, 6). Thus, effective solutions are needed to enhance cardiovascular health and cognition in patients with CVD, while maintaining social distancing during this pandemic period. Furthermore, the pandemic context will likely persist, and full participation in center-based CR or usual exercise habits might take time or be postponed. Maintaining a minimum of physical activity during the COVID-19 crisis is essential for cardiac patients, with the advice of the medical team who could prescribe remote home-based exercise training (6, 7). Remote supervision of exercise is effective on health outcomes and safe in patients with stable CVD (6, 8–10). It is now well established that physical activity positively impacts health outcomes and cognition in older adults with CVD (11, 12). Cognitive training also enhances cognitive performances in older adults (13) and in cardiac patients post-surgery (14). Moreover, cognitive training and exercise could have a synergistic effect on cognition in healthy older adults compared to exercise training alone (15, 16). However, this synergistic effect has never been investigated in older adults with CVD. Furthermore, while there is increasing interest for multidomain interventions combining cognitive and exercise training to improve cognition in older adults, there is still a lack of knowledge on how much benefits can be gained by adding cognitive training to physical exercise, and regarding the optimal dose of each type of intervention. The COVID-19 crisis has put forward the relevance of home-based training. The present context can be viewed as an opportunity to promote a major shift in remote non-pharmacological interventional programs, so that a larger number of individuals could benefit from them.

The COVEPICARDIO project will investigate the effects of home-based physical exercise with or without cognitive training, on cognitive and physical functions in older women and men with stable CVD.

Hypotheses: 1/ After 6 months of home-based physical exercise, cardiac patients will show an improvement in cognitive (primary outcome) and physical measures (secondary outcomes), with larger gains in executive functions. 2/ Cardiac patients randomized in the combined home-based physical exercise and cognitive training group will show greater improvements in cognition and physical functions post-intervention compared to cardiac patients who did not complete the cognitive training.

METHODS AND ANALYSIS

Study Design

The COVEPICARDIO study will be a prospective, single-blind, randomized trial with two parallel intervention arms (1:1): 1/ Physical exercise: remote monitoring and coaching of home-based physical exercise, 2/ Physical exercise, and cognitive training: remote monitoring and coaching of home-based physical exercise and cognitive training. The intervention will last 6 months. Three testing periods lasting 7 to 10 days each will be performed remotely at baseline, three (mid-training) and 6 months (post-training), using videoconference supervision and online questionnaires. Six months after the end of the intervention (12 months), a follow-up assessment will also be performed to explore the potential retention of intervention effects. Since this follow-up does not directly address the main hypotheses, participation will be optional.

Participants

A total of 122 participants with stable CVD will be included.

Inclusion Criteria

Participants will be adults aged 50 and older who have access to Wi-Fi connected to the internet and possess a tablet (e.g., iPad or Android) or a computer. Individuals will be included if they have stable coronary artery disease, stable chronic heart failure, corrected valvular heart disease, or atrial fibrillation, with a low-risk profile, as determined by the physician, and with no contraindication to exercise training.

Exclusion Criteria

1/ Non-cardiopulmonary limitation to exercise (e.g., severe arthritis) or a severe exercise intolerance. 2/ Severe respiratory disease (e.g., COPD, severe COVID-19 related symptoms, severe asthma). 3/ Significant cognitive impairment (i.e., score of 18/23 or lower on the telephone version of Mini-Mental State Examination, MMSE) (17). 4/ Contraindications to exercise testing/training (18) (e.g., uncorrected severe aortic stenosis, severe pulmonary hypertension, severe non-revascularizable coronary disease including left main coronary stenosis, significant myocardial ischemia or arrhythmia during low-intensity exercise).

Interventional Methods

All assessments, exercise monitoring, and cognitive training will be performed remotely. Patients will be identified and recruited by Montreal Heart Institute's (MHI) physicians. During a pre-screening phone call, individuals will be asked about their interest in taking part in the study and whether they possess the proper setup to access the online tools. After a short medical questionnaire, an appointment will be fixed for a preliminary session; they will receive the information and consent form by email prior to this session. During the preliminary session, a staff member will provide details about the consent form. Participants will then be given the opportunity to provide oral consent. They will then be asked to send a written consent by email. If an oral consent is obtained, the cognitive [the telephone version of the Mini Mental State Examination; MMSE (19)] and physical (Physical Activity Readiness Questionnaire; PARQ+) screenings will be performed with a research assistant and will later be reviewed by a neuropsychologist and a kinesiologist, respectively. Once the research team receives the written consent by email and ensures eligibility, the participant will be considered enrolled. A technology tutorial will then be scheduled to ensure that internet access and tools are ready and sufficiently mastered by participants prior to the remote assessments. After baseline assessment, participants will be randomized into one of the two arms. Participants will receive automatic standardized communications by email to transmit study information like appointment scheduling, links to self-reported online questionnaires, and training guidelines. An online platform will centralize all the follow-up information. Staff members will have distinct access to either assessment or training information according to their role, to preserve the study blinding. A non-overwriting process will ensure data integrity: all form submissions will be recorded in a journal fashion.

Exercise and Cognitive Intervention

Participants will receive a group-specific training guide according to their randomization. This training guide contains only recommendations about the nature, intensity, and frequency of training sessions. Participants will then attend an introductory session over the phone given by their training coach.

Exercise Monitoring

Participants will be encouraged to complete a home-based physical exercise using video capsules available via Facebook or Youtube, created by the Centre EPIC of the MHI's team of kinesiologists (20). Aerobic, muscular strengthening, flexibility, and/or balance exercises are proposed in these videos, and do not require any equipment. Each video lasts ∼15 min and includes a 5-min warm-up, followed by a 10-min training, and finally by a 2-min cool-down period. Guidelines are provided by kinesiologists to allow participants to adapt the exercise to their physical capacities. Participants will be invited to perform exercise sessions at least 5 times a week. The exercise sessions can be performed at home using the video capsules, or with other video or web-based training programs. Participants can also engage in outdoor activities (e.g., walking or cycling). To track their adherence, the duration, intensity, and nature of the activity of each training session will be reported by the participant in a journal, that they will transmit to their training coach during their weekly follow-up call. The Borg Rating of Perceived Exertion (RPE) graduated from 0 to 10 will be used by participants to assess the intensity of their exercise sessions.

Cognitive Training

The combined intervention will include home-based cognitive training in addition to physical exercise as described in the previous section. Participants will be asked to complete their home-based cognitive training prior to exercise training or at least 3 h post-exercise session (21). Participants will also be asked to perform their cognitive training in a comfortable position, while limiting distraction. Validated computerized cognitive training tasks, as well as video capsules will be provided. Participants will be encouraged to complete a minimum of 3 sessions per week (15–20 min/session, maximum 1 session/day), i.e., two computerized cognitive training sessions and one video capsule session. Three tasks will be available during the computerized cognitive training sessions: 1/ dual-task training, during which participants must maintain and prepare for several response alternatives and must share attention between two concurrent tasks (i.e., Dual-task), 2/ inhibition training, during which participants must refrain from giving an automatic response (i.e., Stroop task), as well as 3/ working memory training, in which participants must maintain and update information in working memory to recall items presented earlier (i.e., N-Back task). Each cognitive task lasts approximately 15 min and comprises its own sets of visual stimuli (e.g., letters, numbers, symbols) and matching button symbols. Participants will be asked to perform the tasks as fast as possible while maintaining accuracy. The three tasks will be presented in a fixed order for all participants. Task difficulty will increase gradually over the course of the participant's cognitive training to avoid task automatization and to maintain stimulation (22). Live visual feedback will be provided during training, as well as a histogram of daily performances to encourage improvement. Once per week, participants will be asked to complete a strategy-based training, via video capsules. This training is an adapted version of the MEMO+ program, which has been validated previously (23). Participants will learn different mnemotechnics (e.g., face-name association, visual imaging), and will learn about age-related changes in memory. In addition, some video capsules are targeted to help participants develop strategies to cope with

multiple aspects of their daily life, such as sleep, anxiety, and nutrition. To track cognitive training adherence, participants will be asked to complete a follow-up agenda and to indicate days and times when they completed cognitive training sessions. Training adherence for computerized cognitive training tasks will also be monitored online.

Remote Monitoring and Coaching

Once a week, kinesiologists will call participants to (1) motivate them to stay engaged in their training, (2) ensure the safety of the exercise training sessions, and (3) to collect exercise and cognitive training data reported by participants in their follow-up agenda during the previous week. The kinesiologists will also provide updated information about training resources available online, track participant's adherence, and advise participants according to their training characteristics and study training guidelines.

Measurements and Outcomes

Participants will complete videoconference assessments prior to the intervention, mid-intervention (3 months), post-intervention (6 months), as well as 6 months following the end of the intervention (12-month follow-up). All the testing procedures are reported in **Figure 1**. At baseline, demographics, and medical information, including chronic diseases, comorbidities, and medications will be collected by medical questionnaire. A COVID-19 questionnaire (i.e., QCOVID) will address the social and financial consequences of the COVID-19 pandemic on participants, as well as its impact on their physical activity routines.

Primary Outcome: Cognitive Performances

The primary outcome of the study will be cognitive performances. Participants will be tested for general cognitive functioning using a remote version of the Montreal Cognitive Assessment (MoCA) (24). Also, three main components of cognition will be assessed with a comprehensive neuropsychological evaluation, from which composite z-scores will be computed: executive functions, processing speed and episodic memory. The neuropsychological assessment will include the following tests that will be administered in a predetermined order: Rey auditory verbal learning test, Digit Span, oral Trail Making Test (25), Phonological and Semantic Fluency from the D-KEFS battery (26), and Similarities subtest from the WAIS-IV (27). The neuropsychological assessment, including the MoCA, will be performed via videoconference (Zoom). All tests have been validated for remote administration and have previously been used in large participant cohorts (28). Importantly, these tests are validated and normed for an older adult population (17, 21, 29). The similarities subtest will only be completed at baseline to obtain a measure of crystallized intelligence; its completion will be optional and performed according to participant's fatigue. Participants will also realize a computerized or tablet-based cognitive assessment (30). The three computerized cognitive tasks include a dual-task (30), Stroop task, and N-back. Participants will complete trained and transfer versions (same tasks but with different stimuli) that will be presented in a counterbalanced order between participants, via a latin-square procedure. Comparing trained and transfer tasks allows to quantify participants' ability to transfer cognitive training effects to novel tasks. Subcomponents of each task allow dissociating different attentional control mechanisms from mere cognitive speed. Importantly, the tasks record response time in milliseconds, which reduces the likelihood of ceiling effects. These tasks also tend to be more sensitive to training effects. Finally, computer equipment (i.e., computer or tablet) used by participants for the computerized tasks will be documented. Participants will be asked to use the same computer equipment for the four testing periods (including the 12-month follow up).

Secondary Outcome: Physical Functions

Physical functions will be assessed remotely by videoconference. First, balance will be assessed with a one-leg balance test (i.e., stay on one leg as long as possible for a maximum of 60 s; recorded twice for both legs). Lower limb muscle strength will be evaluated with the Five-Time-Sit-to-Stand test (i.e., five consecutive sit-to-stand movements performed as fast as possible without using arms as leverage). The 4-meter walk test and the Timed Up-and-Go (TUG) performed at spontaneous and fast speeds will be used to assess mobility. During the 4-meter walking test, participants' walking speed will be measured in a straight line over 4 meters. During the TUG, participants will be timed for the following sequence: getting up from a chair, walking 3 meters, turning around, and sitting back in the chair. These four tests are commonly used in a clinical setting and have demonstrated their associations with global cognition and specific cognitive domains such as processing speed and executive functions (31). During the physical function session, participants will be first questioned about their history of musculoskeletal and sensory conditions to ensure that they can safely execute the tests. Prior to the physical function assessment, participants will receive by email with a detailed description of each test with its set-up and demonstration videos. Then, at the beginning of the session, the testing set-up will be verified by the research assistant or kinesiologist, to ensure there is sufficient space to perform the tests, as well as a sufficient accuracy for both 4 and 3-m of the walking and TUG tracks measures. During the testing, adequate visual feedback will always be maintained to ensure participant's security and reliability of the measures. Finally, to complete the physical function assessment, the Matthews questionnaire will be used to predict participants' cardiorespiratory fitness (i.e., $VO2_{max}$), which is based on sex, age, anthropometric data, and self-reported physical activity level (32).

Tertiary Outcomes

The following tertiary outcomes will be measured with validated self-reported questionnaires that participants will fill in through online forms: **1/ Sleep:** Participants will complete the Pittsburgh Sleep Quality Index (PSQI) and the Berlin Questionnaire. PSQI scores range from 0 to 21, a lower score indicating better sleep quality. The Berlin Questionnaire will determine the risk of sleep apnea. **2/ Anxiety/mood:** The Geriatric Depression Scale (GDS), the Perceived Stress Scale, the SF-12 (quality of life), the State-Trait Anxiety Inventory, the Anxiety Sensitivity Index, the Intolerance of Uncertainty scale, the Perceived vulnerability

Time point	t_{-1}	t_0	t_1	t_2	t_3
	baseline		3 months	6 months	12 months
Enrolment:					
Eligibility screening	X				
Informed consent	X				
PARQ+ and Mini-Mental State Examination	X				
Tech tutorial		X			
Interventions:					
1/ Exercise monitoring		←	—	→	
2/ Exercise monitoring and cognitive training		←	—	→	
Assessments:					
Neuropsychological assessment[1]		X	X	X	X
iPad Assessments (transfer tasks)		X	X	X	X
iPad Assessment (trained tasks)		X	X	X	X
Physical function assessment[2]		X	X	X	X
Self-reported questionnaires[3]		X	X	X	X

FIGURE 1 | Schedule of enrolment, interventions, and assessments according to SPIRIT guidelines. [1]Montreal Cognitive Assessment, Trail Making Test, Verbal Fluency Test, Digit Span Test, Similarity Test, Rey Auditory Verbal Learning Test. [2]One leg balance test, 5-time Sit to Stand test, Timed up and go test, 4-meter walking speed test. [3]Matthew questionnaire for estimated cardiorespiratory fitness, SF-12, Pittsburgh Sleep Quality Index and Berlin Questionnaire, Short Diet Questionnaire, Stait Trait Anxiety Inventory, Geriatric Depression Scale, Perceived Stress Scale, Perseverative Thinking Questionnaire, Intolerance of Uncertainty Scale, Connor Davidson Resilience Scale 10, Anxiety Sensitivity Index, Social and Community Involvement Questionnaire, Lubben Social Network Scale. The following questionnaires will be completed at baseline only: Medical questionnaire, Q-COVID questionnaire, Physical Activity Scale for the Elderly, Cognitive Reserve Questionnaire, Bem sex-role Inventory.

to disease, the Connor-Davidson Resilience Scale 10, and the Perseverative Thinking Questionnaire, will be used to assess mood and anxiety. **3/ Cognitive reserve:** will be evaluated at baseline. To do so, participants will provide their total years of education and will complete a modified version of the Rami and colleague's cognitive reserve questionnaire (33), adapted for French and English by the CIMA-Q team (17, 34). **4/ Social support:** Social and Community Involvement Questionnaire (35) and the Lubben Social Network Scale will be completed. **5/ Diet:** Participants will complete the Short Diet Questionnaire (36).

6/ Prior physical activity: Participants will fill in the Physical Activity Scale for the Elderly (37). **7/ COVID-19 impact:** The QCOVID questionnaire will be completed at baseline only. **8/ Gender:** The Bem Sex-Role Inventory will be filled at baseline only. All questionnaires will be filled in by participants using anonymous online forms.

Research Plan

Following the preliminary call, five videoconference sessions will be scheduled with the participants. The first session will be a technology tutorial that will ensure that internet connectivity and tools are ready and mastered enough by participants to engage in testing. Then, participants will complete 4 pre-testing sessions (T0), 4 mid-testing sessions (T1 at 3 months), 4 post-testing sessions (T2 at 6 months), and 4 optional follow-up sessions (T3 at 12 months). Total participation in this study is expected to be of a 12-month duration, including 6 months of intervention (**Figure 1**).

Data Analysis

Sample Size Calculation

The team of biostatisticians from the Montreal Health Innovations Coordinating Centre (MHICC) performed the sample size calculation on the primary outcome, i.e., the differential effect of physical exercise training alone vs. the combined physical and cognitive training on cognitive performance (executive functions). The calculation was based on existing values available in the literature for physical exercise training alone, as well as on different plausible effect sizes for the combined physical and cognitive training (no existing values in the literature for this group), and on clinically relevant effect sizes. The calculation revealed that a sample size of 49 subjects in each group will have 80% power to detect a difference in means of−0.186 (difference between physical training alone mean of 0.239 and combined training mean of 0.425, for an effect size of 0.624), assuming that the common standard deviation is 0.325 (slightly larger than in a traditional context because of the home-based administration of neuropsychological tests) using a two-group *t*-test (0.050 two-sided significance level). As we expect a 20% attrition rate based on our previous exercise studies, we will thus recruit 61 participants per intervention arm, for a total of 122 patients with CVD. Participants will be randomized in one of the 2 arms: home-based physical training alone or combined physical and cognitive home-based training. A biostatistician from the MHICC generated the randomization sequence.

Statistical Analysis

The variables in the study will be presented using descriptive statistics. The mean, standard deviation, median, minimum, Q1, Q3 and maximum will be presented for continuous variables. The number and percentage will be presented for nominal/ordinal variables. The assumptions of the statistical tests will be examined, and data transformation or non-parametric analyses may be used as appropriate. SPSS and SAS software version 9.4 or higher will be used to conduct the analyses.

Mean changes in cognitive performance from baseline will be analyzed using a repeated measures analysis of covariance (ANCOVA) model, adjusted for age, sex, education, including the effects of the intervention as between-subject variable (physical exercise alone or combined with cognitive training) and time as within-subject variable (pre at baseline, mid at 3 months, post-intervention at 6 months). Observation of a statistically significant difference in the primary outcome between pre- and post-intervention timepoints will be considered as the evidence of the intervention's efficacy (i.e., primary hypothesis). The interaction between intervention arms (i.e., physical exercise alone or combined training) and performance changes from pre to post-intervention will address the added value of cognitive training compared to physical exercise alone (i.e., secondary hypothesis). Secondary and exploratory outcomes will be analyzed as the primary outcome.

Blinding

Assessors performing the evaluations and investigators will be blinded to group allocation. The statistician will be blinded until completion of the statistical analyses. Only the kinesiologists executing the weekly follow up of exercise/cognitive training program will be aware of the assigned intervention. Kinesiologists will not take part in any assessment.

DISCUSSION

The latest WHO Guidelines on physical activity and sedentary behavior recommends for adults and older adults with chronic conditions, such as CVD, at least 150–300 min of moderate-intensity aerobic physical activity, or at least 75–150 min of vigorous intensity aerobic physical activity, or an equivalent combination of moderate- and vigorous-intensity activity throughout the week (30). With the COVID-19 crisis and the confinement measures in place (e.g., closed gyms), these recommendations are difficult to meet (1, 38, 39). Among the 1,098 Canadian adults included in the study by Lesser et al., 40% of inactive individuals became less active during the restrictions of daily living caused by the COVID-19 pandemic (39). In cardiac patients, who already tend to have a sedentary/inactive profile, the confinement measures excessively increase the level of physical inactivity and sedentary lifestyle, which can further deteriorate cardiovascular health and increase the risk of acute events (4). Frail individuals, older adults, and individuals with chronic conditions such as CVD are at higher risk to suffer from the direct and collateral consequences of the COVID-19 pandemic. Hence, allowing older adults with CVD to maintain and/or improve their cognitive and physical health may help to prevent a possible secondary crisis caused by the long-term alteration of their health condition. Our home prevention strategies allow patients, including those living in remote areas, to have cognitive and physical stimulation for 6 months.

Exercise and cognitive training separately have demonstrated enhancements of multiple and specific aspects of cognitive and physical functions and could have a synergistic effect on cognition in healthy older adults (15). Individuals with CVD who participated in a CR program improve their cognitive

performances (12). In addition, the synergistic effect of exercise associated with cognitive training on cognition has never been shown in older adults with CVD. Thus, some questions need to be addressed regarding how much benefits can be gained by adding cognitive training to physical exercise and regarding the optimal dose of each type of intervention.

This trial proposes crisis-adapted lifestyle interventions and remote assessments. More precisely, to further ensure the continuity and the regularity of the exercise training, the proposed trial offers weekly phone call support and an online video training program, providing a wide range of exercises to help participants maintain their exercise routine even during the lockdown. In CVD, among the patients who complete a phase II center-based CR about half of the patients tend to return to their previous lifestyle habits, become sedentary again after a few months, and are non-adherent to physical activity target levels recommended by physicians (40, 41). Home environment with regular contact with telephone support or videoconference from the medical or research team may be more likely to help sustain positive physical and psychosocial changes over time, compared to institution-based programs (42–44).

Most participants in exercise clinical trials come from urban areas, which likely limits generalization of findings, and individuals from rural communities are still underrepresented (45). Home-based interventions, made available for all, would address this problem, and offer a great solution to counteract sedentary/inactivity behavior in the COVID-19 pandemic situation. A home-based combined intervention with exercise and cognitive training could help to maintain and enhance cognition and cardiovascular health in older adults with CVD who could no longer access training facilities during the COVID-19 lockdown.

Study Limitations

Some limitations of our trial need to be mentioned. First, our design does not include a control group without intervention. Because of the pandemic context, some ethical considerations refrained the research team from implementing a control group without any intervention. However, to partially address this limitation, in our analysis we will take into account the dose response effect as a function of the volume of training performed by participants. Based on the nature of each activity, its intensity and its frequency, a weekly dose of physical exercise (converted in kcal and in METs using the Compendium of Physical Activities) will be computed. The effect of physical exercise dose on cognitive and physical functions will be evaluated according to participant's mean weekly dose during the 6 months of intervention. Based on the overall study sample distribution, participants could be classified into "higher," "medium," and "lower" doses of physical exercise (i.e., tercile split).

Secondly, our participants do not have precise control of the intensity and duration of the exercise sessions (heart rate monitors, pedometers, or other tools). This is only declarative data reported weekly with the kinesiologists. Our study focuses on exercise and cognitive training in CR's phase III and should not be confused with a comprehensive CR program delivered in phase II. Adherence to medication, diet and appropriate teaching about patient's risk factors and CVD are critical aspects of a comprehensive CR program (phase II). Consequently, we will include some patients who have completed a CR program and others who have not benefited from this program. Then, non-exercise prediction of cardiorespiratory fitness with an equation is valid for healthy individuals, but little is known about its reliability in patients with CVD. Moreover, variability was observed in the accuracy between non-exercise prediction equations and the ability of equations to detect changes in cardiorespiratory fitness (46). Therefore, the use of non-exercise prediction equations could lead to a significant error in the estimated change of participants' cardiorespiratory fitness. Finally, sex was not taken into account in recruitment and randomization processes, although sex differences have been reported on cognition (47), as well as in exercise-training effects on cognition in healthy older adults (48). No study to date has documented potential sex-related differences with regards to the effects of exercise, cognitive or combined training on cognition and physical outcomes in older adults with CVD. Given the sex-related differences in cognition, exercise intervention effects and diseases, sex differences are to be expected in the present study.

Trial Status

The COVEPICARDIO study is the fourth version of the protocol validated by the Research Ethics Board of Montreal Heart Institute in October 2020. Recruitment for the study started May 20, 2020 and is planned to be finished in September 2021.

ETHICS STATEMENT

The study protocol was approved by the Montreal Heart Institute's (MHI) Research Ethics Board (FWA00003235; research project: ICM 2020-2785). All participants will provide informed consent prior to starting the study.

AUTHOR CONTRIBUTIONS

LB, FB, ED, CG, MG, TV, C-AB, C-AG, KS, and PV: conception and design of the research. LB: principal investigator. AN: co-investigators. FB, ED, CG, MG, TV, C-AB, C-AG, KS, BB, MO, and PV: collaborators. FB wrote the first version of the manuscript, drafting, and revision of the manuscript. All authors revised it and contributed significantly to write the final version that was accepted.

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ORIGINAL RESEARCH
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Stable Rates of Obstructive Hypertrophic Cardiomyopathy in a Contemporary Era

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Hypertrophic cardiomyopathy is the most common genetic heart disease in the US, with an estimated prevalence of 1 in 500. However, the extent to which obstructive hypertrophic cardiomyopathy is clinically recognized is not well-established. Therefore, the objective of this study was to estimate the annual prevalence of clinically diagnosed oHCM in the US from 2016 to 2018. Data from the MarketScan® database were queried from years 2016 to 2018 to identify patients with ≥1 claim of oHCM (International Statistical Classification of Disease and Related Health Problems diagnosis code: I42.1). Prevalence rates for oHCM were calculated and stratified by sex and age. In 2016, 4,612 unique patients had clinical diagnosis of oHCM, resulting in an estimated oHCM prevalence of 1.65 per 10,000. The prevalence of oHCM in males and females was 2.07 and 1.26, respectively. Prevalence of oHCM was highest in patients 55–64 years of age (4.82). Prevalence of oHCM generally increased with age, from 0.36 per 10,000 in those under 18 to 4.82 per 10,000 in those 55–65. Trends in prevalence of oHCM over time, including by sex and age group, remained similar and consistent in 2017 and 2018. The prevalence of oHCM was stable over the 3-year time period, including higher rates of oHCM in males and patients aged 55–64 years. These results suggest that the majority of privately insured patients with oHCM are undiagnosed in the US and reinforce the need for policies and research to improve the clinical identification of oHCM patients in the US.

Keywords: obstructive hypertrophic cardiomyopathy, hypertrophic cardiomyopathy, prevalence, claims data, sex, age

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease in the United States (US), with an estimated prevalence of 1 in 500 persons from echocardiography-based studies (1). Patients with HCM are at risk for heart failure, stroke and cardiac arrhythmias, including sudden cardiac death (2). Among patients with HCM, approximately two thirds have obstruction (oHCM) either with rest or with provocation (3). Obstruction has been considered the

primary cause of symptoms for patients and is associated with worse clinical outcomes (3, 4). In 2013, the HCM diagnosis rate was estimated at 1 in 3,000 persons (0.03%) in the US, based on an analysis of private practitioner and healthcare system medical claims (5). Maron et al. (5) identified HCM in about 100,000 patients, but it is estimated that 700,000 people in the US may be affected by HCM (1, 5). These existing studies show that the majority of HCM patients in the US are clinically unrecognized and the number of diagnosed HCM patients is significantly lower than the estimated prevalence of HCM in the general population (5).

However, these previous studies in the US have reported on the prevalence of HCM in its entirety and have not specified prevalence rates of patients with obstruction. Patients with obstruction may benefit from guideline directed medical therapy including beta-blockers, calcium channel blockers, and disopyramide (6, 7); and in the future, from emerging medical therapies currently being tested in clinical trials (8–11). Focusing on oHCM prevalence in the US will allow us to estimate the number of patients that are symptomatic and require these contemporary treatments by experienced referral centers. A recent study analyzing obstruction in patients that underwent cardiac magnetic resonance imaging from the United Kingdom (UK) Biobank (UKBB) reported a prevalence of oHCM of 1 in 517 persons (0.19%) (12). Similarly, we sought to close the gap on the unidentified prevalence of patients with obstruction in the US.

No follow-up studies have evaluated prevalence of clinically recognized oHCM in the US in recent years, and trends of oHCM prevalence over time using medical and pharmacy claims data is unknown. Understanding the true prevalence of clinically diagnosed patients with oHCM in the US population may improve the screening, identification and treatment of patients; specifically, those who do not seek specialty care for their disease at an HCM Center of Excellence. Therefore, we estimated the annual prevalence of clinically diagnosed oHCM in the US from 2016 to 2018, including by sex and age.

MATERIALS AND METHODS

Data Source

This retrospective observational study used the MarketScan® Commercial Claims and Encounters Database from IBM Watson Health® (MarketScan), which contains de-identified, patient-specific data on reimbursed healthcare claims for employees, retirees, and their dependents of over 250 medium and large employers and health plans. Individuals included in the database are covered under private insurance plans; no Medicaid or Medicare data are included. These data cover approximately 28 million covered employees and family members per year. MarketScan is divided into subsections including: Inpatient Claims file, Outpatient Claims file, Outpatient Prescription Drug Claims file, Enrollment Information file, and RED BOOK™ Supplement.

Analysis

Analyses were undertaken with SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). Patients with clinically diagnosed oHCM were identified by the presence of at least 1 claim with a diagnosis of oHCM (ICD-10 code I42.1). The number of unique patients with clinically diagnosed oHCM was determined for each year, and the annual prevalence rate for diagnosed oHCM was defined as the number of patients diagnosed with the disease divided by the total number of health plan members at risk during the calendar year (**Figure 1**). Prevalence of oHCM was reported per 10,000 persons and stratified by sex and age group (<18, 18–34, 35–44, 45–54, 55–64, ≥65). Two population proportion tests addressed statistical significance of categorical variables. Tests were 2-sided and $P < 0.05$ was considered statistically significant. This study was approved by the Penn State College of Medicine Institutional Review Board.

RESULTS

In 2016, 4,612 unique patients had a claim with a clinical diagnosis of oHCM, resulting in an estimated oHCM prevalence of 1.65 per 10,000. Prevalence of oHCM in MarketScan shows the prevalence of oHCM remained similar in 2017 and 2018: 1.61 and 1.58, respectively (**Table 1**). Prevalence Rates of oHCM reports the prevalence of oHCM in 2016 was higher in males than in females (2.07 and 1.26, respectively) (**Figure 2**). Trends of prevalence by sex remained similar over the time-period and prevalence of oHCM in males remained higher than in females from 2017 to 2018 ($p < 0.00001$). While the overall prevalence of oHCM decreased slightly over the period, it increased with age.

Prevalence of oHCM by Age Group shows an increased trend of oHCM with age over the time period, beginning with a 2016 oHCM prevalence by age: 0.36 (<18 years) to 2.36 (≥65 years) ($p < 0.00001$) (**Figure 3**). Prevalence of oHCM by age group in 2016 included 0.36 (<18 years), 0.62 (18–34 years), 1.21 (35–44 years), 2.43 (45–54 years), 4.82 (55–64 years), 2.36 (≥65 years). The figure shows consistent trends in oHCM by age group across the latter 2 years ($p < 0.00001$), with oHCM prevalence in 2018 being 0.31 (<18 years), 0.54 (18–34 years), 1.12 (35–44 years), 2.37 (45–54 years), 4.84 (55–64 years), 2.73 (≥65 years). Over the 3-year time period, the prevalence of oHCM remained the greatest in patients 55–64 years of age.

DISCUSSION

The objective of this study was to estimate the annual prevalence of clinically diagnosed oHCM in the US from 2016 to 2018, including by sex and age. To our knowledge, there is limited evidence on the use of real-world data to analyze prevalence of oHCM in the general US population, or trends of oHCM prevalence over time. Using a large, national database of private insurance claims, we found that the prevalence of oHCM was 1.65 per 10,000 individuals in 2016 and remained fairly stable over the 3-year time period. This is considerably lower than the

Abbreviations: HCM, hypertrophic cardiomyopathy; US, United States; oHCM, obstructive hypertrophic cardiomyopathy; ICD-10, International Statistical Classification of Disease and Related Health Problems diagnosis code, version 10.

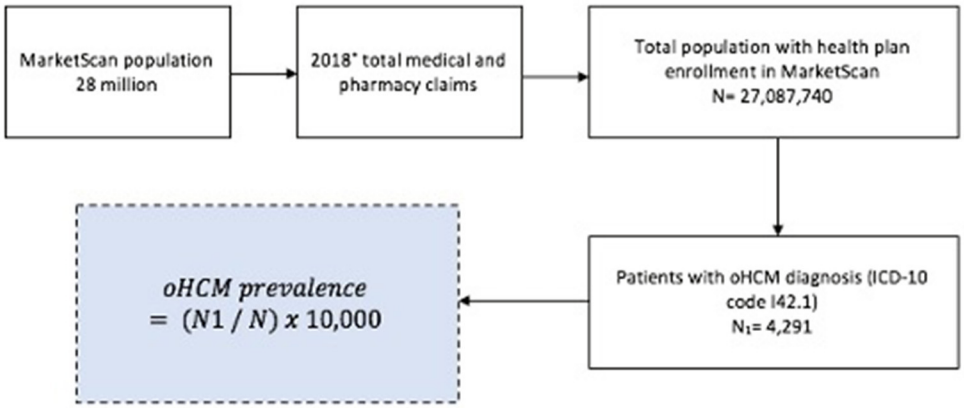

FIGURE 1 | Flow diagram for US prevalence of oHCM in 2018. US prevalence of oHCM in 2018 from MarketScan. oHCM, obstructive hypertrophic cardiomyopathy; ICD- 10, International Classification of Diseases, Clinical Modification Ninth and Tenth. *Prevalence was calculated for all years from 2016–2018.

TABLE 1 | Prevalence of oHCM in MarketScan (per 10,000 persons).

		Sex			Age Group (years)						
Year	**oHCM**	**Male**	**Female**	***P*-value**	**<18**	**18–34**	**35–44**	**45–54**	**55–64**	**≥65**	***P*-value**
2016	1.65	2.07	1.26	<0.00001	0.36	0.62	1.21	2.43	4.82	2.36	<0.00001
2017	1.61	2.00	1.24	<0.00001	0.36	0.57	1.15	2.38	4.75	1.81	<0.00001
2018	1.58	1.97	1.22	<0.00001	0.31	0.54	1.12	2.37	4.84	2.73	<0.00001

oHCM, obstructive hypertrophic cardiomyopathy.

rate of patients with obstruction found in a previous cardiac magnetic resonance-based (1 in 517 persons) (12) representing oHCM patients in the UK. These results provide unique data on trends of oHCM over time and stable rates of oHCM over a 3-year period may suggest the gap on identifying patients with oHCM in the US is not getting smaller.

There are many advantages of using claims data for research. Claims data allow for a large, diverse sample of patients treated in various care settings across all geographic regions in the US. The majority of previous studies analyzing patients with oHCM represent single- or multi-center Centers of Excellence, embodying patients in specialty cardiovascular settings receiving high quality care in specific geographic regions. Studies using claims data allow for longitudinal follow-up of patients to evaluate prevalence, demographics, clinical characteristics, outcomes, and patterns of care and changes over time. Additionally, because claims data are used for billing purposes, they are carefully reviewed for accuracy. Claims data are not limited to services provided at one or a few healthcare centers; therefore, capturing data from over 250 employers and health plans is more generalizable to privately insured patients in the US population.

A recent study using the UKBB encompassing 39,551 participants with cardiac magnetic resonance imaging aged 40–69 years across the UK between 2006 and 2010 analyzed cardiovascular phenotypes according to the presence of rare variants in sarcomere-encoding genes (12). de Marvao and colleagues found the prevalence of phenotypic HCM (defined as wall thickness ≥15 mm in the absence of hypertension and valve disease) was 0.19% (1 in 517) (12). This finding on prevalence from the UKBB is much greater than reported in our results (1.65 per 10,000 individuals). Similar to our study, the UKKB is a population-based cohort of patients with oHCM across the UK, whereas our data source (MarketScan) is generalizable to all patients with commercial health insurance in the US. However, a major difference is de Marvao and colleagues identify prevalence of oHCM using guideline recommended diagnostics using cardiac magnetic resonance (12). Patients in our cohort were defined as clinically diagnosed oHCM based on the presence of at least 1 medical claim with a diagnosis of oHCM (ICD-10 code I42.1). Our methods may not be reliable in the absence of patient level anatomic or genetic confirmation.

The only other study to report on prevalence of HCM (not specifying obstruction) in the US using medical and pharmacy claims was by Maron et al. (5). Similar to our methods, Maron et al. used a large proprietary claims database (Symphony Health Solutions) to identify clinically diagnosed HCM in 2013. Their analysis used ICD-9 diagnosis codes to report prevalence of all HCM types (HCM, oHCM, other HCM including non-obstructive) (5), whereas our analysis used ICD-10 diagnosis codes to capture only HCM patients with obstruction. They estimated an overall prevalence of 0.03% and found that

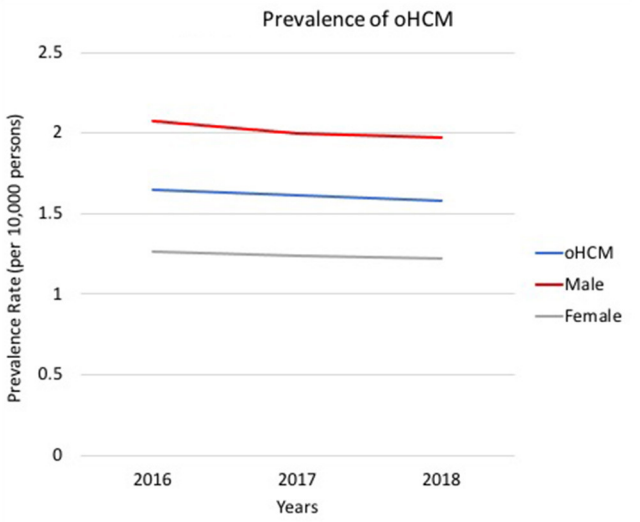

FIGURE 2 | Prevalence of oHCM. Trends of oHCM per 10,000 persons in the US from 2016 – 2018, including by sex. oHCM, obstructive hypertrophic cardiomyopathy; US, United States.

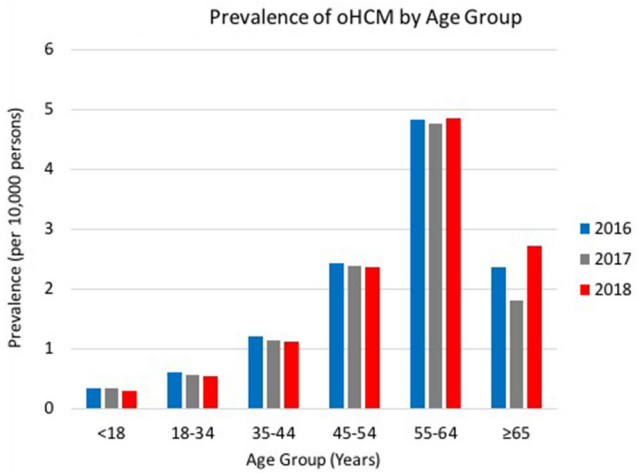

FIGURE 3 | Prevalence of oHCM by Age Group. Trends of oHCM per 10,000 persons in the US by age group from 2016 – 2018. oHCM, obstructive hypertrophic cardiomyopathy; US, United States.

prevalence of HCM was highest in the fifth decade of life, similar to our results. In addition, their cohort was comprised of 43% women (5), similar to our cohort of 40% women. Maron et al. (5) had some important advantages relative to our study. The Symphony Health Solutions database is larger, covering 160 million individual patients (vs. 28 million patients in MarketScan) in the US, and is comprised of all insurance types including Medicare and Medicaid (5). While individuals included in MarketScan are covered under private commercial insurance plans only (no Medicaid or Medicare), our study has unique advantages relative to previous investigations. Our analysis includes contemporary data over a 3-year time period, allowing us to analyze longitudinal trends in oHCM prevalence over time. Additionally, clinical disease identification utilized an updated reporting system of ICD-10 diagnosis codes, which are more specific and generally can be considered a more precise reporting of clinically diagnosed oHCM than previous ICD versions.

The opportunity to access a large national claims database provides a robust and updated presentation of the prevalence of patients with clinically diagnosed oHCM in the general US population. These results suggest that the majority of privately insured patients with oHCM are undiagnosed and are consistent with previous investigations that an overwhelming amount of HCM patients in the US are clinically unrecognized and the number of diagnosed HCM patients is significantly lower than the estimated prevalence of HCM in the general population. This study may reflect actual differences in the underlying prevalence and presentation of oHCM but may also suggest that oHCM is better recognized in families with a history of HCM and patients that have insurance to access specialty healthcare services, including contemporary cardiovascular imaging and genetic testing. The gap of clinical identification remains with patients in the general US population with unknown genetics and/ or cardiovascular medical history. This study reinforces the need for nationwide policies and screening strategies to improve the clinical identification of oHCM patients in the US.

LIMITATIONS

There are several limitations of this analysis that are common in claims data. First, administrative data are primarily collected for billing and reimbursement purposes and may be subject to coding biases, inconsistencies, and missing data. Second, MarketScan includes only individuals with private insurance and does not include Medicaid or Medicare. Thus, the results may not be generalizable to patients with other types of health insurance, who are uninsured, or live outside the US. Third, the use of administrative claims data in this analysis relies on ICD-10 diagnosis codes, which does not include patient level anatomic or genetic confirmation. Fourth, this study did not include patients with non-obstructive HCM. We focused on oHCM in order to estimate the number of patients that are symptomatic and require contemporary treatments by experienced referral centers. Fifth, ICD-10 was not introduced until October 2015, limiting our ability to capture more than 3 full years of data. Sixth, we did not require an additional claim for appropriate drug or septal reduction therapy criteria in our methods which may further distinguish case identification of patients with obstructive HCM; however, this would exclude patients with obstruction that are asymptomatic and not receiving treatment and limit our ability to capture the true prevalence of all patients with obstructive HCM in the MarketScan. A major strength of the study, however, is the ability to observe the healthcare service use of a large, national sample and accurately provide a robust presentation of prevalence trends of patients diagnosed with oHCM in the US.

CONCLUSION

In a large medical and pharmacy claims analysis, the prevalence of clinically recognized oHCM was lower than prevalence rates from echocardiography-based studies, and it remained stable from 2016 to 2018. The prevalence of oHCM was consistently slightly higher in male patients over the study period, with the highest prevalence of oHCM in patients age 55–64 years. These new data support previous studies suggesting that the majority of patients with oHCM are undiagnosed in the US, supporting the need for new strategies to close the gap on unrecognized oHCM. Identifying the true prevalence of clinically diagnosed patients with oHCM may improve early identification and treatment of patients and these results reinforce the imperative need for new policies and future research to improve the clinical identification of oHCM patients in the US.

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

This study was approved by the Penn State College of Medicine Institutional Review Board.

AUTHOR CONTRIBUTIONS

MB and DL conducted the data analysis. MB wrote the first draft of the article with input from the other authors. All authors were involved in the study design, contributed to the interpretation of the results, writing or revision of the manuscript, and approved the decision to submit the article 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.2021.765876/full#supplementary-material

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ORIGINAL RESEARCH
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Serum Chloride Level Is Associated With Abdominal Aortic Calcification

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Background: Abdominal aortic calcification is a potentially important independent risk factor for cardiovascular health. The aim of this study was to determine the relationship between serum chloride level and abdominal artery calcification.

Methods: We obtained the data of 3,018 individuals from the National Health and Nutrition Examination Survey database and analyzed the relationship between serum chloride and abdominal artery calcification. We performed stratified and single factor analysis, multiple equation regression analysis, smooth curve fitting, and threshold effect and saturation effect analysis. R and EmpowerStats were used for data analysis.

Results: Serum chloride is independently related to the AAC total 24 score (AAC-24). The smooth curves fitted were all inverted-U shaped. Below a cutoff value of 92 mmol/L, increase in serum chloride level was associated with increase in AAC-24; however, above that cutoff, increase in serum chloride level was associated with decrease in AAC-24.

Conclusions: At serum levels below 92 mmol/L, chloride is a risk factor for abdominal aortic calcification but levels above 92 mmol/L appear to protect against abdominal aortic calcification.

Keywords: serum chloride, abdominal aortic calcification, association, multiple equation regression analysis, smooth curve fitting

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INTRODUCTION

Aortic calcification is a risk marker for cardiovascular diseases such as coronary artery disease and stroke (1–4). Factors known to be strongly associated with abdominal aortic calcification (AAC) include old age, chronic kidney disease, sex, diabetes mellitus (5), and osteoporosis (6, 7). Several other factors are also probably involved; their identification is essential for effective prevention of AAC.

Serum chloride level has complex effects in humans. It is associated with several diseases, including hypertension (8), heart failure (9), acute pancreatitis (10), acute kidney injury (10), chronic kidney disease (11), pulmonary arterial hypertension (11), cardiorenal syndrome (12), and neuromyelitis optica (13). Changes in serum chloride level—especially increase in serum level—during hospitalization has been shown to be associated with increased in-hospital mortality in patients with chronic kidney disease and pulmonary arterial hypertension (11, 14). In addition, there are studies showing that lanthanum chloride influences bovine vascular smooth muscle cell calcification bi-directionally (15)

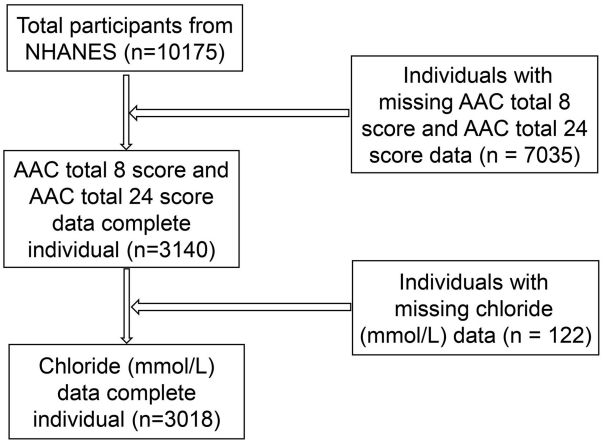

FIGURE 1 | Flowchart of screening participants.

and that magnesium chloride can block tissue valve calcification (16). However, the relationship between serum chloride and AAC in humans has not been well-studied. We speculate that serum chloride could be one such factor.

The aim of this study was to evaluate the relationship between serum chloride and abdominal aortic calcification using a representative sample of elderly individuals from the National Health and Nutrition Examination Survey (NHANES) (17).

MATERIALS AND METHODS

Study Population

The study population was selected from the database of the NHANES—a population-based cross-sectional survey designed to collect information about the health and nutrition of the population of the US. The project surveys a nationally representative sample each year. These populations are located in counties across the country. NHANES interviews collect demographic, socioeconomic, dietary, and health-related information. Physical examinations conducted during the survey include physiological measurements and laboratory examinations (18).

In this study, we retrospectively analyzed the data recorded for the period 2013–2014, which represents one cycle of the NHANES. From among the 10,175 individuals registered in the NHANES database during this period, we eliminated 7,035 because of missing data on AAC Total 24 score (AAC-24) and another 122 because of missing data on serum chloride level. The data of the remaining 3,018 individuals were included in this analysis. **Figure 1** is a flowchart for screening participants.

Abbreviations: AAC-24, AAC total 24 score; ACC, abdominal aortic calcification; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; NHANES, National Health and Nutrition Examination Survey.

Ethics Statement

This study was approved by the Ethics Review Board of the National Center for Health Statistics. Informed consent was not considered necessary as this was an analysis of a database.

Variables

The exposure variable for this study was serum chloride level (mmol/L). Blood specimens were processed and stored in the mobile examination center laboratory, and shipped to the Johns Hopkins University Lipoprotein Analytical Laboratory for analysis. The outcome variable was AAC-24, which was used to describe the amount of calcification. Several studies have shown that lateral spine images obtained with dual-energy X-ray absorptiometry (DXA) for vertebral fracture assessment can detect AAC with reasonably good sensitivity and specificity. The image resolution of lateral spine scans obtained with DXA is close that of a standard radiograph, and radiation exposure is much lower (19, 20). For scoring AAC-24, the anterior and posterior aortic walls were each divided into four segments, corresponding to the areas in front of lumbar vertebrae L1–L4. Within each of the eight segments, aortic calcification was recognized visually as either a diffused white stippling of the anterior and/or posterior aortic walls, or as white linear calcification of the anterior and/or posterior walls. Aortic calcification was scored as "0" if there was no calcification in that segment; "1" if one-third or less of the aortic wall in that segment was calcified; "2" if more than one-third but <two-thirds was calcified; or "3" if more than two-thirds was calcified. The scores were obtained separately for the anterior and posterior aortic wall; thus, the score at each vertebral level ranged from "0" to "6," and the total score from "0" to "24" (20, 21), see **Supplementary Table S1** for details of AAC-24 calculations.

The categorical variables included as covariates in our analysis were sex, race/ethnicity, and education level. The continuous covariates included in our analysis were age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, total serum calcium (mmol/L), serum cholesterol (mmol/L), serum albumin (g/L), serum glucose, and refrigerated serum glucose (mmol/L). All data on serum chloride, AAC-24 score, and the covariates are available at http://www.cdc.gov/nchs/nhanes/.

Statistical Analysis

We performed weighted and variance estimation analyses to account for significant variance in our data set. Weighted multiple logistic regression models were used to assess the association between serum chloride and AAC-24. Weighted chi-square test was used to analyze differences in categorical variables between groups, and a weighted linear regression model was used to analyze differences in continuous variables. Stratified multiple regression was used for subgroup analysis. In addition, smooth curve fitting and generalized additive models were used to address the non-linear relationship between serum chloride and AAC-24. A recursive algorithm was used to calculate the inflection point in the relationship between serum chloride and AAC-24 when non-linearity was detected, with a bi-segmented linear regression model on either side of the inflection point. Data analysis was performed

using R 3.6.3 (http://www.r-project.org) and EmpowerStats (www.empowerstats.net, X&Y Solutions Inc., Boston, MA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Serum chloride was divided into three tertile groups: low: ≥87 mmol/L to 103 mmol/L; medium: ≥103 mmol/L to <105 mmol/L; and high: ≥105 mmol/L to <119 mmol/L. As **Table 1** shows, there were significant differences in baseline characteristics between the three tertile groups. Mean age, systolic blood pressure, serum cholesterol, serum albumin, serum calcium, and serum glucose were all significantly higher in the low tertile group than in the other tertile groups. AAC-24 was lowest in the medium tertile group. Mean BMI was highest in the high tertile group. Race/ethnicity, sex, and diastolic blood pressure were not significantly different between the three tertile groups.

Table 2 shows the results of univariate analysis. Beta value >0 indicates that the factor is a risk factor for AAC, and beta value <0 indicates that the factor is a protective factor. Compared with the low tertile group of serum chlorine levels, the β value (CI) of the serum chlorine level middle tertile group is −1.017 (−1.333, −0.702), and the β value of the serum chlorine level high tertile group is −0.791 (−1.079, −0.502). *P* values are <0.001. The beta value, *P* value, 95% confidence interval and P for trend value of different covariates are shown in **Table 2** (gender, age, race/Hispanic origin, education level recoded, BMI, systolic blood pressure, diastolic blood pressure, total calcium, cholesterol, albumin, glucose, refrigerated serum).

Table 3 shows the results of stratified analysis between serum chloride and AAC-24. The medium and high serum chloride groups were all negative by using the low serum chloride group as a reference. The absolute value of beta for female in the middle and high serum chloride groups were higher than those for male. The absolute value of beta was highest in the other Hispanic group, followed by the non-Hispanic White group and the Mexican-American group. In the stratification of different education levels, the absolute value of beta was highest in the <9th grade group. In the age stratification, the 64–80-year age-group had the highest absolute value of beta, which was significantly higher than the other age-groups. The absolute value of beta was maximum in the 0–64 mmHg diastolic blood pressure group. The absolute value of beta was maximum in the 1.9–2.3

TABLE 1 | Baseline characteristics of participants (*N* = 3,018).

Chloride (mmol/L)	Total	Low (≥87 to 103)	Middle (≥103 to<105)	High (≥105 to ≤119)	*P*-value
AAC total 24 score	1.473 ± 3.285	2.130 ± 3.931	1.112 ± 2.801	1.339 ± 3.120	< 0.00001
Chloride (mmol/L)	104.044 ± 2.814	100.478 ± 2.052	103.528 ± 0.499	106.387 ± 1.475	< 0.00001
Age (years)	57.425 ± 11.550	58.313 ± 11.730	56.888 ± 11.204	57.273 ± 11.640	0.03528
BMI (kg/m^2)	28.541 ± 5.504	28.177 ± 5.285	28.116 ± 5.451	29.024 ± 5.619	0.00007
Systolic blood pressure (mmHg)	125.193 ± 17.671	127.551 ± 18.216	123.346 ± 17.565	125.024 ± 17.260	0.00002
Diastolic blood pressure (mmHg)	71.104 ± 12.334	71.835 ± 12.602	70.940 ± 11.216	70.788 ± 12.839	0.17506
Total calcium (mmol/L)	2.364 ± 0.090	2.385 ± 0.093	2.366 ± 0.077	2.349 ± 0.093	< 0.00001
Cholesterol (mmol/L)	5.085 ± 1.107	5.203 ± 1.173	5.142 ± 1.109	4.982 ± 1.058	0.00001
Albumin (g/L)	42.508 ± 3.015	43.138 ± 3.350	42.809 ± 2.715	41.958 ± 2.901	< 0.00001
Glucose, refrigerated serum (mmol/L)	5.913 ± 2.258	6.603 ± 3.570	5.651 ± 1.573	5.695 ± 1.464	< 0.00001
Gender (%)					0.09330
Male	48.292	51.606	47.769	46.766	
Female	51.708	48.394	52.231	53.234	
Race/Hispanic origin (%)					0.58950
Mexican American	6.985	6.570	6.733	7.383	
Other Hispanic	4.596	4.361	4.233	4.965	
Non-Hispanic white	71.452	72.773	73.102	69.636	
Non-Hispanic black	9.775	9.399	8.353	10.911	
Other races (a)	7.192	6.897	7.578	7.106	
Education level (%)					0.04164
<9th grade	4.997	4.869	4.968	5.088	
9–11th grade (b)	10.229	9.114	9.600	11.265	
High school graduate (c)	21.784	22.156	20.383	22.486	
Some college or AA degree	30.041	30.944	27.488	31.194	
College graduate or above	32.95	32.917	37.562	29.969	

(a) Including Multi-Racial; (b) Includes 12th grade with no diploma; (c) GED or equivalent. BMI, Body mass index. Weighted by: Full sample mobile examination center exam weight. Mean +/- standard deviation for: AAC total 24 score, chloride, age, BMI, systolic blood pressure, diastolic blood pressure, total calcium, cholesterol, albumin, glucose, refrigerated serum. P value was calculated using weighted linear regression model. % For: gender, race/ethnicity, race/Hispanic origin, education level. P value was calculated using weighted chi-square test.

TABLE 2 | Univariate analysis for AAC total 24 score.

	Statistics (%)	AAC total 24 score (β (95%CI) *P*-value)
Chloride (mmol/L) tertiles ≧87 to 103	773 (25.613%)	0
≧103 to <105	851 (28.197%)	−1.017 (−1.333, −0.702) <0.00001
≧105 to ≦119	1,394 (46.190%)	−0.791 (−1.079, −0.502) <0.00001
P for trend		<0.001
Gender Male	1,454 (48.178%)	0
Female	1,564 (51.822%)	0.138 (−0.097, 0.372) 0.25020
P for trend		0.250
Age (years) tertiles 40–50	942 (31.213%)	0
51–63	1,009 (33.433%)	0.574 (0.314, 0.835) 0.00002
64–80	1,067 (35.355%)	2.905 (2.632, 3.179) <0.00001
P for trend		<0.001
Race/Hispanic origin Mexican American	400 (13.254%)	0
Other Hispanic	286 (9.476%)	0.158 (−0.544, 0.861) 0.65815
Non-Hispanic white	1,339 (44.367%)	0.785 (0.322, 1.249) 0.00090
Non-Hispanic black	577 (19.119%)	0.191 (−0.388, 0.770) 0.51888
Other races (a)	416 (13.784%)	0.498 (−0.123, 1.119) 0.11602
P for trend		0.339
Education level recoded		
<9th grade	282 (9.344%)	0
9–11th grade (b)	407 (13.486%)	0.031 (−0.607, 0.668) 0.92446
High school graduate (c)	683 (22.631%)	0.080 (−0.499, 0.660) 0.78611
Some college or AA degree	851 (28.197%)	−0.206 (−0.770, 0.358) 0.47470
College graduate or above	795 (26.342%)	−0.657 (−1.218, −0.096) 0.02174
P for trend		<0.001
BMI (kg/m^2) tertiles		
14.200–25.700	990 (33.044%)	0
25.800–30.200	1,007 (33.611%)	0.072 (−0.217, 0.360) 0.62669
30.300–51.200	999 (33.344%)	−0.469 (−0.757, −0.181) 0.00143
P for trend		0.001
Systolic blood pressure (mmHg) tertiles 74–116	899 (32.490%)	0
118–130	860 (31.081%)	0.272 (−0.018, 0.563) 0.06639
132–228	1,008 (36.429%)	1.484 (1.193, 1.775) <0. 00001
P for trend		<0.001
Diastolic blood pressure (mmHg) tertiles 0–64	760 (27.467%)	0
66–74	981 (35.454%)	−0.929 (−1.234, −0.623) <0.00001
76–122	1,026 (37.080%)	−1.395 (−1.699, −1.091) <0.00001
P for trend		<0.001
Total calcium (mmol/L) tertiles 1.900–2.300	859 (28.643%)	0
2.325–2.375	1,009 (33.645%)	0.148 (-0.152, 0.448) 0.33342
2.400–3.000	1,131 (37.713%)	0.445 (0.151, 0.739) 0.00302
P for trend		0.002
Cholesterol (mmol/L) tertiles 2.069–4.551	1,000 (33.156%)	0
4.577–5.456	997 (33.057%)	−0.571 (−0.860, −0.283) 0.00011

(Continued)

TABLE 2 | Continued

	Statistics (%)	AAC total 24 score (β (95%CI) *P*-value)
5.482–16.525	1,019 (33.786%)	−0.516 (−0.804, −0.229) 0.00043
P for trend		<0.001
Albumin (g/L) tertiles 24.000–40.000	800 (26.508%)	0
41.000–43.000	1,187 (39.331%)	−0.033 (−0.339, 0.274) 0.83492
44.000–54.000	1,031 (34.162%)	−0.200 (−0.509, 0.110) 0.20581
P for trend		0.174
Glucose, refrigerated serum (mmol/L) tertiles 2.720–5.050	955 (31.643%)	0
5.110–5.770	1,048 (34.725%)	0.119 (−0.161, 0.399) 0.40529
5.830–32.030	1,015 (33.632%)	0.946 (0.655, 1.237) <0.00001
P for trend		<0.001

(a) Including Multi-Racial; (b) Includes 12th grade with no diploma; (c) GED or equivalent. Weighted by: Full sample mobile examination center exam weight. AAC, Abdominal Aortic Calcification; CI, confidence interval; BMI, Body mass index.

mmol/L total calcium group. The absolute value of beta was maximum in the 4.577–5.456 mmol/L total cholesterol group. The absolute value of beta was maximum in the 24–40 g/L serum albumin group. The absolute value of beta was maximum in the 5.11–5.77 mmol/L refrigerated serum glucose group.

Table 4 shows the results of the multiple regression equation analysis. The outcome variable was AAC-24, and the exposure variable was serum chloride. Weighted by: Full sample mobile examination center examination. The crude model was unadjusted; model I adjusted for age, sex, and race/Hispanic origin; model II adjusted for age, sex, race/Hispanic origin, education level, BMI, systolic blood pressure, diastolic blood pressure, total calcium, cholesterol, albumin, and refrigerated serum glucose; and model III adjusted for age (smooth), sex, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth), and refrigerated serum glucose (smooth). The beta value for model III was −0.110. We stratified by chloride tertiles and, additionally, by the following covariates: sex, age, race/Hispanic origin, education level, BMI, systolic blood pressure, diastolic blood pressure, total calcium, cholesterol, albumin, and adjusted serum glucose (**Table 4**).

Figure 2 shows the smooth fitting curves of serum chloride and AAC-24. The curve increases first and then decreases gradually, with inflection point being at serum chloride level of 92 mmol/L. We also analyzed the threshold effect and saturation effect using serum chloride of 92 mmol/L as the fold point (**Table 5**). Weighted by: Full sample mobile examination center exam weight. Outcome variable: AAC-24. Exposure variable: serum chloride (mmol/L). **Table 5** shows the adjustment covariates 5. Model I showed a straight-line effect, with a beta value of −0.099 and $P < 0.0001$. Model II: serum chloride equal to 92 mmol/L was used as the fold point, and the results were statistically significant. Log likelihood ratio tests 0.003. The curve rose after 113 mmol/L. We performed the threshold effect and saturation effect analysis with 113, 114, and 115 mmol/L as folding points; the results are presented in **Supplementary Tables S2–S4**, respectively. However, the *P* values after folding point were all >0.05. **Figures 3**, **4** show the smoothed fitted curves of serum chloride and AAC-24, stratified by different covariates. The curves differed across different strata of individual covariates.

DISCUSSION

The relationship between serum chloride and aortic calcification has not received enough attention. Some studies have found that the Wnt/β-Catenin pathway is involved in arterial calcification via regulation of the osteoprotegerin)/receptor activator of NF-κ B ligand system. The Wnt/β-Catenin pathway is activated or inhibited by lithium chloride *in vitro* and *in vivo* (22), implying that chloride may be associated with arterial calcification. Chloride is the cause of arterial calcification. Elastin, a structural protein present in abundance in the arterial wall, is prone to calcification in many diseases; meanwhile, aluminum chloride–pretreated elastin has been shown to be completely resistant to calcification (23, 24). However, none of these earlier studies have directly shown that chloride is associated with arterial calcification; moreover, some of the studies were in animal aortas (23, 24). In this study, we therefore aimed to investigate the association between serum chloride and AAC. We found that serum chloride is independently related to the AAC-24. For serum chloride levels lower than 92 mmol/L, the AAC-24 increased as serum chloride increased, indicating increased risk for calcification; however, for serum chloride levels >92 mmol/L, the AAC-24 decreased as serum chloride increased, indicating a protective effect.

Our large sample size allowed subgroup analysis. When refrigerated serum glucose was 5.38–32.03 mmol/L, the smooth fitting curve between serum chloride and AAC-24 was inverted-U shaped, which is similar to the overall fitting curve

TABLE 3 | Stratified analysis between Chloride (mmol/L) and AAC total 24 score.

Y = AAC total 24 score	Serum chloride tertiles		
X = Chloride (mmol/L)	Low	Middle	High
		β (95%CI) *P* value	β (95%CI) *P* value
Gender			
Male	Reference	−0.990 (−1.412, −0.569) <0.0001	−0.691 (−1.077, −0.305) 0.0005
Female	Reference	−1.063 (−1.531, −0.595) <0.0001	−0.904 (−1.333, −0.476) <0.0001
Race/Hispanic origin			
Mexican American	Reference	−0.681 (−1.272, −0.090) 0.0244	−0.656 (−1.189, −0.123) 0.0163
Other Hispanic	Reference	−1.482 (−2.274, −0.690) 0.0003	−1.633 (−2.337, −0.930) <0.0001
Non-Hispanic white	Reference	−1.129 (−1.628, −0.630) <0.0001	−0.850 (−1.311, −0.388) 0.0003
Non-Hispanic black	Reference	−0.133 (−0.760, 0.494) 0.6777	−0.274 (−0.818, 0.269) 0.3231
Other races (a)	Reference	−1.041 (−1.804, −0.279) 0.0077	−0.175 (−0.884, 0.534) 0.6289
Education level			
<9th grade	Reference	−1.391 (−2.519, −0.263) 0.0163	−1.748 (−2.777, −0.718) 0.0010
9–11th grade (b)	Reference	−1.135 (−2.096, −0.174) 0.0211	−0.622 (−1.481, 0.236) 0.1562
High school graduate (c)	Reference	−0.976 (−1.717, −0.235) 0.0101	−1.079 (−1.742, −0.416) 0.0015
Some college or AA degree	Reference	−1.161 (−1.779, −0.544) 0.0002	−0.939 (−1.488, −0.390) 0.0008
College graduate or above	Reference	−0.747 (−1.251, −0.243) 0.0038	−0.414 (−0.897, 0.069) 0.0934
BMI (kg/m^2) tertiles			
14.200–25.700	Reference	−1.581 (−2.121, −1.041) <0.0001	−0.760 (−1.282, −0.239) 0.0044
25.800–30.200	Reference	−0.825 (−1.403, −0.247) 0.0053	−0.419 (−0.945, 0.106) 0.1180
30.300–51.200	Reference	−0.545 (−1.059, −0.031) 0.0380	−1.016 (−1.467, −0.566) <0.0001
Age (years) tertiles			
40–50	Reference	−0.238 (−0.440, −0.036) 0.0211	−0.121 (−0.307, 0.066) 0.2042
51–63	Reference	−0.676 (−1.033, −0.320) 0.0002	−0.658 (−0.987, −0.328) <0.0001
64–80	Reference	−1.836 (−2.619, −1.052) <0.0001	−1.378 (−2.080, −0.677) 0.0001
Systolic blood pressure (mmHg) tertiles			
74–116	Reference	−0.794 (−1.220, −0.367) 0.0003	−0.480 (−0.883, −0.076) 0.0200
118–130	Reference	−0.928 (−1.451, −0.404) 0.0005	−0.923 (−1.403, −0.442) 0.0002
132–228	Reference	−0.747 (−1.438, −0.057) 0.0342	−0.863 (−1.456, −0.270) 0.0045
Diastolic blood pressure (mmHg) tertiles			
0–64.000	Reference	−2.141 (−2.958, −1.323) <0.0001	−1.925 (−2.662, −1.188) <0.0001
66.000–74.000	Reference	−0.943 (−1.481, −0.404) 0.0006	−0.540 (−1.036, −0.043) 0.0333
76.000–122.000	Reference	−0.339 (−0.698, 0.021) 0.0655	−0.508 (−0.836, −0.180) 0.0025
Total calcium (mmol/L) tertiles			
1.900–2.300	Reference	−1.227 (−1.827, −0.627) <0.0001	−1.013 (−1.533, −0.492) 0.0001
2.325–2.375	Reference	−0.822 (−1.348, −0.296) 0.0023	−0.618 (−1.107, −0.128) 0.0136
2.400–3.000	Reference	−1.039 (−1.566, −0.512) 0.0001	−0.676 (−1.187, −0.165) 0.0097
Cholesterol (mmol/L) tertiles			
2.069–4.551	Reference	−1.009 (−1.688, −0.330) 0.0037	−0.715 (−1.314, −0.117) 0.0193
4.577–5.456	Reference	−1.320 (−1.788, −0.851) <0.0001	−1.264 (−1.699, −0.829) <0.0001
5.482–16.525	Reference	−0.758 (−1.242, −0.273) 0.0022	−0.506 (−0.961, −0.051) 0.0296
Albumin (g/L) tertiles			
24.000–40.000	Reference	−1.671 (−2.339, −1.003) <0.0001	−1.300 (−1.872, −0.728) <0.0001
41.000–43.000	Reference	−1.305 (−1.870, −0.740) <0.0001	−1.045 (−1.564, −0.526) <0.0001
44.000–54.000	Reference	−0.569 (−1.028, −0.111) 0.0151	−0.488 (−0.937, −0.040) 0.0332
Glucose, refrigerated serum (mmol/L) tertiles			
2.720–5.050	Reference	−0.979 (−1.461, −0.497) <0.0001	−0.325 (−0.773, 0.123) 0.1557
5.110–5.770	Reference	−0.998 (−1.487, −0.510) <0.0001	−1.157 (−1.604, −0.711) <0.0001
5.830–32.030	Reference	−0.897 (−1.545, −0.250) 0.0067	−0.661 (−1.247, −0.075) 0.0274

(a) Including Multi-Racial; (b) Includes 12th grade with no diploma; (c) GED or equivalent. BMI, Body mass index. Weighted by: Full sample mobile examination center exam weight.

TABLE 4 | Relationship between serum chloride and abdominal aortic calcification (multiple regression equation analysis).

Outcome	Crude model	Model I	Model II	Model III
	β (95%CI) P-value	β (95%CI) P-value	β (95%CI) P-value	β (95%CI) P-value
Total	−0.170 (−0.250, −0.091) 0.00003	−0.126 (−0.199, −0.053) 0.00074	−0.099 (−0.175, −0.023) 0.01116	−0.110 (-0.185,−0.036) 0.00383
Chloride (mmol/L) tertiles				
≧87 to 103	−0.219 (−0.353, −0.085) 0.00146	−0.092 (−0.213, 0.030) 0.13823	−0.076 (−0.206, 0.054) 0.25306	−0.096 (-0.225, 0.033) 0.14598
≧103 to <105	0.037 (−0.341, 0.414) 0.84873	−0.090 (−0.443, 0.262) 0.61545	−0.076 (−0.456, 0.305) 0.69740	0.006 (-0.352, 0.363) 0.97578
≧105 to ≦119	−0.133 (−0.244, −0.022) 0.01895	−0.130 (−0.232, −0.028) 0.01251	−0.117 (−0.221, −0.012) 0.02872	−0.115 (-0.217,−0.013) 0.02676
Gender				
Male	−0.108 (−0.167, −0.050) 0.00026	−0.131 (−0.184, −0.077) <0.00001	−0.110 (−0.169, −0.052) 0.00024	−0.130 (-0.188,−0.073) 0.00001
Female	−0.153 (−0.211, −0.094) <0.00001	−0.084 (−0.138, −0.030) 0.00235	−0.084 (−0.142, −0.026) 0.00434	−0.074 (-0.130,−0.017) 0.01062
Age (years) tertiles				
40–50	−0.028 (−0.056, 0.001) 0.05629	−0.029 (−0.058, −0.001) 0.04592	−0.029 (−0.061, 0.003) 0.07154	−0.036 (-0.069,−0.004) 0.02966
51–63	−0.116 (−0.164, −0.068) <0.00001	−0.117 (−0.165, −0.069) <0.00001	−0.107 (−0.160, −0.054) 0.00009	−0.113 (-0.166,−0.060) 0.00003
64–80	−0.189 (−0.283, −0.096) 0.00007	−0.185 (−0.279, −0.091) 0.00012	−0.162 (−0.259, −0.064) 0.00117	−0.173 (-0.270,−0.076) 0.00049
Race/Hispanic origin				
Mexican American	−0.100 (−0.175, −0.025) 0.00904	−0.088 (−0.162, −0.015) 0.01854	−0.117 (−0.200, −0.034) 0.00585	−0.114 (-0.196,−0.031) 0.00716
Other Hispanic	−0.236 (−0.336, −0.136) <0.00001	−0.226 (−0.323, −0.128) <0.00001	−0.213 (−0.325, −0.101) 0.00025	−0.203 (-0.309,−0.097) 0.00022
Non-Hispanic white	−0.143 (−0.209, −0.076) 0.00003	−0.122 (−0.182, −0.062) 0.00007	−0.106 (−0.169, −0.042) 0.00117	−0.108 (-0.170,−0.045) 0.00082
Non-Hispanic black	−0.062 (−0.137, 0.012) 0.10317	−0.041 (−0.112, 0.031) 0.26509	−0.087 (−0.167, −0.007) 0.03282	−0.070 (-0.150, 0.010) 0.08795
Other races (a)	−0.030 (−0.141, 0.081) 0.59744	0.001 (−0.101, 0.103) 0.98274	0.066 (−0.047, 0.179) 0.25466	0.068 (-0.040, 0.176) 0.21821
Education level				
<9th grade	−0.172 (−0.310, −0.035) 0.01426	−0.179 (−0.302, −0.057) 0.00434	−0.140 (−0.288, 0.008) 0.06434	−0.128 (-0.267, 0.012) 0.07474
9–11th grade (b)	−0.069 (−0.184, 0.046) 0.24018	0.007 (−0.099, 0.113) 0.89763	0.043 (−0.073, 0.158) 0.46801	0.003 (-0.111, 0.116) 0.96447
High school graduate (c)	−0.217 (−0.315, −0.119) 0.00002	−0.197 (−0.287, −0.108) 0.00002	−0.231 (−0.326, −0.136) <0.00001	−0.237 (-0.330,−0.143) <0.00001
Some college or AA degree	−0.155 (−0.230, −0.081) 0.00005	−0.121 (−0.191, −0.052) 0.00067	−0.097 (−0.172, −0.022) 0.01140	−0.101 (-0.175,−0.027) 0.00768
College graduate or above	−0.066 (−0.141, 0.008) 0.08267	−0.067 (−0.135, 0.000) 0.05176	−0.058 (−0.132, 0.015) 0.12143	−0.072 (-0.140,−0.004) 0.03872
BMI (kg/m^2) tertiles				
14.200–25.700	−0.157 (−0.234, −0.081) 0.00006	−0.133 (−0.202, −0.064) 0.00016	−0.148 (−0.218, −0.078) 0.00004	−0.138 (-0.205,−0.070) 0.00007
25.800–30.200	−0.095 (−0.171, −0.020) 0.01370	−0.081 (−0.150, −0.011) 0.02268	−0.084 (−0.161, −0.007) 0.03195	−0.099 (-0.175,−0.022) 0.01212
30.300–51.200	−0.129 (−0.193, −0.066) 0.00007	−0.106 (−0.166, −0.046) 0.00052	−0.086 (−0.152, −0.020) 0.01088	−0.093 (-0.157,−0.029) 0.00444
Systolic blood pressure (mm Hg) tertiles				
74–116	−0.081 (−0.145, −0.018) 0.01223	−0.062 (−0.120, −0.003) 0.04020	−0.054 (−0.116, 0.009) 0.09191	−0.057 (-0.119, 0.005) 0.07207
118–130	−0.156 (−0.224, −0.088) <0.00001	−0.156 (−0.221, −0.092) <0.00001	−0.117 (−0.184, −0.050) 0.00064	−0.087 (-0.153,−0.022) 0.00892
132–228	−0.130 (−0.210, −0.050) 0.00147	−0.117 (−0.191, −0.043) 0.00191	−0.114 (−0.191, −0.037) 0.00402	−0.129 (-0.206,−0.051) 0.00112
Diastolic blood pressure (mm Hg) tertiles				
0–64.000	−0.315 (−0.419, −0.211) <0.00001	−0.250 (−0.344, −0.155) <0.00001	−0.231 (−0.331, −0.132) <0.00001	−0.243 (-0.340,−0.146) <0.00001
66.000–74.000	−0.075 (−0.147, −0.003) 0.04212	−0.062 (−0.129, 0.005) 0.06895	−0.047 (−0.115, 0.021) 0.17673	−0.040 (-0.108, 0.027) 0.24469
76.000–122.000	−0.076 (−0.124, −0.029) 0.00172	−0.071 (−0.116, −0.026) 0.00208	−0.052 (−0.102, −0.003) 0.03807	−0.053 (-0.102,−0.005) 0.03246

(Continued)

TABLE 4 | Continued

Outcome	Crude model	Model I	Model II	Model III
	β (95%CI) P-value	β (95%CI) P-value	β (95%CI) P-value	β (95%CI) P-value
Total calcium (mmol/L) tertiles				
1.900–2.300	−0.123 (−0.197, −0.048) 0.00132	−0.106 (−0.174, −0.038) 0.00235	−0.069 (−0.141, 0.003) 0.05961	−0.082 (-0.152,−0.013) 0.02061
2.325–2.375	−0.100 (−0.169, −0.031) 0.00476	−0.091 (−0.155, −0.027) 0.00575	−0.099 (−0.167, −0.031) 0.00460	−0.088 (−0.156, −0.021) 0.01034
2.400–3.000	−0.154 (−0.228, −0.080) 0.00004	−0.121 (−0.188, −0.055) 0.00038	−0.111 (−0.184, −0.039) 0.00261	−0.137 (-0.208,−0.066) 0.00017
Cholesterol (mmol/L) tertiles				
2.069–4.551	−0.095 (−0.176, −0.014) 0.02161	−0.052 (−0.125, 0.021) 0.16539	−0.035 (−0.114, 0.043) 0.38012	−0.036 (-0.113, 0.041) 0.36427
4.577–5.456	−0.198 (−0.262, −0.135) <0.00001	−0.173 (−0.231, −0.114) <0.00001	−0.168 (−0.229, −0.107) <0.00001	−0.163 (-0.223,−0.103) <0.00001
5.482–16.525	−0.121 (−0.189, −0.053) 0.00053	−0.104 (−0.168, −0.040) 0.00145	−0.101 (−0.171, −0.031) 0.00485	−0.106 (-0.174,−0.037) 0.00261
Albumin (g/L) tertiles				
24.000–40.000	−0.143 (−0.221, −0.065) 0.00033	−0.112 (−0.184, −0.041) 0.00219	−0.126 (−0.205, −0.047) 0.00175	−0.119 (-0.197,−0.040) 0.00306
41.000–43.000	−0.140 (−0.212, −0.067) 0.00016	−0.101 (−0.167, −0.034) 0.00299	−0.058 (−0.128, 0.012) 0.10603	−0.056 (-0.125, 0.012) 0.10476
44.000–54.000	−0.143 (−0.210, −0.075) 0.00004	−0.112 (−0.174, −0.049) 0.00046	−0.118 (−0.185, −0.052) 0.00052	−0.128 (-0.194,−0.061) 0.00017
Glucose, refrigerated serum (mmol/L) tertiles				
2.720–5.050	−0.092 (−0.158, −0.026) 0.00627	−0.082 (−0.143, −0.021) 0.00814	−0.100 (−0.162, −0.039) 0.00150	−0.083 (-0.142,−0.025) 0.00549
5.110–5.770	−0.160 (−0.224, −0.097) <0.00001	−0.133 (−0.192, −0.074) 0.00001	−0.130 (−0.193, −0.067) 0.00006	−0.134 (-0.198,−0.070) 0.00005
5.830–32.030	−0.108 (−0.190, −0.025) 0.01038	−0.095 (−0.170, −0.020) 0.01305	−0.107 (−0.186, −0.028) 0.00819	−0.103 (-0.183,−0.023) 0.01171

(a) Including Multi-Racial; (b) Includes 12th grade with no diploma; (c) GED or equivalent. Abbreviations: CI, confidence interval; ACC, abdominal aortic calcification; BMI, Body mass index. Weighted by: Full sample mobile examination center exam weight. Outcome variable: AAC total 24 score. Exposure variable: serum chloride. The crude model is not adjusted. Model I adjusted for age, gender, and race/Hispanic origin. Model II adjusted for age, gender, race/Hispanic origin, education level, BMI, systolic blood pressure, diastolic blood pressure, total calcium, cholesterol, albumin and refrigerated serum glucose. Model III adjusted for age (smooth), gender, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth) and refrigerated serum glucose (smooth).

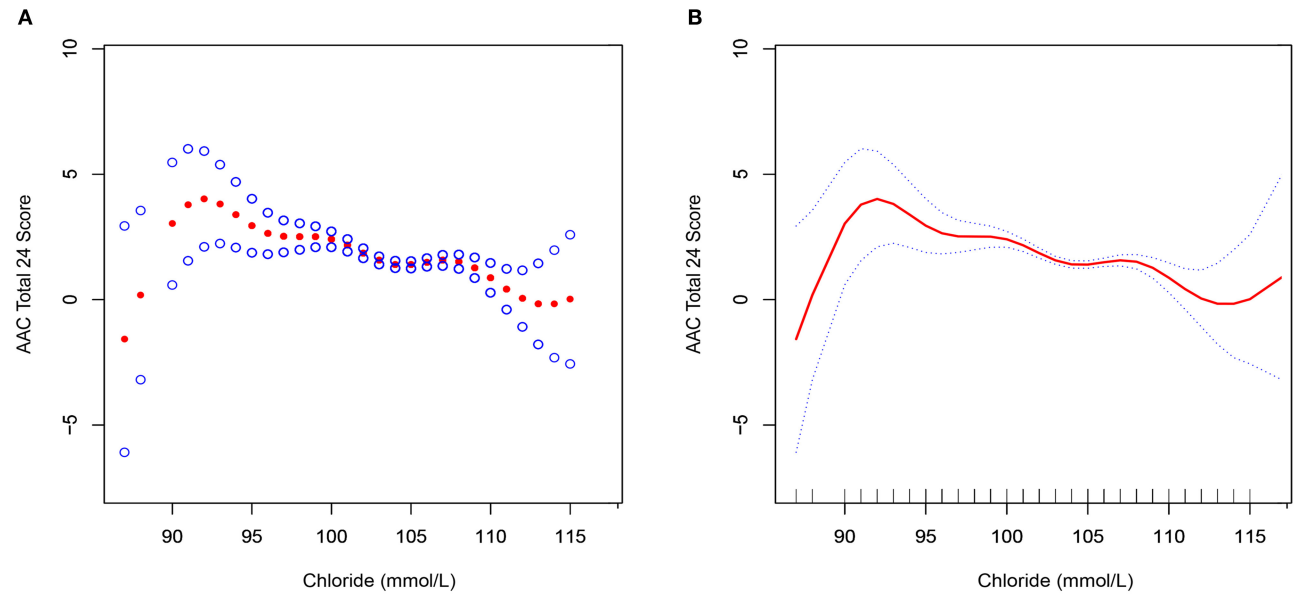

FIGURE 2 | Association between serum chloride and abdominal aortic calcification. The red line represents the smooth curve fit between the variables. The blue bar represents the 95% confidence interval of the fit. Weighted by: full sample mobile examination center examination weight. Adjusted for age (smooth), sex, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth), and refrigerated serum glucose (smooth). **(A)** Scatter plot of curve fit. **(B)** Solid line plot of curve fit.

TABLE 5 | Analysis of threshold effect and saturation effect with 92 mmol/L as cutoff serum chloride value point.

Outcome	AAC total 24 score β (95%CI) *P*-value
Model I	
A straight-line effect	−0.099 (−0.139, −0.059) <0.0001
Model II	
Fold points (K)	92
< K-segment effect 1	1.292 (0.430, 2.154) 0.0033
>K-segment effect 2	−0.114 (−0.155, −0.073) <0.0001
Effect size difference of 2 vs. 1	−1.406 (−2.276, −0.536) 0.0015
Equation predicted values at break points	3.257 (2.710, 3.803)
Log likelihood ratio tests	0.003

CI, confidence interval; ACC, abdominal aortic calcification. Weighted by: Full sample mobile examination center exam weight. Outcome variable: AAC total 24 score. Exposure variable: serum chloride (mmol/L). Adjusted for age (smooth), gender, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth) and refrigerated serum glucose (smooth).

between serum chloride and AAC-24, but the inflection point was >92 mmol/L. In the low serum glucose group (2.72–5.33 mmol/L), there was a downward fitting curve. In the low age triad (40–50 years), the smooth fitting curve between serum chloride and AAC-24 score was flat. The smooth fitting curves of low systolic blood pressure (74–116 mmHg) and high systolic blood pressure (76–122 mmHg) were flat.

Since we used a nationally representative sample, our findings are generalizable to the whole US population. Our results suggest that serum chloride levels in adults should be maintained at an appropriate level, and that control of serum chloride level could be a new method for the prevention and control of peripheral artery calcification. Appropriate and individualized measures for prevention for AAC may have to be developed for different ethnic populations, sexes, and age-groups.

It is important to recognize the limitations of our research. First, because of the cross-sectional design of our study, a causal relationship between adult serum chloride level and AAC-24 cannot be inferred. Second, confounding factors not analyzed in this study may affect the results. Third, no attempt has been made to clarify the mechanism by which serum chloride affects abdominal aortic calcification. Fourth, it would be more convincing if the range of the lowest and highest values of serum chloride among the participants examined were larger.

CONCLUSIONS

Below a cutoff value of 92 mmol/L, serum chloride appears to be a risk factor for development of abdominal aortic calcification, but serum levels >92 mmol/L may protect against abdominal aortic calcification. Thus, detection of serum chlorine detection could be a simple and accurate method for screening individuals for risk of abdominal aorta calcification and cardiovascular disease.

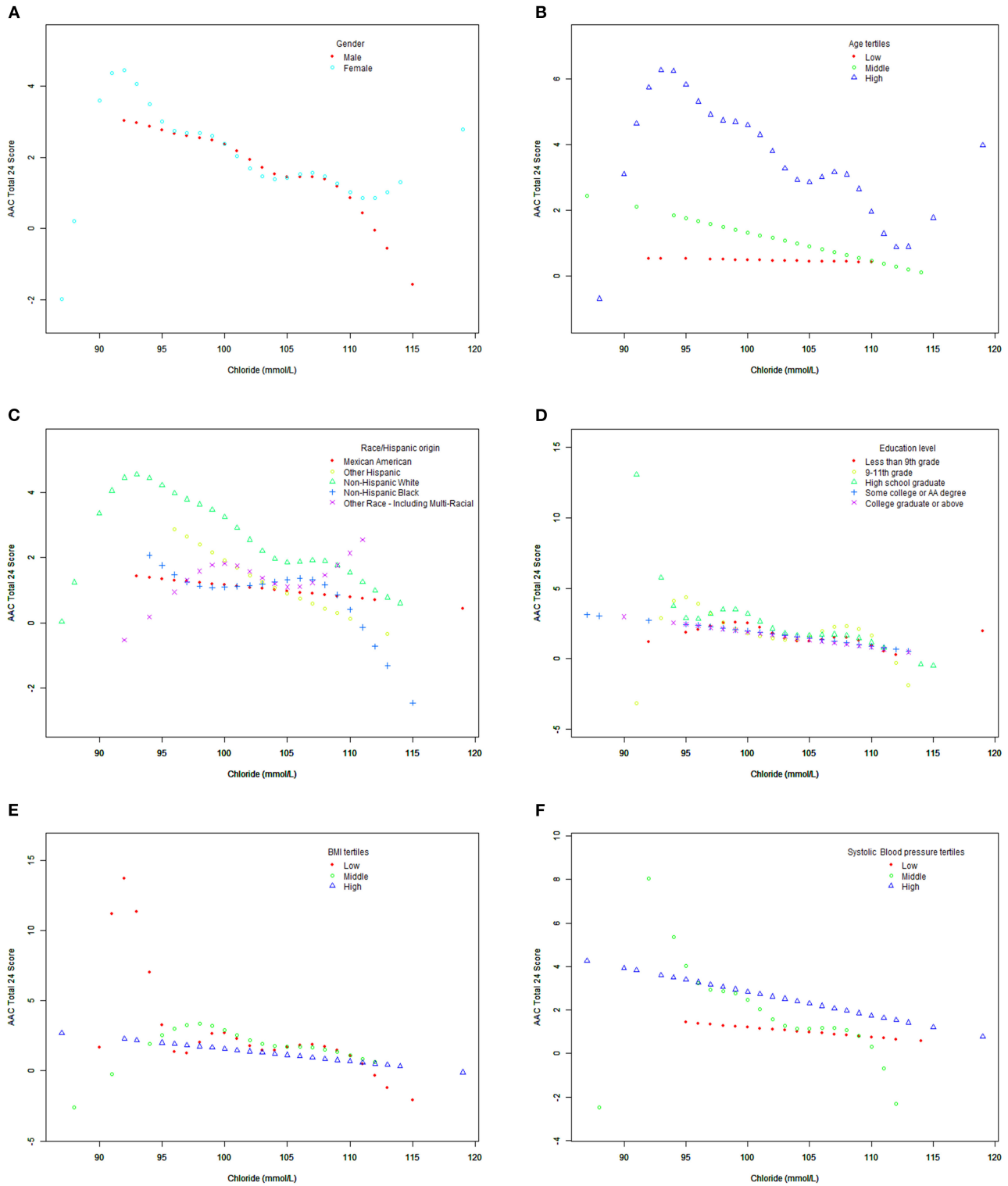

FIGURE 3 | Association between serum chloride and abdominal aortic calcification, stratified by covariates (sex, age, race/Hispanic origin, education level, BMI, systolic blood pressure). Weighted by: full sample mobile examination center examination weight. Adjusted for age (smooth), sex, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth), and refrigerated serum glucose (smooth). **(A)** Stratified by sex. **(B)** Stratified by age tertiles. **(C)** Stratified race/Hispanic origin. **(D)** Stratified by education level. **(E)** Stratified by BMI tertiles. **(F)** Stratified by systolic blood pressure tertiles.

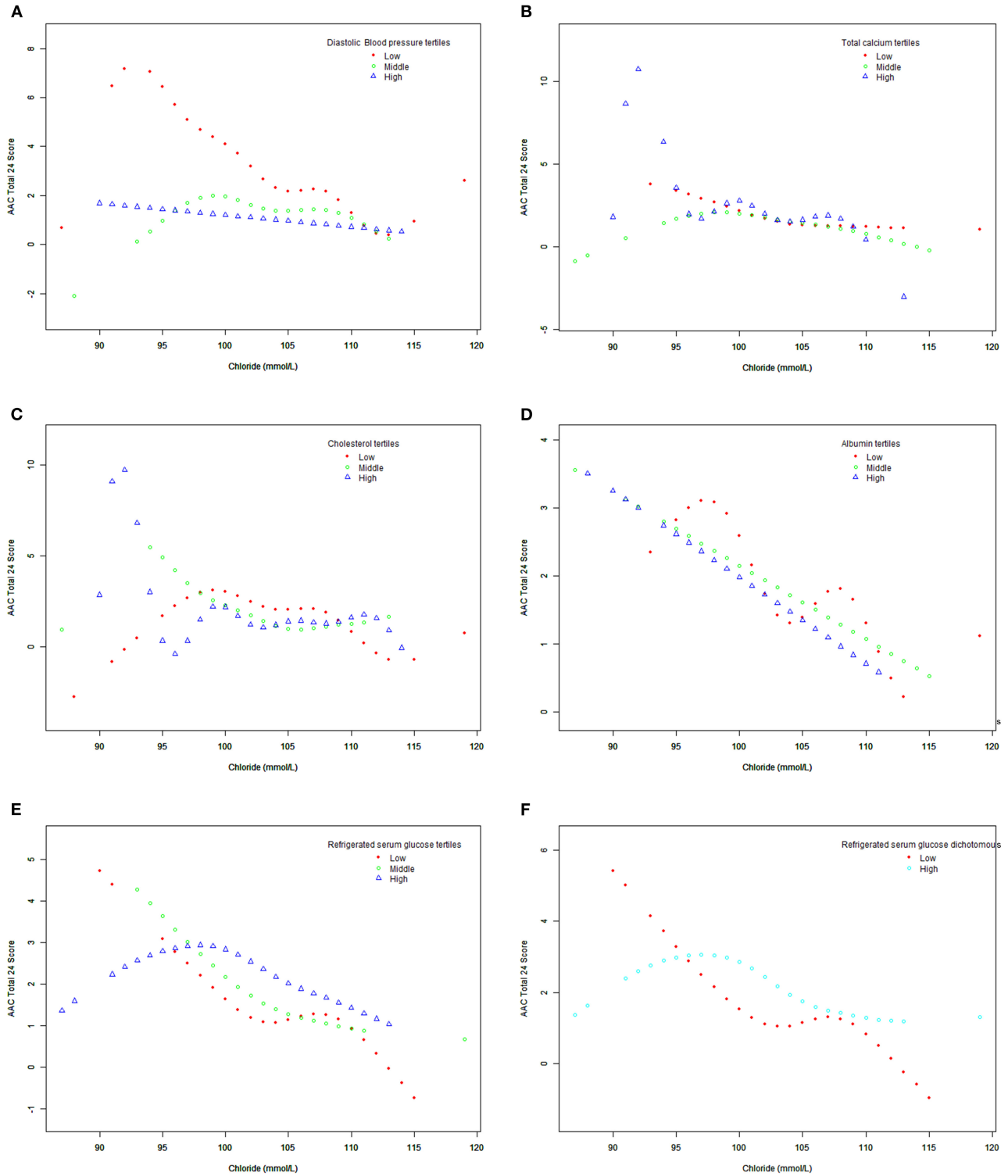

FIGURE 4 | Association between serum chloride and abdominal aortic calcification, stratified by covariates (diastolic blood pressure, total calcium, cholesterol, albumin, refrigerated serum glucose). Weighted by: full sample mobile examination center exam weight. Adjusted for age (smooth), sex, race/Hispanic origin, education level, BMI (smooth), systolic blood pressure (smooth), diastolic blood pressure (smooth), total calcium (smooth), cholesterol (smooth), albumin (smooth), and refrigerated serum glucose (smooth). **(A)** Stratified by diastolic blood pressure tertiles. **(B)** Stratified by total calcium tertiles. **(C)** Stratified cholesterol tertiles. **(D)** Stratified by albumin tertiles. **(E)** Stratified by refrigerated serum glucose tertiles. **(F)** Stratified by refrigerated serum glucose (dichotomous).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved by the Ethics Review Board of the National Center for Health Statistics. Informed consent was not considered necessary as this was an analysis of a database.

AUTHOR CONTRIBUTIONS

SH, JP, and YW contributed to data collection, analysis, and writing of the manuscript. SH, TL, WZ, JY, DZ, YZ, SW, QG, LS, DY, JP, YW, and JX contributed to study design and writing of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.800458/full#supplementary-material

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ORIGINAL RESEARCH
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Standard Modifiable Cardiovascular Risk Factors Mediate the Association Between Elevated Hair Cortisol Concentrations and Coronary Artery Disease

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Background: Increased cortisol exposure is a risk factor for coronary artery disease (CAD). It is not clear to what degree this risk is independent from the standard modifiable risk factors (SMuRFs) dyslipidemia, hypertension, and diabetes.

Aim: To use path analysis to test the direct and indirect association, mediated by SMuRFs, between long-term cortisol levels measured in hair cortisol concentration (HCC) and CAD.

Methods: Hair was sampled from patients admitted with acute myocardial infarction ($n = 203$) and a population-based sample without a diagnosis or symptoms of CAD ($n = 3{,}134$). The HCC was analyzed using radioimmunoassay and all the participants reported whether they were diagnosed with or treated for diabetes, hypertension, and hyperlipidemia. Path analysis was used to test to what degree the association between logarithmized (ln) HCC and CAD was direct or indirect, mediated by SMuRFs.

Results: Participants with CAD had elevated HCC compared to those without elevated HCC [median interquartile range (IQR): 75.2 (167.1) vs. 23.6 (35.0) pg/mg, $p < 0.0001$]. Higher HCC was associated with diabetes, hypertension, and hyperlipidemia, which, in turn, were associated with CAD. In path models, 80% of the association between ln(HCC) and CAD were mediated by SMuRFs, while the direct path between ln(HCC) and CAD was not statistically significant.

Conclusion: The SMuRFs diabetes, hyperlipidemia, and hypertension mediate the association between ln(HCC) and CAD. Some association between ln(HCC) and CAD did not operate via the SMuRFs considered and might have a pathway through atherosclerosis or inflammation.

Keywords: cortisol, cardiovascular risk factors, biological stress, coronary artery disease, path analysis

INTRODUCTION

Coronary artery disease (CAD) is associated with the well-known standard modifiable risk factors (SMuRFs) diabetes, smoking, hypertension, and hyperlipidemia (1, 2). Consequently, prevention of CAD is focused on improving SMuRFs through modified lifestyle and by pharmacological treatment (3). However, about 15% of patients admitted with a primary ST-segment elevation myocardial infarction (STEMI) are SMuRF-less, i.e., nonsmokers without a diagnosis of diabetes, hypertension, or hyperlipidemia (2, 4–6). Notably, these SMuRF-less patients have increased mortality after STEMI compared to patients with SMuRFs (6). Thus, there is a need to find other nonstandard risk factors, which may also contribute to the development of atherosclerosis with subsequent CAD among SMuRF-less patients (4–6).

The glucocorticoid hormone cortisol has both the direct and indirect effects, which may increase the risk of CAD (7). For instance, patients suffering from glucocorticoid excess develop abdominal adiposity, dyslipidemia, hypertension, insulin resistance, and potentially type 2 diabetes (8). Moreover, taking prescribed glucocorticoids increase the risk of cardiovascular disease independent of underlying disease and common cardiovascular risk factors (9).

Cortisol has a considerable diurnal rhythmicity, which complicates sampling of this hormone (10). To overcome this difficulty, methods have been developed to measure cortisol levels in hair, which give a measure of the cumulative cortisol exposure in retrospect (11, 12). Notably, patients suffering from an acute MI (AMI) have increased levels of cortisol in hair prior weeks to the event compared with healthy controls (13) and patients treated for other diseases (14). High hair cortisol concentrations (HCCs) have also been shown among patients with the SMuRFs diabetes (15–17), hypertension (18), and smoking (15), as well as other cardiovascular risk factors including abdominal obesity (19), low high-density lipoprotein (HDL)-cholesterol levels (19), and a history of cardiovascular disease (17). Importantly, longitudinal data suggest that higher HCC is associated with an increased weight gain over 3 years (20). Thus, it may be suggested that increased circulating cortisol levels, either due to Cushing's syndrome (8), glucocorticoid treatment (9), or other more subtle derangements of the hypothalamic-pituitary-adrenal (HPA) axis reflected by increased HCC (20), is a cause rather than a consequence of obesity and SMuRFs.

Even though increased cortisol exposure seems to be a risk factor for CAD, it is not clear to what degree this is an indirect effect mediated by SMuRFs such as dyslipidemia, hypertension, and diabetes or a direct effect on the development of CAD (21). Therefore, we sought to use path analysis to test the direct and indirect association, mediated by SMuRFs, between HCC and CAD.

METHODS

Study Design and Participants

This was a cross-sectional study including participants from two studies—the Stressheart study (13) and the Swedish CArdioPulmonary BioImage Study (SCAPIS) (22). A detailed description and main results from the Stressheart study have been published previously (13). The Stressheart study included 203 patients admitted with a STEMI or non-STEMI (NSTEMI) in the southeast healthcare region of Sweden during year 2016–2019. Patients with a hair length < 1 cm, not speaking Swedish language, and older than 65 years were excluded. The SCAPIS study was conducted during year 2015–2018 at six universities in Sweden. Data in this study were acquired from participants included at Linköping University situated in the same southeast hospital region as in which the Stressheart study was conducted. The SCAPIS study included men and women between 50 and 65 years of age randomly sampled from the population. In total, 5,057 participants were included at Linköping University of whom 3,462 participants provided a hair sample for cortisol analysis. Of these 3,462 participants, 155 participants reported a history of MI ($n = 46$), angina ($n = 25$), previous coronary artery bypass graft surgery or percutaneous coronary artery intervention ($n = 40$), and chest pain when walking on flat ground ($n = 16$) or uphill ($n = 78$), and were, therefore, excluded. Furthermore, another 173 participants who lacked information on history of CAD, chest pain, or smoking status were also excluded leaving 3,134 participants from the SCAPIS study (**Figure 1**). The Stressheart and the SCAPIS studies were approved by the Regional Ethical Review Boards (Dnr 2016-79-31, Dnr 2016-453-32, Dnr 2017-106-32, and Dnr 2010-228-31M). All the participants gave their written informed consent to participate.

Definition of SMuRFs

Standard modifiable risk factors were defined as having a diagnosis of, or pharmacotherapy for, diabetes, hypertension, or hyperlipidemia, which have been used in previous studies on the role of SMuRFs in CAD (4–6). This was reported by the patient in the SCAPIS study and checked in the patient file in the Stressheart study. A total cholesterol ≥ 5.5 mmol/l and/or low-density lipoprotein (LDL)-cholesterol ≥ 3.5 mmol/l was also used to define participants with hyperlipidemia (4–6). Regular smoking of at least one cigarette/day during the last month was also considered as SMuRF (6). The blood pressure level and plasma glucose level were not included in the definition, since single measurements of these parameters were not considered enough to firmly diagnose participants as being hypertensive or having diabetes in either the SCAPIS or the Stressheart studies. In total, 2,141 participants in the SCAPIS study reported having at least one SMuRF. In the Stressheart study, 173 participants had at least one SMuRF at the time of admission with AMI whereas 30 participants were SMuRF-less at the time of admission (**Table 1**).

Outcomes

The primary outcome in this study was the direct and indirect association between HCC and CAD. A detailed description on the hair sampling and analysis of HCC has been published previously (13). At least 1 cm of hair, which represents the previous 4–6 weeks, was sampled from the posterior vertex of the scalp (23). The HCC was analyzed using a competitive radioimmunoassay (RIA), since it is suitable for small samples

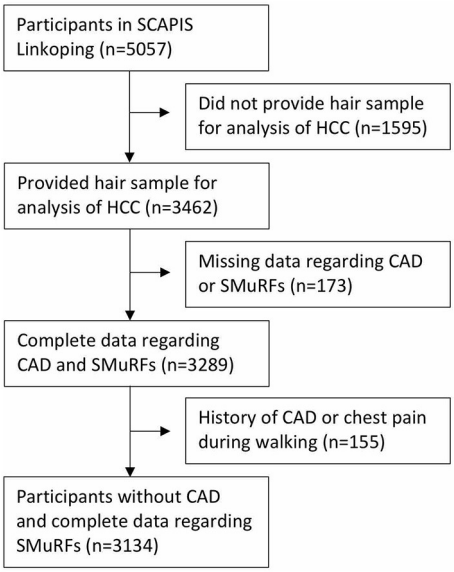

FIGURE 1 | Flow diagram over included participants from the SCAPIS study. HCC, hair cortisol concentration; CAD, coronary artery disease; SMuRF, standard modifiable risk factor; SCAPIS, Swedish CArdioPulmonary BioImage Study.

of hair. Hair samples from both the Stressheart and the SCAPIS studies were analyzed during the same time period (2016–2019) in the same laboratory under the same protocol. All the participants in both studies answered questionnaires with respect to educational level, ethnicity, current pharmacotherapy, present and previous diseases including angina, congestive heart failure, MI, stroke, previous coronary artery bypass grafting or percutaneous coronary intervention, diabetes, hyperlipidemia, and if any first-degree relatives suffered from MI. Smoking status was reported by the participant. Length, weight, waist circumference, and blood pressure on the day of discharge from the hospital in the Stressheart study were measured by a nurse. In the SCAPIS study, a fasting blood sample was drawn and plasma glucose, serum cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were analyzed according to clinical routine (22). In the Stressheart study, data on these biochemical measures were extracted from the Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART), which is a national register of patients treated in coronary care units (24).

Statistical Analysis

All the data were checked for normal distribution. The HCC was not normally distributed and was, therefore, logarithmically transformed using the natural logarithm before inclusion in the statistical models [ln(HCC)]. This transformation also avoids the long tail of raw cortisol values having undue influence on the associations with CAD. All the continuous data were presented as mean with SD, except for HCC, which was presented as

TABLE 1 | Characteristics of included participants grouped according to coronary artery disease (CAD) status.

	Coronary artery disease (n = 203)	No coronary artery disease (n = 3,134)	P-value
Female	56 (28)	1,994 (64)	< 0.0001
Age	57.7 (6.5)	57.3 (4.4)	0.002
Educational level			< 0.0001
Elementary school	47 (23)	251 (8)	
Upper secondary school	113 (56)	1,472 (47)	
University or similar	43 (21)	1,406 (45)	
Born in Sweden	176 (87)	2,957 (94)	< 0.0001
STEMI	112 (57)	NA	
Previous MI	51 (26)	NA	
Heredity for MI	86 (51)	205 (7)	< 0.0001
Hyperlipidemia	56 (32)	255 (8)	< 0.0001
Hypertension	81 (40)	570 (18)	< 0.0001
Diabetes	31 (16)	115 (4)	< 0.0001
Active smoker	56 (28)	271 (9)	< 0.0001
SMuRFs			< 0.0001
0	54 (32)	2,210 (71)	
1	62 (36)	688 (22)	
2	30 (18)	186 (6)	
3	24 (14)	39 (1)	
4	1 (0.6)	8 (0.3)	
BMI (kg/m^2)	27.8 (4.4)	26.8 (4.5)	< 0.0001
Waist circumference (cm)	100 (13)	91 (13)	< 0.0001
P-glucose (mmol/L)	7.2 (3.0)	5.6 (1.1)	< 0.0001
Total cholesterol (mmol/L)	5.1 (1.3)	5.5 (1.0)	< 0.0001
LDL-cholesterol (mmol/L)	3.0 (1.2)	3.3 (0.9)	< 0.0001
HDL-cholesterol (mmol/L)	1.19 (0.40)	1.70 (0.50)	< 0.0001
Triglycerides (mmol/L)	2.1 (1.3)	1.9 (0.7)	< 0.0001
Systolic blood pressure (mmHg)	125 (17)	132 (18)	< 0.0001
Diastolic blood pressure (mmHg)	79 (12)	83 (10)	< 0.0001
Hair cortisol concentration (pg/mg)	75.2 (167.1)	23.6 (35.0)	< 0.0001

Categorical data is presented as n (%) and continuous data as mean (SD) except for the hair cortisol concentration, which is given as median [interquartile range (IQR)]. Prevalence of hyperlipidemia, hypertension, and diabetes were based on either a diagnosis or pharmacological treatment. p-value denotes the significance level of the difference between participants with and without coronary artery disease.

TABLE 2 | Screening of potential mediators to be included in the path analysis.

Predictor	Ln(HCC)		Coronary artery disease	
	Beta	P-value	Odds ratio	P-value
Hypertension	0.15 (0.05–0.24)	0.003	2.99 (2.22–4.01)	< 0.0001
Diabetes	0.41 (0.21–0.62)	< 0.0001	4.82 (3.15–7.37)	< 0.0001
Hyperlipidemia	0.30 (0.17–0.44)	< 0.0001	5.35 (3.80–7.54)	< 0.0001
Smoking	0.01 (−0.12–0.14)	0.86	4.11 (2.95–5.73)	< 0.0001
LDL-cholesterol	−0.02 (−0.06–0.03)	0.45	0.70 (0.59–0.82)	< 0.0001
HDL-cholesterol	−0.31 (−0.38–−0.23)	< 0.0001	0.049 (0.031–0.079)	< 0.0001
Triglycerides	0.14 (0.09–0.20)	< 0.0001	2.16 (1.89–2.47)	< 0.0001
Waist circumference	0.10 (0.07–0.13)	< 0.0001	1.047 (1.037–1.058)	< 0.0001
Female	−0.41 (−0.49–−0.33)	< 0.0001	0.22 (0.16–0.30)	< 0.0001
Educational level	−0.04 (−0.10–0.020)	0.17	0.40 (0.33–0.50)	< 0.0001

The associations were tested using univariate linear regression when logarithmized hair cortisol concentration [ln(HCC)] was the outcome and univariate logistic regression when coronary artery disease was the outcome. Male and lowest level of education (elementary school) was used as reference for these predictors. Raw beta values and hazard ratios are presented with 95% CIs.

median (interquartile range) and discrete data as number (n) with proportion in percent.

Group comparisons were made between participants from the Stressheart and the SCAPIS studies (**Table 1**). Categorical measures were compared using the Pearson's chi-squared test, while quantitative measures were compared between the groups using the nonparametric Mann–Whitney *U* test.

Path analysis was used to test the direct and indirect association between ln(HCC) and CAD. The SMuRFs such as hypertension, hyperlipidemia, and diabetes were included as mediators, while smoking, gender, and educational level were included as confounders. Education was treated as an ordinal measure with elementary school as the reference. The confounders were included, since they were unevenly distributed between participants with and without CAD and are known risk factors for CAD (**Table 1**). Due to the highly parametrized nature of path models, some screening of potential SMuRF measures to be included was undertaken to avoid potential estimation problems. Collinearity was evaluated via the variance inflation factor (VIF), although no clear problems (VIF $>$ 10) were identified. Since model parameter estimation did not converge, measures were omitted one at a time based on the largest VIF values and on a priori strength of evidence To be a potential source of indirect association, a variable needed to be associated with both the ln(HCC) and with CAD. This analysis is reported in **Table 2** using linear regression with statistical inference via the nonparametric bootstrap for ln(HCC) and unconditional logistic regression for CAD. Only SMuRF measures that met both the criteria were included in path models.

The quantitative measures reflecting SMuRFs such as the level of diastolic and systolic blood pressure, fasting plasma glucose, serum triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol, and waist circumference were omitted from the model reported in **Table 3**, despite meeting criteria for inclusion, due to their correlation with SMuRFs inducing multicollinearity. The estimated direct, indirect, and total path coefficients (b) are shown in **Table 3**, along with SE and *p*-value. The percentage of the association between ln(HCC) and CAD that is estimated to be indirect is calculated from indirect ÷ total × 100. Models were fitted in MPlus version 8 using maximum likelihood and with inference derived via the nonparametric bootstrap with 2,000 bootstrap samples. Components of the paths model were estimated using link functions appropriate to the dependent variable measurement scale, identity link for quantitative outcomes, and logit link for binary outcomes. It is noted that due to the cross-sectional design of this study, causal inference cannot be made from these models and purpose of path modeling is to understand the extent to which the associations of interest might be operating via other factors. Any causal modeling would require a future longitudinal study.

A $p < 0.05$ was considered significant in all the analyses. Statistical analyses shown in **Tables 1**, **2** were performed in SPSS version 25 and Stata version 17 for bootstrapping, while the path model shown in **Table 3** were estimated using MPlus version 8.

RESULTS

Age, Gender, Ethnicity, and Educational Level

The mean age was 57 years among participants with and without CAD. A greater proportion of participants with CAD were males (72 vs. 36%, $p < 0.0001$). The educational level was lower among those with CAD and although most participants were born in Sweden, a larger proportion of participants with CAD had another ethnicity (**Table 1**).

Hair Cortisol Concentrations, Cardiovascular Risk Factors, and Anthropometric Measurements

Participant characteristics are given in **Table 1**. Heredity for MI was considerably higher among participants with CAD. Participants with CAD also had a higher body mass index (BMI) and larger waist circumference than participants without CAD. The levels of fasting plasma glucose and triglycerides were higher

TABLE 3 | Direct and indirect associations between ln(HCC) and CAD.

	b	SE	z	P-value
Total	0.349	0.026	13.464	< 0.0001
Total indirect	0.280	0.062	4.508	< 0.0001
Specific indirect				
Hypertension	0.058	0.015	3.812	< 0.0001
Diabetes	0.103	0.031	3.373	0.001
Hyperlipidemia	0.119	0.026	4.505	< 0.0001
Total direct	0.069	0.064	1.070	0.285

Estimates (b) are path coefficients expressing the association between the ln(HCC) as predictor and CAD as outcome either directly or indirectly via several standard modifiable risk factor (SMuRF) measures. The total direct + total indirect = Total.

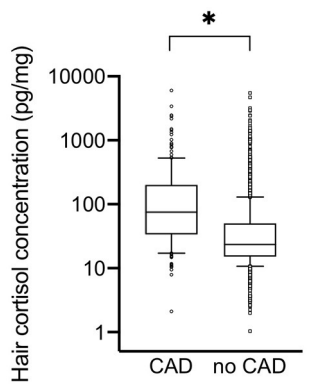

FIGURE 2 | Hair cortisol concentration among participants with or without coronary artery disease (CAD). The hair cortisol concentration given on a logarithmic scale. The line represents the median, box 25th to 75th percentile, and whiskers 10th to 90th percentile. *$p < 0.0001$ for difference between the groups.

among participants with CAD, while the systolic and diastolic blood pressure levels as well as total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were lower. The HCC was three times as high ($p < 0.0001$) among participants with CAD compared to those without CAD (**Figure 2**).

Standard Modifiable Risk Factors

A total of 32% of participants with CAD had no SMuRFs, while 71% of those participants without CAD were SMuRF-less (**Table 1**). Hypertension was the most common SMuRF independently of CAD status (**Table 1**). Participants with CAD had a significantly higher number of SMuRFs compared to participants without CAD (**Table 1**).

Associations Between HCC, Cardiovascular Risk Factors, and CAD

As shown in **Table 2**, higher ln(HCC) was associated with a diagnosis of, or treatment for, hypertension, diabetes, and hyperlipidemia, but not active smoking. Furthermore, a higher ln(HCC) was also associated with lower HDL-cholesterol, higher triglyceride levels, and a greater waist circumference. Being female was associated with lower ln(HCC). Ln(HCC) was

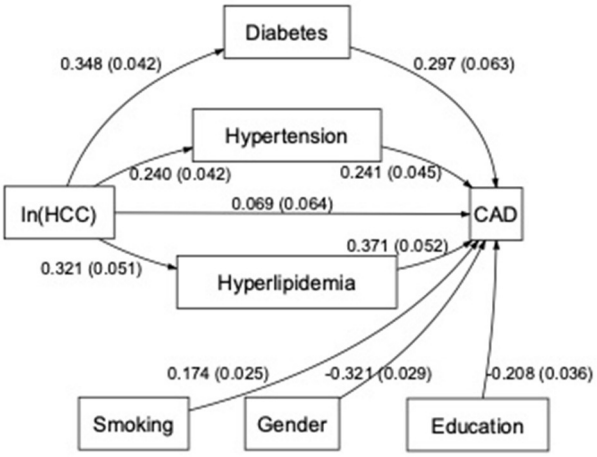

FIGURE 3 | Path diagram over the associations between the HCC, CAD, and standard modifiable cardiovascular risk factors. Boxes represent included variables in the path model and arrows the estimated associations (with the SE in parentheses) between variables in the path model. The arrow from logarithmized HCC [ln(HCC)] to CAD represents the direct association and the others represent the indirect associations. Smoking, gender with male as reference, and education with elementary school as reference were included as confounders in the model.

associated with CAD [hazard ratio (HR) 1.86 (95% CI 1.68–2.06), $p < 0.0001$]. An increased risk of CAD was present among participants with hypertension, diabetes, hyperlipidemia, and active smoking. Lower HDL-cholesterol and higher triglyceride levels were also associated with CAD as well as a large waist circumference, low educational level, and being male were also associated with CAD. Notably, higher LDL-cholesterol levels were associated with lower risk of CAD.

Direct and Indirect Associations Between HCC and CAD

The path model, including the SMuRFs such as diabetes, hypertension, and hyperlipidemia as mediators and smoking, gender, and educational level as confounders, resulted in 80% (0.280/0.349) indirect association between ln(HCC) and CAD (**Figure 3**). Although the direct path between ln(HCC) and CAD was not statistically significant, it was estimated that 20% of the association between ln(HCC) and CAD was direct and, therefore, did not operate via the SMuRFs considered. Specifically, hyperlipidemia ($b = 0.119$), closely followed by diabetes ($b = 0.103$) was the strongest indirect paths between HCC and CAD (**Figure 3**).

DISCUSSION

This study shows that HCC was considerably higher among participants with CAD compared with a healthy population-based sample without a history or symptoms of CAD. The ln(HCC) was also strongly associated with both SMuRFs and other cardiovascular risk factors such as abdominal obesity,

high triglyceride levels, and low HDL-cholesterol levels. Notably, about 80% of the association between ln(HCC) and CAD were indirect, suggesting that these cardiovascular risk factors mediated the association between ln(HCC) and CAD.

The aim of this study was to elucidate upon long-term cortisol exposure as a risk factor for CAD. This was based upon the fact that the SMuRFs such as hypertension, hyperlipidemia, diabetes, and smoking, that are the major contributors of CAD, do not explain development of CAD among all the patients (1). About 15% of patients with a STEMI are SMuRF-less and other risk factors, in addition to SMuRFs, have been sought for (4–6). Since both the iatrogenic and pathological states with cortisol excess increase cardiovascular risk, excessive long-term cortisol exposure is a plausible cardiovascular risk factor in addition to SMuRFs (7, 9, 25). Our results contradict this hypothesis and suggests that the relationship between ln(HCC) and CAD is mainly indirect and mediated by SMuRFs, rather than direct. At first sight, this might seem contradictive to previous studies showing that patients taking prescribed glucocorticoids have increased cardiovascular risk independent of other cardiovascular risk factors (9) and that patients admitted with an AMI have higher HCC prior to the event compared with healthy controls, independent of other cardiovascular risk factors (13, 14). However, estimation of indirect pathways does not equate to an analysis of confounding, but rather estimates how much of the association might be via the SMuRFs considered. Thus, the associations between glucocorticoids and CAD, shown previously that have been independent of cardiovascular risk factors in regression models, do not rule out that the cardiovascular risk factors mediated these associations (9, 13, 14, 26). The clinical implication of this finding is that the increased cortisol levels observed among patients with CAD seems to be more related to the SMuRFs rather than CAD. However, this does not necessarily mean that the role of increased cortisol levels in CAD is unimportant, since it may still increase the risk of SMuRFs. Future longitudinal studies could provide further insight of cause-and-effect in this relationship.

As expected, participants with CAD were mainly males, had lower education, more heredity for MI, a higher number of SMuRFs, larger waist circumference, higher triglyceride, and lower HDL-cholesterol levels as well as higher fasting plasma blood glucose compared to participants without CAD. The total cholesterol level, LDL-cholesterol level, and blood pressure were lower due to a higher rate of pharmacotherapy for hypertension and hyperlipidemia. When screening for potential mediators, several of these quantitative measures were associated with both the ln(HCC) and CAD, qualifying for inclusion in the path model. However, due to multicollinearity, the quantitative measures could not be included as mediators in the final path analysis, but gender, smoking, and educational level were included as confounders.

The strong indirect association between ln(HCC) and CAD does not necessarily equate to a causative effect. A longitudinal or experimental study would be needed to draw such conclusion (26). However, one suggested mechanism may be that glucocorticoids induce insulin resistance and increased blood glucose levels, which are supported by strong associations between increased HCC and the metabolic syndrome and type 2 diabetes (16, 17, 19, 27). Furthermore, several studies imply that individuals with abdominal obesity, which is considered a main cause for insulin resistance in the metabolic syndrome (28), have increased activity in the HPA axis and, therefore, increased cortisol output. Whether the increased cortisol output is a cause or consequence of the abdominal obesity is, however, still debated (29–33).

Data in this article were gathered from two different cohorts. Importantly though, participants in both the cohorts were living in the same geographical region, hair was sampled, and cortisol levels analyzed under the same protocol in the same laboratory during the same period, minimizing the risk for systematic errors in this process. Among participants with CAD, the hair sample was taken during the hospital admission for AMI. Importantly, the first part of the hair strand, reflecting the last week, is located underneath the scalp, and, therefore, not associated with the acute stress from the AMI (34). However, it cannot be excluded that some participants with CAD had angina and chest pain the weeks before the AMI, which could potentially increase stress and, thereby, the HCC. But according to previous studies, the relationship between subjective stress and HCC is limited and only a small proportion of patients with AMI seems to seek medical care due to prodromal symptoms before the actual event (35, 36). Therefore, we consider it unlikely that the increased HCC among patients with CAD were caused by acute stress related to the subsequent AMI, rather than chronically elevated HCC levels. We also note that an analysis of indirect association paths does not literally equate to an analysis of mediation, which has a causal interpretation and, therefore, requires a longitudinal study design.

The diagnosis of CAD was set by the cardiologist at the cardiology department and can, therefore, be considered of high quality. Participants from the SCAPIS study (without CAD) answered a questionnaire including questions on previous MI, angina pectoris, percutaneous coronary intervention, and coronary artery bypass grafting. Furthermore, they were asked about symptoms of chest pain when walking. All the participants answering yes to one of these questions were excluded. Despite being asked explicitly, it cannot be fully granted that none of these participants had been diagnosed with CAD previously. Furthermore, they could have atherosclerotic plaques in coronary arteries despite being asymptomatic. Thus, future studies using noninvasive methods such as coronary computational tomography to examine the prevalence of atherosclerotic coronary artery disease, irrespective of a diagnosis or symptoms of CAD, in relation to cortisol levels could be of great interest. Notably, a recent study suggests that angiographically diagnosed CAD is associated with increased HCC, independent of other cardiovascular risk factors (37). Furthermore, adding information on psychosocial stress would also be interesting, since it is a risk factor for CAD (38) and has been suggested to be linked to increased HCC as well (39).

In conclusion, the association between ln(HCC) and CAD is mainly indirect, mediated by SMuRFs. This suggests that individuals with chronically elevated cortisol levels are more likely to also have cardiovascular risk factors and, thereby, increased cardiovascular risk. However, around 20% of the association between ln(HCC) and CAD is not explained by SMuRFs. Therefore, future studies might also focus on the relation between HCC and atherosclerosis or inflammation (20). Finally, even though studies suggest that increased cortisol levels cause obesity and metabolic dysregulation (8, 9, 20), future longitudinal studies could investigate the cause-and-effect relationship between increased HCC, SMuRFs, and CAD even further.

PREVIOUS PRESENTATIONS

Part of this data focusing on a comparison of the hair cortisol concentration among participants in the Stressheart study and healthy controls have been published previously in Faresjo et al. (13). This article includes more participants with coronary artery disease and addresses the direct and indirect associations between hair cortisol concentrations and coronary artery disease by using path analysis, which was not addressed in the previous article.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available upon request to the authors, if it is in accordance with laws and regulations.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Region Ethical Review Board of Umeå and Linköping. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS: planning and data collection, data analysis, and lead author of manuscript. SS: planning and data collection, data analysis, and co-author of manuscript. ET: development of analysis method and analysis of hair samples, data analysis, and co-author of manuscript. ÅO: design, planning and data collection, and co-author of manuscript. MJ: statistical analysis and co-author of manuscript. TF: principal investigator, design, planning and data collection, data analysis, and co-author of manuscript. All authors contributed to the article and approved the submitted version.

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Association of Central Obesity With All Cause and Cause-Specific Mortality in US Adults: A Prospective Cohort Study

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Background: Previous data on the association between central obesity and mortality are controversial. The aim of this study was to determine the associations between central obesity, as measured by the waist-to-height ratio (WtHR) and waist circumference (WC), with all cause and cause-specific mortality in U.S. adults.

Methods: The study subjects comprised a nationally representative sample of 33,569 adults >20 years of age who were recruited in the National Health and Nutrition Examination Survey between 1999 and 2014. Anthropometric data, including weight, height, and WC, were measured at each of the eight waves using consistent methodology. Death and underlying causes of death were ascertained through 31 December 2015. The association between central obesity and mortality were determined using weighted Cox proportional hazards regression models.

Results: A total of 4013 deaths occurred during a median follow-up of 7.33 years (263,029 person-years). Compared with the subjects in WtHR tertile 1, the subjects in tertiles 2 and 3 were at a higher risk of mortality from all-cause (tertile 2-hazard ratio [HR]: 1.29; 95% confidence interval [CI]: 1.13–1.47; tertile 3-HR: 1.96; 95% CI: 1.64–2.34) and cardiovascular diseases [CVDs] (tertile 2-HR: 1.40; 95% CI: 1.09–1.79; tertile 3-HR: 2.00; 95% CI: 1.47–2.73). Similarly, compared with the subjects in WC tertile 1, the subjects in tertiles 2 and 3 were at a higher risk of mortality from all-cause (tertile 2-HR: 1.15; 95% CI: 1.00–1.31; tertile 3-HR: 1.39; 95% CI: 1.15–1.67) and CVD (tertile 2-HR: 1.48; 95% CI: 1.14–1.93; tertile 3-HR: 1.74; 95% CI: 1.26–2.42). Restricted cubic spline analyses revealed an S-shaped and linear dose-relationship between WtHR and WC with all-cause mortality. Moreover, a WtHR> 0.58 or a WC > 0.98m was shown to be a risk factor for all-cause mortality.

Conclusions: Central obesity was significantly associated with increased risk of all-cause and CVD-related mortality, especially heart diseases-related mortality, even among normal weight adults. In addition to weight control, guideline designer should provide recommendations for people to decrease abdominal fat accumulation, in their effort to reduce mortality risk in later life.

Keywords: central obesity, mortality, cardiovascular diseases, cohort study, NHANES

INTRODUCTION

Central obesity has become a major public health problem in the United States (U.S.), and the estimated prevalence of central obesity in U.S. adults increased from 43.5% in men and 64.7% in women between 2011 and 2012, to 50.1 and 72.5%, respectively, in 2020 (1, 2). Indeed, collective evidence indicated that central obesity, as usually reflected by the waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WtHR), is significantly associated with a higher risk of chronic diseases, such as cardiovascular disease (CVD) or cancer, and the associations are independent of the Body Mass Index (BMI) (3–5); however, the evidence pertaining to the association between central obesity and mortality is conflicting. The majority of studies have shown a positive J-shaped relationship (6–8), but few studies have reported a negative association (9, 10). Furthermore, most studies evaluating the association between central obesity and mortality in select populations such as postmenopausal women, those with chronic health conditions, older adults, or participants located in a given city, rather than a nationally-representative general population (11–14). Additionally, some large scale cohort studies have used self-reported measures rather than technician-measured data to perform analyses, which could result in inaccurate assessment of central obesity because participants tend to over-report their height (6, 15, 16). In addition, several cohort studies did not remove subjects with serious illnesses at baseline to limit the effect of reverse causality (9). Recently, a dose-response meta-analysis involving 72 prospective cohort studies reported a nearly J-shaped association between central obesity indices and all cause mortality (17); however, the potential effects of reverse causality were not restricted. Furthermore, the underlying confounding factors, such as cigarette smoking, were not adjusted in this analysis due an inability to obtain raw data from each of the included studies.

Because the existing evidence is insufficient and controversial regarding to the association between central obesity and mortality, we conducted this study using a nationally representative sample of U.S. adults with precisely measured data to assess the association between central obesity and all-cause and cause-specific mortality.

METHODS

Study Design and Population

We used data from the National Health and Nutrition Examination Survey (NHANES), an ongoing national 2-year-cycle cross-sectional survey conducted by the US Centers for Disease Control and Prevention. Potential participants were selected by a complex, stratified, multistage probability sampling design and were representative of the civilian, non-institutionalized resident population of the U.S. Participants were first interviewed in their homes to collect demographics data and basic health information, then the participants underwent a standardized physical examination in a specially equipped mobile examination center (MEC) for the collection of other health data, such as anthropometric, and laboratory measurements. Written informed consent was provided from all participants or proxies, and the protocol was approved by the Ethics Review Board at the National Center for Health Statistics (NCHS). Detailed information on the design, data collection procedures and weighting are described elsewhere (18). This study included data from representative adults aged >20 years of age who participated in the 8 cycles (1999–2000 to 2013–2014) of the NHANES, with linkage to the National Death Index to 31 December 2015. Among the 43,793 subjects, 10,224 were excluded because of pregnancy (n = 1,416), missing data on height, weight, or WC (n = 4,543), missing data on mortality status (n = 50), and missing data on covariates (demographic information, lifestyle variables, or chronic health conditions, n = 4,215), resulting in a final analytical sample of 33,569 adults.

Anthropometric Measures

Baseline anthropometric information, including weight, height, and WC, were measured during mobile physical examinations in the MEC by trained staff (19). Weight was measured to the nearest 0.1 kg using a digital weight scale with the participants wearing a standard MEC examination gown, consisting of a disposable shirt, pants, and slippers. Height was measured to the nearest 0.1 cm using a stadiometer with a fixed vertical backboard and an adjustable head piece. Participants were instructed to stand with the back of the head, shoulder blades, buttocks, and heels in contact with the vertical backboard. WC was measured to the nearest 0.1 cm using a tape at the uppermost lateral border of the ilium. The WtHR was calculated by dividing the WC by height (both in centimeters).

Mortality

The mortality status of the participants was ascertained by probabilistic matching the NHANES database to the National Death Index records through 31 December, 2015 based on a unique sequence number. Detailed information on linkage methods is available from the NCHS (20).The accuracy of information in the National Death Index records was validated using mortality-linked data from the NHANES I epidemiologic follow-up survey; 98.5% of participants were classified correctly

(21). The 10th revision of the International Classification of Diseases (ICD-10) was used to ascertain the underlying cause of death. We identified deaths from all causes, CVDs (I00–I09, I11, I13, I20–I51, I60–I69), cancers (C00–C97), chronic lower respiratory diseases (J40–J47), diabetes (E10–E14), and other causes. The follow-up duration was defined as the interval from the examination date in the NHANES MEC to the date of death for decedents or to 31 December 2015 for those who were censored.

Covariates Assessment

Covariates of this study involved major demographic characteristics, lifestyle factors, and personal history of chronic diseases, which may be associated with all-cause mortality based on previous literature (22). Demographic and lifestyle variables including age, gender, race/ethnicity, education, marital status, and smoking status, were collected during household interviews, while alcohol intake was obtained during the mobile physical examination in the MEC. Race/ethnicity was classified as Hispanic, non-Hispanic white, non-Hispanic black, or other race. Educational level was categorized as grades 0–12, high school/general equivalency diploma (GED), and some college or above. Marital status was categorized as married, widowed/divorced/separated, never married, and living with a partner. BMI was defined as the weight in kg divided by the height in meters squared, and categorized into normal weight (18.5–<25 kg/m^2), overweight (25–<30 kg/m^2), and obesity (≥30 kg/m^2). Participants were categorized as never, former, and current smokers based on their response to questions about smoking at least 100 cigarettes in their lifetime and whether they were currently smoking. The amount of alcohol intake was classified based on three queries that questioned whether the participant had at least 12 alcohol drinks in any given year (a drink means a 12 ounce beer, a 4 ounce glass of wine, or an ounce of liquor), the amount of days of drinking in the past year and the number of drinks per day on a given drinking day (23). Participants were categorized into the following four alcohol consumption groups: lifetime abstainers (<12 drinks in any given year), former drinkers (≥12 drinks in a previous year), current light to moderate drinkers (current use of <14 drinks/week for men or <7 drinks/week for women), and current heavy drinkers (≥14 drinks/week for men or ≥7 drinks/week for women). Information on personal history of major physician-diagnosed chronic health conditions, including hypertension, diabetes, heart disease (congestive heart failure, coronary heart disease, angina/angina pectoris, or heart attack), stroke, and cancer was collected based on the self-report of participants.

Statistical Analysis

All statistical analyses for the complex sampling design of NHANES by using sample weights, strata, and primary sampling units as specified in the guidelines for analyzing NHANES data (24). The baseline characteristics of participants across the WtHR and WC tertiles were compared using a χ^2 test. We calculated person-years from baseline to the date of death, or 31 December 2015, whichever came first. We tested the proportional hazards assumption by creating interaction terms of exposures and follow-up time and did not identify any violations. We used Cox proportional hazards models to calculated hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of all cause and cause-specific mortality associated with different measures of central obesity. Three multivariable models were constructed: Model 1 was adjusted for age, gender, race/ethnicity, education, marital status, and BMI; Model 2 was additionally adjusted for smoking status, and alcohol intake; and Model 3 was additionally adjusted for chronic health conditions, including hypertension, diabetes, heart disease, stroke, and cancer. Restricted cubic splines (RCSs) with five knots at 5, 25, 50, 75, and 95th percentiles were used to determine the dose-response relationships between the WtHR and WC, and with all-cause mortality after adjusting for all the covariates. We used a stratified analysis to determine whether the association between central obesity and mortality was modified by major baseline variables, including age, gender, race/ethnicity, education, marital status, BMI, smoking status, alcohol intake, and chronic health conditions. Based on sensitivity analysis, we excluded participants who had follow-up evaluations of <2 years duration and removed participants with any chronic health conditions at baseline to minimize potential reverse causation. All analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). A two tailed P-value <0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the baseline characteristics, including demographic factors, lifestyles and personal history of major chronic diseases, of 33,569 eligible adults according to the WtHR and WC tertiles. There were statistically significant differences in each baseline characteristic across the three categories of the WtHR and WC. Participants in WtHR and WC tertile 3 were more likely to be older, non-Hispanic blacks, widowed/divorced/separated, obese, former smokers, lifetime abstainers or former drinkers, have a high school/GED education, and have more chronic conditions when compared with the subjects in tertile 1.

During 263,029 person-years of follow-up (median follow-up, 7.33 years; maximum follow-up, 16.75 years), 4013 deaths occurred, including 863 deaths from CVD, 935 from cancer, 150 from chronic lower respiratory tract diseases, 103 from diabetes, and 1962 from other causes.

The associations between WtHR and WC with all-cause and cause-specific mortality are shown in **Table 2**. Participants in WtHR or WC tertiles 2 or 3 were at higher risk for all cause and CVD mortality compared with the subjects in tertile 1 across the 3 models. In the fully adjusted model, the multivariable-adjusted HRs for all-cause and CVD-related mortality for participants in WtHR tertile 3 were 1.96 (95% CI, 1.64–2.34) and 2.00 (95% CI, 1.47–2.73), respectively, while the HRs for the subjects in WtHR tertile 2 were 1.29 (95% CI, 1.13–1.47) and 1.40 (95% CI, 1.09–1.79), respectively, compared with the subjects in WtHR tertile 1. After adjustment for all confounders, the HRs for all-cause and CVD mortality for the subjects in WC tertile 3 were 1.39

TABLE 1 | Characteristics of study adults according to baseline measures of the WtHR and WC in the National Health and Nutrition Examination Survey, 1999–2014.

Characteristics	WtHR			*P*-values	WC			*P*-values
	Tertile 1	Tertile 2	Tertile 3		Tertile 1	Tertile 2	Tertile 3	
Age group (years)								
20–39	5,521(51.66)	2,998(29.70)	2,435(24.92)	<0.001	5,331(51.32)	2,996(30.80)	2,627(26.13)	<0.001
40–59	3,435(34.92)	3,925(42.91)	3,762(41.09)		3,295(33.75)	3,879(41.87)	3,948(42.98)	
≥60	2,233(13.42)	4,265(27.39)	4,993(33.98)		2,549(14.93)	4,326(27.33)	4,618(30.89)	
Sex								
Men	6,048(50.74)	6,314(55.54)	4,633(42.12)	<0.001	5,664(46.60)	5,678(50.69)	5,653(52.15)	<0.001
Women	5,141(49.26)	4,876(44.46)	6,557(57.88)		5,511(53.40)	5,523(49.31)	5,540(47.85)	
Race/ethnicity								
Hispanic	2,092(10.23)	3,208(14.93)	3,132(14.03)	<0.001	2,650(12.93)	3,230(14.62)	2,552(11.14)	<0.001
Non-Hispanic White	5,624(72.32)	5,233(70.33)	5,216(69.95)		5,143(68.85)	5,267(70.85)	5,663(73.39)	
Non-Hispanic Black	2,384(10.43)	2,036(9.32)	2,432(12.23)		2,191(10.19)	2,050(9.49)	2,611(12.18)	
Other race	1,089(7.02)	713(5.42)	410(3.79)		1,191(8.03)	654(5.04)	367(3.29)	
Education								
Grades 0–12	2,246(13.34)	3,243(18.75)	3,640(21.70)	<0.001	2,684(15.69)	3,300(18.81)	3,145(18.46)	<0.001
High school/GED	2,458(21.37)	2,623(24.48)	2,788(27.27)		2,435(21.73)	2,582(23.77)	2,852(27.15)	
Some college or above	6,485(65.29)	5,324(56.77)	4,762(51.03)		6,056(62.58)	5,319(57.42)	5,196(54.39)	
Marital status								
Married	5,503(52.70)	6,623(62.94)	5,974(57.67)	<0.001	5,464(51.81)	6,445(61.44)	6,191(59.78)	<0.001
Widowed/Divorced/Separated	1,918(14.43)	2,475(18.67)	3,086(23.11)		2,057(15.36)	2,626(19.29)	2,796(20.83)	
Never married	2,819(24.32)	1,361(12.10)	1,523(13.53)		2,731(24.38)	1,401(12.80)	1,571(13.55)	
Living with partner	949(8.55)	731(6.29)	607(5.69)		923(8.45)	729(6.47)	635(5.84)	
Body mass index category								
Normal	8,319(73.49)	1,507(10.80)	50(0.31)	<0.001	8,415(76.90)	1,446(12.58)	15(0.14)	<0.001
Overweight	2,789(25.84)	7,274(65.14)	1,686(12.21)		2,692(22.61)	7,378(67.03)	1,679(14.93)	
Obesity	81(0.67)	2,409(24.06)	9,454(87.48)		68(0.49)	2,377(20.39)	9,499(84.93)	
Smoking status								
Never	6,044(54.22)	5,781(51.36)	5,903(51.73)	<0.001	6,194(54.65)	5,899(52.82)	5,635(49.97)	<0.001
Former	2,150(19.66)	3,176(27.99)	3,270(28.92)		2,132(19.63)	3,039(25.95)	3,425(30.32)	
Current	2,995(26.12)	2,233(20.65)	2,017(19.35)		2,849(25.72)	2,263(21.23)	2,133(19.71)	
Alcohol intake								
Lifetime abstainers	2,630(19.42)	3,048(23.36)	3,932(31.19)	<0.001	2,913(21.29)	3,237(24.50)	3,460(27.14)	<0.001
Former drinker	826(6.06)	1,324(9.54)	1,674(13.41)		886(6.17)	1,283(9.22)	1,655(13.07)	
Light to moderate	6,522(62.52)	5,776(56.20)	4,876(48.22)		6,228(60.73)	5,687(55.77)	5,259(51.65)	
Heavy	1211(12.00)	1042(10.90)	708(7.18)		1148(11.81)	994(10.51)	819(8.14)	
Chronic conditions								
Hypertension	2,034(14.99)	3,909(31.60)	5,776(48.56)	<0.001	2,124(15.36)	3,935(30.42)	5,660(46.87)	<0.001
Diabetes	411(2.30)	1,084(6.56)	2,339(17.21)	<0.001	473(2.67)	1,135(6.37)	2,226(15.88)	<0.001
Heart disease	472(3.01)	960(6.93)	1,375(10.61)	<0.001	520(3.30)	941(6.70)	1,346(9.97)	<0.001
Stroke	190(1.23)	379(2.50)	565(4.00)	<0.001	209(1.33)	417(2.75)	508(3.42)	<0.001
Cancer	760(6.89)	1,133(10.00)	1,243(11.52)	<0.001	774(7.03)	1,131(9.88)	1,231(11.13)	<0.001

Values are n (percentages) unless stated otherwise.
WtHR, waist-to-height ratio; WC, waist circumference; GED, general equivalency diploma.
WtHR: Tertile 1, ≤0.54; Tertile 2, 0.55-0.62; Tertile 3, ≥0.63.
WC: Tertile 1, Men ≤0.93 cm, Women ≤0.88cm; Tertile 2, Men 0.94-1.05 cm, Women 0.89–1.02 cm; Tertile 3, Men ≥1.06cm, Women ≥1.03 cm.

(95% CI, 1.15–1.67) and 1.74 (95% CI, 1.26–2.42), respectively, while the HRs for the subjects in WC tertile 2 were 1.15 (95% CI, 1.00–1.31) and 1.48 (95% CI, 1.14–1.93), respectively, compared with WC tertile 1. In Model 3, participants in WtHR tertile 3 had a 71% higher risk of death from cancer (HR, 1.71, 95% CI, 1.18–2.47) and a 2.52-fold higher risk of death from chronic lower respiratory tract diseases (HR, 2.52, 95% CI, 1.13–5.63), compared with the subjects in WtHR tertile 1. The individuals in WC tertile 3 had a 3.62-fold higher risk of death from chronic lower respiratory tract diseases (HR, 3.62, 95% CI, 1.72–7.59),

TABLE 2 | Associations between the WtHR and WC with all-cause and cause-specific mortality.

Cause of death	WtHR				WC			
	Tertile 1	Tertile 2	Tertile 3	P for trend	Tertile 1	Tertile 2	Tertile 3	P for trend
All causes								
No. of deaths	935	1,433	1,645		1,035	1,476	1,502	
Model 1	1.00	1.37(1.21–1.55)	2.37(2.01–2.79)	<0.001	1.00	1.19(1.06–1.35)	1.63(1.37–1.94)	<0.001
Model 2	1.00	1.37(1.21–1.55)	2.32(1.97–2.73)	<0.001	1.00	1.19(1.05–1.34)	1.57(1.32–1.88)	<0.001
Model 3	1.00	1.29(1.13–1.47)	1.96(1.64–2.34)	<0.001	1.00	1.15(1.00–1.31)	1.39(1.15–1.67)	<0.001
Cardiovascular diseases								
No. of deaths	169	325	369		188	341	334	
Model 1	1.00	1.55(1.22–1.96)	2.70(2.03–3.57)	<0.001	1.00	1.52(1.19–1.95)	2.10(1.52–2.91)	<0.001
Model 2	1.00	1.53(1.21–1.94)	2.59(1.95–3.45)	<0.001	1.00	1.52(1.18–1.94)	2.06(1.49–2.85)	<0.001
Model 3	1.00	1.40(1.09–1.79)	2.00(1.47–2.73)	<0.001	1.00	1.48(1.14–1.93)	1.74(1.26–2.42)	0.001
Cancer								
No. of deaths	232	330	373		241	345	349	
Model 1	1.00	1.13(0.86–1.49)	1.78(1.24–2.56)	0.002	1.00	1.03(0.80–1.32)	1.15(0.80–1.67)	0.432
Model 2	1.00	1.15(0.88–1.51)	1.79(1.24–2.58)	0.002	1.00	1.02(0.79–1.30)	1.09(0.74–1.59)	0.656
Model 3	1.00	1.12(0.85–1.47)	1.71(1.18–2.47)	0.003	1.00	0.98(0.76–1.27)	1.01(0.68–1.50)	0.947
Chronic lower respiratory tract diseases								
No. of deaths	42	53	55		42	56	52	
Model 1	1.00	0.98(0.59–1.64)	2.42(1.15–5.11)	0.041	1.00	1.30(0.76–2.20)	4.26(2.10–8.62)	<0.001
Model 2	1.00	1.09(0.64–1.84)	2.73(1.27–5.86)	0.022	1.00	1.28(0.77–2.15)	3.89(1.90–7.94)	<0.001
Model 3	1.00	1.05(0.62–1.78)	2.52(1.13–5.63)	0.043	1.00	1.26(0.75–2.12)	3.62(1.72–7.59)	0.002
Diabetes mellitus								
No. of deaths	11	32	60		17	39	47	
Model 1	1.00	2.68(1.00–7.13)	6.04(2.15–16.96)	<0.001	1.00	1.76(0.72–4.31)	2.27(0.98–5.24)	0.051
Model 2	1.00	2.56(0.97–6.76)	5.41(0.94–15.13)	0.001	1.00	1.73(0.71–4.24)	2.18(0.93–5.14)	0.069
Model 3	1.00	1.44(0.53–3.96)	1.59(0.50–5.10)	0.505	1.00	1.33(0.51–3.47)	0.97(0.37–2.57)	0.673
Other causes								
No. of deaths	481	693	788		547	695	720	
Model 1	1.00	1.45(1.20–1.74)	2.48(1.92–3.22)	<0.001	1.00	1.14(0.95–1.36)	1.57(1.24–2.00)	<0.001
Model 2	1.00	1.44(1.19–1.73)	2.40(1.85–3.13)	<0.001	1.00	1.13(0.95–1.36)	1.55(1.22–1.97)	<0.001
Model 3	1.00	1.36(1.12–1.65)	2.05(1.58–2.67)	<0.001	1.00	1.10(0.91–1.32)	1.37(1.08–1.74)	0.009

Values are n or weighted hazard ratio (95% confidence interval) unless stated otherwise.
Model 1: Adjusted for sex, age, race/ethnicity, education, marital status and BMI.
Model 2: Model 1+smoking status, and alcohol intake.
Model 3: Model 2+chronic conditions (including hypertension, diabetes, heart disease, stroke, and cancer).
WtHR, waist-to-height ratio; WC, waist circumference.
WtHR: Tertile 1, ≤0.54; Tertile 2, 0.55-0.62; Tertile 3, ≥0.63.
WC: Tertile 1, Men ≤0.93 cm, Women ≤0.88cm; Tertile 2, Men 0.94–1.05 cm, Women 0.89–1.02 cm; Tertile 3, Men ≥1.06cm, Women ≥1.03 cm.

compared with those in WC tertile 1. A similar association existed between the WtHR and other causes of mortality across the three models, as well as WC and other causes of mortality in Model 1. The increasing trend in HRs with the increase in the WtHR or WC was demonstrated by the above models; however, the associations between the WtHR and WC with diabetes were not consistently significant across the three models.

We further divided CVDs into heart diseases (I00–I09, I11, I13, I20–I51) and cerebrovascular diseases (I60–I69). The association between WtHR and heart disease-related mortality was significant across the 3 models, and the effect magnitude increased with elevated WtHR (**Table 3**). In the fully-adjusted model, the HRs for heart diseases-related mortality for participants in tertile 2 or 3 were 1.41 (95% CI, 1.06–1.87) and 2.16 (95% CI, 1.51–3.11), respectively, compared with the subjects in WtHR tertile 1. The relationships between WC and heart, and cerebrovascular disease-related mortality were significant across the 3 models, and the HRs increased in parallel with the increase in WC (**Table 3**). After adjustment for all variables, the effect sizes for heart and cerebrovascular diseases-related mortality for the subjects in WC tertile 3 of were 1.39 (95% CI, 1.15–1.67) and 1.74 (95% CI, 1.26–2.42), respectively, and the HRs for the subjects in WC tertile 2 were 1.15 (95% CI, 1.00–1.31) and 1.48 (95% CI, 1.14–1.93), respectively, compared with the subjects in WC tertile 1.

The RCS analyses showed that the WtHR was associated with all-cause mortality in an S-shaped non-linear manner in the fully-adjusted model ($P_{overall} < 0.01$, $P_{nonlinear} < 0.01$; **Figure 1**).

TABLE 3 | Associations between theWtHR and WC with heart and cerebrovascular diseases-specific mortality.

Cause of death	WtHR				WC			
	Tertile 1	Tertile 2	Tertile 3	*P* for trend	Tertile 1	Tertile 2	Tertile 3	*P* for trend
Heart diseases								
No. of deaths	135	265	305		156	271	278	
Model 1	1.00	1.56(1.19–2.04)	2.95(2.11–4.12)	<0.001	1.00	1.19(1.06–1.35)	1.63(1.37–1.94)	<0.001
Model 2	1.00	1.56(1.19–2.04)	2.85(2.04–4.00)	<0.001	1.00	1.19(1.05–1.34)	1.57(1.32–1.88)	<0.001
Model 3	1.00	1.41(1.06–1.87)	2.16(1.51–3.11)	<0.001	1.00	1.15(1.00–1.31)	1.39(1.15–1.67)	0.007
Cerebrovascular diseases								
No. of deaths	34	60	64		32	70	56	
Model 1	1.00	1.49(0.98–2.28)	1.81(0.94–3.47)	0.080	1.00	1.52(1.19–1.95)	2.10(1.52–2.91)	0.029
Model 2	1.00	1.45(0.95–2.21)	1.69(0.88–3.25)	0.117	1.00	1.52(1.18–1.94)	2.06(1.49–2.85)	0.026
Model 3	1.00	1.36(0.88–2.09)	1.41(0.72–2.78)	0.318	1.00	1.48(1.14–1.93)	1.74(1.26–2.42)	0.053

Values are n or weighted hazard ratio (95% confidence interval) unless stated otherwise.
Model 1: Adjusted for sex, age, race/ethnicity, education, marital status and BMI.
Model 2: Model 1+smoking status, and alcohol intake.
Model 3: Model 2+chronic conditions (including hypertension, diabetes, heart disease, stroke, and cancer).
WtHR, waist-to-height ratio; WC, waist circumference.
WtHR: Tertile 1, ≤0.54; Tertile 2, 0.55–0.62; Tertile 3, ≥0.63.
WC: Tertile 1, Men ≤0.93 cm, Women ≤0.88cm; Tertile 2, Men 0.94-1.05 cm, Women 0.89-1.02 cm; Tertile 3, Men ≥1.06cm, Women ≥1.03 cm.
Heart diseases:I00–I09, I11, I13, I20–I51.
Cerebrovascular diseases: I60–I69.

The risk of all-cause mortality increased with the increase in the WtHR. Specifically, a WtHR>0.58 was shown to be a risk factor of all-cause mortality. The HRs of all-cause mortality increased with the increase in WC in a linear dose-responsive manner ($P_{overall} < 0.01$, $P_{nonlinear} = 0.52$; **Figure 2**). Specifically, a WC > 0.98 m was shown to be a risk factor for all-cause mortality. These results were consistent with the results obtained when the WtHR and WC were treated as category variables in **Table 2**.

Based on the stratified analyses (**Table 4**), the association between WtHR tertile 3 and all-cause mortality was stronger among middle-aged adults (vs. younger or older adults), adults with a high school education (vs. adults with a grade 0–12 education or some college or above education), healthy adults (vs. adults with hypertension or stroke or cancer).The effect sizes between WC tertile 3 and all-cause mortality were stronger among middle-aged adults (vs. younger or older adults), adults with a higher education (vs. lower education), healthy adults (vs. adults with hypertension or cancer). The increasing trend in HRs with the increase in the WtHR or WC was present in most of the subgroups. In addition, the forest plots for the WtHR or WC and all-cause and cause-specific mortality in the normal weight and overweight subgroups, as measured by BMI, are shown in **Supplementary Figures 1**, **2**. The forest plots for the obesity subgroup are not shown because the number of deaths in WtHR or WC tertile 1 in obese subjects was small, which resulted in infinite HRs. In the normal weight subgroup, we found positive associations between WtHR tertile 2 or 3 with all-cause mortality, and CVD, diabetes, and other causes of mortality. The associations between WC tertile 2 or 3 with all-cause mortality, and CVD, and chronic lower respiratory tract diseases-related mortality were positive. In the overweight subgroup, positive associations were shown between WtHR tertile 2 or 3 with all-cause mortality, and CVD, cancer, diabetes, and other causes of mortality. The association between WC tertile 3 and chronic lower respiratory tract disease-related mortality was positive.

Two sensitivity analyses were performed to validate the findings. First, when we removed participants who had <2 years of follow-up time in the lag analysis, the results were nearly unchanged for all-cause mortality (WtHR: tertile 2, 1.28 [1.11–1.48]; tertile 3, 1.92 [1.58–2.33]; WC: tertile 2, 1.19 [1.03–1.37]; tertile 3, 1.39 [1.13–1.70]). Secondly, excluding participants with chronic conditions at baseline, the effect sizes between WC Tertile 3 or WtHR and all-cause mortality were even stronger (WtHR: tertile 2, 1.34 [1.03–1.75]; tertile 3, 2.55 [1.72–3.76]; WC: tertile 3, 1.76 [1.21–2.55]),however, the association between WC tertile 2 and all-cause mortality was no longer significant (1.11 [0.86–1.43]).

DISCUSSION

Summary of Findings

In the present large prospective study of a nationally-representative cohort of U.S. adults, we showed that central obesity, as determined by the WtHR and WC, is associated with an increased risk of all-cause and CVD-related mortality, especially heart diseases-related mortality, independent of demographics, lifestyle factors, and BMI. In addition, the WtHR and WC were shown to be associated with all-cause mortality in an S-shaped non-linear and a linear dose-responsive manner, respectively, and a WtHR> 0.58 or WC > 0.98 m was shown to be a risk factor for all-cause mortality. The distribution of WtHR and WC appeared statistically significantly different between different categories of BMI

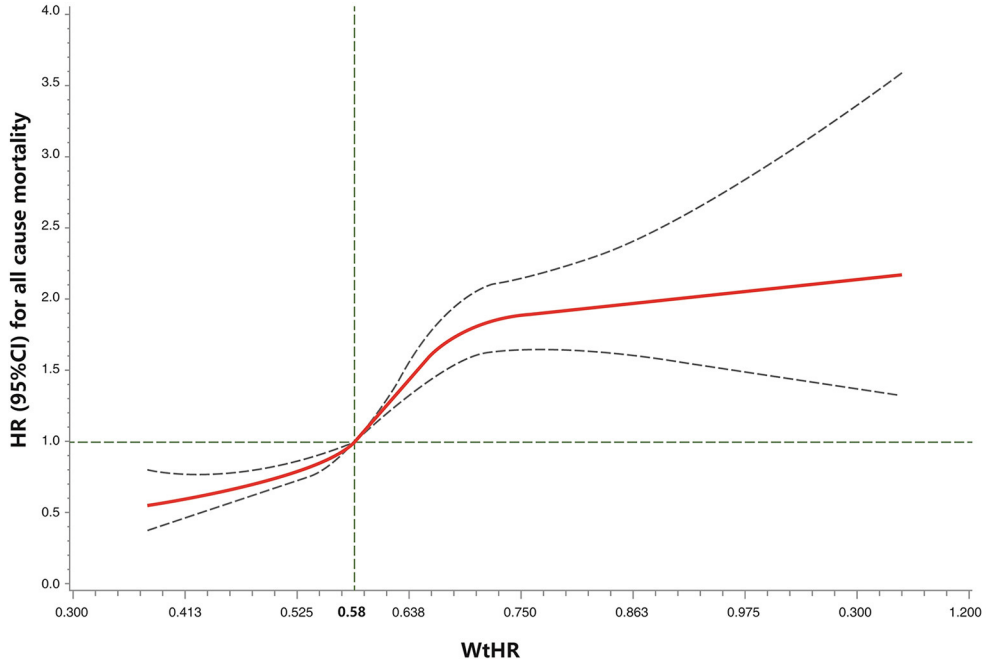

FIGURE 1 | Restricted cubic spline model of the association between the WtHR and all-cause mortality. Legend: Adjusted for demographics, lifestyle factors, body mass index, and chronic health conditions. The solid curve represents the HRs, and the dashed curves represent the 95% CIs. $P_{overall} < 0.01$, $P_{nonlinear} < 0.01$. WtHR, waist-to-height ratio.

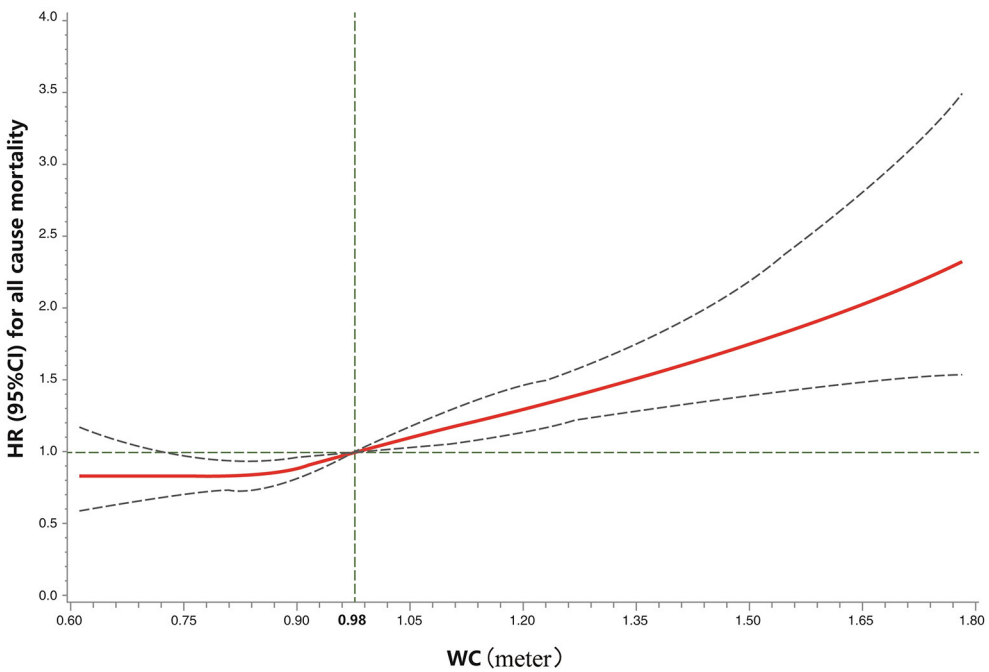

FIGURE 2 | Restricted cubic spline model of the association between the WC and all-cause mortality. Legend: Adjusted for demographics, lifestyle factors, body mass index, and chronic health conditions. The solid curve represents the HRs, and the dashed curves represent the 95% CIs. $P_{overall} < 0.01$, $P_{nonlinear} = 0.52$. WC, waist circumference (meter).

TABLE 4 | Stratified analysis of associations between the WtHR and WC with all-cause mortality.

Subgroups	WtHR					WC				
	Tertile 1	Tertile 2	Tertile 3	*P* for trend	*P* for interaction	Tertile 1	Tertile 2	Tertile 3	*P* for trend	*P* for interaction
Age group (years)										
20–39	1.00	1.09 (0.59–2.01)	1.25 (0.56–2.82)	0.580	<0.001	1.00	1.06 (0.53–2.12)	1.58 (0.75–3.33)	0.190	<0.001
40–59	1.00	1.31 (1.01–1.70)	2.48 (1.66–3.68)	<0.001		1.00	1.17 (0.87–1.57)	1.77 (1.19–2.64)	0.004	
≥60	1.00	1.19 (1.03–1.37)	1.74 (1.43–2.13)	<0.001		1.00	1.09 (0.96–1.25)	1.22 (1.02–1.47)	0.031	
Sex										
Men	1.00	1.40 (1.18–1.66)	2.37 (1.82–3.08)	<0.001	0.312	1.00	1.22 (1.01–1.47)	1.57 (1.23–2.01)	<0.001	0.507
Women	1.00	1.22 (1.03–1.45)	1.62 (1.29–2.04)	<0.001		1.00	1.09 (0.92–1.29)	1.25 (0.98–1.59)	0.070	
Race/ethnicity										
Hispanic	1.00	1.04 (0.77–1.40)	1.59 (1.04–2.44)	0.021	0.491	1.00	0.96 (0.75–1.21)	1.22 (0.86–1.72)	0.230	0.345
Non-Hispanic White	1.00	1.37 (1.17–1.60)	2.16 (1.74–2.68)	<0.001		1.00	1.18 (1.02–1.37)	1.42 (1.15–1.75)	0.001	
Non-Hispanic Black	1.00	1.25 (0.93–1.68)	2.01 (1.35–3.00)	<0.001		1.00	1.23 (0.95–1.60)	1.43 (1.04–1.97)	0.030	
Other race	1.00	0.88 (0.46–1.69)	0.71 (0.26–1.97)	0.525		1.00	0.85 (0.46–1.56)	1.06 (0.36–3.15)	0.951	
Education										
Grades 0–12	1.00	0.97 (0.81–1.16)	1.48 (1.13–1.94)	0.003	<0.001	1.00	1.00 (0.83–1.20)	1.16 (0.92–1.45)	0.199	0.002
High school/GED	1.00	1.45 (1.11–1.89)	2.52 (1.67–3.79)	<0.001		1.00	1.11 (0.83–1.47)	1.41 (0.97–2.05)	0.056	
Some college or above	1.00	1.47 (1.21–1.80)	2.13 (1.63–2.77)	<0.001		1.00	1.32 (1.07–1.64)	1.70 (1.26–2.29)	<0.001	
Marital status										
Married	1.00	1.32 (1.09–1.59)	2.02 (1.57–2.60)	<0.001	0.635	1.00	1.21 (1.00–1.46)	1.51 (1.17–1.95)	0.001	0.349
Widowed/Divorced/Separated	1.00	1.15 (0.96–1.38)	1.77 (1.42–2.19)	<0.001		1.00	1.05 (0.88–1.25)	1.31 (1.01–1.70)	0.044	
Never married	1.00	1.39 (0.84–2.29)	1.96 (0.91–4.21)	0.086		1.00	1.12 (0.66–1.88)	1.08 (0.56–2.09)	0.804	
Living with partner	1.00	1.69 (0.90–3.17)	2.07 (0.81–5.34)	0.114		1.00	1.70 (0.93–3.10)	1.48 (0.57–3.88)	0.389	
Body mass index category										
Normal weight	1.00	1.18 (1.00–1.39)	1.66 (1.04–2.64)	0.021	0.308	1.00	1.20 (1.04–1.40)	0.86 (0.48–1.52)	0.034	0.197
Overweight	1.00	1.36 (1.07–1.74)	2.01 (1.53–2.63)	<0.001		1.00	1.09 (0.88–1.35)	1.24 (0.97–1.58)	0.046	
Obesity	1.00	0.85 (0.11–6.45)	1.46 (0.20–10.81)	<0.001		1.00	0.49 (0.17–1.48)	0.68 (0.22–2.15)	0.023	
Smoking status										
Never	1.00	1.44 (1.19–1.73)	1.92 (1.54–2.39)	<0.001	0.849	1.00	1.13 (0.94–1.35)	1.28 (1.01–1.61)	0.036	0.197
Former	1.00	1.26 (1.04–1.53)	1.96 (1.49–2.59)	<0.001		1.00	1.30 (1.03–1.64)	1.60 (1.18–2.17)	0.003	
Current	1.00	1.02 (0.75–1.39)	1.79 (1.09–2.95)	0.023		1.00	0.93 (0.68–2.26)	1.31 (0.80–2.14)	0.306	
Alcohol intake										
Lifetime abstainers	1.00	1.19 (0.97–1.47)	1.68 (1.26–2.23)	<0.001	0.263	1.00	1.05 (0.85–1.28)	1.26 (0.95–1.66)	0.098	0.484

(Continued)

TABLE 4 | Continued

Subgroups	WtHR					WC				
	Tertile 1	Tertile 2	Tertile 3	*P* for trend	*P* for interaction	Tertile 1	Tertile 2	Tertile 3	*P* for trend	*P* for interaction
Former drinker	1.00	1.41 (1.08–1.85)	2.06 (1.46–2.92)	<0.001		1.00	1.30 (0.99–1.70)	1.76 (1.18–2.60)	0.006	
Light to moderate	1.00	1.26 (1.04–1.52)	1.97 (1.43–2.70)	<0.001		1.00	1.18 (0.96–1.45)	1.45 (1.08–1.96)	0.014	
Heavy	1.00	1.27 (0.86–1.87)	2.14 (1.16–3.94)	0.016		1.00	0.94 (0.64–1.38)	0.90 (0.50–1.60)	0.705	
Hypertension										
Yes	1.00	1.18 (0.99–1.40)	1.68 (1.32–2.15)	<0.001	<0.001	1.00	1.08 (0.89–1.31)	1.26 (0.99–1.60)	0.046	0.001
No	1.00	1.30 (1.09–1.57)	2.20 (1.69–2.87)	<0.001		1.00	1.15 (0.99–1.34)	1.53 (1.21–1.93)	<0.001	
Diabetes										
Yes	1.00	0.77 (0.53–1.13)	1.02 (0.65–1.59)	0.571	0.120	1.00	1.12 (0.81–1.54)	1.22 (0.82–1.81)	0.318	0.838
No	1.00	1.39 (1.23–1.57)	2.20 (1.83–2.63)	<0.001		1.00	1.15 (1.01–1.31)	1.41 (1.14–1.74)	0.002	
Heart disease										
Yes	1.00	1.45 (1.17–1.80)	1.86 (1.39–2.50)	<0.001	0.245	1.00	1.48 (1.14–1.93)	1.51 (1.08–2.12)	0.032	0.434
No	1.00	1.24 (1.08–1.43)	2.00 (1.65–2.42)	<0.001		1.00	1.05 (0.92–1.20)	1.35 (1.09–1.68)	0.006	
Stroke										
Yes	1.00	1.29 (0.89–1.86)	1.35 (0.87–2.10)	0.209	0.033	1.00	1.23 (0.84–1.81)	1.22 (0.75–2.01)	0.473	0.195
No	1.00	1.29 (1.11–1.48)	2.05 (1.68–2.51)	<0.001		1.00	1.13 (0.98–1.30)	1.41 (0.17–1.71)	<0.001	
Cancer										
Yes	1.00	1.21 (0.96–1.52)	1.77 (1.30–2.41)	<0.001	0.009	1.00	1.11 (0.86–1.41)	1.08 (0.74–1.57)	0.698	<0.001
No	1.00	1.31 (1.12–1.53)	2.01 (1.62–2.49)	<0.001		1.00	1.16 (1.00–1.34)	1.50 (1.22–1.84)	<0.001	

Values are weighted hazard ratio (95% confidence interval) unless stated otherwise.
WtHR, waist-to-height ratio; WC, waist circumference; GED, general equivalency diploma.
WtHR: Tertile 1, ≤0.54; Tertile 2, 0.55-0.62; Tertile 3, ≥0.63.
WC: Tertile 1, Men ≤0.93 cm, Women ≤0.88cm; Tertile 2, Men 0.94-1.05 cm, Women 0.89-1.02 cm; Tertile 3, Men ≥1.06cm, Women ≥1.03 cm.

(**Table 1**), which supported the differences between BMI and central obesity measures, therefore further corroborating the importance of measuring central obesity for the risk assessment of mortality. Our findings underscore the importance of decreasing abdominal fat accumulation to avoid central obesity, even among adults with a normal BMI, for reducing mortality risk in later life.

Comparison With Previous Studies

Our findings are generally consistent with several previous studies (7, 13, 17). A recent dose-response meta-analysis involving 72 prospective cohort studies showed that indices of central fatness were positively and significantly associated with a higher all-cause mortality risk (17). Another meta-regression analysis involving 18 prospective studies suggested a J-shaped association between abdominal obesity, as measured by WC, and all-cause mortality (7). A meta-analysis of 82,864 participants from 9 cohort studies showed that a 1 standard deviation increase in the WHR and WC was related to a higher risk of CVD-related mortality (HR [95% CI]: 1.15 [1.05–1.25] and 1.15 [1.04–1.27], respectively) after adjusting for potential confounders (25). These findings were similar to the findings herein; however, several studies obtained different results (9, 10). One such study was a 22-years of prospective population-based cohort study from Netherlands that shown WC and the WtHR are not associated with CVD, cancer or all-cause mortality (10).Of note, the negative relationship may be due to the small sample size; only 6,366 (62.3%) persons were included in the analysis because of missing data or withdrawal (10). Additionally, the subjects were older adults (> 55 years of age) who had a higher prevalence of baseline life-threatening conditions compared with young adults or the general population; however, the information on the disease conditions at baseline were not collected and adjusted, which may have led to reverse causality for the results. Another 22-years

of prospective cohort study involving 15,582 participants from China reported that central obesity was associated with lower all-cause mortality in females >60 years of age (9).The heterogeneity of results might be a reflection of different leading causes of death among the Chinese and Americans. Indeed, obesity-related CVD is the leading cause of death in the U.S. population (26), while underweight-related morbidities, such as cancer and respiratory diseases are the major causes of death in China during the study period (27). Thus, the Chinese cohort had an opposite association between central obesity and mortality in older females. Furthermore, the Chinese study did not remove participants with major diseases at baseline, and did not adjust for the history of diseases, which may have introduced confounding bias for the association.

Interpretation of Results and Implications

There are several possible explanations for our findings. First, central obesity, reflected mainly by a large WC or WtHR, is highly associated with detrimental visceral fat and is a reflection of visceral fat accumulation (17). Excessive visceral fat is related to a variety of adverse metabolic outcomes, including insulin resistance, hyperinsulinemia, hypertension, dyslipidemia, and inflammation, which are known risk factors for CVDs and cancer (11, 28, 29). Second, the association between central obesity and mortality might reflect the characteristics of subjects with abdominal adiposity. It is possible that those individuals might be sedentary or consume more alcohol, which are confirmed risk factors for all-cause mortality (30, 31). Third, central obesity measured by a large WtHR suggests a larger WC or shorter height, or both. Previous studies have reported that taller height is inversely associated with cardiometabolic risk, such as a lower blood cholesterol concentration and systolic blood pressure, a lower plasma glucose levels, and decreased insulin resistance, which might be cardioprotective (32, 33). Thus, central obesity characterized by an increased WtHR or shorter height, resulted in high morbidity and mortality due to CVD.

Our findings may have significant clinical and public health implications. According to the 2013 AHA/ACC/TOS guideline for obesity, clinicians are recommended to use a BMI $\geq$ 25 kg/m^2 as cut-off point to identify patients who need to lose weight, because the WC is measured only in overweight and obese adults (34). Thus, normal weight patients with central obesity are considered free of any particular adiposity-related risk and are not given advice or enrolled in intervention programs to lose weight. Our findings suggest that the guidelines for obesity need to be updated to recognize the potential high-risk subgroup population. In addition, public education and promotion by the Center for Disease Control and Prevention is necessary to guide people with central obesity to exercise more, change the sedentary lifestyle, and restrict diet to reduce calorie intake to decrease abdominal fat.

Strengths and Limitations

This study had several strengths. First, the study was a prospective cohort study that used a nationally-representative sample, which facilitated generalization of the findings to U.S. adults. Second, the anthropometric data, including WC, weight, and height, were measured using precise instruments by trained staff rather than self-report, which reduced information bias. Third, a variety of demographic, lifestyle, and chronic conditional factors were available during each wave, thus we were able to control these potential confounding factors in the analyses.

Our study also had several limitations. First, anthropometric data were only measured at baseline, so we were unable to evaluate the effects of changes in central obesity during follow-up evaluations on all-cause and cause-specific mortality. Second, a total of 10,224 subjects were excluded because of pregnancy or missing data on exposure or covariates, which may have introduced bias if there were differences between those excluded and included. Third, although we adjusted for 13 potential confounders, unmeasured confounding by unmeasured variables cannot be entirely ruled out. Fourth, hip circumferences were not collected in the NHANES study, thus we cannot assess the association between the WHR and mortality. Fifth, participants who had a follow-up time of <2 years or with any chronic health conditions at baseline were excluded in the sensitivity analysis, thus reverse causation could be partially, but not completely overcome. Finally, the number of deaths cases in some subgroups such as the obese subjects in WtHR or WC tertile 1 was insufficient to generate precise estimations.

CONCLUSIONS

This prospective cohort study of U.S. adults showed that central obesity is significantly associated with an increased risk of all-cause and CVD mortality, especially heart diseases-related mortality, even among normal adults, as weight measured by the BMI. In addition to weight control, guideline designers should provide advice and intervention programs for people to decrease abdominal fat and avoid central obesity, in an effort to reduce mortality risk in later life.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/.

ETHICS STATEMENT

This National Health and Nutrition Examination Survey was reviewed and approved by the Ethics Review Board at the National Center for Health Statistics (NCHS). The patients/participants and proxies provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PH and W-QL: conceived and design the study. JL and XY: performed the acquisition of data. PH, XY, and W-QL: conducted the statistical analysis of data. PH, JL, and XY: drafted the manuscript and all authors critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.816144/full#supplementary-material

Supplementary Figure 1 | Associationsbetwen the WtHR and WC wtih all-cause and cause-specific mortality in the normal weight subgroup. Legend: Points represent the HRs, and the transverse lines represent the 95% CIs. CVD, cardiovascular disease; WtHR, waist-to-height ratio; WC, waist circumference (meter).

Supplementary Figure 2 | Associations betwen the WtHR and WC wtih all-cause and cause-specific mortality in the overweight subgroup. Legend: Points represent the HRs, and the transverse lines represent the 95% CIs. CVD, cardiovascular disease; WtHR, waist-to-height ratio; WC, waist circumference (meter).

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Combined Lifestyle Factors and Risk of All-Cause and Cause-Specific Mortality Among Participants in the Linxian Nutrition Intervention Trial: A Cohort, Observational Study

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Background: Several studies have indicated that combinations of lifestyle and dietary factors are associated with risk of total mortality and death from cardiovascular disease and cancer, but limited data are available from long-term follow-up studies in China.

Methods: This study was a observational cohort study. We prospectively examined the associations of combined lifestyle factors and risk of total and cause-specific mortality in the Linxian General Population Nutrition Intervention Trial (NIT) cohort that included 29,584 healthy adults. A points system method was used to calculate a combined risk score of five lifestyle factors, including smoking, alcohol drinking, body mass index, vegetable intake and fruit intake. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs).

Results: Overall, adjusted hazard ratios for mortality increased progressively with an increasing combined risk score. Compared to individuals with a score of zero or one, HRs (95%CIs) for a score of five or above were 1.59 (1.44–1.75) for all-cause mortality, 1.67 (1.48–1.88) for heart disease, 1.69 (1.52–1.88) for stroke, and 1.34 (1.21, 1.47) for cancer. This association for mortality was seen consistently, regardless of gender and age at baseline.

Conclusions: A higher combined risk score was positively associated with risk of total, heart disease, stroke, and cancer mortality. These findings could provide further evidence for the idea that healthy lifestyle is the optimal way to reduce the risk of premature death, and encourage behavior change.

Keywords: combined risk factors, mortality, prospective study, linxian nutrition intervention trial, China

INTRODUCTION

A large body of evidence has suggested that lifestyle-related factors are associated with a higher risk of multiple chronic diseases and premature death (1–6). However, limited studies have examined the combined effects of lifestyle-related factors on risk of mortality (7–15). Quantifying the magnitude of effects of lifestyle-related factors on risk of mortality individually and in combination will help identify priorities for clinical and public health efforts. A previous prospective cohort study of 74,942 women in the Shanghai Women's Health Study indicated a 33% reduction in all-cause mortality and a 59% reduction in cardiovascular mortality among women with four to five healthy factors (including never exposed to spouse's smoking, lower waist-hip ratio, daily exercise, normal weight, and higher daily fruit and vegetable intake), as compared with women with none of these healthy factors (7). Other prospective cohort studies, such as the Nurse's Health Study (13), the EPIC-Norfolk Study (14) and the JACC study (10), similarly reported an inverse association between combinations of healthy lifestyle factors and all-cause mortality. However, most studies for the association of combined lifestyle factors with risk of mortality have been conducted in the Western countries. Little evidence is available from long-term follow-up studies in the Chinese population, whose lifestyles may differ from the Western populations.

The Linxian General Population Nutrition Intervention Trial (NIT) prospectively collected data on all-cause mortality outcomes. The selected lifestyle factors included body mass index (BMI), smoking, alcohol drinking, vegetable intake and fruit intake. Herein we reported the association of the combination of these lifestyle factors and risk of all-cause and cause-specific mortality over 30 years of follow-up in the NIT Cohort in China.

METHODS

Study Population

This study was a observational study based on NIT cohort. The design of the NIT study has been described previously (16, 17). Briefly, the NIT enrolled healthy individuals between the ages of 40 and 69 years from four communes in northern Linxian. All participants have provided written informed consent. Finally, a total of 29,584 subjects were randomly assigned to one of eight intervention groups which received daily vitamin/mineral supplement combinations or placebo according to a one-half replicate of a 2^4 fractional factorial design. The intervention began in March 1986 and lasted for 5.25 years.

We excluded participants ($n = 133$) with missing values of five included lifestyle factors. Finally, a total of 29,451 subjects were included in the analysis (13,129 men and 16,322 women) (**Supplementary Figure 1**).

This study was approved by the Institutional Review Boards of the US NIH and the Chinese Academy of Medical Sciences. All participants gave informed consent for the use of their data.

Data Collection

Demographic characteristics and lifestyle factors at baseline were collected using a self-designed questionnaire. All participants had their basic physical examination by trained staff using a standard protocol. Body weight and height were measured while subjects were not wearing shoes. Body mass index was then calculated as weight in kilograms divided by height in meters squared (kg/m^2). Smoking was defined as regular cigarette or pipe use for at least 6 months (including ever or current smokers), and alcohol use was defined as any alcohol consumption in the past 12 months (including ever or current alcohol drinkers). For vegetable and fruit intake, participants were asked how often, on average, they consumed during the last year before the interview date. The possible responses ranged from "never" to "several times per day." The responses were then converted to different units according to the distribution of our data, e.g., times per week for vegetables and times per year for fruit. Family history of cancer was considered positive if participants reported a cancer in at least one first-degree relative.

Follow-Up and Outcome

During the trial period (1986–1991), village doctors visited all participants monthly, and all endpoints were confirmed by an International Endpoints Review Committee consisting of

TABLE 1 | Baseline characteristics in the Linxian General Population Nutrition Intervention Trial Cohort.

Lifestyle factors	All subjects	Men	Women
No. of participants, *n* (%)	29,451 (100.0)	13,129 (44.6)	16,322 (55.4)
Age [Mean (SD), years]	51.9 (8.9)	52.6 (9.0)	51.3 (8.7)
Person-years	629,144.1	258,773.4	370,370.7
BMI [Mean (SD), kg/m^2]	22.0 (2.5)	21.7 (2.1)	22.2 (2.7)
Smoking, *n* (%)			
No	20,557 (69.8)	4,274 (32.5)	16,283 (99.8)
Yes	8,894 (30.2)	8,855 (67.5)	39 (0.2)
Alcohol drinking, *n* (%)			
No	22,536 (76.5)	7,879 (60.0)	14,657 (89.8)
Yes	6,915 (23.5)	5,250 (40.0)	1,665 (10.2)
Vegetable intake, *n* (%), (times/week)			
Tertile 1, <14	9,473 (32.2)	4,075 (31.0)	5,398 (33.1)
Tertile 2, ≥14 to <17.5	8,461 (28.7)	3,702 (28.2)	4,759 (29.1)
Tertile 3, ≥17. 5	11,517 (39.1)	5,352 (40.8)	6,165 (37.8)
Fruit intake, *n* (%), (times/year)			
Tertile 1, <2.4	10,462 (35.5)	3,960 (30.2)	6,502 (39.8)
Tertile 2, ≥2.4 to <24	8,609 (29.2)	3,850 (29.3)	4,759 (29.2)
Tertile 3, ≥24	10,380 (35.3)	5,319 (40.5)	5,061 (31.0)
Education level, n (%)			
Never	11,808 (40.1)	2,400 (18.3)	9,408 (57.6)
One to 5 years	9,190 (31.2)	5,848 (44.5)	3,342 (20.5)
Primary school	3,148 (10.7)	2,172 (16.5)	976 (6.0)
High school or higher education	2,705 (9.2)	2,097 (16.0)	608 (3.7)
Others	2,600 (8.8)	612 (4.7)	1,988 (12.2)
Family history of cancer, n (%)			
No	19315 (65.6)	8,500 (64.7)	10,815 (66.3)
Yes	10,136 (34.4)	4,629 (35.3)	5,507 (33.7)

American and Chinese experts in cytology, pathology, surgery, and radiology. In the post-trial follow-up (after 1991), village doctors continued to contact all living participants monthly, and new cancer cases and all-cause deaths were verified by a panel of American and Chinese experts (1991–1996) or senior Chinese doctors (1996–2018). Diagnostic materials included case records, pathology and cytology slides, X rays, biochemical results, and ultrasound, endoscopy and surgery reports. Death outcomes were examined with death registration quarterly. The causes of death were coded according to the International Disease Classification Codes, version 10 (ICD-10). Cause-specific mortality included heart disease mortality, stroke mortality, and cancer mortality. Heart disease mortality were defined as death caused by coronary heart disease, hypertensive heart disease, rheumatic heart disease, pulmonary heart disease, or other cardiovascular disease.

Statistical Analysis

Frequencies and percentages of demographic and other participant characteristics were calculated by gender. Participants were censored at the date of death, last known follow-up date, or January 31, 2018, whichever occurred first.

Two combined risk scores of five lifestyle factors were created to examine the combined effects of the individual risk factors. A combined risk score 1 (CRS1) was created using a points system method based on the selected risk factors. This method was originally developed by Sullivan et al. to evaluate the combined effects of several risk factors on chronic diseases (18). Briefly, we created the CRS1 through the following three steps: (1) running the multivariable Cox regression model that includes all baseline characteristics and selected lifestyle factors, and defining the regression coefficient of age (β_0) as the constant, representing the regression coefficient for one-year increase in age with risk of mortality; (2) calculating the individual risk point for each level of each lifestyle factor by dividing the respective regression coefficient (β_i) with the constant (β_0); (3) rounding the risk points to the nearest integers and calculating the CRS1 by summing individual risk points for each level of each risk factor. We also created a CRS2 by summing the number of five risk factors (total score: range of 0–5 points). Subjects were assigned one point for

TABLE 2 | Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the associations between individual lifestyle factors and risk of all-cause and cause-specific mortality.

Lifestyle factors	All-cause		Heart disease		Stroke		Cancer	
	No. of death cases	Multivariable HR (95% CI) *	No. of death cases	Multivariable HR (95% CI) *	No. of death cases	Multivariable HR (95% CI) *	No. of death cases	Multivariable HR (95% CI) *
Body mass index, kg/m^2								
<18.5	1,273	**1.12 (1.05, 1.18)**	414	**1.34 (1.21, 1.48)**	328	**0.87 (0.77, 0.97)**	324	1.06 (0.95, 1.19)
≥18.5 to <25.0	16,981	1.00 (Ref)	4,128	1.00 (Ref)	5,311	1.00 (Ref)	5,162	1.00 (Ref)
≥25.0 to <30.0	2,088	**1.14 (1.09, 1.19)**	458	1.03 (0.93, 1.13)	870	**1.47 (1.37, 1.58)**	490	0.91 (0.83, 1.00)
≥ 30.0	124	**1.43 (1.20, 1.70)**	26	1.12 (0.76, 1.65)	55	**1.87 (1.43, 2.44)**	20	0.86 (0.56, 1.34)
Smoking								
Ever or current	6,900	**1.16 (1.11, 1.21)**	1,673	**1.35 (1.24, 1.48)**	1,913	1.02 (0.95, 1.10)	2,290	**1.20 (1.11, 1.29)**
Never	13,566	1.00 (Ref)	3,353	1.00 (Ref)	4,651	1.00 (Ref)	3,706	1.00 (Ref)
Alcohol drinking								
Never	15,900	**1.13 (1.03, 1.24)**	4,003	1.19 (0.97, 1.46)	5,206	0.98 (0.83, 1.16)	4,491	**1.31 (1.10, 1.56)**
Ever or current	4,566	1.00 (Ref)	1,023	1.00 (Ref)	1,358	1.00 (Ref)	1,505	1.00 (Ref)
Vegetable intake, times/week								
Tertile 1, <14	6,753	1.02 (0.99, 1.06)	1,675	1.03 (0.96, 1.10)	2,158	1.00 (0.94, 1.06)	1,905	1.00 (0.94, 1.07)
Tertile 2, ≥14 to <17.5	5,795	1.00 (0.96, 1.03)	1,434	1.00 (0.94, 1.08)	1,880	1.00 (0.94, 1.06)	1,681	0.99 (0.93, 1.06)
Tertile 3, ≥17.5	7,918	1.00 (Ref)	1,917	1.00 (Ref)	2,526	1.00 (Ref)	2,410	1.00 (Ref)
Fruit intake, times/year								
Tertile 1, <2.4	7,943	**1.10 (1.07, 1.14)**	2,127	**1.17 (1.01, 1.25)**	2,536	**1.06 (1.00, 1.13)**	2,172	1.05 (0.99, 1.12)
Tertile 2, ≥2.4 to <24	5,841	**1.05 (1.02, 1.09)**	1,400	**1.09 (1.01, 1.17)**	1,866	1.01 (0.95, 1.07)	1,704	1.02 (0.96, 1.09)
Tertile 3, ≥24	6,682	1.00 (Ref)	1,499	1.00 (Ref)	2,162	1.00 (Ref)	2,120	1.00 (Ref)

Adjusted for age at baseline, sex, commune, education level, and family history of cancer. Bold text indicates statistical significance.

having the unhealthy factor and zero point for having the healthy factor (more details see **Supplementary Table 1**).

Cox proportional hazards regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the associations between the individual lifestyle factors / CRS and risk of all-cause and cause-specific mortality. CRS1 were treated as both continuous and categorical variables (CRS1≤1 as the reference group, based on the distribution) in the Cox models. Proportional hazards assumption was tested by including an interaction term between time and risk factor ($P>0.05$ for all tests) in the Cox models. Potential confounders included age at baseline (continuous variable), sex (men or women), commune (Rencun,Yaocun, Hengshui or Donggang),education level (never, <5 years, primary school, high school or higher education, or other), and family history of cancer (positive or negative). We also performed sensitivity analyses by excluding individuals who were followed up <3 years.

All statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc. Cary, NC, USA). All tests were two-sided and associations were considered significant for $p < 0.05$.

RESULTS

During 629,144 person-years of follow-up, we identified 20,466 deaths, including 5,026 from heart disease, 6,564 from stroke and 5,996 from cancer. Approximately 1% of participants were lost to follow-up. **Table 1** shows characteristics of the participants at baseline by men and women. Women were more likely to be older, less educated (higher education, 16.0 vs. 3.7%), and less commonly smokers and alcohol drinkers.

Table 2 summaries the hazard ratios for the associations of the individual lifestyle factors and risk of total, heart disease, stroke and cancer mortality. Each of the unhealthy lifestyle factors: being underweight, overweight or obese, smoking, no alcohol intake, having a low vegetable intake, and having a low fruit intake, was associated with a significantly higher risk of all-cause mortality. As expected, there was more variability in the risks observed for the associations of the different health behaviors and cause-specific mortalities.

Table 3 presents the HRs for the CRS1 of multiple lifestyle factors and overall and cause-specific mortality. As shown in the table, all categories of mortality elevated with an increasing risk score (all P_{trend} values<0.05). As compared with subjects who had a CRS1 of 0–1 point, the HR for individuals with five or more points was 1.59 (95% CI: 1.44, 1.75) for all-cause mortality, 1.67 (1.48, 1.88) for heart disease mortality, 1.69 (1.52, 1.88) for stroke mortality and 1.34 (1.21, 1.47) for cancer mortality. Subgroup analyses by age at baseline indicated similar associations among younger (<55 years) and older participants (≥55 years). The strongest association was for deaths due to stroke among younger individuals who had a score of 5 or more points (HR = 1.96, 95% CI: 1.67, 2.30) (**Figure 1**). Results for men and women were similar to each other, with moderate increases in risk for all-cause, heart disease, and stroke mortality and relatively small associations for cancer mortality (**Figure 2**). Sensitivity analyses by exclusion of individuals who died the first 3 years of follow-up did not materially alter our results (**Supplementary Figure 2**). Similar patterns were generally seen for the association between CRS 2 and total, cause-specific mortality (**Supplementary Table 3**). Cumulative mortality curves of total, heart disease, stroke, and cancer by CRS1 categories are presented in **Supplementary Figure 3.**

TABLE 3 | HRs and 95% CIs for the associations between CRS 1 and risk of all-cause and cause-specific mortality.

Mortality	HR and 95% CI*						P_{trend}
	Continuous	CRS1≤1	2	3	4	≥5	
All-cause							
No. of deaths	20,466	3,398	7,995	4,809	3,793	471	
Age- and sex-adjusted HR (95%CI)	**1.10 (1.09, 1.12)**	1.00 (Ref)	**1.19 (1.05, 1.14)**	**1.18 (1.13, 1.23)**	**1.32 (1.26, 1.38)**	**1.60 (1.45, 1.76)**	<0.001
Multivariate adjusted HR (95%CI)*	**1.10 (1.08, 1.11)**	1.00 (Ref)	**1.10 (1.05, 1.14)**	**1.18 (1.13, 1.23)**	**1.31 (1.25, 1.37)**	**1.59 (1.44, 1.75)**	<0.001
Heart disease							
No. of deaths	5,026	978	2,154	660	764	470	
Age- and sex-adjusted HR (95%CI)	**1.15 (1.12, 1.18)**	1.00 (Ref)	**1.14 (1.05, 1.23)**	**1.26 (1.14, 1.40)**	**1.52 (1.38, 1.68)**	**1.74 (1.55, 1.96)**	<0.001
Multivariate adjusted HR (95%CI)*	**1.14 (1.11, 1.17)**	1.00 (Ref)	**1.13 (1.05, 1.22)**	**1.24 (1.18, 1.37)**	**1.51 (1.37, 1.67)**	**1.67 (1.48, 1.88)**	<0.001
Stroke							
No. of deaths	6,564	3,259	2,380	144	396	385	
Age- and sex-adjusted HR (95%CI)	**1.14 (1.12, 1.16)**	1.00 (Ref)	**1.12 (1.07, 1.19)**	**1.45 (1.23, 1.72)**	**1.49 (1.34, 1.66)**	**1.66 (1.49, 1.84)**	<0.001
Multivariate adjusted HR (95%CI)*	**1.15 (1.12, 1.17)**	1.00 (Ref)	**1.14 (1.08, 1.20)**	**1.51 (1.28, 1.79)**	**1.51 (1.36, 1.68)**	**1.69 (1.52, 1.88)**	<0.001
Cancer							
No. of deaths	5,996	867	1,870	1,054	921	1,284	
Age- and sex-adjusted HR (95%CI)	**1.09 (1.07, 1.11)**	1.00 (Ref)	**1.11 (1.02, 1.20)**	**1.19 (1.09, 1.31)**	**1.30 (1.18, 1.44)**	**1.41 (1.28, 1.56)**	<0.001
Multivariate adjusted HR (95%CI)*	**1.07 (1.05, 1.10)**	1.00 (Ref)	**1.10 (1.02, 1.19)**	**1.14 (1.04, 1.25)**	**1.25 (1.13, 1.38)**	**1.34 (1.21, 1.47)**	<0.001

HR, hazard ratio; 95% CI, 95% confidence interval; CRS, combined risk score.

**Adjusted for age at baseline, sex, commune, education level, and family history of cancer. Bold text indicates statistical significance.*

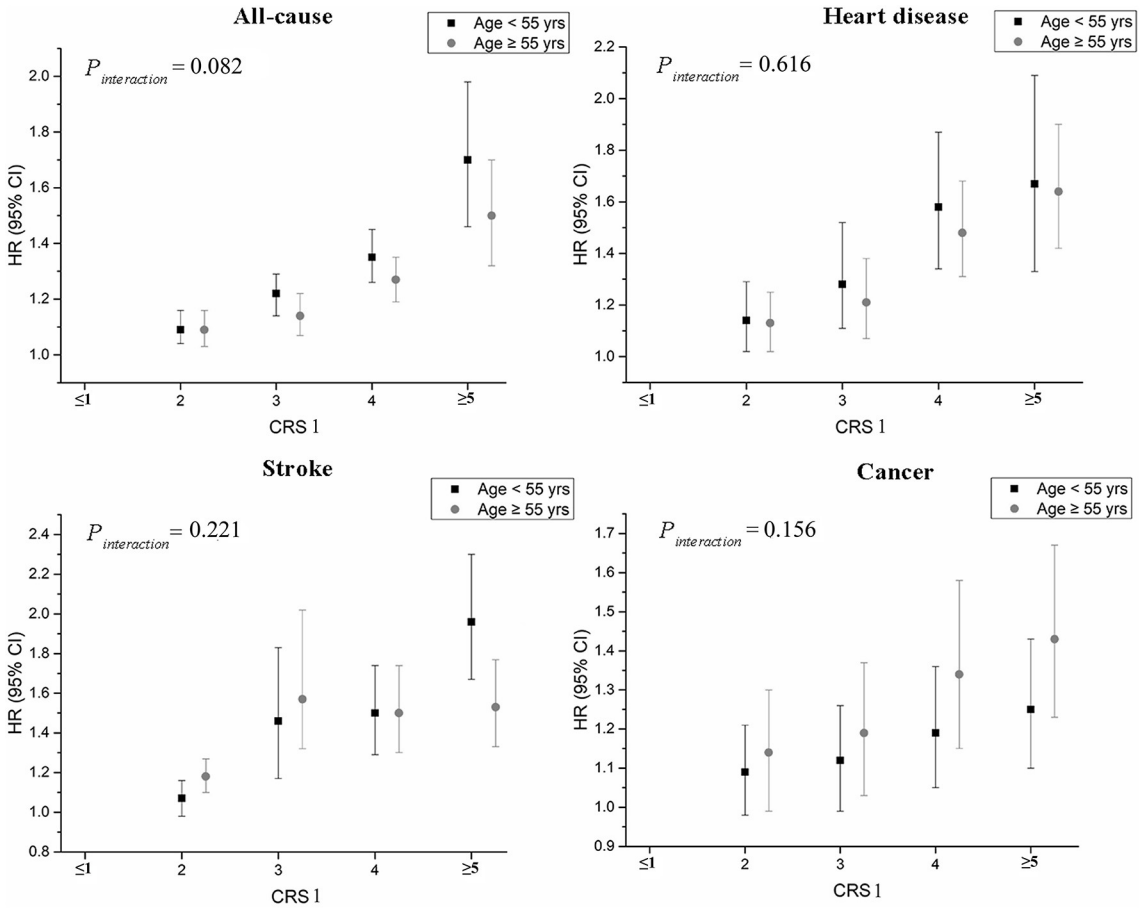

FIGURE 1 | Hazard ratios and 95% confidence intervals for the associations between CRS1 and risk of all-cause and cause-specific mortality by age at baseline in the Linxian Nutrition Intervention Trial Cohort. Age at baseline was divided into two groups (<55 years and ≥55 years), based on the median value. Multivariable hazard ratios were adjusted for age at baseline, sex, commune, education level, and family history of cancer. CRS, combined risk score.

DISCUSSION

In this large population-based cohort study, we found that being underweight, overweight or obese, smoking, no alcohol intake, having a low vegetable intake, and having a low fruit intake were independently associated with higher risk of all-cause, heart disease, stroke and cancer mortality. There was also a significant trend of increasing mortality risk with increasing values of the combined risk score. Significant associations were observed for total and cause-specific mortality, regardless of gender and age at baseline. After excluding subjects who were followed up <3 years, our results did not materially change.

In previous prospective cohort studies, smoking, overweight or obesity, and dietary patterns have consistently been associated with increased risk of chronic diseases (1, 2, 5). Not all of these risk factors could be studied in randomized controlled trials with end points of disease due to ethical or feasibility concerns. However, evidence from randomized controlled trials indicates that Mediterranean-style diet could be a protective factor of coronary heart disease (19). In addition, there is also evidence support the protective effect of a healthy diet, the combination of physical activity, and moderate weight loss for type 2 diabetes (20); and of smoking cessation for premature mortality (21).

A number of studies have demonstrated associations of heavy alcohol consumption with a higher risk of liver cirrhosis, stroke, coronary artery disease, various cancers, and hypertension (22, 23). Consistent with previous studies from Linxian, however, we found that alcohol consumption was associated with lower total and cancer mortality, and a nearly significant reduction in heart disease mortality as well. One possible reason for this was that alcohol consumption in Linxian, in 1985, was rare and quite modest, due to the expense of alcohol, and drinking alcohol was probably a marker for higher socioeconomic status (24). Indeed, one limitation of our study was the lack of information on the amount of alcohol consumption, which could have helped explain the observed mortality associations. In western studies, moderate alcohol consumption has been reported to have debated benefits, indicating either beneficial or harmful effects (25, 26). Nonetheless, determination of beneficial effects could be biased from healthy lifestyle behaviors, and harmful effects could also be overestimated due to related unhealthy factors, such as smoking, uncontrolled diet, and physical inactivity. Thus, the

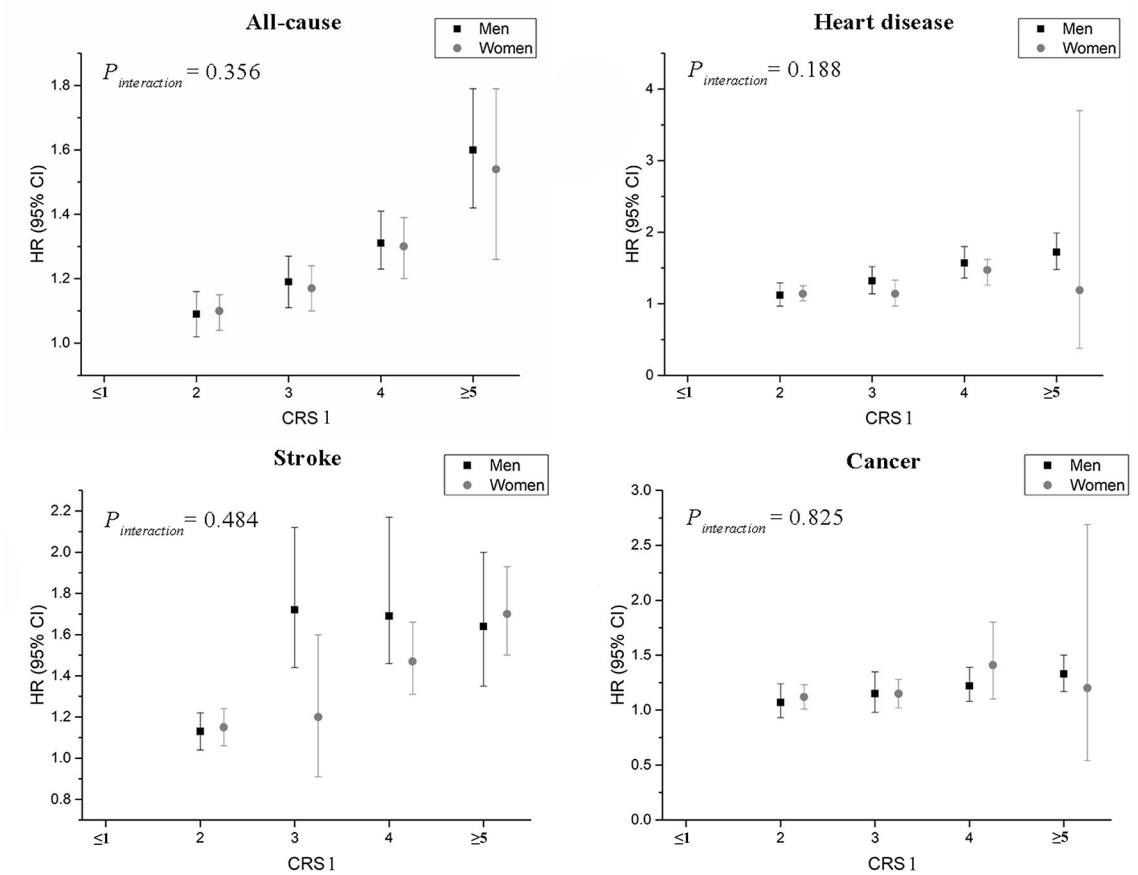

FIGURE 2 | Hazard ratios and 95% confidence intervals for the associations between CRS1 and risk of all-cause and cause-specific mortality by men and women in the Linxian Nutrition Intervention Trial Cohort. Multivariable hazard ratios were adjusted for age at baseline, commune, education level, and family history of cancer. CRS, combined risk score.

overall balance of beneficial and harmful effects of alcohol should be considered when making recommendations.

The effect of lifestyle factors such as smoking, alcohol drinking, BMI, and diet on health is overwhelming. Previous studies have also reported the protective effects of combinations of lifestyle factors on mortality (7–15, 27–30). Results from the Nurses' Health Study (NHS) of 77,782 women aged 34–59 years during 24 years of follow-up indicated that RRs for five lifestyle factors (being overweight, cigarette smoking, no light-to-moderate alcohol intake, taking little moderate-to-vigorous physical activity, and low diet quality score) were 4.31 for total mortality, 3.26 for cancer mortality, and 8.17 for cardiovascular mortality. They estimated that these lifestyle factors could be responsible for ∼55% of the deaths occurring in this cohort (13). In addition, the US Health Professionals Study of 42,847 men aged 40–75 years, followed for 16 years, suggested that men with five healthy behaviors, including not smoking, BMI<25 kg/m^2, moderate-to-vigorous activity, moderate alcohol consumption, and being in the top 40% of a healthy diet score, had an 87% lower risk of coronary heart disease compared with men who had none of these factors (31). More recently, Veronese et al. examined the combined associations of physical activity, diet, smoking, and moderate alcohol drinking with body weight on risk of mortality in the NHS and the Health Professionals Follow-up Study after 30-years follow-up. In each of the four categories of BMI studied, individuals who had healthy lifestyle factors had a significantly lower risk of total, cardiovascular, and cancer mortality as compared with individuals who had no healthy lifestyle factors. For BMI between 18.5–22.4, subjects with a combination of at least three healthy lifestyle factors had the lowest risk of all cause (HR = 0.39, 95% CI: 0.35, 0.43), cancer (0.40, 95% CI: 0.34, 0.47), and cardiovascular (0.37, 95% CI: 0.29, 0.46) mortality, as compared with those with BMI of 22.5–24.9 and none of the healthy lifestyle factors (12). Most of the previously published studies of the effects of multiple lifestyle factors on mortality have been conducted in western countries, but a few studies have examined these associations in Asian populations, including one study conducted in China (7) and three conducted in Japan (10, 32, 33). Each of these studies indicated that healthier lifestyles, defined by several lifestyle-related factors, were associated with substantial reductions in death in Asian populations, consistent with our findings.

Effective recommendations for preventing cardiovascular disease and cancer are very important for the public health. General recommendations for diet have been released which are supported by strong evidence. In addition, Scicchitano P et al. observed the impact of nutraceuticals in managing lipid disorders, which could play an important role in the occurrence of cardiovascular disease (34). However, the previous survey showed that because of the lower socio-economic status, dietary pattern in the Linxian population had the characteristics of single variety, and great seasonal effects (35), and the obtain of nutraceuticals was difficult. Therefore, the effect of nutraceuticals on the results was not evaluated in current study. Nonetheless, many people take dietary supplements, even though several studies have reported no effects of multivitamin supplementation on mortality or cardiovascular disease (36–38). These findings suggest that supplementing the diet with vitamin/mineral supplements has no clear benefit for well-nourished adults and may even be harmful, and that therefore these supplements should not be applied to chronic disease prevention in populations that do not have vitamin or mineral deficiencies.

Strengths and Limitations

Our study had several important strengths, including its prospective design, large sample size, homogeneous ethnic makeup, and over 30 years of follow-up. Furthermore, we used a points system method to calculate the combined risk score of lifestyle factors, which was originally developed and validated by Sullivan et al. (18) based on The Framingham Heart Study. This method was a weighted approach based on the effect size of each factor which could improve the estimates of the overall impact of lifestyle factors on mortality. We also calculated a simple score that could be conceptually easy to understand and could be used in clinical practice and the development of public guidelines.

A number of limitations need to be noted regarding the present study. First, we only had a single baseline questionnaire to characterize individuals and thus could not take into account likely changes in lifestyles during the follow-up period, a time of dramatic changes in Chinese society, and this could contribute to misclassification of lifestyle factors. Second, there was no mention about the occurrence of well-known cardiovascular-specific risk factors such as hypertension, abnormal blood lipids, and adverse emotional states, which may impact results independently from lifestyle. Third, as noted above, no data were collected at baseline on the place and type of work of participants, air pollution, and the amount of alcohol, we cannot exclude the residual confounding due to these unmeasured factors. Fourth, although individuals who had ever been diagnosed with cancer or severe diseases (such as liver disease or severe kidney) were excluded from the main analyses, our results still may have been influenced by the presence of prevalent subclinical disease. Finally, our subjects were entirely composed of rural Chinese adults whose lifestyles and diets were assessed in 1985, which may affect the generalizability of our results to other populations today.

CONCLUSIONS

In summary, we found that a higher combined risk score, based on five factors, was associated with risk of total, heart disease, stroke and cancer mortality. These results may indicate that even small differences in lifestyle may make a large difference to health. Future studies are needed to design appropriate interventions to reduce these unhealthy lifestyle factors in Asian populations.

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 Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-lQ, J-hF, PT, and CA: study concepts. Y-lQ, J-hF, PT, CA, and SD: study design. Y-lQ and J-hF: data acquisition and quality control of data and algorithms. J-bW and SD: data analysis and interpretation. HY and J-bW: statistical analysis and manuscript editing. J-hF, J-bW, and HY: manuscript preparation. HY, J-bW, J-hF, Y-lQ, PT, and CA: manuscript review. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.772617/full#supplementary-material

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Similarities in Hypertension Status but Differences in Mortality Risk: A Comparison of 2017 ACC/AHA and 2018 Chinese Hypertension Guidelines

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Background: Few studies investigated the concordance in hypertension status and antihypertensive treatment recommendations between the 2018 Chinese Hypertension League (CHL) guidelines and the 2017 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines and assessed the change of premature mortality risk with hypertension defined by the ACC/AHA guidelines.

Methods: We used the baseline data of the China Health and Retirement Longitudinal Study (CHARLS) to estimate the population impact on hypertension management between CHL and ACC/AHA guidelines. Mortality risk from hypertension was estimated using the data from China Health and Nutrition Survey (CHNS). Cox proportional hazards model was used to estimate the hazard ratios (HRs) and their 95% confidence intervals(CIs).

Results: Among 13,704 participants analyzed from the nationally representative data of CHARLS, 42.64% (95% CI: 40.35, 44.96) of Chinese adults were diagnosed by both CHL and ACC/AHA guidelines. 41.25% (39.17, 43.36) did not have hypertension according to either guideline. Overall, the concordance in hypertension status was 83.89% (81.69, 85.57). A high percentage of agreement was also found for recommendation to initiate treatment among untreated subjects (87.62% [86.67, 88.51]) and blood pressure (BP) above the goal among treated subjects (71.68% [68.16, 74.95]). Among 23,063 adults from CHNS, subjects with hypertension by CHL had a higher risk of premature mortality (1.75 [1.50, 2.04]) compared with those without hypertension. The association diminished for hypertension by ACC/AHA (1.46 [1.07, 1.30]). Moreover, the excess risk was not significant for the newly defined Grade 1 hypertension by ACC/AHA (1.15 [0.95, 1.38]) when compared with BP <120/80 mmHg. This contrasted with the estimate from CHL (1.54 [1.25, 1.89]). The same pattern was observed for total mortality.

Conclusions: If ACC/AHA guidelines were adopted, a high degree of concordance in hypertension status and initiation of antihypertensive treatment was found with CHL guidelines. However, the mortality risk with hypertension was reduced with a non-significant risk for Grade 1 hypertension defined by the ACC/AHA.

Keywords: blood pressure, mortality, hypertension, premature mortality, cohort study

INTRODUCTION

In 2017, the American College of Cardiology/American Heart Association (ACC/AHA) released an updated blood pressure (BP) guidelines and reduced the diagnostic threshold for hypertension to systolic blood pressure (SBP)/diastolic blood pressure (DBP) ≥130/80 mmHg (1). This contrasts with the 2018 Chinese Hypertension League (CHL) BP guidelines and the 2018 European Society of Cardiology (ESC)/European Society of Hypertension (ESH) BP guidelines, where hypertension is diagnosed based on a threshold of ≥140/90 mmHg (2, 3). This new change substantially increases the prevalence and number of subjects with hypertension and extensively revised the treatment recommendations and goal of antihypertensive treatment (4–7). However, few of them investigated the concordances in hypertension management between 2018 CHL and 2017 ACC/AHA. This is of particular interest for the treatment recommendations as the ACC/AHA employs a different strategy to guide the antihypertensive medication treatment through predicted cardiovascular disease (CVD) risk in conjunction with BP (1).

Several studies have assessed the impact of a lower BP threshold on the risk of a cardiovascular event and/or all-cause mortality (8–12). However, a few of them compared the effect of different BP thresholds (e.g., from ACC/AHA and Chinese guidelines) simultaneously. Also, there are uncertainties as to whether either definition of hypertension is associated with premature mortality, which is a very important index for public health surveillance (13). Although some studies demonstrated the positive associations between hypertension (i.e., ≥140/90 mmHg) and premature mortality (14–16), few of them detailed the relationship of premature mortality with different hypertension categories (e.g., stage 1 hypertension) and did not include SBP or DBP to evaluate their specific associations with premature mortality.

In our analysis, we aim to (1) compare the agreement on the definition of hypertension, treatment initiation recommendation, and control status of hypertension between 2017 ACC/AHA and 2018 CHL guideline; and (2) determine whether hypertension or different hypertension categories, as defined by either 2017 ACC/AHA or 2018 CHL guideline, is associated with premature mortality.

METHODS

Study Populations

We used the baseline data of China Health and Retirement Longitudinal Study (CHARLS) (2011–2012) to estimate the population-level impact of utilizing 2017 ACC/AHA on the prevalence or number of subjects with hypertension, recommendation for antihypertensive treatment, and intensification of therapy, in comparison with the 2018 CHL guidelines. The profile and data quality of this study has been described elsewhere (17). Briefly, CHARLS is a nationally representative survey for 17,708 subjects aged 45 years and older in China and was conducted from 2011 to 2012. The participants were selected through a multistage probability sampling method and can be weighted to obtain national estimates.

To further explore the mortality risk of different subgroups of hypertension defined by ACC/AHA and CHL guidelines, we analyzed the data from China Health and Nutrition Survey (CHNS). CHNS started in 1989 from 9 China provinces that varied substantially in geography, economic development, and followed up every 2–4 years (i.e., in 1989, 1991, 1993, 1997, 2000, 2004, 2006, 2009, and 2011) (18). Since the conflicting results have been repeatedly reported between BP and all-cause mortality among the elderly population (19, 20) and disease burden from early death is relevant to young and middle-aged adults, we only included participants aged 18–75 years with the key information available (e.g., measurements of BP).

The CHARLS and CHNS obtained each participant's written informed consent and were approved by institutional review board of Peking university, and the University of North Carolina at Chapel Hill and the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, respectively. This analysis has been approved by the Human Research Ethics Committee of the Xi'an Jiaotong University Health Science Center (No: 2021-6).

BP Measurement and Definition

Seated SBP and DBP for each participant were taken using calibrated mercury sphygmomanometers in CHNS or the attended automated BP device (Omron model HEM-7200) in CHARLS following the standardized procedure of each study. The mean value of 3 available BP measurements was used in the current analysis.

The definitions of hypertension, initiation of antihypertensive medication, and BP goals referred to the 2017 ACC/AHA and the 2018 CHL guidelines are displayed in Appendix Table 1 (**Supplementary Material**). According to the 2018 CHL guidelines, participants were classified into five categories: Normal (untreated SBP <120 mmHg and DBP <80 mmHg); High normal (untreated SBP 120–139 mmHg and/or DBP 80–89 mmHg); Grade 1 hypertension (SBP 140–159 mmHg and/or DBP 90–99 mmHg); Grade 2 hypertension (SBP 160–179 mmHg and/or DBP 100–109 mmHg); Grade 3 hypertension (SBP ≥180

mmHg and/or DBP ≥110 mmHg) (2). Similarly, according to the 2017 ACC/AHA guideline, participants were reclassified into four categories: Normal (untreated SBP <120 mmHg and DBP <80 mmHg); Elevated (untreated SBP 120–129 mmHg and/or DBP <80 mmHg); Grade 1 hypertension (SBP 130–139 mmHg and/or DBP 80–89 mmHg); Grade 2 hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) (1).

Mortality

Data from the CHNS cohort study were used to estimate the mortality risk from different BP categories defined by ACC/AHA and Chinese guidelines. In the present study, premature death is defined as mortality before age 73.64 years in men and 79.43 years in women, which was the average life expectancies in China in 2015 (21). To overcome the issues related to the arbitrary selection of age threshold for premature mortality, we also adopted the definition from the Global Burden of Disease (GBD) study, where a death that occurred before the potential maximum life expectancy [refer to the standard life expectancy (SLE)] observed at the age of the person who died. Here, the SLE is intended to represent the potential achievable human life spans of an individual at a given age. It is calculated based on the highest national life expectancy projected for the year 2050 (22).

Assessment of Covariables

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Some other variables included educational level (illiterate, primary, middle/high school, bachelor, or above), marital status (married, never), residence status (rural, urban), smoking status (non-smoker, current smoker, ex-smoker), drinking status (non-drinker, drinker), self-reported health status in excellent/very good condition (yes, no), and self-reported CVD (yes, no), diabetes (yes, no), cancer (yes, no).

Statistical Analysis

The percentage and number (95% confidence interval [CI]) of adults with hypertension, recommended antihypertensive treatment and control status of hypertension based on 2017 ACC/AHA guidelines only, 2018 CHL guidelines only, and both or either guideline was estimated using CHARLS sampling weights to extrapolate to Chinese population (≥45 years).

Baseline characteristics of participants from CHNS were compared between hypertension and non-hypertension groups by *t*-tests for continuous variables expressed as mean (standard deviation) or Chi-square tests for categorical variables expressed as *n* (%). Kaplan–Meier (KM) curves were employed to estimate the survival probabilities across different hypertension categories and compared by the log-rank test. The incidence rate (per 1,000 person-years) for all-cause mortality and premature death was calculated. The hazard ratios (HRs) and 95% CIs of death among different BP categories were estimated by Cox proportional hazards model. To test the reliability of our analysis, we fitted three models, with the first model adjusting for age and gender (model 1). The second model included the variables model 1 plus educational level, marital status, rural residents, drinking, or smoking status (model 2). The third model included the variables in model 2 plus BMI, history of diabetes mellitus, CVD, or cancer (model 3). *p*-values for trends were calculated using the quartile median values of BP categories. To validate the association between BP with premature mortality, we repeated the main analyses but redefined the premature death by the GBD approach.

All *p*-values are two-sided and statistical analyses were done with Stata 15.0.

RESULTS

Percentage and Number of Hypertension, Recommended Initiating Antihypertensive Medication, Above-The-Goal BP Defined by Chinese and ACC/AHA Guidelines

Our current analysis was based on 13,704 subjects after excluding 4,541 subjects who did not participate in the physical examination with missing BP. Overall, we estimated 42.64% (95%CI: 40.35, 44.96; representing 227.04 million) of Chinese adults aged ≥45 years had hypertension according to the CHL, but this would be 16.11% higher (14.13, 18.31; representing 85.79 million) if ACC/AHA guidelines were adopted. Also, 41.25% (39.17, 43.36; representing 219.63 million) did not have hypertension according to either definition. As such, the overall concordance in hypertension status was 83.89% (81.69, 85.57) (**Figure 1**—Left; **Figure 2** and Appendix Table 2 in the **Supplementary Material**).

Among participants who reported not taking antihypertensive medication (N = 11,126), 12.38% (11.49, 13.33; representing 52.89 million) were recommended antihypertensive medication by 2017 ACC/AHA guidelines only. However, 62.70% (60.65, 64.70; representing 267.83 million) were not recommended to initiate antihypertensive medication by either ACC/AHA or CHL guidelines and 24.92% (22.90, 27.06; representing 106.46 million) were recommended to initiate treatment by both guidelines (overall concordance 87.62% [86.67, 88.51]) (**Figure 1**—Middle; **Figure 2** and Appendix Table 2 in the **Supplementary Material**).

Among those with hypertension taking antihypertensive medication (N = 2,578), 28.32% (25.05, 31.84; representing 29.82 million) and 51.93% (47.10, 56.72; representing 54.67 million), 19.75% (17.22, 22.54; representing 20.79 million) was above the ACC/AHA goal only, both and neither guideline, respectively (overall concordance 71.68% [68.16, 74.95]) (**Figure 1**—Right; **Figure 2** and Appendix Table 2 in the **Supplementary Material**).

Baseline Information of Participants Included in CHNS Study

A total of 23,063 participants were included in the present analysis. The mean age was 41.08 (range: 18–75) years; 45.24% were male. Over a median of 6.06 years of follow-up, there were 1,673 deaths. Of these deaths, 74.06% (1,239/1,673) were defined as premature. The prevalence of hypertension was 20.22% (4,663/23,063) by CHL definition and 46.85% (10,805/23,063) by ACC/AHA definition. Subjects with hypertension by both definitions tended to be older, received lower education, had a higher proportion of smoking and drinking, higher BMI levels,

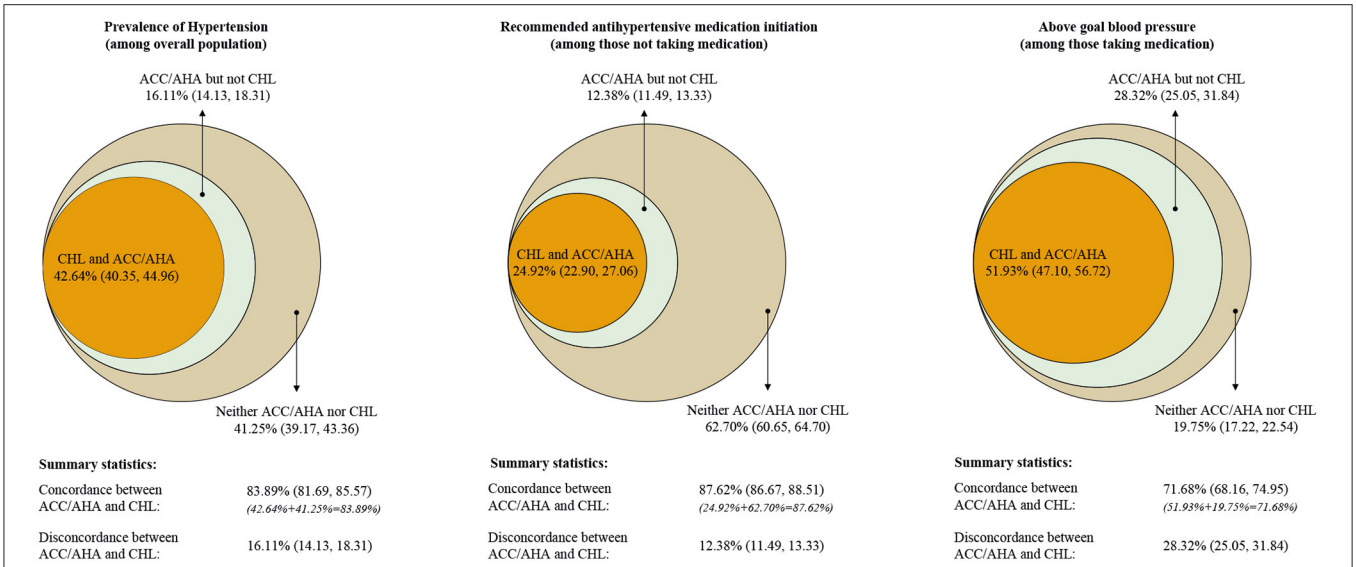

FIGURE 1 | Percentage of hypertension, recommended to initiate antihypertensive medication, above-the goal blood pressure. Data used for estimating was from CHARLS 2011–2012 baseline survey. (Left) Percentage of Chinese adults who have hypertension; (Middle) percentage of Chinese adults who are recommended to initiate antihypertensive medication among those not taking antihypertensive medication; (Right) percentage of Chinese adults with above the goal blood pressure among those taking antihypertensive medication.

higher prevalence of CVD, diabetes, and cancer (Appendix Table 3 in the **Supplementary Material**).

Associations Between Hypertension Categories and Mortality

Kaplan–Meier curves indicated that the cumulative rate of premature mortality was significantly different between participants with and without hypertension defined by CHL ($P < 0.001$) in **Figure 3A**. A similar result was found for hypertension defined by ACC/AHA in **Figure 3B**. However, we could graphically identify that the difference in the cumulative rate between hypertension and non-hypertension groups defined by ACC/AHA was smaller than those defined by CHL. This was supported by the adjusted Cox regression model. We found that hypertension by CHL significantly increased the risk of premature death by 75% (Model 3: 1.75, 1.50–2.04), whereas this risk reduced to 46% (Model 3: 1.46, 1.26–1.69) for hypertension by ACC/AHA (**Table 1**).

While further exploring the BP categories with premature mortality, we found the curves for each BP categorized by CHL diverged over time but not substantial for ACC/AHA, although differences were significant across hypertension categories from **Figures 3C,D** (both $P < 0.001$). This observation from KM curves was proved by HRs of premature death in the Cox model. Grade 1 hypertension (Model 3: 1.54, 1.25–1.89), Grade 2 hypertension (Model 3: 2.01, 1.52–2.66), and Grade 3 hypertension (Model 3: 3.76, 2.81–5.04) by CHL could significantly increase the risk of premature mortality over normal BP (<120/80 mmHg), although not for high normal (Model 3: 1.10, 0.92–1.32). On the contrary, we only found Grade 2 hypertension by ACC/AHA reached statistical significance (Model 3: 1.89, 1.58–2.28) but not for Grade 1 (Model 3: 1.15, 0.95–1.38) and elevated BP (Model 3: 0.96, 0.71–1.28), compared with normal BP (<120/80 mmHg) (**Table 1**).

Like the estimates for premature mortality, we found a weak but significant association with all-cause mortality for hypertension by ACC/AHA (Model 3: 1.39, 1.23–1.57), in comparison with the result by CHL (Model 3: 1.65, 1.45–1.87). Also, a significant association was observed for Grade 1 hypertension by CHL (Model 3: 1.49, 1.26–1.77) but not by ACC/AHA (Model 3: 1.14, 0.97–1.33) (**Table 1**). This could be supported from our further analyses by SBP or DBP levels, where positive association started from SBP ≥140 or DBP ≥90 (Appendix Table 4 in the **Supplementary Material**).

Sensitivity analyses for the association with premature mortality defined by the GBD study revealed similar findings as those using age threshold from life expectancy for defining premature mortality, although the percentage of premature mortality differs [99.04% (1,657/1,673) by GBD study vs. 74.06% (1,239/1,673) by the threshold of the expected life year] (Appendix Table 5 in the **Supplementary Material**).

DISCUSSION

Our study identified that 2017 ACC/AHA guidelines substantially increased the percentage and number of subjects with hypertension, eligibility for antihypertensive medication compared to 2018 CHL guidelines. Furthermore, we found that the degree of concordance was high between CHL and ACC/AHA guidelines with a few small differences. In addition, we found that hypertension, both by CHL or ACC/AHA definition, would significantly increase the risk of premature

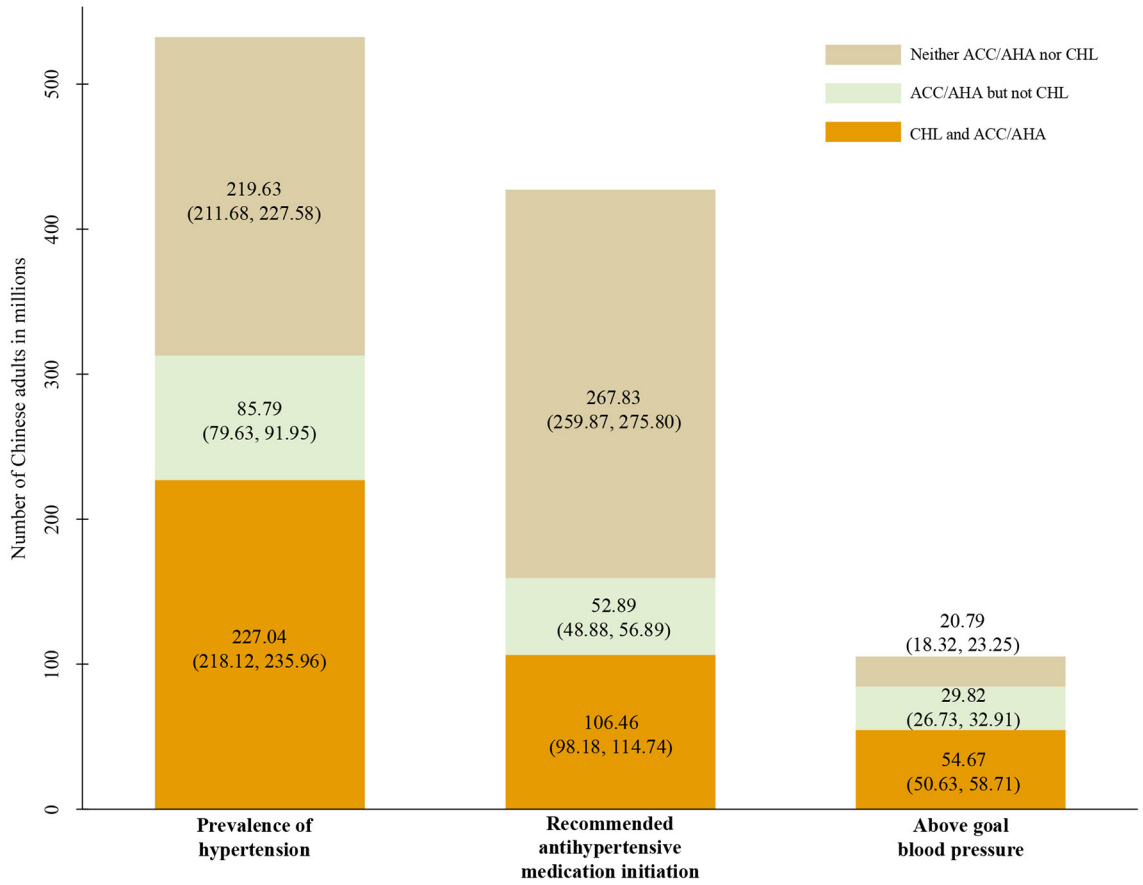

FIGURE 2 | Number of hypertension cases, recommended to initiate antihypertensive medication, above the goal blood pressure. Data used for estimating were collected from CHARLS 2011–2012 baseline survey.

death or total death among participants aged 18–75 years. However, this excess risk was diminished for hypertension and not observed for the newly defined Grade 1 hypertension by ACC/AHA.

The lower SBP and DBP levels used to define hypertension and the goal of antihypertensive treatment in the 2017 ACC/AHA is the obvious reason for the substantial increase of the percentage of hypertension and not reaching the goal of antihypertensive treatment. A similar increase was also reported in Bangladesh (23) and Korea (24). Our results also emphasized that a high percentage of Chinese adults was provided identical antihypertensive treatment recommendations by ACC/AHA and CHL guidelines. This is in line with the data reported in adults with diabetes in the United States (25). However, the decision to initiate and intensify antihypertensive medication should consider both the benefit and harm as well as the cost of drugs. Therefore, a careful evaluation of the cost-effectiveness of 2017 ACC/AHA guidelines should be performed to provide evidence for hypertension management, especially in China with a large absolute number of hypertensive subjects. Also, we should note that evidence for developing ACC/AHA guidelines as well as ESC/ESH guidelines are mainly from studies in the Caucasian population, which were interpreted carefully while developing CHL guidelines with limited data among Chinese or Asians.

The positive associations have been repeatedly reported between hypertension with cardiovascular incidence and total mortality among young or middle-aged adults (20, 26–30). This is consistent with our study using the traditional BP threshold for hypertension (140/90 mmHg). However, a few studies have explored whether the excess risk persisted if a lower threshold for hypertension by ACC/AHA was adopted. In our analysis, we found a high risk of total mortality with the newly defined hypertension but not stage 1 hypertension. This finding is generally in line with results from a meta-analysis (31) and a recent pooled analysis among the Chinese population (8). It is noted that our analysis further found that the risk of all-cause mortality significantly increased with higher systolic or diastolic BP, in a dose-dependent manner. However, this increased risk only reached statistical significance from SBP $\geq$140 or DBP $\geq$90, respectively. This partly supported our finding on the null association for the lower BP cut points for stage 1 hypertension from ACC/AHA with mortality.

Although death is inevitable, evidence has shown that most premature deaths in young or middle-aged adults could be highly

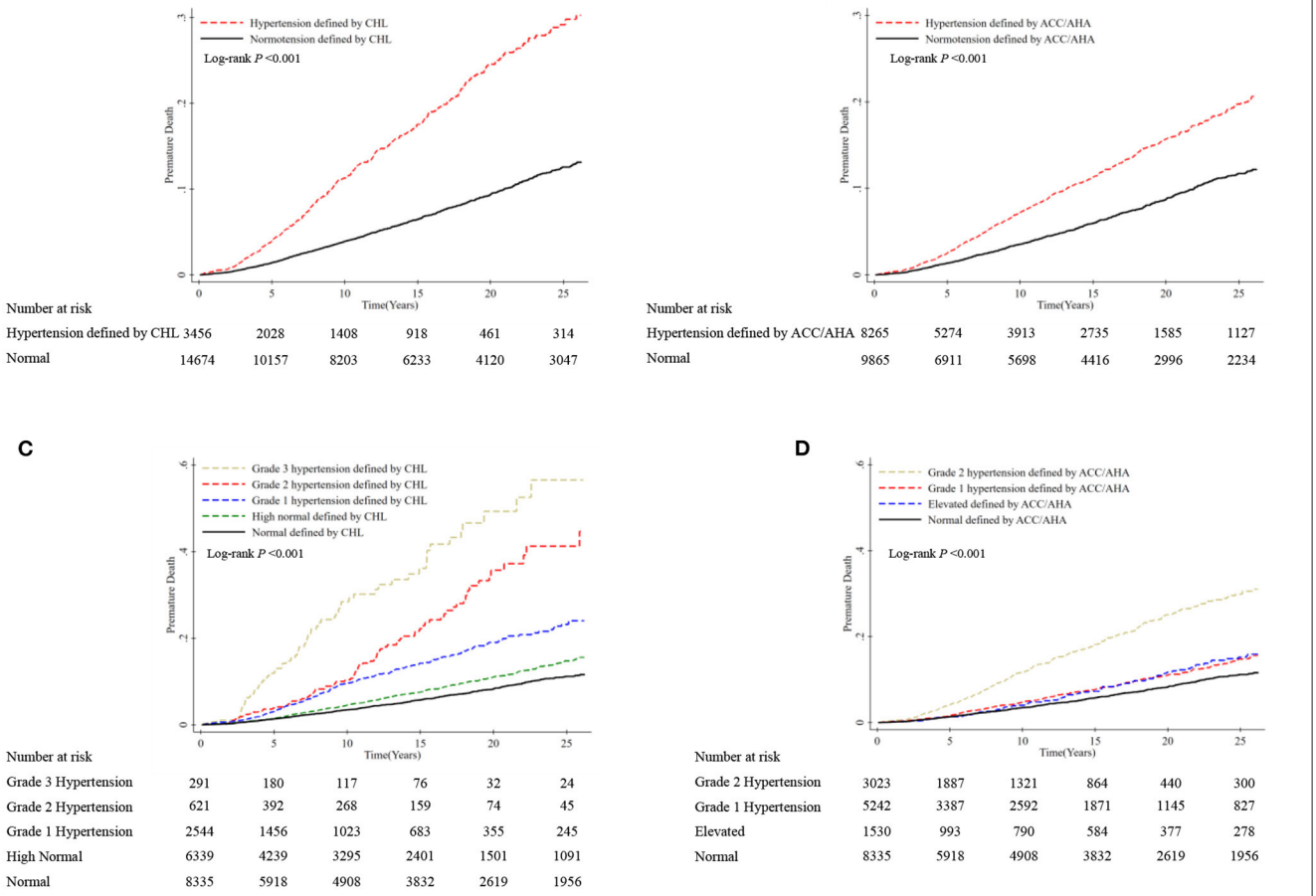

FIGURE 3 | Cumulative incidence of premature mortality according to hypertension categories by CHL and ACC/AHA definition. Premature death is defined as deaths before the age of 73.64 years in men and 79.43 years in women. **(A)** Hypertension was defined as SBP ≥140 mmHg, DBP ≥90 mmHg, or taking antihypertensive medication according to the CHL guidelines; **(B)** hypertension was defined as SBP ≥130 mmHg, DBP ≥80 mmHg, or taking antihypertensive medication according to the 2017 ACC/AHA guidelines; **(C)** participants were categorized as having normal BP (untreated SBP <120 mmHg and DBP <80 mmHg); high normal BP (untreated SBP 120–139 mmHg and/or DBP 80–89 mmHg); Grade 1 hypertension (SBP 140–159 mmHg and/or DBP 90–99 mmHg); Grade 2 hypertension (SBP 160–179 mmHg and/or DBP 100–109 mmHg); and Grade 3 hypertension (SBP ≥180 mmHg and/or DBP ≥110 mmHg) according to the CHL guidelines; **(D)** participants were categorized as having normal BP (untreated SBP <120 mmHg and DBP <80 mmHg); elevated BP (untreated SBP 120–129 mmHg and/or DBP <80 mmHg); Grade 1 hypertension (SBP 130–139 mmHg and/or DBP 80–89 mmHg); and Grade 2 hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) according to the 2017 ACC/AHA guidelines.

preventable (13). A few studies have assessed the association between BP categories and premature mortality. In our study, we found that 74.06% of all deaths were premature, which is higher than studies from He et al. (14) in China (55.3%) and Nalini et al. (16) in Iran (63.6%). Furthermore, consistent with the findings for the all-cause mortality, our study demonstrated that hypertension would significantly increase the risk of early death with an adjusted HR of 1.75. This was supported by our additional analyses using the GBD definition for premature death. Our study was consistent with a previous study in a sample of 169,871 Chinese adults aged 40 years and older (14). Our study further indicated a moderate but significantly increased risk of premature mortality with the lower BP criterion for hypertension by ACC/AHA and a linear trend with the increasing SBP and DBP. Meanwhile, we also found that this positive relationship could not be observed for the newly defined stage 1 hypertension.

The limitations of our study need to be acknowledged. First, our study did not report the association between hypertension with cause-specific mortality, particularly cardiovascular death. This will make the interpretation of hypertension effect on total mortality not intuitive. However, we believed that selection of all-cause mortality as an endpoint could avoid misclassification issues compared with disease-specific ones. Meanwhile, our examination of total or premature mortality could provide substantial information from a public health perspective. Second, BP was measured at a single visit for CHARLS, which is different from the requirement from both guidelines about the diagnosis of hypertension. Third, some covariates, such as cancer treatments,

TABLE 1 | Hazard ratios of all-cause mortality and premature mortality according to hypertension categories by Chinese Hypertension League (CHL) and European Society of Cardiology (ESC)/European Society of Hypertension (ESH) and American College of Cardiology (ACC)/American Heart Association (AHA) definitions[a,b].

	N	Death or premature	Incidence rate per 1,000	Model 1*		Model 2†		Model 3‡	
			Person-years	HR	95%CI	HR	95%CI	HR	95%CI
ALL-CAUSE MORTALITY									
CHL[a]									
Normal	18,400	1,113	5.75	Reference		Reference		Reference	
Hypertension	4,663	560	16.10	1.35	1.21–1.50	1.49	1.33–1.69	1.65	1.45–1.87
P for trend				<0.001		<0.001		<0.001	
CHL[a]									
Normal	10,229	574	4.98	Reference		Reference		Reference	
High Normal	8,171	539	6.90	1.03	0.92–1.16	1.05	0.90–1.21	1.12	0.97–1.30
Grade 1 Hypertension	3,453	330	12.91	1.17	1.02–1.35	1.31	1.11–1.54	1.49	1.26–1.77
Grade 2 Hypertension	833	140	22.05	1.61	1.33–1.95	1.78	1.45–2.20	2.07	1.66–2.57
Grade 3 Hypertension	377	90	31.38	2.35	1.87–2.96	2.46	1.92–3.15	2.96	2.30–3.81
P for trend				<0.001		<0.001		<0.001	
ACC/AHA[b]									
Normal	12,258	716	5.33	Reference		Reference		Reference	
Hypertension	10,805	957	10.18	1.15	1.04–1.27	1.28	1.14–1.44	1.39	1.23–1.57
P for trend				<0.001		<0.001		<0.001	
ACC/AHA[b]									
Normal	10,229	574	4.98	Reference		Reference		Reference	
Elevated	2,029	142	7.50	1.11	0.92–1.33	1.03	0.82–1.30	1.08	0.86–1.35
Grade 1 Hypertension	6,801	427	6.87	0.99	0.88–1.13	1.06	0.91–1.23	1.14	0.97–1.33
Grade 2 Hypertension	4,004	530	16.68	1.42	1.25–1.60	1.56	1.35–1.80	1.78	1.54–2.07
P for trend				<0.001		<0.001		<0.001	
PREMATURE DEATH[C]									
CHL[a]									
Normal	18,400	865	4.47	Reference		Reference		Reference	
Hypertension	4,663	374	10.75	1.40	1.23–1.59	1.57	1.36–1.82	1.75	1.50–2.04
P for trend				<0.001		<0.001		<0.001	
CHL[a]									
Normal	10,229	463	4.02	Reference		Reference		Reference	
High Normal	8,171	402	5.15	1.03	0.90–1.18	1.01	0.85–1.21	1.10	0.92–1.32
Grade 1 Hypertension	3,453	218	8.53	1.18	0.99–1.40	1.33	1.09–1.62	1.54	1.25–1.89
Grade 2 Hypertension	833	87	13.70	1.60	1.26–2.04	1.71	1.31–2.24	2.01	1.52–2.66
Grade 3 Hypertension	377	69	24.06	2.90	2.23–3.76	3.06	2.31–4.07	3.76	2.81–5.04
P for trend				<0.001		<0.001		<0.001	
ACC/AHA[b]									
Normal	12,258	561	4.18	Reference		Reference		Reference	
Hypertension	10,805	678	7.22	1.18	1.05–1.33	1.33	1.15–1.53	1.46	1.26–1.69
P for trend				<0.001		<0.001		<0.001	
ACC/AHA[b]									
Normal	10,229	463	4.02	Reference		Reference		Reference	
Elevated	2,029	98	5.18	1.03	0.83–1.29	0.90	0.68–1.21	0.96	0.71–1.28
Grade 1 Hypertension	6,801	322	5.18	1.01	0.87–1.17	1.05	0.88–1.26	1.15	0.95–1.38
Grade 2 Hypertension	4,004	356	11.20	1.47	1.27–1.71	1.62	1.36–1.93	1.89	1.58–2.28
P for trend				<0.001		<0.001		<0.001	

[a]*CHL classification of blood pressure: normal BP (untreated SBP <120 mmHg and DBP <80 mmHg); high normal BP (untreated SBP 120–149 mmHg and/or DBP 80–89 mmHg); Grade 1 hypertension (SBP 140–159 mmHg and/or DBP 90–99 mmHg); Grade 2 hypertension (SBP 160–179 mmHg and/or DBP 100–109 mmHg); Grade 3 hypertension (SBP ≥180 mmHg and/or DBP ≥110).*

[b]*2017 ACC/AHA classification of blood pressure: Normal BP (untreated SBP <120 mmHg and DBP <80 mmHg); elevated BP (untreated SBP 120–129 mmHg and/or DBP <80 mmHg); Grade 1 hypertension (SBP 130–139 mmHg and/or DBP 80–89 mmHg); Grade 2 hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg).*

[c]*Premature death is defined as deaths before the age of 73.64 years in men and 79.43 years in women.*

** Model 1: adjusted for age, gender.*

† Model 2: adjusted for age, gender, educational level, marital status, whether rural residents, history of drinking, or smoking.

‡Model 3: adjusted for age, gender, educational level, marital status, whether rural residents, history of drinking or smoking, body mass index, previous history of diabetes mellitus, CVD, or cancer.

glucose or lipid level, or nutraceuticals, which have been reported to be associated with participants' health outcomes, were not collected in the CHNS study, thus, we could not include them in our analysis. However, we assessed our estimates by adding the variable sequentially and found that they were insensitive to the variables included.

CONCLUSION

In summary, our study indicated a high degree of concordance between CHL and ACC/AHA guidelines in terms of hypertension status and antihypertensive treatment recommendations. Moreover, this study provided evidence of attenuated mortality risk from hypertension and non-significant risk from newly defined Grade 1 hypertension by ACC/AHA.

Therefore, we should pay more attention to the impact of the 2017 ACC/AHA guidelines and carefully consider the need to increase the appropriate use of antihypertensive medication to reduce hypertension-related burden in China.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available upon application. The two public-access study data can be downloaded from http://charls.pku.edu.cn/index/en.html (For CHARLS) and https://www.cpc.unc.edu/projects/china (for CHNS).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Xi'an Jiaotong University Health Science Center. The patients/participants provided their written informed consent to participate in the CHARLS and CHNS study.

AUTHOR CONTRIBUTIONS

KC was responsible for drafting the article and for the overall content. HS and QW assisted with the drafting and revision of the article. RS, FY, JY, XY, RQ, ZZ, and ZH provided inputs to the study concept and design, critically reviewed the results of analyses, and reviewed and contributed significantly to article revision. CL and ZW performed the statistical analyses. TC and CL provided full access to the study data as well as study oversight and article revision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.784433/full#supplementary-material

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Age-Related Utilization of Thrombus Aspiration in Patients With ST-Segment Elevation Myocardial Infarction: Findings From the Improving Care for Cardiovascular Disease in China Project

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Background: There are some controversies on the utilization and benefits of thrombus aspiration in patients with ST-segment elevation myocardial infarction (STEMI). However, a few studies investigated this issue and the age-associated effects among the large population in China. Hence, we aimed to figure out the age-associated utilization and in-hospital outcomes of thrombus aspiration to improve therapeutic decisions in clinical routine.

Methods: We retrospectively recruited 13,655 eligible STEMI patients from the database of the Improving Care for Cardiovascular Disease in China-Acute Coronary Syndrome project. These subjects were allocated into primary percutaneous coronary intervention (PPCI)-only group and thrombus aspiration group after being subdivided into three age groups (G_{21-50}, G_{51-75}, and G_{76-95}). After 1:1 propensity score matching for PPCI-only and thrombus aspiration groups, a total of 8,815 matched patients were enrolled for the subsequent analysis. The primary outcome was in-hospital cardiovascular death, and the key safety outcome was in-hospital stroke.

Results: We observed that the ratio of STEMI patients undergoing thrombus aspiration to PPCI-only reduced with aging. For patients $\leq$ 75 years, the culprit lesion suffered from thrombus aspiration was mainly located in the left anterior descending branch, and left-ventricular ejection fraction (LVEF) was lower (G_{21-50}: 54.9 $\pm$ 8.9 vs. 56.0 $\pm$ 8.7%, $P = 0.01$; G_{51-75}: 53.9 $\pm$ 9.6 vs. 54.8 $\pm$ 9.0%, $P = 0.001$) and the rate of regional wall motion abnormality was higher (G_{21-50}: 75.7 vs. 66.5%, $P < 0.001$; G_{51-75}: 75.4 vs. 69.1%, $P < 0.001$) in the thrombus aspiration group. By contrast, for patients > 75 years, the right coronary artery was the predominant culprit lesion undergoing thrombus aspiration, LVEF (63.1 $\pm$ 10.5 vs. 53.1 $\pm$ 9.5%, $P = 0.985$) and the regional wall motion abnormality (79.2 vs. 74.2%, $P = 0.089$) were comparable between the two treatment groups. Thrombus aspiration neither reduced the in-hospital risk of cardiovascular death, all-cause death, recurrent myocardial infarction, acute stent thrombosis, heart failure,

cardiogenic shock, and sudden cardiac arrest nor increased stroke risk compared with the PPCI-only group. However, after adjustment for age, thrombus aspiration presented the tendency to reduce the incidence of sudden cardiac arrest (4.9 vs. 2.5%, $P = 0.06$) and in-hospital cardiovascular death at 3 days (hazard ratio 0.46; 95% CI, 0.20–1.06; log-rank $P = 0.08$) in G_{76-95} group and tended to increase the incidence of heart failure in G_{51-75} (5.7 vs. 6.9%, $P = 0.07$).

Conclusion: The thrombus aspiration neither significantly reduced the in-hospital incidence of major adverse cardiac events nor increased stroke risk. However, it might play a protective role in reducing in-hospital sudden cardiac arrest and increasing survival from cardiovascular death at 3 days for the elderly.

Keywords: thrombus aspiration, primary percutaneous coronary intervention, ST-segment elevation myocardial infarction, age, adverse cardiac events, stroke

BACKGROUND

ST-segment elevation myocardial infarction (STEMI) is a severe cardiovascular disease, the major cause of which is a complete coronary artery occlusion due to the formation of the thrombus (1). Primary percutaneous coronary intervention (PPCI) within the first 12 h of the symptom onset has been proven as the most efficient therapeutic means to reperfuse the infarcted myocardium in the clinical routine (1, 2). However, the manipulation of atherectomy catheters, balloons, and stents during the PPCI procedure has the chance to cause distal embolization of thrombus. The subsequent obstructed microvasculature, no-reflow phenomenon, impaired tissue perfusion and increased infarct size may lead to cardiovascular death (3).

The routine use of manual thrombus aspiration during the percutaneous coronary intervention (PCI) could improve the primary outcome of microvascular perfusion at 30 days and decrease 1-year cardiac death and non-fatal reinfarction, which was reported by Thrombus Aspiration during Percutaneous Coronary Intervention in Acute Myocardial Infarction Study (TAPAS) (4, 5). However, there were some debates on this issue. Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE trial) revealed that thrombus aspiration could not significantly reduce the mortality either at 30 days or 1 year (6, 7). As a larger registry enrolling 10,732 patients at 87 hospitals in 20 countries, Trial of Routine Aspiration Thrombectomy with PCI vs. PCI Alone in Patients with STEMI (TOTAL) disclosed that thrombus aspiration could not reduce the 180-day and 1-year risk of cardiovascular death, recurrent myocardial infarction, cardiogenic shock, or heart failure. However, the rates of stroke within 30 and 180 days were higher in the thrombus aspiration group (2, 8). Even though thrombus aspiration was not recommended as a routine procedure by TOTAL and TATSE trials, it is considered in case of a large residual thrombus burden (9, 10).

To figure out the nationwide utilization and clinical outcomes of thrombus aspiration to provide evidence for therapeutic strategy in clinical routine, we analyzed the real-world data from the Improving Care for Cardiovascular Disease in China–Acute Coronary Syndrome (CCC–ACS) project, which is the largest project and quality improvement registry program for ACS in China (11). Considering the utilization of thrombus aspiration differed for patients at different ages, age subgroups were established for the subsequent investigation.

METHODS

CCC–ACS Project and Data Collection

CCC–ACS project was co-launched by the American Heart Association (AHA) and Chinese Society of Cardiology (CSC) and collected the information of ACS patients. Unlike TAPAS, TASTE, and TOTAL, which were prospective trials (12–14), the CCC–ACS project did nothing to intervene in the treatment in clinical practice. The principal purpose of this project was to describe the baseline characteristics, in-hospital treatment, and outcomes of ACS patients in China, and then make efforts to optimize the therapeutic strategies and improve therapeutic efficacy accordingly (15). A total of 150 tertiary hospitals from different geographic and economic regions were recruited. The detailed rationale and design of this project have been published before (15). Briefly, in each hospital, the information of the first 20–30 ACS patients was consecutively reported by a well-trained physician on a web-based data collection platform (Oracle Clinical Remote Data Capture; Oracle Corporation, Redwood City, CA, United States) on the official website

Abbreviations: ACEI, Angiotensin-converting enzyme inhibitor; AHA, American Heart Association; ARB, Angiotensin receptor blocker; CCC-ACS project, The Improving Care for Cardiovascular Disease in China-Acute Coronary Syndrome project; CI, Confidence interval; CSC, Chinese Society of Cardiology; DAPT, Dual antiplatelet therapy; DES, Drug-eluting stent; GP IIb/IIIa inhibitor, Glycoprotein IIb/IIIa inhibitor; HR, Hazard ratio; LAD, Left anterior descending branch; LCX, Left circumflex branch; LM, Left main coronary artery; LVEF, Left-ventricular ejection fraction; OR, Odds ratio; PCI, Percutaneous coronary intervention; PPCI, Primary percutaneous coronary intervention; RCA, Right coronary artery; SBP, Systolic blood pressure; SD, Standard deviation; STEMI, ST-segment elevation myocardial infarction; TAPAS, The Thrombus Aspiration during Percutaneous Coronary Intervention in Acute Myocardial Infarction Study; TASTE, The Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia; TOTAL, Trial of Routine Aspiration Thrombectomy with PCI vs. PCI Alone in Patients with STEMI.

(www.ccc-heart.com) month by month (11). The comprehensive information includes demography, medical history, risk factors, symptoms on arrival, in-hospital laboratory results, reperfusion, medication treatment, and events, as well as medications and counseling at discharge. This project included both unstable angina pectoris and acute myocardial infarction cases, acute myocardial infarction cases have reporting priority. Finally, 63,641 patients diagnosed as the ACS based on the symptoms such as chest pain or distress, alterations of myocardial injury biomarkers, and anomalous results of an electrocardiogram (16, 17) were enrolled from November 2014 to July 2017.

To ensure the accuracy of data collection, the data collection platform was equipped with automatic checks for invalid values. Moreover, the clinical research associates were designated to perform a regular on-site quality inspection. A 5% of reported cases would be randomly selected for the comparisons between original medical records and reported data. This study was approved by the Ethics Committee of Beijing Anzhen Hospital, Capital Medical University, and it has been registered at www.ClinicalTrials.gov (Unique identifier: NCT02306616).

Recruitment of Study Population

STEMI was defined according to the guideline issued by the CSC in 2010 (16). In this study, the inclusion criteria of patients were (i) undergoing PPCI within 12 h of the onset of the symptoms and (ii) placement of at least one drug-eluting stent (DES). The exclusion criteria were (i) placement of a bare-metal stent; (ii) subject to coronary artery bypass graft surgery before; (iii) undergoing fibrinolytic therapy at admission; and (iv) lacking information on age, thrombus aspiration treatment (yes / no), or Killip classification. Considering the potential age-associated utilization and dissimilarity of clinical outcomes of thrombus aspiration, the recruited subjects were divided into three age subgroups: G_{21-50} (range 21–50 years), G_{51-75} (range 51–75 years), and G_{76-95} (range 76–95 years) for the subsequent analysis.

Study Outcomes

The occurrence of in-hospital major adverse cardiac cerebrovascular events was evaluated in this study. The primary efficacy outcome was cardiovascular death. The secondary outcomes were all-cause death, recurrent myocardial infarction, acute stent thrombosis, heart failure, cardiogenic shock and sudden cardiac arrest. The key safety outcome was stroke during hospitalization. LV ejection fraction (LVEF) and regional wall motion were examined with echocardiography before discharge.

Statistical Analysis

Categorical variables were presented as numbers and percentages. Continuous variables were expressed as mean ± SD or median (interquartile ranges), as appropriate. A chi-squared test was used to evaluate the differences for categorical variables. Unpaired *t*-test or Mann–Whitney *U*-test was used to test the differences of continuous variables between PPCI-only and thrombus aspiration groups, as applicable. The connection between patients' age and thrombus aspiration treatment was assessed with a logistic regression model and expressed as OR with 95% CI. All recruited patients were matched between PPCI-only and thrombus aspiration groups with 1:1 propensity score matching in each age subgroup to diminish the influence of confounders. The prespecified covariates used to calculate propensity scores were age, gender, heart rate, systolic blood pressure (SBP), Killip classification, smoking, medical history (prior myocardial infarction, prior PCI, heart failure, hypertension, hyperlipemia, diabetes mellitus, renal failure, cerebral infarction, and cerebral hemorrhage), culprit lesions (left main coronary artery [LM], left anterior descending branch [LAD], left circumflex branch [LCX], right coronary artery [RCA]), in-hospital medications (dual antiplatelet therapy [DAPT, aspirin, and clopidogrel/ticagrelor], angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker [ARB], statin, β receptor blocker, glycoprotein IIb/IIIa [GP IIb/IIIa] inhibitor, and low molecular heparin), as well as gross domestic product. The match tolerance was set as 0.02. For variables with missing data, we imputed the missing values with the sequential regression multiple imputation method implemented by the IVEware software version 0.2 (Survey Research Center, University of Michigan, Ann Arbor, MI, United States). The Cox proportional hazards regression model was used to evaluate time-to-event data of all adverse cardiovascular events after the adjustment of age, and then generate hazard ratio (HR) and 95% CIs. Kaplan–Meier analysis was performed for assessing survival from in-hospital cardiovascular death at 3, 7, and 10 days, and the statistical significance was tested with the log-rank test. In all cases, two-tailed tests were applied and significance was determined as $P < 0.05$.

RESULTS

Recruitment of Study Population

A total of 63,641 ACS patients were reported in the database from November 2014 to July 2017, of whom 39,915 (62.7%) were diagnosed as STEMI; 14,953 of 39,915 (37.5%) STEMI patients underwent PPCI treatment within 12 h of the onset of symptoms and implanted at least one DES in the culprit lesion. After excluding patients with coronary artery bypass grafting history, undergoing fibrinolytic therapy at admission, or lacking information on age, thrombus aspiration and Killip classification, 13,655 eligible patients were enrolled for this study (**Figure 1**).

Age-Associated Utilization of Thrombus Aspiration

Among the whole enrolled population, the patients in the PPCI-only group were significantly older than those in PPCI combining thrombus aspiration group (61.2 ± 12.1 years vs. 59.7 ± 12.2 years, $P < 0.001$) (**Figure 2A**). Logistic regression analysis also revealed that thrombus aspiration was less likely to be conducted with the increase of age (OR = 0.990; 95% CI, 0.987 to 0.993; $P < 0.001$) (**Supplementary Figure 1**). We divided the patients into three age subgroups for the subsequent analysis. The patients between 51 and 75 years accounted for a substantial part of the study population (n = 9,097, 66.6%). The proportions of

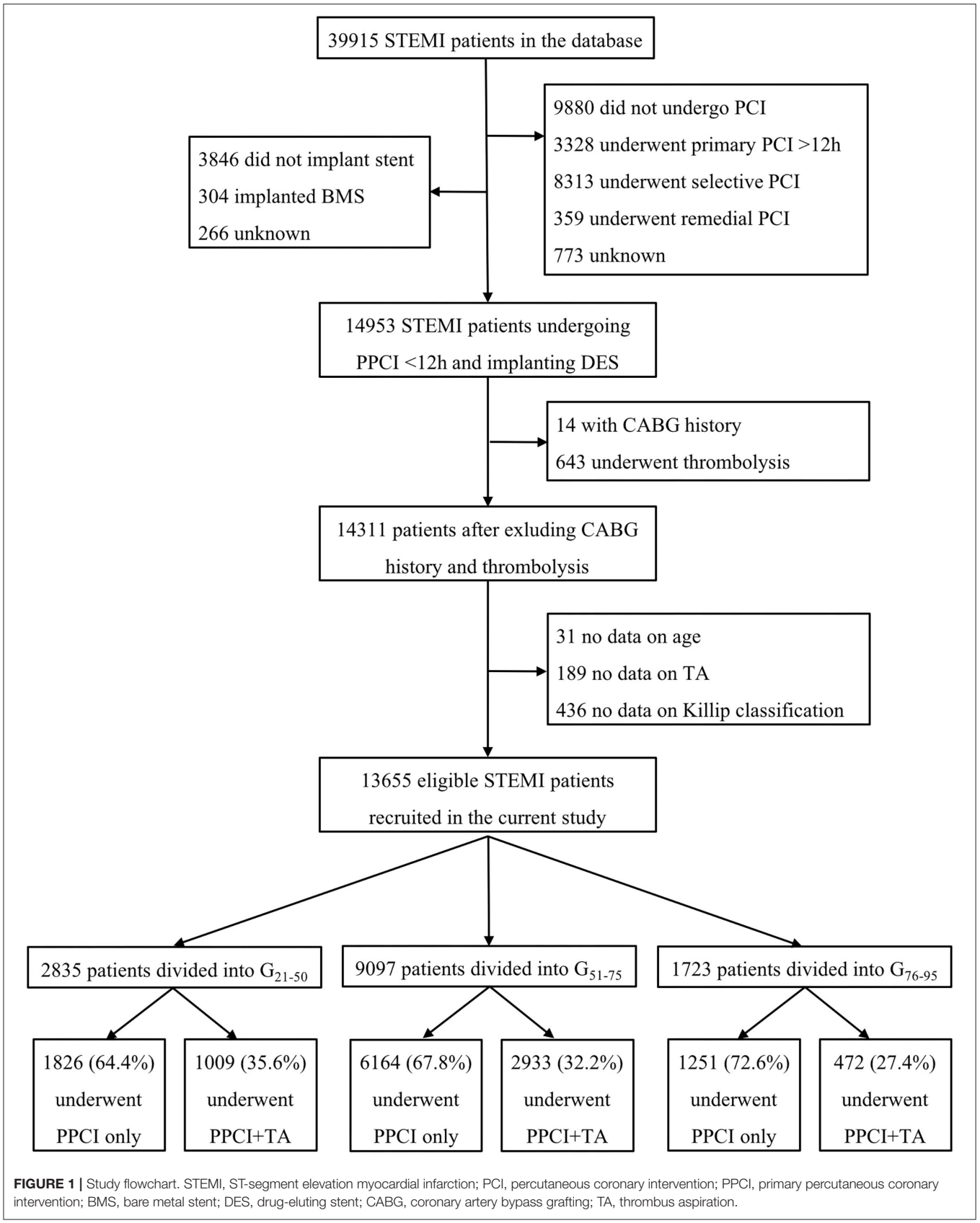

FIGURE 1 | Study flowchart. STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PPCI, primary percutaneous coronary intervention; BMS, bare metal stent; DES, drug-eluting stent; CABG, coronary artery bypass grafting; TA, thrombus aspiration.

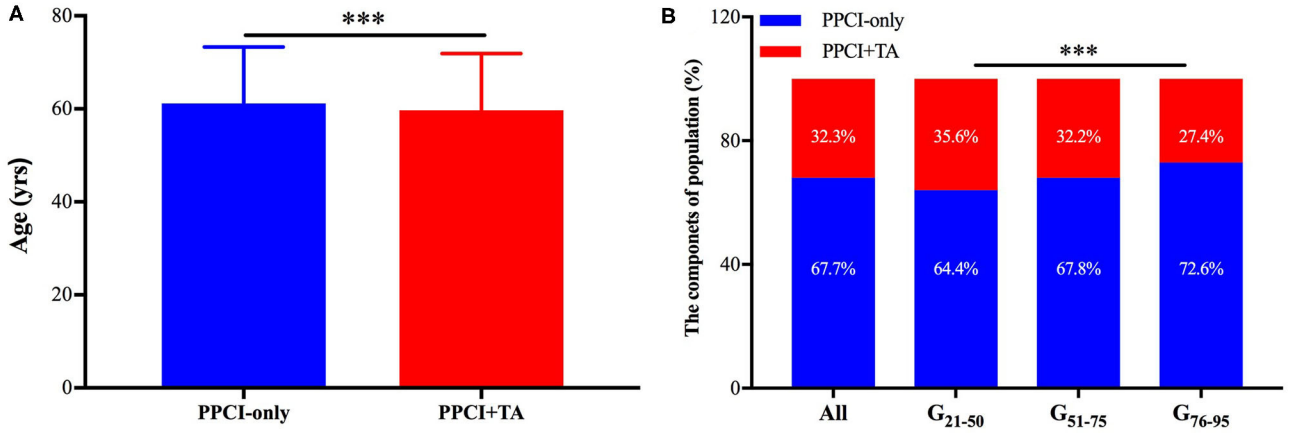

FIGURE 2 | The age-associated utilization of thrombus aspiration. **(A)** The patients in the PPCI-only group were significantly older than those in PPCI combining thrombus aspiration group. ***$P < 0.001$. **(B)** The percentages of STEMI patients undergoing PPCI-only and thrombus aspiration were presented. PPCI, primary percutaneous coronary intervention; TA, thrombus aspiration; CI, confidence interval.

patients ≤50 years and >75 years reached 20.8% ($n = 2,835$) and 12.6% ($n = 1,723$), respectively. The percentages of patients undergoing thrombus aspiration in G_{21-50} and G_{51-75} groups were discernibly higher than that in the G_{76-95} group (35.6% in G_{21-50}, 32.2% in G_{51-75}, and 27.4% in G_{76-95}, $P < 0.001$), in which the percentage of STEMI patients undergoing PPCI only was almost 1.6 times higher than that of patients undergoing thrombus aspiration (**Figure 2B**).

Clinical Characteristics of the Study Population

Age, the proportion of males, body mass index, and heart rate were comparable between PPCI-only and thrombus aspiration groups except for patients in the G_{51-75} subgroup (age: 62.4 ± 6.7 years *vs.* 62.0 ± 6.8 years, $P = 0.020$). The systolic and diastolic blood pressure were higher in the PPCI-only group. The percentage of Killip Class I decreased and the percentage of Killip classes II–IV increased with aging. The most frequently encountered risk factors for the development of STEMI were smoking and hypertension in the whole population. In addition, G_{76-95} patients showed a lower prevalence of smoking history and hyperlipemia as well as a higher incidence of prior myocardial infarction, PCI history, hypertension, diabetes mellitus and stroke compared with the youngest patients. In the G_{21-50} subgroup, the medical history was well-balanced between the two groups except for the proportion of smokers (71.8% in the PPCI-only group *vs.* 68.0% in the thrombus aspiration group, $P = 0.036$) (**Table 1**).

The culprit lesion differed between PPCI-only and thrombus aspiration groups. Meanwhile, the culprit lesion undergoing thrombus aspiration was more likely to be LAD in G_{21-50} and G_{51-75} subgroups, and RCA in the G_{75-95} subgroup. The percentages of patients accepting DAPT, statin, β receptor blocker, ACEI or ARB treatment were comparable between PPCI-only and thrombus aspiration groups except for the use of DAPT in the G_{76-95} group (96.6 vs. 98.5%, $P = 0.027$). The utilization of anticoagulation therapy including GP IIb/IIIa inhibitor and low molecular heparin increased in the thrombus aspiration group ($P < 0.05$). The hospitalization day was prolonged with the increase of age and remained comparable between PPCI-only and thrombus aspiration groups (**Table 2**).

Propensity Score Matching for Patients

After 1:1 propensity score matching for PPCI-only and thrombus aspiration groups, a total of 8,815 matched patients were obtained for the subsequent analysis. The detailed information was presented in **Supplementary Tables 1, 2**. In the G_{21-50} subgroup, only age (42.9 ± 5.3 years *vs.* 43.5 ± 5.8 years) and the utilization of DAPT (97.7 vs. 98.7%) were not well-matched (both $P < 0.001$). In the G_{51-75} subgroup, age and Killip class turned out to be comparable after matching. However, because of the possible complexity of characteristics presented in the G_{51-75} group, there were still differences in blood pressure, culprit lesion, and the utilization of DAPT, statin and anticoagulants. The matching worked excellently in the G_{76-95} subgroup, in which no significant difference of parameters remained between PPCI-only and thrombus aspiration groups.

In-Hospital Clinical Outcomes

The occurrence of in-hospital adverse cardiovascular events among the matched population was analyzed. The rates of the primary outcome, namely in-hospital cardiovascular death, were 0.5, 1.3 and 4.0% in each age subgroup, showing no significant difference between PPCI-only and thrombus aspiration groups. Nevertheless, thrombus aspiration reduced the occurrence of cardiovascular death by 27.7% (**Figure 3A**). Although the rates of cardiovascular death at 3, 7, and 10 days were comparable between PPCI-only and thrombus aspiration groups in all age subgroups (**Figures 3B–D**), thrombus aspiration presented the tendency to reduce the occurrence of in-hospital cardiovascular death at 3 days (HR 0.46; 95% CI, 0.20–1.06; log-rank $P = 0.08$). Additionally, the rates of secondary outcomes including all-cause

TABLE 1 | The baseline characteristics of the patients (n = 13,655).

	G_{21-50}			G_{51-75}			G_{76-95}		
	PPCI-only (n = 1,826)	**PPCI+TA (n = 1,009)**	***P*-value**	**PPCI-only (n = 6,164)**	**PPCI+TA (n = 2,933)**	***P*-value**	**PPCI-only (n = 1,251)**	**PPCI+TA (n = 472)**	***P*-value**
BASELINE INFORMATION									
Age (years)	44.0 ± 5.2	43.5 ± 5.8	0.067	62.4 ± 6.7	62.0 ± 6.8	**0.020**	80.4 ± 3.7	80.2 ± 3.7	0.348
Male, n (%)	1,748 (95.7%)	970 (96.1%)	0.602	4,959 (80.5%)	2,398 (81.8%)	0.138	780 (62.4%)	295 (62.5%)	0.954
BMI (kg/m^2)	25.4 ± 3.3	25.2 ± 3.4	0.351	24.4 ± 3.0	24.5 ± 2.9	0.111	23.4 ± 3.0	23.5 ± 3.1	0.653
Heart Rate (bpm)	81.0 ± 15.2	81.2 ± 15.2	0.535	77.1 ± 15.9	76.8 ± 15.7	0.680	76.7 ± 17.2	76.2 ± 18.0	0.693
SBP (mmHg)	129.0 ± 22.7	127.0 ± 22.6	**0.019**	127.6 ± 23.6	123.9 ± 23.1	**<0.001**	126.8 ± 25.5	123.6 ± 24.1	**0.027**
DBP (mmHg)	82.0 ± 16.2	80.9 ± 16.0	**0.035**	78.0 ± 14.6	76.2 ± 14.6	**<0.001**	72.9 ± 14.7	72.3 ± 13.8	0.543
Killip class									
I, n (%)	1,485 (81.3%)	843 (83.5%)	0.155	4,752 (77.1%)	2,329 (79.4%)	**0.013**	866 (69.2%)	327 (69.3%)	0.982
II, n (%)	266 (14.6%)	129 (12.8%)	0.189	1,070 (17.4%)	437 (14.9%)	**0.003**	274 (21.9%)	105 (22.2%)	0.878
III, n (%)	24 (1.3%)	13 (1.3%)	0.958	125 (2.0%)	49 (1.7%)	0.245	39 (3.1%)	15 (3.2%)	0.949
IV, n (%)	51 (2.8%)	24 (2.4%)	0.510	217 (3.5%)	118 (4.0%)	0.234	72 (5.8%)	25 (5.3%)	0.713
MEDICAL HISTORY									
Smoking	1,241 (68.0%)	724 (71.8%)	**0.036**	3,188 (51.7%)	1,542 (52.6%)	0.446	340 (27.2%)	130 (27.5%)	0.880
Prior myocardial infarction	60 (3.3%)	25 (2.5%)	0.227	268 (4.3%)	136 (4.6%)	0.532	59 (4.7%)	19 (4.0%)	0.538
Prior PCI	59 (3.2%)	26 (2.6%)	0.328	281 (4.6%)	143 (4.9%)	0.503	74 (5.9%)	23 (4.9%)	0.402
Hypertension	668 (36.6%)	378 (37.5%)	0.642	3,026 (49.1%)	1,474 (50.3%)	0.299	718 (57.4%)	267 (56.6%)	0.757
Hyperlipemia	127 (7.0%)	87 (8.6%)	0.108	384 (6.2%)	212 (7.2%)	0.072	58 (4.6%)	27 (5.7%)	0.354
Diabetes Mellitus	236 (12.9%)	136 (13.5%)	0.676	1,288 (20.9%)	599 (20.4%)	0.603	240 (19.2%)	88 (18.6%)	0.799
Stroke	38 (2.1%)	20 (2.0%)	0.859	475 (7.7%)	258 (8.8%)	0.074	149 (11.9%)	61 (12.9%)	0.566

Results are reported as mean ± SD or n (%). PPCI, primary percutaneous coronary intervention; TA, thrombus aspiration; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. The bold values indicate that the values smaller than 0.5 implying significant difference.

TABLE 2 | The culprit lesion and in-hospital medication (n = 13,655).

	G_{21-50}			G_{51-75}			G_{76-95}		
	PPCI-only (n = 1,826)	PPCI+TA (n = 1,009)	P-value	PPCI-only (n = 6,164)	PPCI+TA (n = 2,933)	P-value	PPCI-only (n = 1,251)	PPCI+TA (n = 472)	P-value
CULPRIT LESION									
LM	27 (1.5%)	10 (1.0%)	0.273	103 (1.7%)	53 (1.8%)	0.640	29 (2.3%)	8 (1.7%)	0.426
LAD	1,084 (59.4%)	553 (54.8%)	**0.019**	3,470 (56.3%)	1,477 (50.4%)	**<0.001**	679 (54.3%)	207 (43.9%)	**<0.001**
LCX	307 (16.8%)	131 (13.0%)	**0.007**	999 (16.2%)	401 (13.7%)	**0.002**	183 (14.6%)	69 (14.6%)	0.996
RCA	629 (34.4%)	370 (36.7%)	0.235	2,377 (38.6%)	1,325 (45.2%)	**<0.001**	555 (44.4%)	253 (53.6%)	**0.001**
IN-HOSPITAL MEDICATION									
DAPT	1,788 (97.9%)	998 (98.9%)	0.053	6,042 (98.0%)	2,862 (97.6%)	0.172	1,209 (96.6%)	465 (98.5%)	**0.027**
Statin	1,752 (95.9%)	970 (96.1%)	0.807	5,868 (95.2%)	2,802 (95.5%)	0.479	1,186 (94.8%)	453 (96.0%)	0.314
β Receptor Blocker	1,082 (59.3%)	590 (58.5%)	0.685	3,111 (50.5%)	1,472 (50.2%)	0.801	534 (42.7%)	205 (43.4%)	0.780
ACE I or ARB	911 (49.9%)	481 (47.7%)	0.258	2,770 (44.9%)	1,336 (45.6%)	0.583	543 (43.4%)	197 (41.7%)	0.533
Glycoprotein IIb/IIIa inhibitor	1,093 (59.9%)	698 (69.2%)	**<0.001**	3,487 (56.6%)	1,877 (64.0%)	**<0.001**	538 (43.0%)	254 (53.8%)	**<0.001**
Low molecular heparin	1,355 (74.2%)	785 (77.8%)	**0.033**	4,454 (72.3%)	2,192 (74.7%)	**0.013**	797 (63.7%)	350 (74.2%)	**<0.001**
Hospitalization day	9 (7, 11)	9 (7, 11)	0.506	9 (7, 12)	9 (7, 12)	0.391	10 (8, 13)	10 (8, 13)	0.086

Results are reported as mean ± SD, medians (25th–75th percentiles) or n (%), as appropriate. PPCI, primary percutaneous coronary intervention; TA, thrombus aspiration; LM, left main coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery; DAPT, dual antiplatelet therapy; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker. The bold values indicate that the values smaller than 0.5 implying significant difference.

death, recurrent myocardial infarction, acute stent thrombosis, heart failure, cardiogenic shock, sudden cardiac arrest and stroke were comparable between two treatment groups in three age subgroups (all $P > 0.05$ after adjustment for age, **Figure 4**). While, in the G_{51-75} group, we observed a marginally higher rate of heart failure in patients undergoing thrombus aspiration (5.7 vs. 6.9%, adjusted $P = 0.07$). Meanwhile, the rate of sudden cardiac arrest was insignificantly lower in the thrombus aspiration group (4.9 vs. 2.5%, adjusted $P = 0.06$) for the elderly.

Stroke was regarded as the key safety outcome, of which the incidence increased with aging and sustained the similarity between PPCI-only and thrombus aspiration groups (all $P > 0.05$, **Figure 5**).

The last echocardiography examination before discharge presented that among patients ≤ 75 years old, LVEF was lower and the rate of regional wall motion abnormality was higher in the thrombus aspiration group (both $P < 0.05$), while no significant difference was observed in the G_{76-95} subgroup. Also, the incidence of regional wall motion abnormality raised with the increase of age (**Supplementary Figure 2**).

DISCUSSION

To the best of our knowledge, it is the largest nationwide, multicenter, retrospective epidemiological study to evaluate the age-associated utilization and clinical outcomes of thrombus aspiration among STEMI patients undergoing PPCI within 12 h of symptoms onset and implanting DES in China. We demonstrated that thrombus aspiration did not increase the rate of in-hospital major adverse cardiac cerebrovascular events (e.g., cardiovascular death and stroke). However, the utilization of thrombus aspiration in patients older than 75 years helped maintain LVEF and regional wall motion normality and presented the tendency toward diminishing the risk of in-hospital cardiovascular death at 3 days and sudden cardiac arrest.

According to the data from the CCC–ACS database, thrombus aspiration is still being used in a substantial number of STEMI patients. A 32.3% the STEMI patients undergoing PPCI and implanting DES were treated with upfront or bailout thrombus aspiration. STEMI patients were less likely to be treated with thrombus aspiration with the increase of age. We found that the most frequently encountered risk factor of STEMI was smoking in patients ≤ 75 years and hypertension in patients >75 years, this phenomenon was partially in line with the previous findings (18, 19). For patients aged 21–50 years, the percentage of smokers was higher in the thrombus aspiration group.

In the entire population, the incidences of in-hospital adverse cardiovascular events were comparable between PPCI-only and thrombus aspiration groups. This finding was sort of consistent with the results in TASTE, TOTAL and a large observational study (n = 42,829) using available data from the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) at 30 days (7, 8, 20). Due to the ventricular remodeling, diastolic dysfunction and decreased vascular compliance with aging, the elderly presented higher mortality when suffering from the sudden occlusion of the coronary artery (21). Nevertheless, we

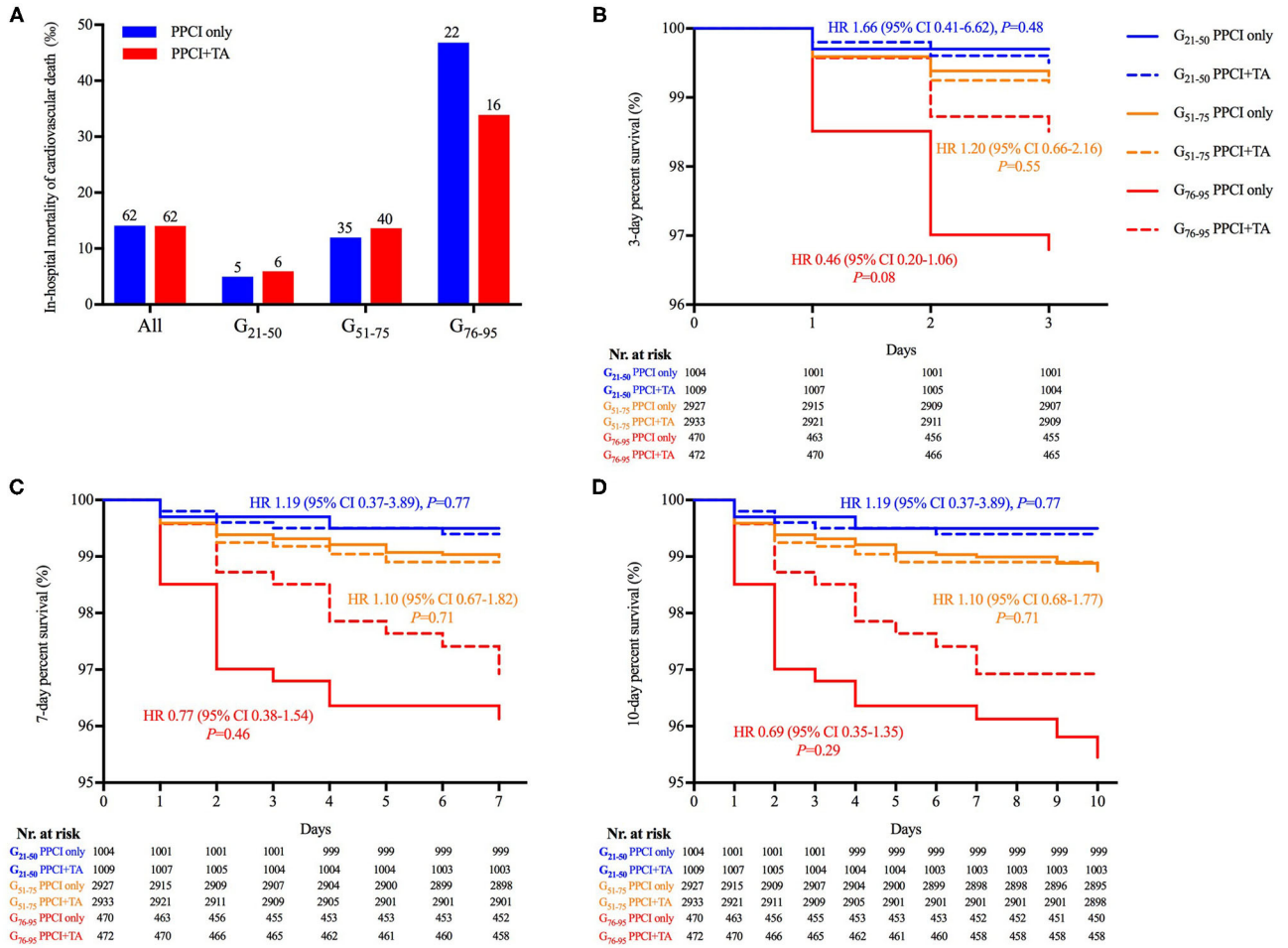

FIGURE 3 | The in-hospital mortality and Kaplan–Meier curves of cardiovascular death. **(A)** The in-hospital mortality of cardiovascular death was comparable between PPCI-only and thrombus aspiration groups ($P > 0.05$). **(B–D)** Kaplan–Meier curve revealed that the in-hospital occurrence of cardiovascular death was comparable between PPCI-only and thrombus aspiration groups in each age subgroup. However, thrombus aspiration presented a tendency to decrease cardiovascular death at 3 days in the G_{76-95} subgroup (log-rank $P = 0.08$).

detected that thrombus aspiration tended to reduce the risk of in-hospital cardiovascular death at 3 days by 27.6% in the G_{76-95} group. It is noteworthy that thrombus aspiration also tended to decline the rate of rehospitalization due to reinfarction (0.5 vs. 0.9%; HR 0.61; 95% CI, 0.34–1.07; $P = 0.09$) and stent thrombosis (0.2 vs. 0.5%; HR 0.47; 95% CI, 0.20–1.02; $P = 0.06$) at 30 days in TASTE (7). Meanwhile, we noticed that the incidence of stent thrombosis in the PPCI-only group was 1.5–3 times compared to that in the thrombus aspiration group, although the difference was insignificant. Considering the extremely low incidence of stent thrombosis, whether thrombus aspiration can reduce the occurrence of this event needs to be further investigated in a larger population. What is more, thrombus aspiration showed a protective impact on reducing the prevalence of sudden cardiac death in the elderly (4.9 vs. 2.5%, $P = 0.06$). While, in the G_{51-75} group, we observed a higher rate of heart failure in patients undergoing thrombus aspiration, which may raise physicians' attention in the clinical practice.

A part of the information derived from angiography and the last echocardiography before discharge was recorded in the CCC–ACS database. Among patients ≤ 75 years, the culprit lesion suffered from thrombus aspiration was mainly located in LAD, LVEF was lower and the rate of regional wall motion abnormality was higher in the thrombus aspiration group. STEMI patients with a high thrombus burden are more likely to benefit from thrombus aspiration compared with patients with a low thrombus burden, suggesting the more frequent utilization of thrombus aspiration in patients with high thrombus burden (10). Ahmed et al. uncovered that the higher thrombus grade was associated with larger infarct size and slightly worse LV function (22). Hence, we hypothesized that the deterioration of cardiac function and myocardial wall motion might be associated with the burden of coronary thrombus rather than the treatment. Unfortunately, the severe impairment of cardiac performance cannot be simply renovated by thrombus aspiration in a short time. By contrast, among patients > 75 years, the culprit lesion

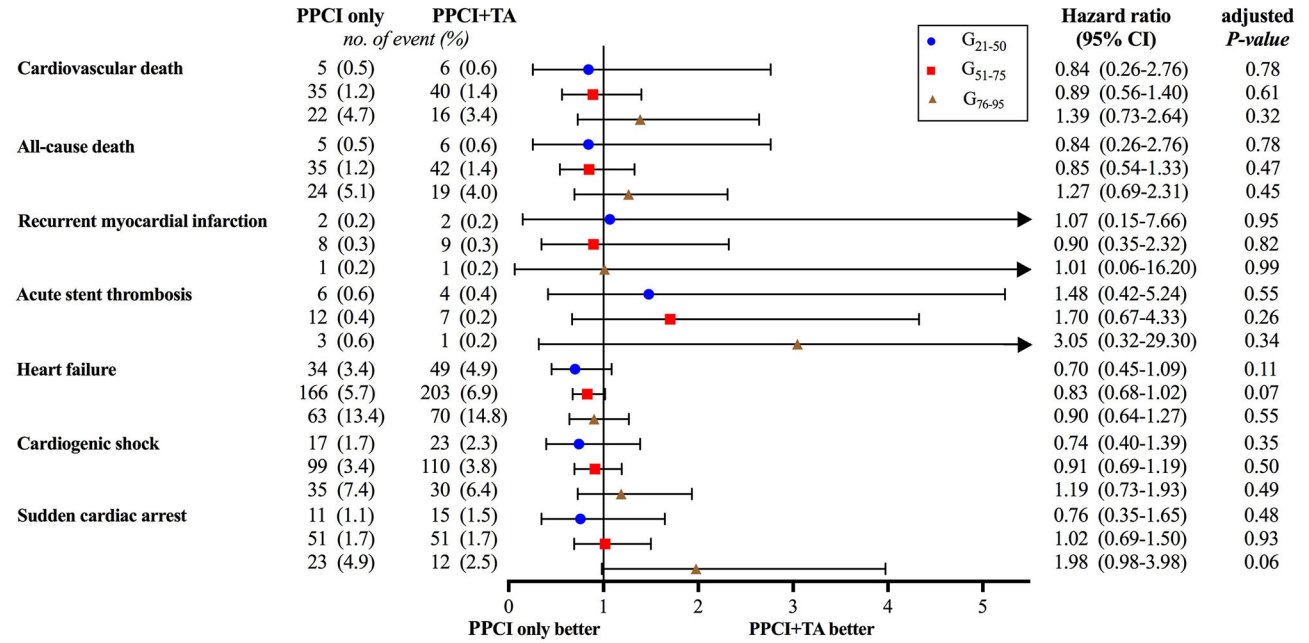

FIGURE 4 | Forest plot of in-hospital adverse cardiovascular events. No significant differences were observed between PPCI-only and thrombus aspiration groups after adjustment for age.

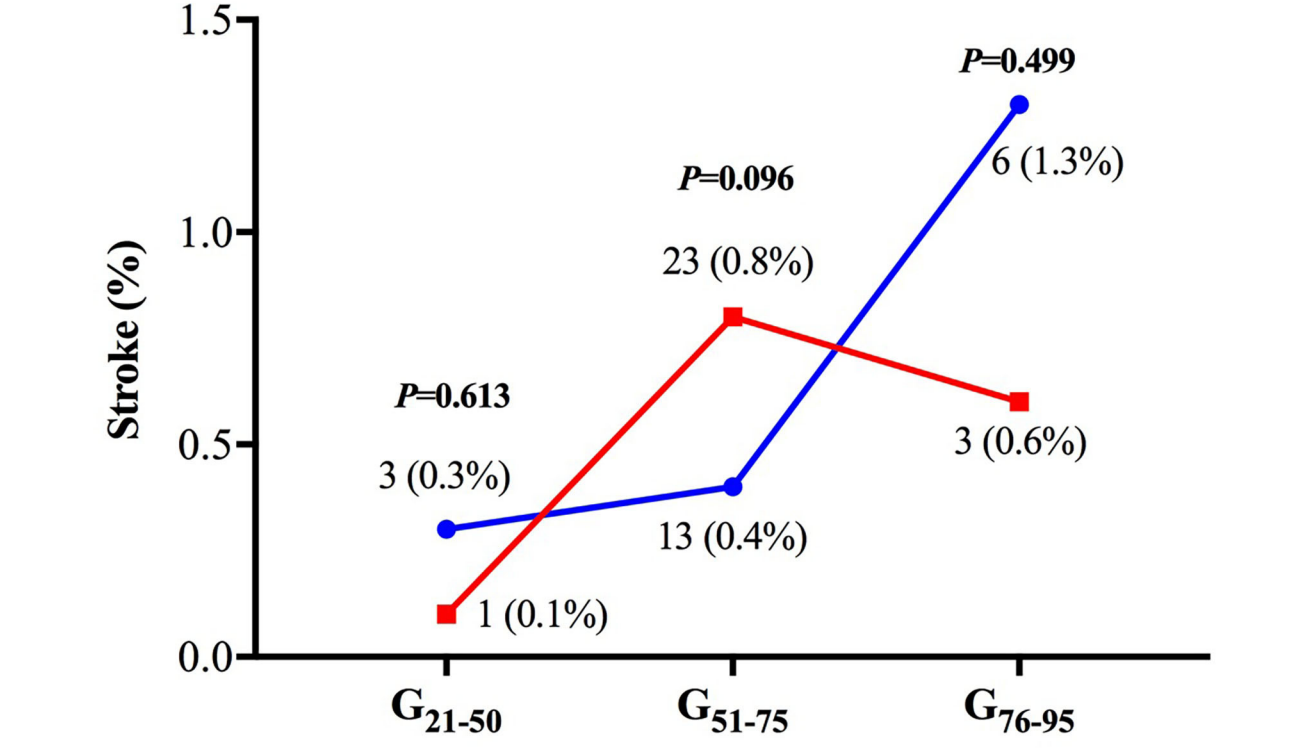

FIGURE 5 | The incidence of in-hospital stroke. There was no significant difference in the risk of in-hospital stroke between the two treatment groups in all age subgroups.

suffered from thrombus aspiration was mainly located in RCA, and LVEF and the incidence of regional wall motion abnormality were comparable between the two treatment groups. Unlike LAD predominantly supplying blood to the anterior and septal proportions of LV myocardium, RCA principally supplies the right ventricle and atrium (23). Meanwhile, the elderly patients suffering from persistent angina have robust collateral circulation to restore flow, which might help remain the blood supply and cardiac function to some extent (24).

There are still debates on whether thrombus aspiration can increase the rate of stroke. In this study, we did not find significantly increased in-hospital occurrence of the stroke in the thrombus aspiration group, which was similar in TASTE and other research studies (7, 20). Although TOTAL propagated the attitude that thrombus aspiration could increase the occurrence of a stroke at 30 and 180 days ($P = 0.020$ and 0.002, respectively), the conclusion was drawn among the randomized population with crossover from their initial treatment allocation to the alternative therapy in both groups. Looking back on the on-treatment analysis among patients who received upfront or bailout thrombus aspiration irrespective of randomization and convention PCI in TOTAL, the thrombus aspiration could not increase the rate of stroke at 30 and 180 days ($P = 0.193$ and 0.068, respectively) (8). TOTAL also indicated that the application of thrombus aspiration among patients with high thrombus burden (thrombolysis in myocardial infarction [TIMI] thrombus Grade $\geq$3) rather than those with low thrombus burden could increase the rate of stroke at 30 days (0.7% in thrombus aspiration group *vs*. 0.4% in PCI-only group; HR = 1.90; 95% CI, 1.04–3.08; $P = 0.03$) (25). In the TOTAL trial, the minimal volume of thrombus aspiration procedures required for operators only reached five in the last 2 years (14). The utilization of upfront bailout GP IIb/IIIa inhibitor was more frequent among the patients undergoing thrombus aspiration treatment than those in the PCI-only group (25). Hence, we cannot rule out that the higher rate of stroke was caused by procedures during thrombus aspiration therapy or more intensive anticoagulation therapy after thrombus aspiration. It is a consensus that the inappropriate operation of thrombus aspiration devices may cause systemic embolization of thrombotic material or air embolism (26). So, the development of aspiration catheters and improvement of thrombus aspiration skills need to be emphasized to avoid the increased ischemic stroke risk, especially among patients with a high thrombus burden. Meanwhile, the patients with a high thrombus burden are more likely to suffer from hemorrhagic stroke due to the intensive anticoagulants and need to be closely monitored in intensive or cardiac care units post procedurally.

LIMITATIONS

There were several limitations in this study. First, the CCC–ACS project is a retrospective and observational real-world study based on the medical records uploaded in the database, limited data were gathered. Hence, the detailed information of properties of thrombus (red/white), location of thrombus (proximal/distal), TIMI thrombus grade, size and length of the stent, bifurcation, collateral circulation and so on were inaccessible. However, this study still provides physicians with a better understanding of the utilization and clinical benefit of thrombus aspiration. Second, due to the numerous factors considered, there were still differences with the potential confounders between PPCI-only and thrombus aspiration groups after 1:1 propensity score matching. While, most variables were well-matched, the difference of age in the unmatched population was taken into consideration during the analysis of clinical outcomes to increase the reliability of results.

CONCLUSION

In this large nationwide observational study, thrombus aspiration did not significantly reduce the in-hospital risk of cardiovascular death, all-cause death, recurrent myocardial infarction, acute stent thrombosis, heart failure, cardiogenic shock, sudden cardiac arrest and stroke compared with conventional PPCI among the whole STEMI patients. However, it presented the tendency to reduce the occurrence of in-hospital cardiovascular death at 3 days and sudden cardiac arrest among patients older than 75 years, and increase the risk of heart failure in patients aged 51–75 years.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the datasets analyzed in this study are not publicly available according to the regulation of CCC-ACS project, but the reliability and accuracy can be validated with the assistance of Prof. Dong Zhao who is the principle investigator of this project. Requests to access the datasets should be directed to Prof. Dong Zhao, deezhao@vip.sina.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Beijing Anzhen Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

G-SM and L-JC contributed to the design and overall investigation. Y-YQ was responsible for the data collection, statistical analysis, and manuscript. X-GZ, C-WJ, Y-MS, RZ, W-JZ, and Z-JJ have made substantial contributions to the analysis and interpretation of data or revising the manuscript. All authors read and approved the final manuscript.

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We acknowledge the contribution of all investigators, including the physicians participating in the project from 150 hospitals (**Supplementary Table 3**).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.791007/full#supplementary-material

Supplementary Figure 1 | Logistic regression analysis revealed that thrombus aspiration was less likely to be conducted with the increase of age. PPCI, primary percutaneous coronary intervention; TA, thrombus aspiration; CI, confidence interval.

Supplementary Figure 2 | LVEF and the rate of region wall motion abnormality before discharge. **(A,B)** For patients ≤ 75 years, patients undergoing thrombus aspiration treatment presented with lower LVEF and a higher rate of regional wall motion abnormality. While for patients > 75 years, no significant difference was observed between the two treatment groups. RWMA, regional wall motion abnormality.

Supplementary Table 1 | The baseline characteristics of the matched patients ($n = 8,815$).

Supplementary Table 2 | The culprit lesion and in-hospital medication of the matched patients ($n = 8,815$).

Supplementary Table 3 | One hundred and fifty hospitals and investigators participating in the CCC-ACS project during 2014–2017.

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The Prescription Characteristics, Efficacy and Safety of Spironolactone in Real-World Patients With Acute Heart Failure Syndrome: A Prospective Nationwide Cohort Study

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Background: Randomized clinical trials of spironolactone showed significant mortality reduction in patients with heart failure with reduced ejection fraction. However, its role in acute heart failure syndrome (AHFS) is largely unknown.

Aim: To investigate the prescription characteristics, efficacy and safety of spironolactone in real-world patients with AHFS.

Methods: 5,136 AHFS patients who survived to hospital discharge using a nationwide prospective registry in Korea were analyzed. The primary efficacy outcome was 3-year all-cause mortality.

Results: Spironolactone was prescribed in 2,402 (46.8%) at discharge: <25 mg in 890 patients (37.1%), ≥25 mg, and <50 mg in 1,154 patients (48.0%), and ≥50 mg in 358 patients (14.9%). Patients treated with spironolactone had a lower proportion of chronic renal failure and renal replacement therapy during hospitalization and had lower serum creatinine level than those who did not. In overall patients, 3-year mortality was not different in both groups (35.9 vs. 34.5%, $P = 0.279$). The incidence of renal injury and hyperkalemia was 2.2% and 4.3%, respectively, at the first follow-up visit. The treatment effect of spironolactone on mortality was different across subpopulations according to

LVEF. The use of spironolactone was associated with a significant reduction in 3-year morality in patients with LVEF ≤ 26% (33.8 vs. 44.3%, $P < 0.001$; adjusted HR 0.79, 95% CI 0.64–0.97, $P = 0.023$), but not in patients with LVEF > 26%.

Conclusions: Although spironolactone was frequently used at lower doses in real-world practice, use of spironolactone significantly reduced 3-year mortality in patients with severely reduced LVEF with acceptable safety profile. However, our findings remain prone to various biases and further prospective randomized controlled studies are needed to confirm these findings.

Keywords: acute heart failure syndrome, spironolactone, mineralocorticoid receptor antagonists, drug therapy, outcome

INTRODUCTION

Aldosterone has gained interest as a therapeutic target due to its independent and significant role in the pathophysiology of heart failure (HF). Beyond maintaining sodium and water homeostasis, aldosterone is involved in myocardial hypertrophy, fibrosis, and endothelial dysfunction (1). After the results of the Randomized Aldactone Evaluation Study (RALES) trial, which demonstrated an association between spironolactone and considerable mortality risk reduction in patients with severe HF, mineralocorticoid antagonists became a component of treatment for HF with reduced ejection fraction (HFrEF) (2–4). There was an attempt to reconsider for spironolactone to expand its therapeutic range to HF with preserved ejection fraction (HFpEF), and recently the U.S Food and Drug Administration's advisory committee reviewed a labeled indication for spironolactone in the treatment of adults with HFpEF (5).

However, data on the efficacy and safety of spironolactone in patients with acute heart failure syndrome (AHFS) including HFpEF are still limited. Even in the Aldosterone Antagonist Therapy for Adults with Heart Failure and Preserved Systolic Function (TOPCAT) trial, which investigated the use of spironolactone in HFpEF, outcome improvement was identified only in patients enrolled in the Americas, not all participants (6, 7). In addition, spironolactone showed conflicting results in a broad unselected population with HF outside of clinical trials (8). Considering the potential risk of adverse effects of spironolactone, such as renal impairment and hyperkalemia (9, 10), it is necessary to collect data on the efficacy and safety of spironolactone in real clinical AHFS practice to establish guidance for the use of spironolactone.

Therefore, we aimed to present the current spironolactone prescription pattern, efficacy, and safety, and to evaluate whether the efficacy of spironolactone could be varied depending on the left ventricular ejection fraction (LVEF) in Korean patients with AHFS.

METHODS

Study Design and Population

Data for this study were from the Korean Acute Heart Failure (KorAHF) registry. Details on the study design and rationale of the KorAHF registry were previously reported (11, 12). The KorAHF registry is a nationwide prospective multicenter cohort study that evaluates the clinical characteristics, management, and outcomes of patients hospitalized for AHFS in Korea. Patients were enrolled at 10 tertiary university-affiliated hospitals from March 2011 to February 2014. Patients with signs or symptoms of HF and either (1) lung congestion defined as congestion on a chest X-ray or as rales on physical examination or (2) objective findings of LV systolic dysfunction or structural heart disease were eligible for the registry. A total of 5,625 consecutive patients were enrolled in the registry. Because our study aimed to identify whether spironolactone have a homogeneous treatment effect among patient subpopulations with different ejection fraction, we included the whole range of LVEF. Among them, patients without documented data of LVEF and patients who died during index hospitalization were excluded in this study (**Supplementary Figure S1**). The institutional review board or ethics committee at each participating hospital approved the study protocol and waived the need for written informed consent. This study complied with the Declaration of Helsinki principles.

Data Collection and Clinical Outcomes

Data were collected by attending physicians in each participating center using a web-based case-report form in the Clinical Research and Trial Management System (iCReaT) supported by the Korean National Institute of Health with the assistance of a clinical research coordinator. Information about patient demographic characteristics including comorbidities, etiology of HF, vital signs, laboratory and echocardiographic measurements, treatments, and clinical outcomes were obtained prospectively at the time of admission, discharge, and during the follow-up period. Data were periodically reviewed by an independent data monitoring team.

For C-reactive protein (CRP)/ high sensitivity CRP (hs-CRP) and brain natriuretic peptide (BNP)/ N-terminal pro-BNP (NT-proBNP), only one of the two variables was measured for each hospital. Therefore, these variables were classified as follows, referring to previous publications, in order to reduce missing values: BNP < 100 pg/mL or NT-proBNP < 360 pg/mL and BNP ≥ 100 pg/mL or NT-proBNP ≥ 360 pg/mL, and CRP level ≤ 10 mg/L or hsCRP ≤ 3.0 mg/L and CRP level > 10 mg/L or hsCRP > 3.0 mg/L (6, 13, 14).

The primary outcome in this study was all-cause mortality 3 years after hospital discharge. The mortality data for patients who were lost to follow-up was collected from the National Insurance data or National Death Records. Mortality and cause of death were verified by a Clinical Event Committee, which was composed of independent experts in HF who have not participated in patient enrolment.

Definitions

The use of spironolactone was assessed at hospital discharge. The prescription for initiation, dose adjustment or discontinuation of medications including spironolactone was left to the discretion of the physician in charge, but the decision-making generally followed the guidelines (3, 4). The safety of spironolactone treatment was evaluated at the first post-discharge outpatient follow-up visit. Renal injury was defined as a doubling of serum creatinine based on the Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE) classification creatinine doubling, and hyperkalemia was defined as potassium greater than 5.5 mmol/L (15).

Echocardiography was performed by a board-certified cardiologist or echocardiography technician. Quantitative assessment of LVEF using the modified Simpson's method was recommended, but LVEF that measured by M-mode or visual estimation was also used for HF categorization when the accuracy of the biplane method was limited due to a poor acoustic window (16). We defined the HF classification based on LVEF using the criteria of American College of Cardiology/American Heart Association (ACC/AHA) and the European Society of Cardiology (ESC) guidelines as follows: HFrEF, HF with LVEF $\leq$40%; HF with mid-range ejection fraction (HFmrEF), HF with LVEF 41–49%, HFpEF, HF with LVEF $\geq$50% (4, 17).

Statistical Analysis

To compare clinical characteristics and outcomes between the two groups, we analyzed categorical variables as numbers and percentages using the χ^2 test or Fisher's exact test. Continuous variables are reported as mean $\pm$ standard deviation and were compared using the *t*-test. We performed subpopulation treatment effect pattern plot (STEPP) and the Contal and O'Quigley to evaluate whether the effect of spironolactone varies according to LVEF and to identify the cut-off value. To evaluate the effect of the spironolactone use and to identify risk factors for the 3-year all-cause mortality, we performed Cox proportional hazard regression analysis. Variables deemed clinically relevant from previous studies were considered candidate variables in multivariable Cox regression models. The hazard ratio (HR) of each variable is reported with the 95% confidence interval (CI). Survival curves were constructed by the Kaplan-Meier method, and the significance level was assessed using the log rank test to assess the effect of spironolactone with respect to the primary outcome according to classification of HF. To reduce the effects of potential confounders and selection bias, we performed a sensitivity analysis using propensity score matching. Propensity scores were estimated using a logistic regression model of the treatment on the covariate included in the Cox regression models. The patients were matched 1:1 by propensity scores. For all analyses, a two-tailed test with a *P*-value less than 0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R software package (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Prescription Pattern of Spironolactone

Of the 5,136 eligible patients, 2,402 (46.8%) patients were treated with spironolactone. The proportion of patients prescribed spironolactone decreased as the degree of renal function worsened (**Supplementary Figure S2**). The prescribed doses of spironolactone were $<$ 25 mg in 890 (37.1%), $\geq$ 25 mg and $<$ 50 mg in 1,154 (48.0%), and $\geq$ 50 mg in 358 (14.9%) in overall patients. About 75% of survivors in the spironolactone group were followed up until 3 years after hospital discharge, and 51.8% of them maintained spironolactone treatment during the 3-year follow-up period (**Supplementary Figure S3**).

There were significant differences in clinical and in-hospital treatment characteristics between patients treated with spironolactone and without spironolactone (**Table 1**). The proportion of de novo HF, hypertension, diabetes, and ischemic heart disease were higher in the no spironolactone group, and the proportion of dilated cardiomyopathy, and atrial fibrillation were higher in the spironolactone group. In the spironolactone group, chronic renal failure and need of renal replacement therapy during hospitalization were less common and serum creatinine was significantly lower than in the no spironolactone group. In addition, the spironolactone group had lower LVEF than the no spironolactone group.

Mortality and Adverse Events

Overall, 1,810 (35.2%) patients died during the 3-year follow-up after discharge and there was no significant difference in 3-year (35.9% vs. 34.5%, $P = 0.279$) all-cause mortality between the two groups (**Figure 1**). As renal function worsened, the 3-year mortality tended to increase and the difference in 3-year mortality between the two groups was not significant except for patients with glomerular filtration rate $<$15 mL/min/1.73m^2 (57/132 vs. 2/14, $P = 0.036$) (**Supplementary Table S1**).

Among the spironolactone group, 1,324 (55.1%) patients had post-discharge outpatient follow-up visits with blood sampling within an average of 14.9 days. There were significant differences in systolic blood pressure (113.1 mmHg vs. 109.9 mmHg, $P < 0.001$), serum creatinine (1.08 mg/dL vs. 1.18 mg/dL, $P < 0.001$), and serum potassium (4.2 mmol/L vs. 4.6 mmol/L, $P < 0.001$) compared with those at hospital discharge, but the incidence of renal injury and hyperkalemia was 2.2 and 4.3%, respectively (**Supplementary Table S2**). There was no case of discontinuing spironolactone due to gynecomastia in the transition period (**Supplementary Table S3**).

Effect of Spironolactone According to LVEF

STEPP analysis of the treatment effect of spironolactone across subpopulations according to LVEF showed that the spironolactone treatment was associated with lower 3-year

TABLE 1 | Clinical and treatment characteristics in overall patients.

Variables	Overall (*n* = 5,136)	No SPR (*n* = 2,734)	SPR (*n* = 2,402)	*P*-Value
Age, years	68.4 ± 14.4	69.0 ± 14.4	67.7 ± 14.4	<0.001
Male	2728 (53.1)	1463 (53.5)	1265 (52.7)	0.544
De novo HF	2748 (53.5)	1509 (55.2)	1239 (51.6)	0.010
Past medical history				
Hypertension	3025 (58.9)	1682 (61.5)	1343 (55.9)	<0.001
Diabetes mellitus	1799 (35.0)	1015 (37.1)	784 (32.6)	0.001
Ischemic heart disease	1415 (27.6)	793 (29.0)	622 (25.9)	0.014
Dilated cardiomyopathy	411 (8.0)	172 (6.3)	239 (10.0)	<0.001
Valvular heart disease	724 (14.1)	390 (14.3)	334 (13.9)	0.716
Atrial fibrillation	1456 (28.4)	739 (27.0)	717 (29.9)	0.025
Chronic lung disease	567 (11.0)	314 (11.5)	253 (10.5)	0.280
Chronic renal failure	698 (13.6)	515 (18.8)	183 (7.6)	<0.001
Cerebrovascular disease	764 (14.9)	428 (15.7)	336 (14.0)	0.093
Treatment during hospitalization				
Parenteral diuretics	3843 (74.8)	1979 (72.4)	1864 (77.6)	<0.001
Parenteral inotropes	1463 (28.5)	764 (27.9)	699 (29.1)	0.360
Parenteral vasodilators	2124 (41.4)	1232 (45.1)	892 (37.1)	<0.001
Intensive care unit admission	2412 (47.0)	1345 (49.2)	1067 (44.4)	0.001
Mechanical ventilation	670 (13.1)	387 (14.2)	283 (11.8)	0.012
Renal replacement therapy	286 (5.6)	231 (8.5)	55 (2.3)	<0.001
Vital signs at discharge				
Systolic blood pressure, mmHg	114.9 ± 17.6	117.3 ± 17.7	112.2 ± 17.1	<0.001
Heart rate, /min	76.8 ± 14.1	77.5 ± 14.3	76.0 ± 13.9	0.062
NYHA class II-IV	4224 (82.2)	2242 (82.0)	1982 (82.5)	0.633
Laboratory measurements at discharge				
Sodium, mmol/L	137.9 ± 3.9	138.1 ± 3.9	137.7 ± 4.0	0.161
Potassium, mmol/L	4.2 ± 0.5	4.2 ± 0.5	4.2 ± 0.5	0.154
Hemoglobin, g/dL	12.1 ± 2.1	11.9 ± 2.1	12.4 ± 2.1	<0.001
Creatinine, mg/dL	1.35 ± 1.31	1.58 ± 1.66	1.09 ± 0.64	<0.001
CRP > 3mg/dL or hs-CRP > 10 mg/dL	555 (11.4)	332 (12.6)	223 (10.0)	0.004
BNP > 100pg/mL or NT-proBNP > 360 pg/mL	4425 (94.9)	2328 (93.7)	2097 (96.2)	<0.001
Echocardiographic parameters				
LVEDV, mL	151.8 ± 71.8	142.0 ± 65.0	164.9 ± 78.1	<0.001
LVESV, mL	99.2 ± 62.6	91.1 ± 57.4	110.1 ± 67.5	<0.001
Ejection fraction, %	37.9 ± 15.5	39.9 ± 15.5	35.7 ± 15.1	<0.001
LA volume index, mL/m^2	63.7 ± 42.2	61.2 ± 33.9	66.1 ± 48.8	0.002
E, m/sec	0.94 ± 0.39	0.94 ± 0.40	0.95 ± 0.37	0.250
A, m/sec	0.76 ± 1.51	0.76 ± 0.37	0.76 ± 2.24	0.981
E/A ratio	1.6 ± 3.8	1.5 ± 2.9	1.8 ± 4.6	0.018
Deceleration time, msec	170.7 ± 82.9	176.3 ± 88.2	164.3 ± 76.0	<0.001
e', cm/sec	5.01 ± 2.32	5.05 ± 2.13	4.96 ± 2.52	0.249
a', cm/sec	6.16 ± 2.76	6.44 ± 2.70	5.79 ± 2.79	<0.001
E/e' ratio	21.2 ± 11.4	20.9 ± 11.4	21.5 ± 11.5	0.070
TR Vmax, m/s	2.90 ± 0.59	2.88 ± 0.57	2.91 ± 0.61	0.202

Values are mean ± standard deviation and median with interquartile range or n (%).
BNP, brain natriuretic peptide; CRP, C-reactive protein; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SPR, spironolactone.

mortality only in subpopulations where LVEF was less than 28% (**Figure 2** and **Supplementary Table S4**). The most significant cut-off value of LVEF by Contal and O'Quigley method was 26.1%, which discriminated patients with different survival according to use of spironolactone. In patients with LVEF < 26.1%, the spironolactone group had significantly lower 3-year

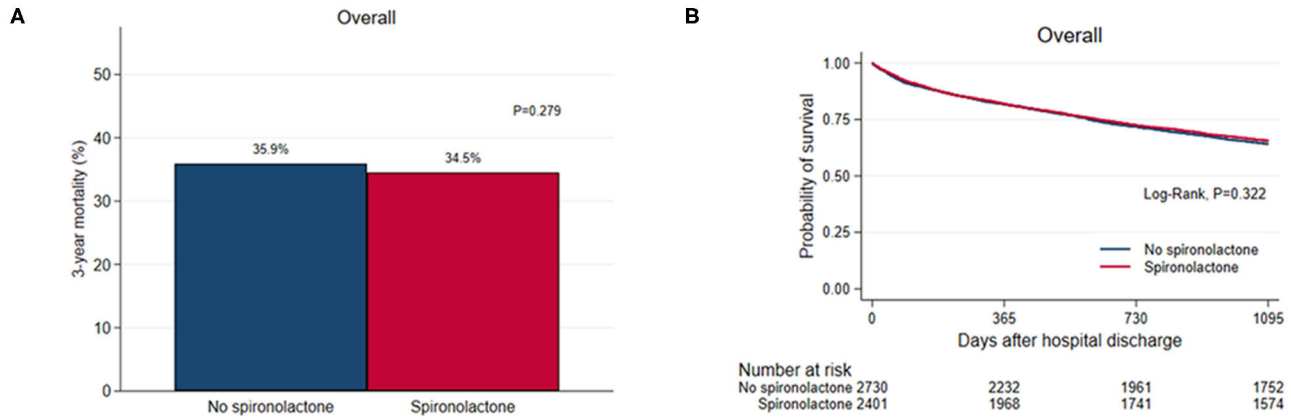

FIGURE 1 | (A) Bar graph and **(B)** Kaplan–Meier curves of the 3-year all-cause mortality after hospital discharge according to spironolactone treatment in overall patients.

(33.8 vs. 44.3%, $P < 0.001$) mortality than the no spironolactone group, but there was no difference in mortality between the two groups in patients with LVEF > 26% (**Figure 3**). Cox regression analysis revealed that spironolactone treatment was independently associated with a reduction in 3-year mortality in patients with LVEF ≤ 26% (HR 0.71, adjusted HR 0.79, 95% CI 0.64–0.97, $P = 0.023$) (**Table 2**).

When overall patients were divided into three classifications of HF based on LVEF, there was no significant difference in 3-year mortality between the two groups among patients with HFmrEF (32.9 vs. 33.1%, $P = 0.945$) and HFpEF (32.3 vs. 36.3%, $P = 0.109$) (**Figure 4**). In patients with HFrEF, the 3-year mortality rate was significantly reduced in the spironolactone group (34.0 vs. 39.6%, $P = 0.002$).

Sensitivity Analysis

Clinical and in-hospital treatment characteristics of the two groups in propensity score matched cohort are shown in the **Supplementary Table S5**. The no spironolactone group had a higher proportion of chronic renal failure and renal replacement therapy during hospitalization than the spironolactone group. Potassium and creatinine levels and LVEF at discharge were also slightly higher in the no spironolactone group. Similar to the results of our main analysis, the Kaplan-Meier survival curve showed a significant difference between the survival rates of the two groups during a 3-year follow up in patients with LVEF ≤ 26% (log rank test, $P = 0.043$), but the survival rates were similar between the two groups in patients with LVEF > 26% (log rank test, $P = 0.824$) (**Supplementary Figure S4**). In Cox regression analysis, spironolactone was related to 3-year mortality in patients with LVEF ≤ 26% (adjusted HR 0.78, 95% CI 0.62–0.99, $P = 0.044$), but not in patients with LVEF > 26% (adjusted HR 1.01, 95% CI 0.89–1.15, $P = 0.824$).

DISCUSSION

In the present study, we identified the prescription pattern of spironolactone and evaluated the efficacy and safety of spironolactone in patients with AFHS using a large prospective nationwide cohort in Korea. We found that (1) spironolactone was used in 46.8% of Korean patients with AHFS and spironolactone was often used at a dose lower than the dose recommended in the guidelines; (2) use of spironolactone was not associated with reduction of 3-year all-cause mortality and occurrence of significant renal injury or hyperkalemia in overall patients; and (3) the effect of spironolactone on mortality was different depending on the LVEF, and the survival benefit was particularly remarkable in patients with severely reduced LVEF.

Mineralocorticoid antagonists, including spironolactone, are commonly prescribed in HFrEF patients, which ranges from about 50 to 70% in recent studies (18–21). Spironolactone has emerged as an important treatment option for HF since the 1990s because of its ability to attenuate the neurohormonal signals, which play a central role in the progression of HF, and to reverse remodeling. Spironolactone treatment significantly reduces plasma procollagen type III aminoterminal peptide (PIIINP), a biochemical marker of myocardial fibrosis and/or remodeling, and BNP, a prognostic marker of HF, and improves endothelial function, which is associated with cardiovascular events in patients with HF of varying severity (22–24). Studies evaluating the effect of spironolactone with echocardiographic assessment showed improvement of LV systolic and diastolic function and ventricular-arterial coupling, as well as reduction of LV volume and mass in patients treated with spironolactone (25, 26). In addition to the improvement of these laboratory and echocardiographic parameters, positive results from three large-scale, multi-center, placebo-controlled clinical trials, RALES, the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), and the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF), empowered MRA treatment in HFrEF (2, 27, 28). Furthermore, the TOPCAT trial suggested that the effect of spironolactone are not limited to HFrEF, but may extend to patients with HFpEF (6).

Contrary to the favorable results of the previous studies, spironolactone was not associated with a reduced mortality

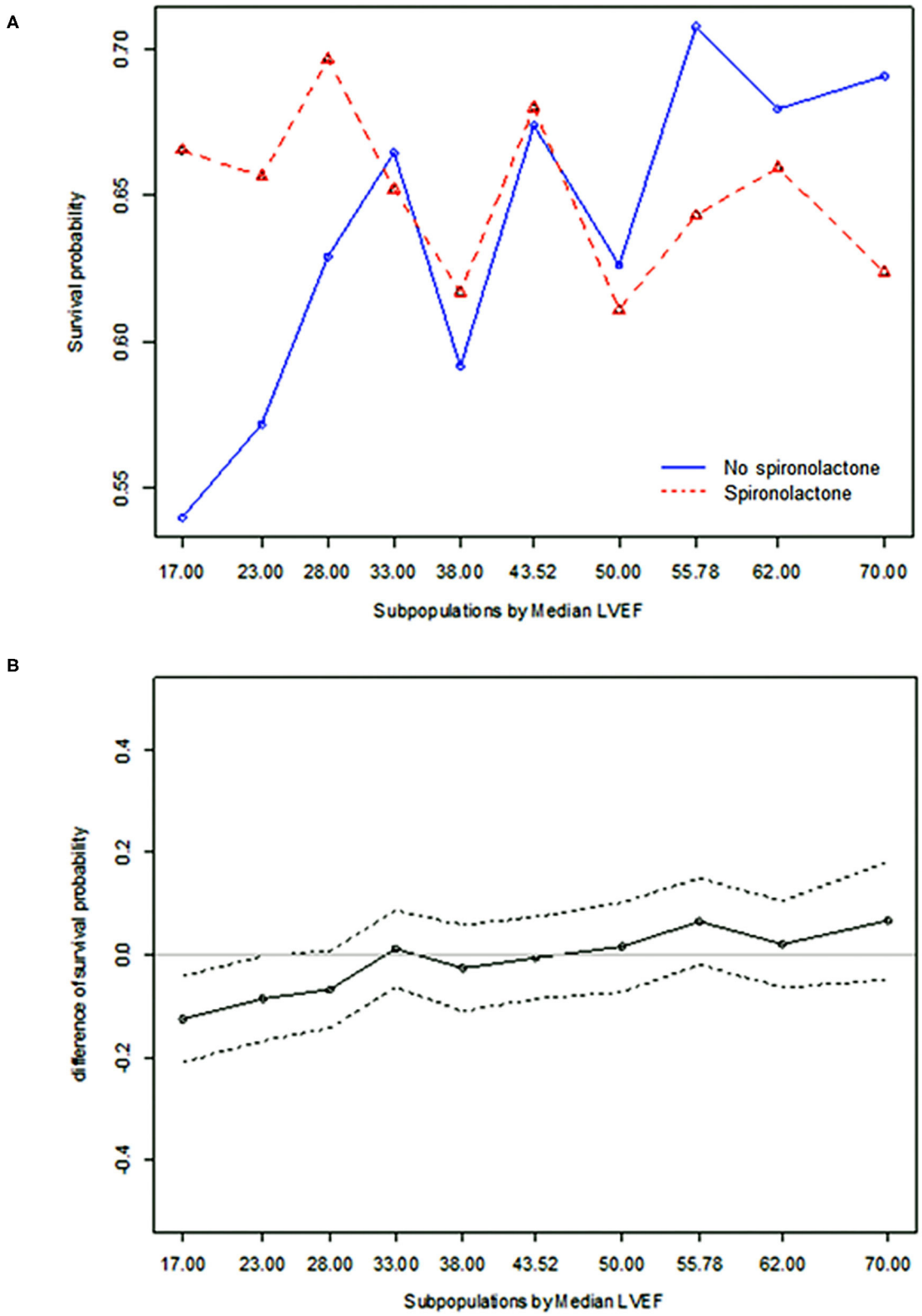

FIGURE 2 | Subpopulation treatment effect pattern plot analysis of the treatment effect of spironolactone as measured by **(A)** 3-year all-cause mortality, **(B)** difference in 3-year all-cause mortality.

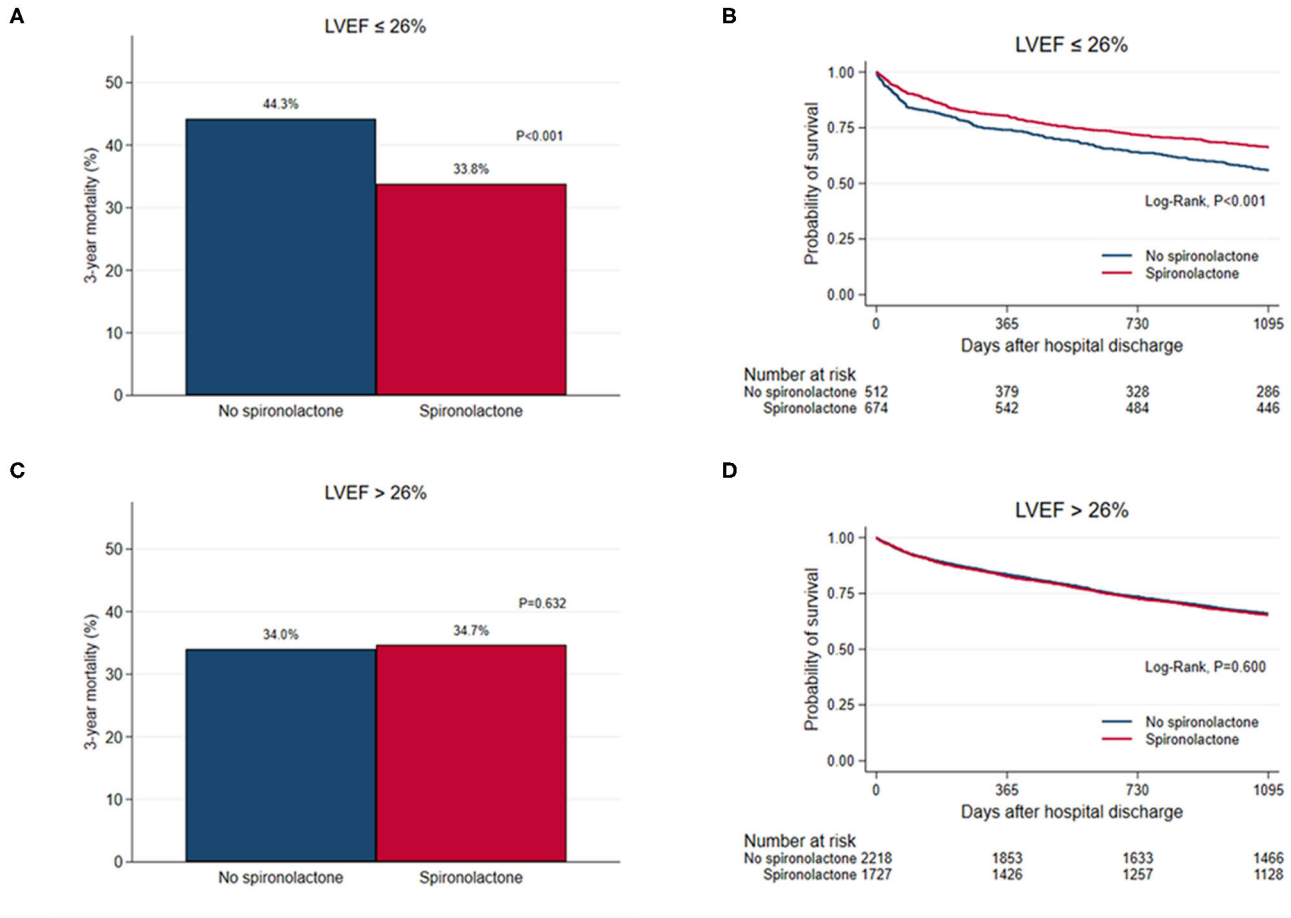

FIGURE 3 | (A,C) Bar graph and **(B,D)** Kaplan–Meier curves of the 3-year all-cause mortality after hospital discharge according to spironolactone treatment in patients with left ventricular ejection fraction (LVEF) ≤ 26% and in patients with LVEF > 26%.

in our entire cohort. Our findings are consistent with a study of Lund et al. who failed to show differences in mortality according to mineralocorticoid antagonist treatment in a large general HF population of the Swedish Heart Failure Registry (8). It suggests that there may be a gap between the randomized clinical trials and real-world practice. In our study, patients who received spironolactone treatment had substantially different characteristics from those who did not, suggesting that spironolactone was selectively prescribed. Considering the *post-hoc* analysis of the TOPCAT trial, which demonstrated the the response to spironolactone was significantly different according to clinical phenogroups (6, 29), differences in patient selection and patient characteristics may be one explanation. In particular, we did not limit our analysis to patients with HFrEF or HFpEF because our study was interested in evaluating the efficacy of spironolactone in real-world practice, but the efficacy of spironolactone was different depending on the LVEF. The survival benefit of spironolactone was significant only in patient with severely reduced LVEF. Although our findings cannot explain the underlying mechanism of the relationship between spironolactone and LVEF, it supports the current guidelines recommending the use of spironolactone in patients with LVEF ≤ 35% (17, 30).

In our study, spironolactone was prescribed in only about half of overall patients. In particular, the proportion of patients prescribed spironolactone was significantly reduced in patients with severe renal dysfunction. Our findings are similar to a recent study by Patel et al. which demonstrated that spironolactone was infrequently used compared to other guideline recommended, especially in patients with renal dysfunction (31). It is presumably the result of concerns about spironolactone-related complications, such as worsening of renal function and hyperkalemia. However, selective use of spironolactone did not increase the incidence of mortality or adverse events in patients with renal dysfunction in our cohort. Furthermore, Oh et al. showed the survival benefit of spironolactone in patients with stage 3b chronic kidney disease (32). Therefore, further studies should be conducted for the proper use of spironolactone in patients with renal dysfunction.

Also, the that about 40% of patients treated with spironolactone in our study were not prescribed a guideline-recommended dose may have may have influenced the efficacy

TABLE 2 | Predictors for 3-year all-cause mortality in patients according to LVEF.

	In patients with LVEF ≤ 26%					
	Univariable			Multivariable*		
Variables	**HR**	**95% CI**	***P*-value**	**Adjusted HR**	**95% CI**	***P*-value**
Spironolactone use	0.71	0.59–0.86	<0.001	0.79	0.64–0.97	0.023
Age ≥67	3.20	2.59–3.94	<0.001	2.78	2.16–3.56	<0.001
Male	0.89	0.73–1.08	0.232	0.86	0.69–1.06	0.157
De novo HF	0.44	0.36–0.54	<0.001	0.66	0.53–0.82	<0.001
Hypertension	1.73	1.43–2.09	<0.001	1.33	1.07–1.67	0.012
Diabetes mellitus	1.62	1.35–1.95	<0.001	1.35	1.09–1.66	0.005
Cerebrovascular disease	1.75	1.39–2.21	<0.001	1.18	0.91–1.53	0.209
Use of parenteral inotropes	1.75	1.46–2.10	<0.001	1.32	1.06–1.63	0.011
Systolic blood pressure	1.00	0.99–1.00	0.353	0.99	0.98–1.00	0.015
Heart rate	1.01	1.00–1.02	0.001	1.01	1.01–1.02	0.001
Sodium	0.92	0.91–0.94	<0.001	0.95	0.93–0.97	<0.001
CRP > 3 mg/dL or hs-CRP > 10 mg/dL	1.96	1.48–2.60	<0.001	1.51	1.12–2.04	0.007
BNP > 100 pg/mL or NT-proBNP > 360 pg/mL	20.43	1.28–326.50	0.033	13.55	0.84–218.77	0.066
	In patients with LVEF > 26%					
	Univariable			**Multivariable***		
Variables	**HR**	**95% CI**	***P*-value**	**Adjusted HR**	**95% CI**	***P*-value**
Spironolactone use	1.03	0.92–1.14	0.653	1.00	0.89–1.12	0.995
Age ≥67	2.36	2.11–2.64	<0.001	2.13	1.88–2.42	<0.001
Male	0.99	0.89–1.10	0.870	1.09	0.97–1.23	0.142
De novo HF	0.61	0.55–0.68	<0.001	0.66	0.59–0.74	<0.001
Hypertension	1.41	1.26–1.59	<0.001	1.09	0.95–1.24	0.216
Diabetes mellitus	1.33	1.19–1.48	<0.001	1.12	0.99–1.26	0.065
Cerebrovascular disease	1.58	1.38–1.80	<0.001	1.35	1.18–1.56	<0.001
Use of parenteral inotropes	1.02	0.90–1.15	0.739	0.99	0.87–1.13	0.895
Systolic blood pressure	1.00	1.00–1.00	0.118	1.00	1.00–1.00	0.730
Heart rate	1.01	1.00–1.01	<0.001	1.01	1.00–1.01	<0.001
Sodium	0.93	0.92–0.95	<0.001	0.95	0.93–0.96	<0.001
CRP > 3 mg/dL or hs-CRP > 10 mg/dL	1.47	1.26–1.72	<0.001	1.36	1.15–1.60	<0.001
BNP > 100 pg/mL or NT-proBNP > 360 pg/mL	2.56	1.83–3.58	<0.001	2.00	1.42–2.84	<0.001

**All variables shown in table were entered into the multivariable Cox proportional hazard regression model.*

BNP, brain natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; HF, heart failure; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide.

of spironolactone treatment. Although current guidelines recommend 25 mg spironolactone once daily with titration up to 50 mg once daily for patients with HFrEF based on landmark trials (17, 30), a large number of patients are treated with spironolactone doses of less than 25 mg in real-world practice (19, 33). The dose response relationship between spironolactone and survival has not yet been clearly identified. In the Aldosterone Targeted Neurohormonal Combined with Natriuresis Therapy in Heart Failure (ATHENA-HF) trial, 100 mg of spironolactone was not associated with an improved outcome compared to placebo or 25 mg of spironolactone in patients with AHFS (34). On the other hand, in the ASIAN-HF registry, patients who received at least 100% of guideline-recommended dose had better composite outcomes of all-cause deaths or hospitalization for HF than did those who received lower doses (19). To properly assess the characteristics of patients who are likely to benefit from spironolactone treatment, the effects of under-dosing should be further elucidated.

Although our study provided information regarding the association between spironolactone treatment and long-term survival in a large population of Korean patients with AHFS, there are several limitations that should be considered. First, because of the observational study design, our findings remain prone to various biases and potential confounding factors. Although we used regression modeling and propensity score matching to control for confounders, unmeasured confounders may have been present. In particular, our study did not control the effect of standard medical treatment for HF with reduced

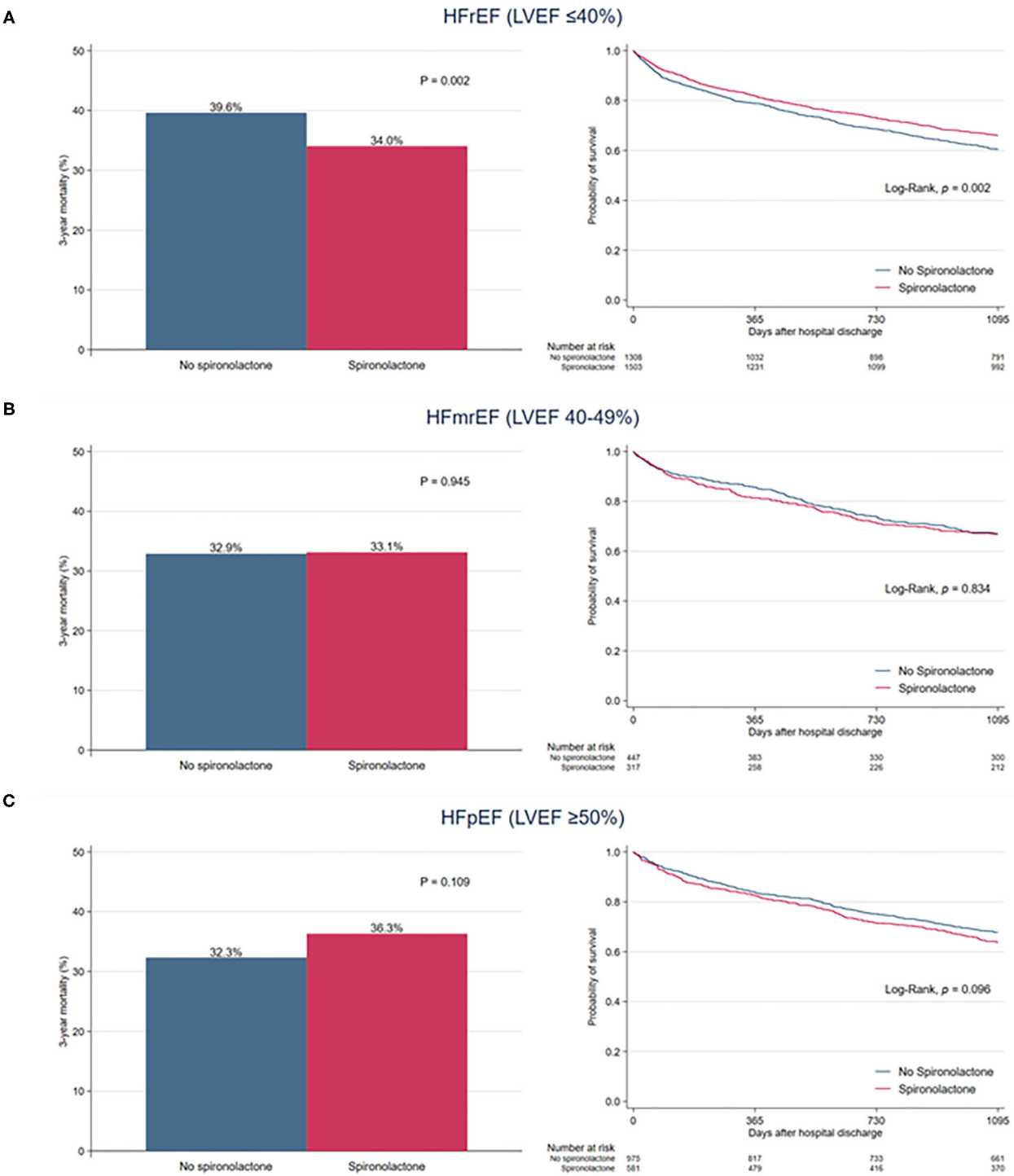

FIGURE 4 | Bar graph and Kaplan–Meier curves of the 3-year all-cause mortality after hospital discharge according to spironolactone treatment in patients with **(A)** heart failure with reduced ejection fraction (HFrEF), **(B)** heart failure with mid-range ejection fraction (HFmrEF), and **(C)** heart failure with preserved ejection fraction (HFpEF).

EF such as renin-angiotensin system blockade or beta blocker. Second, it is possible that more severe and complex patients were included in this study because only tertiary university-affiliated hospitals participated in the registry. In addition, since risk factors for mortality and the efficacy of medication in patients with HF may vary depending on regional differences, our findings have limitations in their generalizability to other populations (7). Third, we determined whether the patient was treated with spironolactone only by prescription at hospital discharge. Because the total treatment duration and changes in dosage of spironolactone and medication adherence were not evaluated, we could not exclude the possibility that insufficient treatment duration and adherence may have affected our findings. In addition, we could not assess the effect of valvular and right ventricular function on clinical outcomes. Further prospective randomized controlled studies are needed to confirm these findings.

CONCLUSION

Spironolactone was prescribed in selective patients and under-dosing was common for treatment of AHFS in real-world clinical practice. Although spironolactone was used in patients with a wide range of LVEF, the effect of spironolactone on mortality differed according to the LVEF and spironolactone was associated with a reduction of 3-year mortality only in patients with severely reduced LVEF. Further studies to identify patients who are likely to benefit from spironolactone treatment are necessary for the expansion of the therapeutic field of spironolactone and the optimal use in patient with AHFS.

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 Institutional Review Board of the Catholic Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SJN, J-CY, and SHB conceived and designed the study and drafted the manuscript for intellectual content. SJN, J-CY, HSL, SJ, and SHB analyzed and interpreted the data. SJN, J-CY, H-YL, H-JC, J-OC, E-SJ, SEL, M-SK, J-JK, K-KH, M-CC, SCC, S-MK, D-JC, B-SY, KHK, B-HO, and SHB revised the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.791446/full#supplementary-material

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A High Triglyceride-Glucose Index Value Is Associated With an Increased Risk of Carotid Plaque Burden in Subjects With Prediabetes and New-Onset Type 2 Diabetes: A Real-World Study

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Background: The triglyceride-glucose (TyG) index has been proposed as a convincing indicator of insulin resistance and has been found to be associated with atherosclerosis among diabetic patients. However, the relationship between the TyG index and arteriosclerosis in subjects with prediabetes and new-onset type 2 diabetes (T2D) remains uncertain. The purpose of this study was to assess the degree of carotid plaque burden in patients with prediabetes and new-onset T2D and to investigate the association between the TyG index and the degree of carotid plaque burden in this population.

Methods: This was a cross-sectional observational study that included 716 subjects aged 40–70 years old with prediabetes or new-onset T2D. Demographic, anthropometric, and laboratory measurements were collected. Participants underwent carotid arteriosclerosis evaluation by ultrasonography, and the degree of atherosclerosis was evaluated according to the carotid plaque burden. The TyG index was calculated.

Results: The population was stratified into high or low TyG index groups according to the median TyG index value. Higher values were associated with a higher BMI and waist circumference as well as higher total cholesterol, triglyceride, low-density lipoprotein cholesterol, plasma glucose, glycated hemoglobin, fasting C-peptide, and C-reactive protein levels ($P < 0.001$). The high TyG index group had a higher atherosclerotic plaque burden than the low TyG index group ($P < 0.001$). Multiclassification logistic regression analysis showed that the TyG index was positively associated with a high plaque burden [odds ratio (OR): 16.706, 95% confidence interval (CI): 3.988–69.978, $P = 0.000$], while no association was found between the TyG index and a low/moderate plaque burden. This association remained consistent in the subgroup analysis. In multiple

linear regression analysis, sex, age, and the TyG index were found to be independently associated with carotid plaque burden. For each unit increase in the TyG index, the risk of a high carotid plaque burden increased 1.595-fold.

Conclusion: A high TyG index was positively associated with a high carotid plaque burden in subjects with prediabetes and new-onset T2D. Clinicians should pay close attention to the TyG index to help these patients receive the greatest benefit from early intervention.

Keywords: triglyceride-glucose index, atherosclerosis, carotid plaque, prediabetes, new-onset type 2 diabetes

INTRODUCTION

Diabetes mellitus, a leading cause of disability worldwide, is a well-known risk factor for atherosclerotic cardiovascular disease (ASCVD) (1). A previous study indicated that early identification of diabetic individuals at high risk for cardiovascular events should be of a clinical priority because timely prevention and subsequent rapid interventions may reduce the disability and mortality rates in these patients (2).

Indeed, in the prediabetic state, the risk of cardiovascular events is persistent, and atherosclerosis might appear prior to a diagnosis of diabetes mellitus (3, 4). According to previous studies, subjects with prediabetes, and new-onset type 2 diabetes (T2D) may represent a specific population with increased cardiovascular risk, and thus, different prevention programs should be applied (5). Therefore, assessment of the progression of atherosclerosis in patients with prediabetes and new-onset T2D is critical for optimizing and personalizing treatment strategies.

Carotid plaque burden plays an essential role in the progression of ASCVD. For the early diagnosis of arteriosclerosis, imaging modalities such as ultrasound and coronary angiography are usually needed, and performing such procedures during routine monitoring of an asymptomatic population on a large scale may be difficult as they are expensive and time-consuming. Therefore, early recognition of simple and accurate indicators of carotid plaque burden that can be applied in daily clinical practice could aid in the early identification of patients at high risk of ASCVDs (6). Insulin resistance (IR) has been confirmed to be generally present before the onset of T2D and plays a major role in the development of atherosclerosis (7, 8). Triglyceride-glucose (TyG) index, which has been proposed as a convincing indicator of IR (9), has been demonstrated to be associated with the prevalence of ASCVD (10, 11). However, the study populations have mainly consisted of patients with diabetes (12–14). To the best of our knowledge, there is little evidence on the correlation between the TyG index and carotid plaque burden in patients with prediabetes and new-onset T2D to date.

Based on the above background, the current study evaluated whether and how the simple calculated TyG index was associated with the carotid plaque burden in subjects with prediabetes and new-onset T2D without any ASCVDs. The results of this work can contribute to the early recognition of patients at high risk of cerebrovascular accidents.

MATERIALS AND METHODS

Study Design and Subjects

Between January 2018 and January 2021, a total of 4,394 hospitalized patients with abnormal blood glucose were screened. After a review of clinical information consisting of medication usage data, self-reported medical history, glycated hemoglobin (HbA1c), fasting blood glucose, and oral glucose tolerance test (OGTT) results, 992 patients with a diagnosis of prediabetes or new-onset T2D at our hospital were included. The inclusion criteria were as follows: (1) diagnosis of prediabetes or new-onset T2D; (2) age between 40 and 70 years old; (3) no history of atherosclerotic cardiovascular disease; (4) no severe renal dysfunction; (5) no previous lipid-lowering treatment; and (6) carotid ultrasonography.

The criteria for prediabetes included a fasting plasma glucose (FPG) level between 100 mg/dL (5.6 mmol/l) and 125 mg/dL (6.9 mmol/l) and/or an HbA1c ranging between 5.7 and 6.4%, without a history of diabetes or the use of any antidiabetic drugs. Criteria for new-onset T2D included an incidental finding of FPG $\geq$ 126 mg/dL (7.0 mmol/l) and/or HbA1c $\geq$ 6.5% for no more than 6 months, without a history of diabetes or the use of any antidiabetic drugs (15, 16). Atherosclerotic cardiovascular disease included ischemic stroke, transient ischemic attack, coronary artery disease, heart failure, and arteriosclerosis obliterans. Severe renal dysfunction was defined as an estimated glomerular filtration rate (eGFR) $<$ 30 mL/min.

Based on the inclusion criteria, 276 patients were excluded, and 716 patients were ultimately included in the analysis (**Figure 1**). The study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Shaoxing Hospital, Zhejiang University School of Medicine. The data were anonymous, and the requirement for informed consent was therefore waived.

Data Collection and Laboratory Measurements

Demographic information (including sex and age) and anthropometric measures (including height, weight, and waist circumference) were collected. Body mass index (BMI) was calculated using the equation weight (kg)/squared value of height (m^2).

Clinical data such as tobacco use, alcohol use and history of hypertension were also recorded. Hypertension was defined as

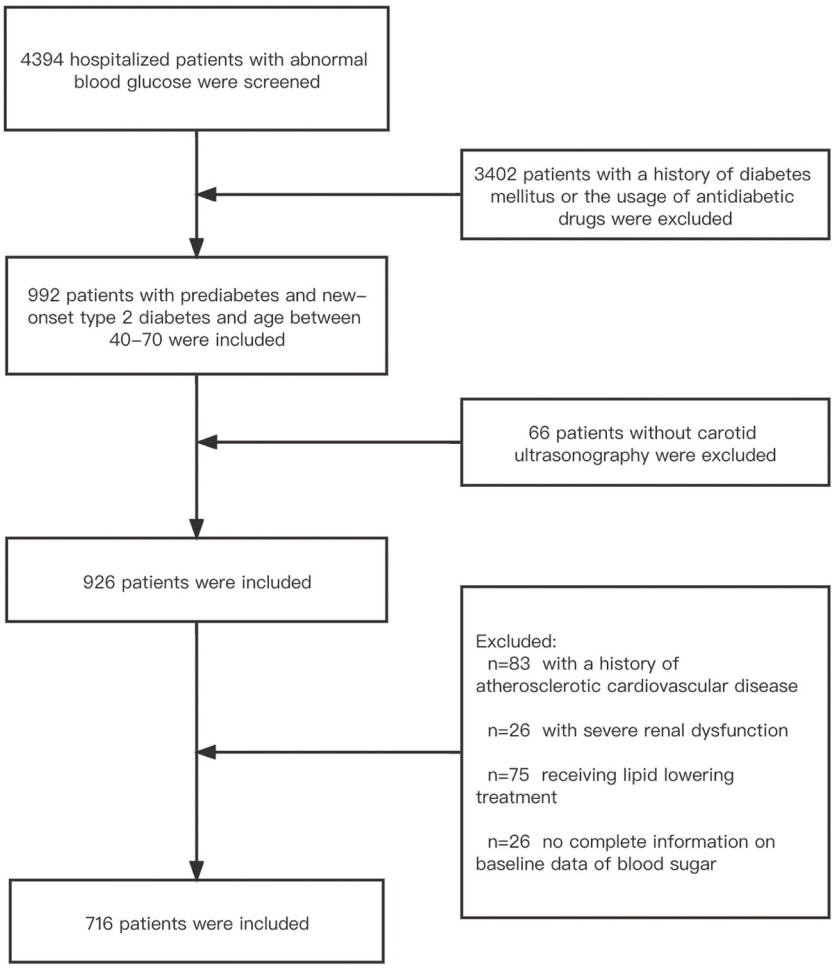

FIGURE 1 | Enrollment flowchart of the study population.

systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg on two different occasions or a history of antihypertensive therapy.

Venous blood samples were collected from the subjects after an overnight fasting for the analysis of total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), FPG, HbA1c, and high-sensitivity C-reactive protein (hs-CRP). We calculated the TyG index using the equation log [(fasting TG (mg/dl) $\times$ FPG (mg/dl)/2] (17).

Assessment of Carotid Plaques

Carotid ultrasonography was performed by trained and experienced sonographers using a color ultrasound diagnostic apparatus equipped with a linear array transducer. The common, bifurcation, external, and internal carotid arteries were examined bilaterally from the transverse and longitudinal orientations to evaluate the carotid intima-media thickness (IMT), the presence and morphology of atherosclerotic plaque, and the presence of carotid stenosis.

Carotid intimal thickening was defined as 1.0 $\leq$ IMT $\leq$ 1.5 mm, and atherosclerotic plaque was defined as a localized IMT $\geq$ 1.5 mm or a relative focal thickening of more than 50% of the IMT of the surrounding tissue. A finding of two or more plaques was defined as multiple plaques. According to morphology and echo characteristics, we divided the plaques into stable plaques or unstable plaques. Plaques with hyperechoic, homogeneous echoes and a smooth surface were defined as stable plaques. Hypoechoics plaques and those with mixed echoes or ulcerations were defined as unstable plaques (18). Carotid stenosis was defined as any degree of narrowing resulting from plaques. The carotid plaque burden of each subject was evaluated using a modified plaque scoring system (19). Briefly, for each subject, the unilateral carotid artery was divided into four regions: the common carotid artery region, carotid bulb and bifurcating region, internal carotid region, and external carotid region. Each region received a score from 1 to 3, with 1 being a single plaque, 2 being multiple plaques, and 3 being stenosis. A total score was obtained by adding the scores of the eight regions together, and then the carotid plaque burden could be assessed. The plaque score (PS) was classified into PS_1: low

TABLE 1 | Baseline characteristics of the subjects stratified according to TyG index.

	Overall ($n = 716$)	High TyG index group ($n = 358$)	Low TyG index group ($n = 358$)	*P*-value
Age (years)	55 (49–61)	54 (48–60)	56 (50–62)	0.002*
Men (*n*, %)	469 (65.50)	245 (68.44)	224 (62.57)	0.099
New-onset T2D (*n*, %)	559 (78.07)	335 (93.58)	224 (62.57)	<0.001*
BMI (kg/m^2)	24.70 (22.40–26.90)	25.30 (22.95–27.40)	24.29 (22.05–26.43)	<0.001*
Waist circumference (cm)	88.00 (82.00–94.00)	90.00 (84.00–95.88)	85.00 (80.00–92.75)	<0.001*
SBP (mmHg)	131.00 (119.25–144.00)	130.00 (119.00–143.00)	132.00 (119.75–145.00)	0.655
DBP (mmHg)	83.00 (75.00–90.00)	84.50 (77.00–90.00)	81.00 (74.00–89.00)	0.003*
Hypertension (*n*, %)	336 (46.93)	162 (45.25)	174 (48.60)	0.369
Current smoking (*n*, %)	309 (43.16)	163 (45.53)	146 (40.78)	0.200
Alcohol consumption (*n*, %)	267 (37.29)	134 (37.43)	133 (37.15)	0.938
Total cholesterol (mmol/L)	4.72 (4.01–5.52)	5.21 (4.38–6.10)	4.31 (3.69–4.93)	<0.001*
Triglyceride (mmol/L)	1.51 (1.12–2.25)	2.20 (1.68–2.97)	1.14 (0.94–1.44)	<0.001*
HDL cholesterol (mmol/L)	1.08 (0.92–1.30)	1.05 (0.91–1.24)	1.13 (0.97–1.34)	0.001*
LDL cholesterol (mmol/L)	3.06 (2.47–3.69)	3.44 (2.74–4.01)	2.78 (2.28–3.25)	<0.001*
Hs-CRP (mg/L)	1.56 (0.76–3.31)	1.88 (1.02–3.47)	1.26 (0.55–3.21)	<0.001*
Fasting blood glucose (mmol/L)	10.20 (7.34–13.22)	12.09 (9.60–14.52)	8.12 (6.21–10.66)	<0.001*
TyG index	7.84 (7.39–8.28)	8.28 (8.05–8.62)	7.39 (7.11–7.61)	<0.001*
Fasting C-peptide (pmol/L)	489.31 (345.41–646.16)	512.00 (369.10–666.41)	470.00(333.52–617.87)	0.047*
Fasting insulin (pmol/L)	35.01 (24.40–52.40)	36.41 (24.99–53.30)	34.00(23.93–51.02)	0.159
HbA1c (%)	10.30 (8.20–12.10)	10.80 (9.30–12.50)	9.50 (7.05–11.70)	<0.001*

*Data are presented as number (%) or median (P_{25}-P_{75}). TyG, triglyceride-glucose; T2D, type 2 diabetes; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; hs-CRP, high sensitivity C-reactive protein; HbA1c, hemoglobin. *indicates data with statistical significance ($P \leq 0.05$).*

plaque burden ($0 < PS < 3$, single plaque in no more than three regions or multiple plaques in no more than two regions); PS_2: moderate plaque burden ($3 \leq PS \leq 6$, multiple plaques in no more than three regions or carotid stenosis in no more than two regions); and PS_3: high plaque burden ($PS > 6$, multiple plaques in more than three regions or carotid stenosis in more than two regions). The carotid ultrasonography results were reviewed by two independent experienced sonographers blinded to the clinical data. Discrepancies were resolved by consensus.

Statistical Analysis

The subjects were divided into two groups based on their TyG index values (high or low), and the baseline clinical data and ultrasound findings of the two groups were compared. The Kolmogorov–Smirnov test was used to evaluate the normality of the distribution of continuous variables. Data are presented as medians (interquartile ranges) for continuous variables with a non-normal distribution and as percentages for categorical variables. Group differences were analyzed using the Wilcoxon rank sum test for continuous variables and the chi-square test for categorical variables. A chi-square test for trend was used to assess the trend of carotid plaque burden prevalence with a high TyG index. To assess the possible influencing factors of carotid plaque burden of the subjects, multiclassification logistic regression analysis was performed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). Finally, we developed a linear regression model to explore the correlation between the change in the TyG index and the carotid plaque burden after adjusting for sex, age, tobacco use, TGs, HDL-C, FPG, and HbA1c. A $P \leq 0.05$ was considered indicative of statistical significance. All statistical analyses were performed by using SPSS version 21.0 (SPSS Inc., Chicago, IL).

RESULTS

Characteristics of the Subjects

The demographic and baseline data are presented in **Table 1**. The median age of the subjects was 55 years (interquartile range: 49–61 years), and 469 (65.50%) subjects were male. A total of 559 (78.07%) subjects had new-onset T2D. Regarding arteriosclerosis risk factors among the participants, 46.93% had hypertension, 43.16% were current smokers, and 37.29% currently consumed alcohol.

The median value of the TyG index ($M = 7.84$) was used to divide the participants into high and low TyG index groups, and subgroup analyses of the data were performed. Subjects with a higher TyG index value were more likely to have higher BMI and waist circumference (25.30 [22.95–27.40] vs. 24.29 [22.05–26.43)] kg/m^2, $P < 0.001$ and 90.00 [84.00–95.88] vs. 85.00 [80.00–92.75] cm, $P < 0.001$, respectively) than subjects with a lower TyG index value. TC and TG values were higher in the high TyG index group than in the low TyG index group (5.21 [4.38–6.10] vs. 4.31 [3.69–4.93)] mmol/L, $P < 0.001$ and 2.20 [1.68–2.97] vs. 1.14 [0.94–1.44] mmol/L, $P < 0.001$, respectively). Accordingly, the high TyG index group exhibited a higher level of LDL-C (3.44 [2.74–4.01] vs. 2.78 [2.28–3.25)] mmol/L, $P < 0.001$) and a lower level of HDL-C (1.05 [0.91–1.24] vs. 1.13 [0.97–1.34] mmol/L, $P = 0.001$) than the low TyG index group. Moreover, FPG, HbA1c,

TABLE 2 | Degree of carotid arteriosclerosis of the subjects.

Degree of carotid arteriosclerosis	High TyG index group (n = 358)	Low TyG index group (n = 358)	P-value
CIMT (mm)	0.80 (0.70–0.90)	0.80 (0.70–0.90)	0.693
CIMT thickening (n, %)			0.150
No	313 (87.43)	325 (90.78)	
Yes	45 (12.57)	33 (9.22)	
Carotid plaque (n, %)			0.881
No	185 (51.68)	187 (52.23)	
Yes	173 (48.32)	171 (47.77)	
Number of plaques, n (%)			<0.001*
=0	185 (51.68)	187 (52.23)	
=1	79 (22.07)	119 (33.24)	
≥2	94 (26.26)	52 (14.53)	
Plaque stability (n, %)			0.001*
No plaque	185 (51.68)	187 (52.23)	
Stable plaque	30 (8.38)	59 (16.48)	
Unstable plaque	143 (39.94)	112 (31.28)	
Plaque thickness (mm)	2.10 (1.80–2.70)	2.10 (1.60–2.50)	0.219
Plaque score	2.00 (1.00–6.00)	2.00 (1.00–4.00)	<0.001*
Number of PS, n (%)			0.004*
PS_1	96 (96/219, 43.84)	123 (123/219, 56.16)	
PS_2	35 (35/80, 43.75)	45 (45/80, 56.25)	
PS_3	42 (42/45, 93.33)	3 (3/45, 6.67)	

*Data are presented as number (%) or median (P_{25}-P_{75}). CIMT, carotid intima-media thickness; TyG, triglyceride-glucose; PS_1, low plaque burden; PS_2, moderate plaque burden; PS_3, high plaque burden. *indicates data with statistical significance ($P \leq 0.05$).*

and fasting C-peptide levels were higher in the high TyG index group than in the low TyG index group (12.09 [9.60–14.52] vs. 8.12 [6.21–10.66] mmol/L, $P < 0.001$, 10.80 [9.30–12.50] vs. 9.50 [7.05–11.70] %, $P < 0.001$, and 512.00 [369.10–666.41] vs. 470.00 [333.52–617.87] pmol/L, $P < 0.05$, respectively). Finally, subjects in the high TyG index group had higher levels of hs-CRP than those in the low TyG index group (1.88 [1.02–3.47] vs. 1.26 [0.55–3.21] mg/L, $P < 0.001$).

Degree of Carotid Arteriosclerosis of the Subjects

The degree of carotid arteriosclerosis for the subjects is listed in **Table 2**. The present study found that there was no difference in carotid IMT measurements or the prevalence of IMT thickening and plaques between the high and low TyG index groups. However, the high TyG index group had a higher atherosclerotic plaque burden than the low TyG index group, with more patients with multiple plaques (26.26 vs. 14.53%, $P < 0.001$), more unstable plaques (39.94 vs. 31.28%, $P < 0.001$), and a higher PS (2.00 [1.00–6.00] vs. 2.00 [1.00–4.00], $P < 0.001$; **Figure 2**).

With an increasing PS, the proportion of subjects with a high TyG index increased significantly, reaching 93.33% in the PS_3 category (P for trend =0.004). On the other hand, the proportions of subjects with a high TyG index were similar between PS_1 and PS_2 (43.84 vs. 43.75%, $P > 0.001$, **Figure 2**).

Association of the TyG Index With Carotid Plaque Burden

Univariate logistic regression analysis was conducted with the possible influencing factors of carotid plaque burden as independent variables and PS as the dependent variable. Factors that were statistically significant in the univariate analysis were introduced into the unordered multiclassification logistic regression model, and the analysis results are shown in **Table 3**. Compared with subjects without a carotid plaque burden, those with a low plaque burden were more likely to be older, and the OR was 1.763 (95% CI, 1.238–2.511). Those with a moderate plaque burden were more likely to be older and current smokers and had a history of hypertension, with ORs of 4.960 (95% CI, 2.728–9.018), 1.896 (95% CI, 1.000–3.594), and 2.155 (95% CI, 1.267–3.666), respectively. Those with a high plaque burden were more likely to be older, had a history of hypertension, and had a high TyG index, with ORs of 3.183 (95% CI, 1.572–6.444), 2.477 (95% CI, 1.236–4.965), and 16.706 (95% CI, 3.988–69.978), respectively. The TyG index was positively associated with a high plaque burden (P =0.000), while no association between the TyG index and a low/moderate plaque burden was found in the study population.

Even when subjects were stratified by age (<55 or ≥55 years), sex (male or female), BMI (<25 or ≥25 kg/m^2), and hypertension (no or yes), the association between the TyG index and incident high plaque burden remained consistent (all P for interactions > 0.05, **Figure 3**). The ORs of incident high plaque burden tended to be higher in populations who were elderly, female, lean, or

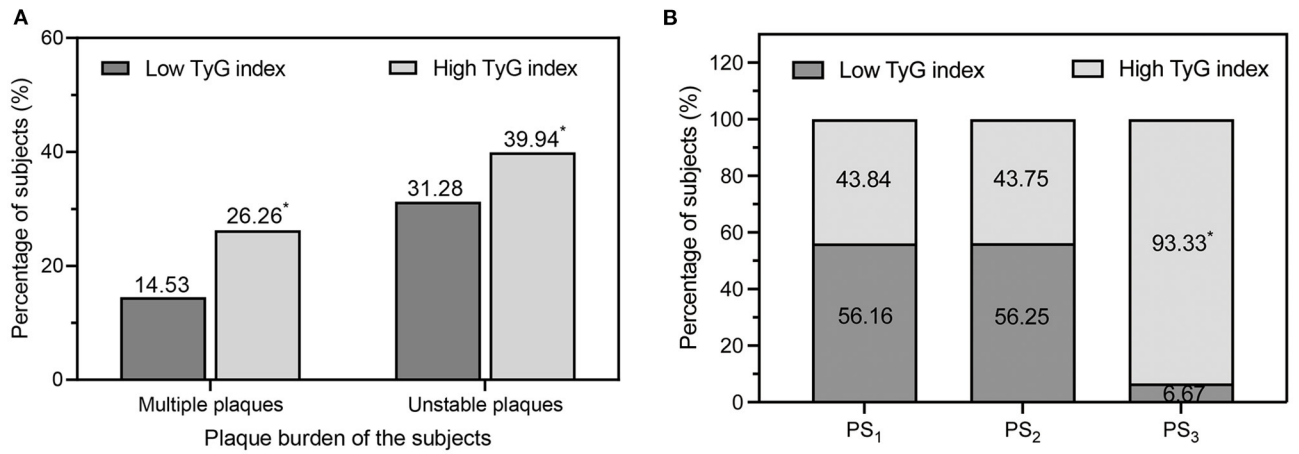

FIGURE 2 | (A) Comparison of the percentage of multiple plaques and unstable plaques in the low TyG index group and high TyG index group. **(B)** Percentages of low/high TyG index values in different categories of carotid plaque burden degree. PS_1, low plaque burden; PS_2, moderate plaque burden; PS_3, high plaque burden. PS, plaque score; TyG, triglyceride-glucose. *$P < 0.01$ vs. low TyG index.

hypertensive than in those who were younger, male, obese, or non-hypertensive (OR [95% CI] = 3.364 [1.814–6.237], 2.735 [0.984–7.599], 2.974 [1.699–5.209], and 2.653 [1.452–4.848], respectively, $P \leq 0.05$; **Figure 3**).

To further evaluate the extent to which the TyG index was associated with carotid plaque burden in our population, multiple linear regression analysis was performed. First, possible risk or protective parameters were included in the univariate analysis, and the results showed that sex, age, smoking, triglycerides, HDL cholesterol, fasting blood glucose, HbA1c, and the TyG index were meaningful. These parameters were then used to construct a regression model by using a multiple linear regression equation, and the results are displayed in **Table 4**. Sex, age, and the TyG index had a positive correlation with the carotid plaque burden ($b = 0.848$, $t = 2.139$, $P = 0.033$; $b = 0.077$, $t = 4.000$, $P < 0.001$; $b = 1.595$, $t = 2.748$, $P = 0.006$, respectively). TyG index showed the strongest association with the carotid plaque burden. For each unit increase in the TyG index value during this period, the plaque burden score increased 1.595-fold.

DISCUSSION

This cross-sectional study shows for the first time that a high TyG index value in subjects with prediabetes and new-onset T2D is significantly associated with an increased risk of high carotid plaque burden as assessed by ultrasound. The association remained consistent even when the subjects were stratified by age, sex, BMI, and hypertension. No association between the TyG index and a low/moderate plaque burden was found. Furthermore, we found that compared with other factors, the TyG index showed the strongest association with the carotid plaque burden. For each unit increase in the TyG index, the risk of a high carotid plaque burden increased 1.595-fold.

Prediabetes is an intermediate stage in which glucose tolerance progresses from normal to uncontrolled intolerance. As a multifactorial metabolic disorder, prediabetes has been confirmed to have a causal relationship with ASCVD (20, 21). In addition, new-onset diabetes negatively affects the morbidity and mortality rates of ASCVD to the same extent as diabetes (22). Studies have shown that arteriosclerosis is more pronounced in patients with prediabetes and new-onset diabetes than in patients with normal glucose tolerance (23). Therefore, early personalized management of subjects with prediabetes and new-onset diabetes is an important issue. However, few studies about the arteriosclerosis in these populations are available, which may hinder the identification of who should be fast-tracked into a prevention programmer during the early stage of diabetes (24). In the present study, 157 prediabetes patients and 559 new-onset diabetes inpatients were selected as research subjects for the evaluation of possible biological indicators for the detection of arteriosclerosis. Most of these people found abnormal blood glucose accidentally in the health check-up or other physical discomfort. Before the diagnosis of prediabetes or new-onset diabetes was made, none of them had ever taken any antidiabetic treatments. We united prediabetes and new-onset T2D in one group because they may represent the early stage of diabetes. The recognition of an economical and practical marker for the plaque burden in this population could help these patients receive the greatest benefit from early intervention.

In previous studies, one of the difficulties in identifying the risk factors involved in arteriosclerosis progression was the quantification of arteriosclerotic severity. In the present study, carotid plaque burden was used as a marker of atherosclerosis, which manifested as the change in the plaque score obtained from different carotid districts. As reported in the literature, the prevalence of carotid artery plaque shows a good correlation with the presence of atherosclerosis and can well reflect the overall severity of atherosclerosis in the vasculature (25, 26). The association between the TyG index and arteriosclerosis has been demonstrated in some previous studies. Guo et al. reported that the TyG index was independently associated with the arterial stiffness of peripheral arteries evaluated by

TABLE 3 | Multiclassification logistic regression analysis.

	Carotid plaque burden								
	PS_1			PS_2			PS_3		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Age (Ref < 55)	1.763	1.238–2.511	0.002*	4.960	2.728–9.018	0.000*	3.183	1.572–6.444	0.001*
Smoking (Ref no)	1.073	0.696–1.654	0.749	1.896	1.000–3.594	0.050*	1.900	0.844–4.276	0.121
Hypertension (Ref no)	1.402	0.986–1.996	0.060	2.155	1.267–3.666	0.005*	2.477	1.236–4.965	0.011*
TyG index (Ref < 7.84)	0.899	0.534–1.513	0.689	0.782	0.361–1.693	0.536	16.706	3.988–69.978	0.000*

*Multiclassification logistic regression model was used to estimate Odds ratio (OR) and 95% confidence interval (CI). Dependent variable: carotid plaque burden. PS_1, low plaque burden; PS_2, moderate plaque burden; PS_3, high plaque burden. Model was adjusted for sex, age, smoking, hypertension, triglyceride, and fasting blood glucose. PS, plaque score; Ref, reference; TyG, triglyceride-glucose. *indicates data with statistical significance ($P \leq 0.05$).*

brachial-ankle pulse wave velocity (27). Research has also shown that the TyG index, as a surrogate for IR, is a marker of the presence of non-calcified or mixed plaques in coronary arteries in asymptomatic individuals (28). Other studies support that a higher TyG index value is associated with the severity of coronary artery stenosis in not only patients with acute ST-elevated myocardial infarction but also asymptomatic patients with T2D (26, 29). However, their focus of these studies falls outside the areas of carotid arteries. The results from a Spanish study that included 191 participants showed that IR assessed by the TyG index was independently associated with preclinical carotid atherosclerosis in type 1 diabetes subjects (30). In addition, the TG-to-HDL ratio, another novel and simple indicator of IR, can evaluate atherosclerotic extension in prediabetic subjects and may be useful for identifying subjects at high cardiovascular risk. Nevertheless, this study did not further investigate the possible association between IR and atherosclerosis extension (31). In addition, the aforementioned studies did not evaluate the role of the TyG index in assessing the carotid plaque burden among subjects with prediabetes and new-onset T2D. In our population, the differences in carotid IMT measurements and the prevalence of IMT thickening, which are subclinical indicators of arteriosclerosis (32), were no significant between the high and low TyG index groups. Also, the percentages of subjects suffering carotid plaque between high and low TyG index group were similar. However, in the population with carotid plaques, more patients with multiple plaques and more unstable plaques were found in the high TyG index group than in the low TyG index group, indicating that the TyG index may be a more specific indicator of arteriosclerotic plaque burden in subjects with prediabetes and new-onset T2D.

In the present study, we observed that advanced age is a risk factor for different degrees of carotid arteriosclerosis. Compared with no plaque burden, the risk was increased by 1.763 times in the low plaque burden group, 4.960 times in the moderate plaque burden group, and 3.183 times in the high plaque burden group, which was consistent with previous reports, emphasizing the importance of age as a clinical risk factor for ASCVD events (33). Considering smoking as a risk factor, the association between smoking and plaque burden was not substantial, with only a weak correlation observed between smoking and moderate plaque burden in the carotid artery ($P = 0.05$). This could be explained by the possibility that plaque erosion, but not plaque burden, is the intermediate between smoking and ASCVD (34). Hypertension was found to be correlated with moderate and high plaque burden, with the risks of prevalence increasing to 2.155-fold for patients with a moderate plaque burden and 2.477-fold for those with a high plaque burden. Hypertension plays a significant role in the development of atherosclerosis. For those with uncontrolled hypertension, standard risk management programs have been effective (35), which implies that early consideration of hypertension-induced systematic chronic damage earlier could improve the current standard clinical management. The TyG index was positively associated with a high plaque burden, and no association was found with a low/moderate plaque burden in our study population. In the subjects with a high TyG index value, the prevalence of a high plaque burden was increased between 3.988- and 69.978-fold compared with that of no plaque burden, indicating that this index may be a specific indicator of a high plaque burden in asymptomatic subjects with prediabetes and new-onset T2D. We subsequently analyzed the extent to which the TyG index was associated with carotid arteriosclerosis. For each one-unit increase in the TyG index, the plaque burden score increased by 1.595-fold, which further confirmed that the TyG index may serve as a low-cost and simple indicator in evaluating the prevalence of carotid plaque burden for patients with prediabetes and new-onset T2D.

In our subgroup analysis, we found that the positive relationship between the TyG index and high plaque burden was consistent across all subgroup variables and seemed to be more evident in individuals who were elderly, female, lean or hypertensive. Age, sex, BMI, and blood pressure are well-known traditional risk factors that affecting arteriosclerosis. Interestingly, in our BMI stratified subgroup, non-obese subjects showed a higher risk of TyG-related plaque burden than obese subjects. Similar results were found in some other studies. An investigation conducted in 4,285 Korean non-obese adults over 12 years showed that a higher TyG index value significantly predicted T2D among community-dwelling lean people (36). In addition, Zhang et al. revealed that even for non-obese individuals, the TyG index can predict the risk of diabetes (37). Unlike the prevalence of obesity-related diabetes in Western countries, the proportion of non-obese subjects with diabetes is

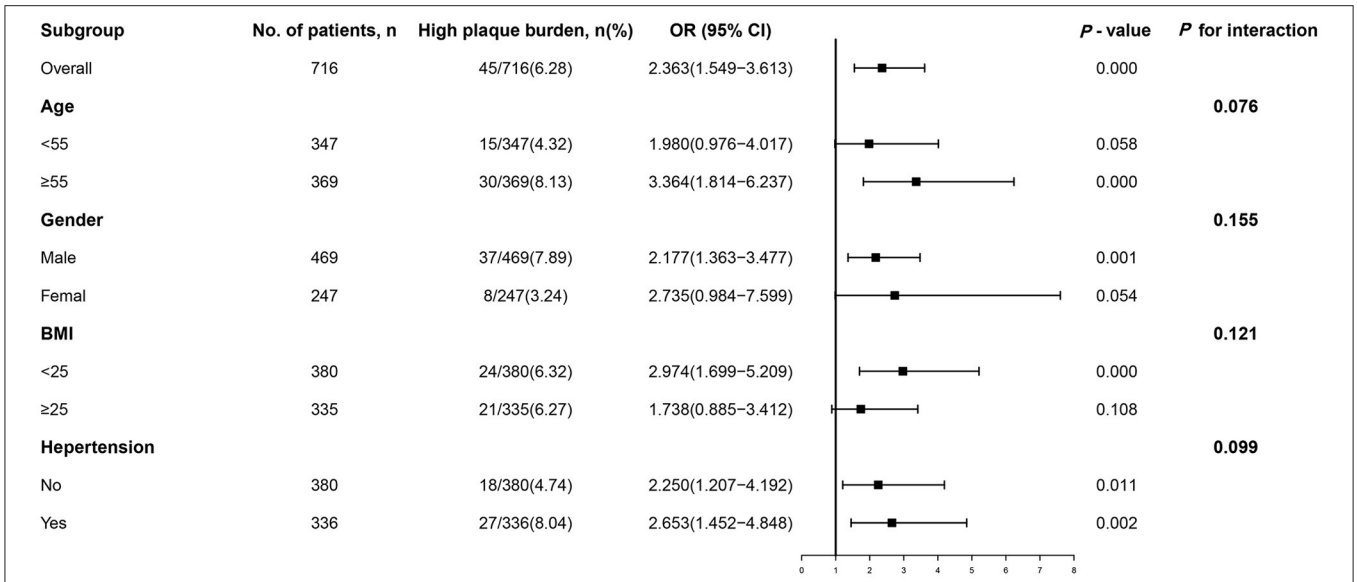

FIGURE 3 | Subgroup analysis of the association between the TyG index and high plaque burden. Logistic regression after adjustment for triglycerides and fasting blood glucose was performed in subgroups according to age, sex, BMI, hypertension, smoking, and alcohol consumption. OR, odds ratio; CI, confidence interval; BMI, body mass index; TyG, triglyceride-glucose.

TABLE 4 | Multiple linear regression analysis.

	B	SE	T	95% CI	*P-value*
Sex	0.848	0.397	2.139	0.068 to 1.628	0.033*
Age	0.077	0.019	4.000	0.039 to 0.116	0.000*
Smoking	0.258	0.359	0.720	−0.448 to 0.964	0.472
Triglyceride (mmol/L)	−0.045	0.243	−0.184	−0.524 to 0.434	0.854
HDL cholesterol (mmol/L)	−0.416	0.490	−0.848	−1.380 to 0.549	0.397
Fasting blood glucose (mmol/L)	−0.606	0.072	−0.834	−0.201 to 0.081	0.405
HbA1c (%)	0.015	0.078	0.194	−0.138 to 0.168	0.847
TyG index	1.595	0.580	2.748	0.453 to 2.737	0.006*

*Dependent variable: carotid plaque burden. B, linear regression coefficient; SE, standard error; CI, confidence interval; Ref, reference; HDL, high density lipoprotein; TyG, triglyceride-glucose. *indicates data with statistical significance (P ≤ 0.05).*

higher in Asian populations, which may be due to factors related to ethnic and lifestyle differences (36). Insulin sensitivity may also be affected by adipose tissue. The lack of subcutaneous fat makes lean people more susceptible to hypertriglyceridemia, which may lead to dysfunctional β-cell dysfunction and IR (38). As a result, lean individuals may be more sensitive to an increased TyG index value (39).

Although the potential mechanism of the relationship between the TyG index and carotid plaque burden is unclear, it may be relevant to IR. An increasing TG level may lead to the elevation of free fatty acids and more extravasation of free fatty acids from fat to non-fat tissue, which may result in IR (13). Furthermore, the insulin sensitivity of skeletal muscle decreases as FPG increases (6, 40, 41). Therefore, the combination of TG and FPG has a high sensitivity for diagnosing IR. As IR promotes endothelial dysfunction, increases the proinflammatory state, and leads to the release of reactive oxygen species, it plays a critical role in the promotion of atherosclerosis progression and plaque formation (42–44).

LIMITATIONS

Several limitations in the current study should be considered. First, this study is a cross-sectional observational study. Although many confounding factors were adjusted, there may be some potential selection bias. Further prospective cohort studies and the inclusion of a control group may provide more precise evidence in future research. Second, the result of 2-h oral glucose tolerance test was not considered in the diagnosis of prediabetes or new-onset T2D, which might limit the sample size that included. Third, the source of the study subjects was a population of asymptomatic, but not healthy, hospitalized patients. Nonetheless, patients with underlying disease and those who received blood glucose-lowering and lipid-lowering therapy

were excluded to minimize the effects of the interfering factors. Finally, the study was a single-center study, and multicenter studies may be needed. However, a positive correlation was observed between the TyG index and carotid plaque burden, suggesting that the TyG index may be used as a tool to assess carotid plaque burden in asymptomatic prediabetes and new-onset T2D subjects.

CONCLUSIONS

In conclusion, a high TyG index value was positively associated with a high carotid plaque burden in subjects with prediabetes and new-onset T2D. Clinicians should pay close attention to the TyG index in these populations, as it could help such patients receive the greatest benefit from early intervention.

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 Academic Ethics Committee of Shaoxing People's Hospital. Written informed consent for participation was not required for this study in accordance with the National Legislation and the Institutional Requirements.

AUTHOR CONTRIBUTIONS

Z-zJ was responsible for conceptualization, investigation, and writing the original draft. J-bZ, H-lS, S-sZ, S-qT, and Y-yT contributed to data interpretation and data collection. T-aJ and X-tL designed the study and contributed to critically revising the manuscript. All authors have read and approved the final manuscript.

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Negative Risk Markers for Cardiovascular Risk Evaluation in Chinese Adults

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Background: The atherosclerotic cardiovascular disease (ASCVD) risk predicted by traditional risk factors is used to guide preventive treatment. We aimed to investigate whether preferable levels of non-traditional emerging risk factors (i.e., negative risk markers) could downgrade the predicted ASCVD risk beyond traditional risk factors.

Methods: A total of 7,568 Chinese adults aged ≥ 40 years were followed up during 2010–2015. Negative risk markers including non-traditional lipids, urinary albumin-to-creatinine ratio, electrocardiogram (ECG), and measurements of atherosclerosis were evaluated using diagnostic likelihood ratio (DLR) and continuous net reclassification index (NRI) for their ability to downshift predicted CVD risk in the overall study population and in participants with intermediate (traditional risk factor predicted ASCVD risk 7.5% to 19.9%) or high risk (≥20%).

Results: During a median follow-up of 4.5 years, 416 participants developed CVD events including non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death. Among negative risk markers examined, lipoprotein(a) ≤10th percentile (5 mg/dL), normal ECG, and carotid intima-media thickness (CIMT) ≤25th percentile (0.5 mm) provided moderate CVD risk reclassification and downward changes in pre- to post-test risk on top of the traditional CVD risk factors, especially in high-risk participants. The DLRs were 0.41, 0.75, and 0.41, and the NRIs were 18, 22, and 14% for lipoprotein(a), ECG, and CIMT, respectively in high-risk participants.

Conclusions: Lipoprotein(a) ≤5 mg/dL, normal ECG, and CIMT ≤0.5 mm might be used as negative non-traditional risk markers to correctly downgrade predicted ASCVD risk in Chinese adults.

Keywords: cardiovascular disease, negative risk marker, lipoprotein(a), electrocardiogram, carotid intima-media thickness

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INTRODUCTION

Cardiovascular disease (CVD) is a major health challenge for the modern society. The total prevalent cases of CVD are ∼523 million globally in 2019 and 18.6 million people die of CVD each year (1). The heavy burden of CVD is attributable to the increasing prevalence of hypertension, diabetes, dyslipidemia, and chronic kidney disease, et al. (1). Risk assessment tools for CVD development are important in predicting future CVD events for early intervention (2, 3). A well-known example is the Pooled Cohort Equations (PCEs) for 10-year atherosclerotic CVD (ASCVD) risk calculated using traditional CVD risk factors recommended by the American College of Cardiology/American Heart Association (ACC/AHA) (2). Preventive medications such as statins are recommended in people with elevated ASCVD risk predicted using the PCEs (4, 5). However, the absence of non-traditional CVD risk factors often predicted a low risk of CVD, regardless of PCE score (6). A previous study found that a coronary artery calcium (CAC) score of 0 was associated with a reduced CVD risk and thus defined CAC = 0 as a negative risk marker (7). For individuals at intermediate ASCVD risk estimated by the PCE score (7.5–19.9%) and without coronary artery calcium, statin therapy may be withheld or delayed (8). Therefore, negative risk markers can be used to reduce overtreatment.

China has the largest number of people suffering from CVD worldwide (1). Because the PCEs were developed in US cohorts, it may overestimate ASCVD risk in Chinese adults (9). Chinese adults might receive preventive treatment based on an overestimated risk by PCEs. Therefore, it is demanded to identify individuals with high-predicted risk but low-observed risk in Chinese adults. Negative risk markers have shown significant potentials in downgrading the predicted 10-year ASCVD risk, raising the question whether there are other negative risk markers to help identify the true "low risk" population in addition to a CAC=0. Therefore, in the current study, we aimed to examine the ability of several potential negative risk markers to downgrade the predicted 10-year ASCVD risk in Chinese adults.

METHOD

Study Population

A total of 10,375 community residents aged 40 years or older were recruited from Jia Ding District, Shanghai, China between March and August 2010. During August 2014 and May 2015, participants were invited to take part in a follow-up examination for the development of CVD events including myocardial infarction (MI), stroke, and cardiovascular death. The study design and data collection procedures were described previously (10, 11). We excluded 306 participants with a history of CVD, 147 participants aged 80 years and older, 756 participants with missing data on negative risk markers at baseline and 1,580 participants with missing data on CVD outcomes at follow-up. Therefore, 7,586 participants were included in the current analysis.

The study protocol was approved by the Institutional Review Board of the Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine. Written informed consent was obtained from each participant before data collection.

Baseline Examination

Baseline characteristics including sociodemographic variables, history of chronic diseases and medications, and lifestyle habits, et al. were evaluated using a standard questionnaire administered during a face-to-face interview. Blood pressure was measured on the non-dominant arm of each participant after at least 10 min of sitting rest using an automated device (OMRON Model HEM- 752 FUZZY; Omron Co., Dalian, China) three times consecutively with 1-min intervals, and the mean of the three measurements was used for analysis.

Venous blood samples were drawn in the morning from each participant after at least a 10-h overnight fast. An oral glucose tolerance test was conducted in participants without diabetes history and 2-h post-load blood samples were collected. Fasting and post-load blood glucose levels were measured on an autoanalyzer (Modular P800; Roche, Basel, Switzerland) using the glucose oxidation method. Diabetes was defined as a fasting blood glucose level ≥126 mg/dL and/or a post-load glucose level ≥200 mg/dL and/or taking any glucose-lowering medications. Concentrations of fasting serum total cholesterol, high-density lipoprotein cholesterol (HDL-c), lipoprotein(a), apoB, apoA-I were measured on an autoanalyzer (Modular E170; Roche) using the chemiluminescence method. More details on lipid measurements are described previously (12).

The first morning spot urine samples were obtained from each participant. The urinary albumin levels were measured by the immunoturbidimetric method (Beijing Atom High-Tech, Beijing, China) and urinary creatinine levels were measured by the Jaffe's kinetic method on an automatic analyzer (Hitachi 7600-020, Tokyo, Japan). Urinary albumin-to-creatinine ratio (UACR) (mg/g) was calculated as the urinary albumin concentration divided by the urinary creatinine concentration.

Electrocardiograms (ECG) were recorded according to a standard protocol with participants in the supine position using a 12-lead ECG machine (CAM14, GE, US). Computer-assigned Minnesota Code (MC) to electrocardiographs of each participant was used for categorization. Carotid intima-media thickness (CIMT) measurements were carried out by an experienced sonographer using a high-resolution B-mode tomographic ultrasound system (Esaote Biomedica SpA, Italy) with a linear 7.5-MHz transducer. The CIMT was measured on the far wall of the right and left common carotid arteries, 1.5 cm proximal to the bifurcation. The distance from the leading edge of the first echogenic line to that of the second echogenic line at the end of diastole was calculated as the CIMT of either side. The brachial-ankle pulse wave velocity (baPWV) and the ankle-brachial index (ABI) were measured using a Colin VP-1000 device (Model BP203RPE II, form PWV/ABI, China) after 10 min of rest. After placing the cuffs on the right and left upper arms and the right and left ankles, pulse waves were obtained simultaneously. The time delay in obtaining pulse waves and the distance of the right and left upper arms to the right and left ankles were included in the calculation of the right and left baPWVs. ABI was calculated as the ratio of ankle systolic BP to

arm systolic BP. The larger CIMT or baPWV at either side was used for analysis.

Negative Risk Markers

Negative risk markers were chosen based on previous evidence of significant associations with CVD risks. Several negative risk markers were examined in the current study, including non-traditional lipids, UACR, ECG, and measurements of atherosclerosis. For risk markers that have definite clinical cutoff points such as UACR, ECG, and ABI, we used these cutoffs to define "normal" or "negative." For example, UACR <30 mg/g is regarded as normal according to the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline (13). In addition, because previous studies indicate that the risk extends below this level, we also categorized UACR as "optimal" values below 10 mg/g (14). For risk markers that do not have definite clinical cutoff points such as non-traditional lipids, PWV, and CIMT, we used the lowest or highest quartile to indicate an optimal level. In addition, for lipids, we also used the lowest or highest 10 percentile to indicate a more stringent optimal level. Specifically, they were: (1) lipoprotein(a) ≤25th percentile (9 mg/dL) or ≤10th percentile (5 mg/dL), (2) apoB ≤25th percentile (0.81 g/L) or ≤10th percentile (0.69 g/L), (3) apoA-I ≥75th percentile (1.43 g/L) or ≥90th percentile (1.60 g/L), (4) UACR < 30 mg/g or < 10 mg/g, (5) normal ECG, (6) normal corrected QT interval (men: 390–450 ms, women: 390–460 ms), (7) CIMT ≤25th percentile (0.5 mm), (8) baPWV ≤ 25th percentile (1,350 cm/s), and (9) normal ABI (≥0.90 and ≤1.30) at both sides.

CVD Outcomes

The development of CVD events including non-fatal MI, non-fatal stroke, and cardiovascular death was recorded by self-report from participants or collected from the national insurance system and local death registries. Photocopies of the participant's inpatient record, discharge summary, electrocardiogram, and imaging reports were obtained. Myocardial infarction was defined by changes in levels of troponin T and Creatine-Kinase-MB isoform, symptoms of myocardial ischemia, changes in ECG results, et al. Stroke was defined by a fixed neurological deficit lasting >24 h because of a presumed vascular cause. Cardiovascular death was defined as death due to cardiovascular causes. Two physicians independently reviewed medical charts and verified each CVD event with discrepancies resolved through discussion (15).

Statistical Analysis

Baseline characteristics of the overall study population and participants who did or did not develop CVD events are described with continuous variables in means ± standard deviations (SD) or medians (25–75% quartile) and categorical variables in numbers (percentages). Differences between participants with and without incident CVD events were compared using student's *t*-test for continuous variables and chi-square test for categorical variables. Numbers (percentages) of CVD events in participants with and without each individual negative risk markers were calculated and compared using chi-square test. Cox regression models were used to assess the associations (hazard ratios [HRs] and 95% confidence interval [CI]) of negative risk markers with the development of CVD in the overall study population as well as in individuals with intermediate ASCVD risk (10-year predicted ASCVD risk of 7.5 to 19.9%) or high ASCVD risk (10-year predicted ASCVD risk ≥ 20%) calculated using the PCEs. Model 1 was adjusted for age and sex. Model 2 was further adjusted for other traditional CVD risk factors including current smoking, diabetes, total cholesterol, HDL-c, systolic blood pressure, and antihypertensive drug treatment.

The continuous net reclassification index (NRI) was used to measure the ability of each negative risk markers to improve the CVD risk classification beyond traditional risk factors. To do this, we established a basic logistic regression model that included traditional risk factors. The predicted CVD risks by the basic model with and without negative risk markers were compared. Because the negative risk markers are able to downgrade risk, the positive NRIs will be driven by improvements in specificity (indicating less medication-overuse).

Finally, we calculated the diagnostic likelihood ratio (DLR), which measures the impact of negative risk markers by comparing pre-test and post-test risks. We constructed logistic regression models including baseline predictors X (e.g., traditional CVD risk factors) to predict pre-test CVD risk, and logistic regression models including predictors X and predictor Y (e.g., individual negative risk markers) to predict post-test CVD risk. The multivariable-adjusted DLR is then calculated by subtracting the pre-test risk model from the post-test risk model. More details about the DLR calculation have been reported in the previous literature (7). A DLR > 1 indicates that post-test risk is higher than pre-test risk (i.e., test risk markers have no effect on risk reduction), whereas a DLR < 1 indicates that post-test risk is lower than pre-test risk (i.e., test risk markers may have effect on risk reduction).

All statistical analyses were performed using R 4.0.2 (http://www.r-project.org/) and SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). All analyses were two-sided and a *p-value* of <0.05 was considered statistically significant.

RESULTS

General Characteristics

A total of 416 participants developed CVD events during a median of 4.5 years of follow-up. **Table 1** shows the baseline characteristics of the study participants. Overall, the average age was 57.9 ± 9.0 years and 37.8% were men. Approximately 23.7% of the participants were current smokers, 17.7% had diabetes, and the median (interquartile range) estimated 10-year ASCVD risk was 6.7% (2.7 to 15.1%). Generally, levels of systolic blood pressure, lipoprotein(a), baPWV, CIMT, QTc interval, and UACR, and proportions of participants with diabetes, or abnormal ECG increased significantly in participants who developed CVD events compared with those who did not (all *p-value*s <0.05).

TABLE 1 | Characteristics of the study population at baseline.

Characteristics	Total	Incident CVD events		
	(*n* = 7,586)	Without (*n* = 7,170)	With (*n* = 416)	*p-value*
Age, years	57.9 ± 9.0	57.6 ± 8.9	63.0 ± 9.2	<0.001
Men, *n* (%)	2,867 (37.8)	2,697 (37.6)	170 (40.9)	0.202
Current smoker, *n* (%)	1,800 (23.7)	1,695 (2,367)	105 (25.2)	0.492
Type 2 diabetes, *n* (%)	1,345 (17.7)	1,218 (17.0)	127 (30.5)	<0.001
Systolic blood pressure, mmHg	141.0 ± 19.9	140.5 ± 19.7	148.9 ± 21.3	<0.001
Total cholesterol, mg/dL	206.4 ± 39.0	206.2 ± 39.0	209.9 ± 39.1	0.062
HDL cholesterol, mg/dL	51.4 ± 12.4	51.4 ± 12.5	51.6 ± 11.8	0.866
Use of antihypertensive drugs, *n* (%)	2,059 (27.1)	1,888(26.3)	171(41.1)	<0.001
10-year ASCVD risk *	6.7 (2.7, 15.1)	6.5 (2.6, 14.4)	14.5 (5.1, 27.2)	<0.001
Lp(a), mg/dL	20.9 ± 15.5	20.8 ± 15.5	22.6 ± 15.5	0.024
apoB, mg/dL	0.97 ± 0.24	0.97 ± 0.24	0.99 ± 0.24	0.087
apoA-I, mg/dL	1.26 ± 0.28	1.25 ± 0.28	1.26 ± 0.27	0.549
UACR, mg/g	4.87 (2.77, 9.06)	4.81 (2.77, 8.89)	6.00 (2.89, 13.14)	<0.001
Normal ECG, *n* (%)	3,063 (40.4)	2,930 (40.9)	133 (32.0)	<0.001
QTc interval, ms	433.2 ± 32.5	432.8 ± 32.4	439.4 ± 34.0	<0.001
baPWV, cm/s	1,599.4 ± 356.7	1,589.6 ± 349.6	1,766.8 ± 429.4	<0.001
CIMT, mm	0.58 ± 0.11	0.58 ± 0.11	0.63 ± 0.11	<0.001
Normal ABI, *n* (%)	6,898 (90.9)	6,520 (90.9)	378 (90.9)	1.000

Data are means ± standard deviations, numbers (percentages), or medians (interquartile ranges).
**Estimated using the PCEs.*
CVD, cardiovascular diseases; HDL, high-density lipoprotein; Lp (a), lipoprotein(a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; UACR, urinary albumin-to-creatinine ratio; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; ABI, ankle-brachial index; ECG, electrocardiogram; QTc interval, corrected QT interval; ASCVD, atherosclerotic cardiovascular disease; PCEs, Pooled Cohort Equations.

Negative Risk Markers in Association With CVD Risk

Most participants had normal (93.55%) or low UACR (77.51%), or normal ABI (90.93%). Less than half participants had other negative risk markers (**Table 2**). The development of incident CVD events was the lowest in participants with CIMT ≤25th percentile (3.14%), followed by that in participants with baPWV ≤25th percentile (3.72%). The development of incident CVD events were significantly different in participants with *vs.* without lipoprotein(a) ≤10th percentile, UACR < 30 mg/g or <10 mg/g, normal ECG, normal corrected QT interval, or CIMT ≤ 25th percentile.

Table 3 shows the associations of negative risk markers with CVD risks. In the overall study population, lipoprotein(a) ≤10th percentile, UACR <30 mg/g or <10 mg/g, normal QTc, and CIMT ≤25th percentile were all associated with a significantly reduced risk of CVD events after adjustment for traditional CVD risk factors. They were also associated with a significantly reduced CVD risk in participants with high risk (10-year predicted ASCVD risk ≥20%). In addition, lipoprotein(a) ≤25th percentile and normal ECG also demonstrated associations with a significantly reduced CVD risk in participants with high risk. None of the negative risk markers were associated with reduced CVD risks in participants with intermediate risk.

CVD Risk Reclassification

We evaluated CVD risk reclassifications by negative risk markers in addition to traditional ASCVD risk factors in **Table 4**. Moderate and significant risk reclassifications were observed in the overall study population for negative risk markers such as UACR <10 mg/g, normal ECG, normal QTc, and CIMT ≤25th percentile (continuous NRI from 0.18 to 0.25). In addition to these negative risk markers, lipoprotein(a) ≤25th percentile or ≤ 10th percentile, UACR < 30 mg/g, normal ECG, normal QTc and CIMT ≤25th percentile also demonstrated moderate risk reclassifications in participants with high predicted ASCVD risk (continuous NRI from 0.14 to 0.24). Only urinary ACR <10 mg/g had significant CVD risk reclassifications in participants with moderate predicted ASCVD risk.

As shown in **Table 5**, lipoprotein(a) ≤10th percentile and CIMT ≤25th percentile provided the greatest downward change in pre-test to post-test risk, both with a mean multivariable-adjusted DLR of 0.41 in participants with high predicted ASCVD risk. This equates to a 59% reduction in relative risk compared with that expected from traditional risk factors. Among participants at high risk, lipoprotein(a) ≤25th percentile (DLR 0.67) and normal ECG (DLR 0.75) also provided significant downward changes in pre-test to post-test risk. In participants at intermediate risk, lipoprotein(a) ≤10th (DLR 0.78) also

TABLE 2 | The development of CVD events in participants with and without negative risk markers.

Negative risk markers	Number of participants (%)	Incident CVD events, *n* (%)		
		In participants with negative risk markers	In participants without negative risk markers	*p-value*[a]
Non-traditional lipids				
Lp(a) ≤ 25th percentile (9 mg/dL)	2,074 (27.34)	98 (4.73)	318 (5.77)	0.187
Lp(a) ≤ 10th percentile (5 mg/dL)	923 (12.17)	35 (3.80)	381 (5.72)	0.060
apoB ≤ 25th percentile (0.81 g/L)	1,991 (26.25)	96 (4.82)	320 (5.72)	0.340
apoB ≤ 10th percentile (0.69 g/L)	819 (10.80)	43 (5.25)	373 (5.51)	0.933
apoA-I ≥ 75th percentile (1.43 g/L)	1,915 (25.24)	110 (5.74)	306 (5.40)	0.760
apoA-I ≥ 90th percentile (1.60 g/L)	784 (10.33)	47 (5.99)	369 (5.42)	0.886
Urinary ACR				
<30 mg/g	7,097 (93.55)	364 (5.14)	52 (10.63)	<0.001
<10 mg/g	5,880 (77.51)	283 (4.81)	133 (7.80)	<0.001
Electrocardiogram				
Normal ECG	3,063 (40.38)	133 (4.34)	283 (6.26)	0.018
Normal QTc (men: 390–450 ms, women: 390–460 ms)	6,087 (80.23)	293 (4.81)	123 (7.41)	<0.001
Measurements of atherosclerosis				
baPWV ≤ 25th percentile (1,350 cm/s)	1,909 (25.16)	71 (3.72)	345 (6.08)	0.770
CIMT ≤ 25th percentile (0.5 mm)	2,991 (39.43)	94 (3.14)	322 (7.01)	<0.002
Normal ABI (0.9-1.3)	6,898 (90.93)	378 (5.48)	38 (5.52)	0.752

Data are numbers (percentages) unless otherwise indicated.

[a]*P-values were adjusted for age and sex.*

CVD, cardiovascular diseases; HDL, high-density lipoprotein; Lp (a), lipoprotein(a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; UACR, urinary albumin-to-creatinine ratio; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; ABI, ankle-brachial index; ECG, electrocardiogram; QTc interval, corrected QT interval; ASCVD, atherosclerotic cardiovascular disease; PCEs, Pooled Cohort Equations.

showed a significant downregulation of CVD risk, followed by apoA-I ≥75th percentile (DLR 0.80) and CIMT ≤25th percentile (DLR 0.87).

Finally, we did a sensitivity analysis using tertiles of PCE score in the study population to define intermediate (3.6 to 11.3%) and high (≥11.4%) risk groups. Results were largely unchanged (**Supplementary Tables 1**–**3**). In addition, we combined the 3 potential negative risk markers including lipoprotein(a) ≤5 mg/dL, normal ECG, and CIMT ≤0.5 mm and re-did the analysis using the number of negative risk markers (**Supplementary Tables 4**–**7**). When having ≥2 negative risk markers, the associations, post- vs. pre-test risks, and down-reclassifications of CVD risks were all improved significantly in both intermediate- and high-risk participants.

DISCUSSION

Using data from a large cohort of community adults aged ≥40 years followed up for a median of 4.5 years, we evaluated several non-traditional negative risk markers in associations with the development of incident CVD and in risk downgrade of CVD events. We found that lipoprotein(a) ≤10th percentile (5 mg/dL), normal ECG, normal QTc, and CIMT ≤25th percentile (0.5 mm) were significantly associated with a reduced CVD risk, had moderate CVD risk reclassification, and provided downward changes in pre- to post-test risk on top of the traditional CVD risk factors, especially in high-risk population. Therefore, CVD risk assessment should take into account both traditional and non-traditional risk factors and negative risk markers such as a low lipoprotein(a) level, normal ECG, and a low CIMT might be considered in adults categorized as high risk by traditional risk evaluation tools before making decisions of preventive treatment.

Lipoprotein(a) is a major oxidized phospholipids (OxPL) carrier in human circulation and mediates clearance of these proinflammatory factors. However, high levels of lipoprotein(a) with its OxPL load recirculating into the vasculature may turn into harm (16). Previous epidemiological studies have reported that elevated plasma lipoprotein(a) was an independent risk factor for cardiovascular disease (17, 18). In addition, studies have shown that lipoprotein(a) improved the reclassification of predicted CVD risk when added to the PCE score or traditional risk factors (18, 19). Therefore, the 2019 ACC/AHA guideline on the primary prevention of cardiovascular diseases defined a high level of lipoprotein(a) as one of the risk enhancers (20). Although previous studies have long focused on the association of high levels of lipoprotein(a) with increased cardiovascular risk, a recent study found that 1-SD genetically lowered lipoprotein(a) level was associated with a 29% lower

TABLE 3 | The associations of negative risk markers with CVD risk.

Negative risk markers	Total (n = 7,586)		Intermediate risk (n = 2,248)		High risk (n = 1,299)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Non-traditional lipids						
Lp(a) ≤ 25th percentile (9 mg/dL)	0.87 (0.70, 1.10)	0.82 (0.65, 1.03)	0.94 (0.63, 1.42)	0.89 (0.59, 1.34)	**0.62 (0.42, 0.91)**	**0.62 (0.42, 0.92)**
Lp(a) ≤ 10th percentile (5 mg/dL)	0.73 (0.51, 1.03)	**0.67 (0.47, 0.95)**	0.89 (0.50, 1.60)	0.80 (0.44, 1.43)	**0.41 (0.21, 0.80)**	**0.41 (0.21, 0.80)**
apoB ≤ 25th percentile (0.81 g/L)	0.87 (0.70, 1.10)	0.97 (0.74, 1.28)	1.15 (0.75, 1.77)	0.89 (0.53, 1.51)	0.68 (0.45, 1.03)	0.77 (0.48, 1.25)
apoB ≤ 10th percentile (0.69 g/L)	0.97 (0.71, 1.33)	1.12 (0.79, 1.58)	1.22 (0.68, 2.19)	0.98 (0.51, 1.90)	0.78 (0.42, 1.45)	0.90 (0.47, 1.74)
apoA-I ≥ 75th percentile (1.43 g/L)	0.95 (0.76, 1.18)	0.94 (0.72, 1.23)	0.83 (0.54, 1.28)	0.76 (0.45, 1.28)	1.25 (0.89, 1.76)	1.20 (0.79, 1.81)
apoA-I ≥ 90th percentile (1.60 g/L)	1.01 (0.74, 1.37)	1.02 (0.72, 1.46)	1.04 (0.58, 1.87)	1.02 (0.52, 2.00)	1.30 (0.80, 2.10)	1.16 (0.65, 2.07)
Urinary ACR						
<30 mg/g	**0.54 (0.41, 0.73)**	**0.64 (0.47, 0.86)**	0.64 (0.35, 1.18)	0.71 (0.38, 1.30)	**0.52 (0.35, 0.77)**	**0.55 (0.37, 0.82)**
<10 mg/g	**0.71 (0.57, 0.87)**	**0.80 (0.65, 0.99)**	0.70 (0.47, 1.06)	0.75 (0.50, 1.13)	0.74 (0.54, 1.03)	0.78 (0.56, 1.08)
Electrocardiogram						
Normal ECG	**0.80 (0.65, 0.98)**	0.85 (0.69, 1.05)	0.79 (0.53, 1.17)	0.84 (0.57, 1.26)	**0.67 (0.47, 0.96)**	**0.70 (0.49, 1.01)**
Normal QTc (men: 390–450 ms, women: 390–460 ms)	**0.65 (0.53, 0.81)**	**0.71 (0.57, 0.88)**	0.69 (0.46, 1.03)	0.74 (0.50, 1.11)	**0.61 (0.45, 0.84)**	**0.63 (0.46, 0.87)**
Measurements of atherosclerosis						
baPWV ≤ 25th percentile (1,350 cm/s)	1.03 (0.78, 1.36)	1.44 (1.07, 1.94)	1.04 (0.61, 1.77)	1.41 (0.80, 2.48)	/*	/*
CIMT ≤ 25th percentile (0.5 mm)	**0.67 (0.52, 0.86)**	**0.71 (0.55, 0.91)**	0.85 (0.55, 1.32)	0.84 (0.54, 1.30)	**0.38 (0.19, 0.78)**	**0.39 (0.19, 0.79)**
Normal ABI (0.9–1.3)	1.07 (0.77, 1.49)	1.09 (0.78, 1.52)	1.25 (0.63, 2.47)	1.26 (0.63, 2.48)	0.90 (0.57, 1.45)	0.94 (0.59, 1.50)

Data are hazard ratios (95% confidence intervals). The bold values indicate statistical significance.
Intermediate risk: 10-year ASCVD risk 7.5% to 19.9% by the PCEs; High risk: 10-year ASCVD risk ≥ 20% by the PCEs.
Model 1: adjusted for age and sex.
Model 2: further adjusted for smoking status, diabetes, total cholesterol, HDL-c, systolic blood pressure, antihypertensive drugs.
**Data cannot be shown due to a limited number of participants with high risk and baPWV ≤ 25th percentile (n = 45).*
CVD, cardiovascular diseases; HDL, high-density lipoprotein; Lp (a), lipoprotein(a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; UACR, urinary albumin-to-creatinine ratio; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; ABI, ankle-brachial index; ECG, electrocardiogram; QTc interval, corrected QT interval; ASCVD, atherosclerotic cardiovascular disease; PCEs, Pooled Cohort Equations.

TABLE 4 | Continuous NRI for CVD risk by adding each negative risk markers to a basic model with traditional CVD risk factors.

Negative risk markers	Total (n = 7,586)	Intermediate risk (n = 2,248)	High risk (n = 1,299)
Non-traditional lipids			
Lp(a) ≤ 25th percentile (9 mg/dL)	**0.08 (0.00, 0.17)**	0.04 (−0.12, 0.21)	**0.23 (0.10, 0.37)**
Lp(a) ≤ 10th percentile (5 mg/dL)	**0.09 (0.03, 0.14)**	0.06 (−0.06, 0.17)	**0.18 (0.10, 0.27)**
apoB ≤ 25th percentile (0.81 g/L)	0.02 (−0.08, 0.11)	−0.01 (−0.19, 0.17)	0.06 (−0.10, 0.21)
apoB ≤ 10th percentile (0.69 g/L)	0.07 (−0.02, 0.16)	0.02 (−0.15, 0.20)	−0.07 (−0.22, 0.08)
apoA-I ≥ 75th percentile (1.43 g/L)	0.08 (−0.02, 0.17)	0.13 (−0.05, 0.30)	0.00 (−0.16, 0.16)
apoA-I ≥ 90th percentile (1.60 g/L)	−0.06 (−0.15, 0.04)	−0.11 (−0.29, 0.07)	−0.05 (−0.21, 0.12)
Urinary ACR			
<30 mg/g	0.03 (−0.04, 0.10)	0.08 (−0.04, 0.21)	**0.20 (0.07, 0.33)**
<10 mg/g	**0.18 (0.09, 0.27)**	**0.18 (0.01, 0.35)**	**0.18 (0.02, 0.34)**
Electrocardiogram			
Normal ECG	**0.18 (0.09, 0.27)**	0.15 (−0.02, 0.32)	**0.22 (0.08, 0.37)**
Normal QTc (men: 390–450 ms, women: 390–460 ms)	**0.21 (0.12, 0.30)**	0.15 (−0.01, 0.32)	**0.24 (0.08, 0.39)**
Measurements of atherosclerosis			
baPWV ≤ 25th percentile (1,350 cm/s)	0.07 (−0.03, 0.16)	0.14 (−0.04, 0.31)	/*
CIMT ≤ 25th percentile (0.5 mm)	**0.25 (0.16, 0.34)**	0.08 (−0.08, 0.23)	**0.14 (0.03, 0.24)**
Normal ABI (0.9–1.3)	0.00 (−0.06, 0.06)	0.03 (−0.07, 0.13)	0.03 (−0.08, 0.13)

Data are continuous NRI (95% confidence intervals). The bold values indicate statistical significance.
Intermediate risk: 10-year ASCVD risk 7.5% to 19.9% by the PCEs; High risk: 10-year ASCVD risk ≥ 20% by the PCEs.
**Data cannot be shown due to a limited number of participants with high risk and baPWV ≤ 25th percentile (n = 45).*
NRI, net reclassification index; CVD, cardiovascular diseases; HDL, high-density lipoprotein; Lp (a), lipoprotein(a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; UACR, urinary albumin-to-creatinine ratio; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; ABI, ankle-brachial index; ECG, electrocardiogram; QTc interval, corrected QT interval; ASCVD, atherosclerotic cardiovascular disease; PCEs, Pooled Cohort Equations.

TABLE 5 | Multivariable adjusted DLR.

Negative risk markers	Total	Intermediate risk	High risk
Non-traditional lipids			
Lp(a) ≤ 25th percentile (9 mg/dL)	0.85 ± 0.01	0.91 ± 0.01	0.67 ± 0.03
Lp(a) ≤ 10th percentile (5 mg/dL)	0.68 ± 0.01	0.78 ± 0.01	0.41 ± 0.02
apoB ≤ 25th percentile (0.81 g/L)	0.99 ± 0.00	0.91 ± 0.03	0.81 ± 0.04
apoB ≤ 10th percentile (0.69 g/L)	1.12 ± 0.02	1.00 ± 0.00	0.88 ± 0.01
apoA-I ≥ 75th percentile (1.43 g/L)	0.95 ± 0.02	0.80 ± 0.06	1.17 ± 0.06
apoA-I ≥ 90th percentile (1.60 g/L)	1.00 ± 0.00	1.03 ± 0.00	1.12 ± 0.02
Urinary ACR			
<30 mg/g	0.96 ± 0.03	0.98 ± 0.02	0.91 ± 0.04
<10 mg/g	0.94 ± 0.03	0.93 ± 0.03	0.91 ± 0.03
Electrocardiogram			
Normal ECG	0.89 ± 0.01	0.88 ± 0.02	0.75 ± 0.03
Normal QTc (men: 390–450 ms, women: 390–460 ms)	0.92 ± 0.02	0.93 ± 0.02	0.86 ± 0.03
Measurements of atherosclerosis			
baPWV ≤ 25th percentile (1,350 cm/s)	1.34 ± 0.09	1.35 ± 0.07	/*
CIMT ≤ 25th percentile (0.5 mm)	0.80 ± 0.05	0.87 ± 0.01	0.41 ± 0.02
Normal ABI (0.9–1.3)	1.01 ± 0.00	1.02 ± 0.00	0.99 ± 0.00

Data are means ± standard deviations.
Intermediate risk: 10-year ASCVD risk 7.5% to 19.9% by the PCEs; High risk: 10-year ASCVD risk ≥ 20% by the PCEs.
**Data cannot be shown due to a limited number of participants with high risk and baPWV ≤ 25th percentile (n = 45).*
DLR, diagnostic likelihood ratios; CVD, cardiovascular diseases; HDL, high-density lipoprotein; Lp (a), lipoprotein(a); apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; UACR, urinary albumin-to-creatinine ratio; baPWV, brachial-ankle pulse wave velocity; CIMT, carotid intima-media thickness; ABI, ankle-brachial index; ECG, electrocardiogram; QTc interval, corrected QT interval; ASCVD, atherosclerotic cardiovascular disease; PCEs, Pooled Cohort Equations.

risk of coronary heart disease and a 13% lower risk of stroke (21). However, a lipoprotein(a) <25th percentile (6 mg/dl) failed to downshift the predicted risk of CVD in an elderly US population (22). A similar finding was observed in the current study for a lipoprotein(a) ≤25th percentile (9 mg/dl), whereas the predicted risk of CVD could be moderately downshifted when lipoprotein(a) was at a lower level (i.e., ≤5 mg/dl at 10th percentile). Pronounced differences across ethnicities with regard to lipoprotein(a) levels have been well-demonstrated (23) and further studies are warranted in other ethnic populations.

Previous studies have reported that ECG abnormalities among older adults were associated with an increased risk of CVD outcomes (24, 25). The US Preventive Services Task Force (USPSTF) recently updated its recommendations on ECG and recommended against screening with resting or exercise ECG for the prevention of CVD in asymptomatic adults at low risk (26). Findings from the current study may have provided a new angle at this by demonstrating that in the high-risk population predicted by traditional risk factors, normal ECG could correctly reclassify some participants to a lower predicted CVD risk category. In addition, we previously reported that a prolonged QTc interval was associated with an increased risk of CVD (27). We went further in the current study to demonstrate that a normal QTc interval could correctly downshift the predicted CVD risk beyond traditional risk factors in Chinese adults, which has important implications in risk prediction to guide preventive treatment.

We found that CIMT ≤0.5 mm resulted in a significant change in pre-test to post-test risk for CVD. CIMT is the thickness of the intimal and medial layer of the carotid artery wall. It can be measured non-invasively using ultrasound imaging and is considered a marker for the early stage of atherosclerosis. High CIMT independently predicts cardiovascular events (28). However, the reclassification of CVD risk by CIMT is inconsistent between studies. One study revealed that adding CIMT to traditional risk assessment tool did not lead to a clinically important improvement in risk prediction of myocardial infarction or stroke (29). However, another study demonstrated that adding CIMT to traditional risk factors significantly improved risk classification in older women, but not in older men (30). In addition, it was reported that CIMT modestly improved risk prediction for CVD in older adults mainly by down-classifying risk in those without CVD (31). Findings from the Multi-Ethnic Study of Atherosclerosis (MESA) revealed that low CIMT showed the best performance in reducing predicted CVD risk only after CAC = 0 (7). Findings from the current study also demonstrated better risk reduction by low CIMT compared with other potential negative risk markers.

The strengths of the current study included the large sample of community adults with well-characterized CVD risk and the comprehensive evaluation of multiple potential negative risk markers being compared directly for risk prediction in a single cohort. It also has several limitations. First, a limited number of CVD events were recorded due to a relatively short duration of follow-up. Second, all the tests and measurements were conducted only once. Although CIMT was measured by an experienced ultrasound examiner, two examinations for each participant should have been conducted by two independent examiners. The CIMT was measured only at the common carotid arteries. However, a comprehensive evaluation of CIMT at the common carotid arteries, the carotid bifurcation, and the internal carotid artery is the best way to display atherosclerosis burden (32). Third, the PCEs used in the current study to predict ASCVD risk by traditional risk factors were developed in western populations. The prediction for ASCVD risk in China (China-PAR) equations should have been used to estimate the CVD risk in Chinese population (9). However, we did not have information on family history of CVD which is required in the China-PAR equations. Fourth, other potential negative risk markers such as CAC = 0 were not evaluated in the current study due to lack of information. Finally, the generalizability of the findings is limited to Chinese community residents aged ≥40 years.

In conclusion, lipoprotein(a) ≤5 mg/dL, normal ECG, and CIMT ≤0.5 mm moderately downshifted predicted ASCVD risk in Chinese adults with high risk predicted based on traditional risk factors. The role of negative non-traditional risk markers in CVD risk prediction warrants further investigation.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the IRB has requested that currently, the dataset should be used by the research team members only. If the dataset has to be accessed to verify the results, the request can be directed to the corresponding author. Requests to access the datasets should be directed to YX, jane.yuxu@gmail.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB, JN, and SWu: conceptualization, formal analysis, and writing—original draft. RZ, MX, JL, TW, ZZ, SWa, HL, MD, DZ, and YC: acquisition of data and revision of the manuscript for intellectual content. WW and GN: supervision, funding acquisition, and revision of the manuscript for intellectual content. YB, ML, and YX: conceptualization, formal analysis, funding acquisition, and critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.800671/full#supplementary-material

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Folate Status and Mortality in US Adults With Diabetes: A Nationally Representative Cohort Study

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Background: Public health concerns have gradually shifted from inadequate intakes to potential adverse effects associated with excessive folate intakes following the full implementation of mandatory folate fortification. This study aimed to examine the associations of red blood cell (RBC) folate with all-cause and cardiovascular disease (CVD) mortality among patients with diabetes.

Methods: Data of 15,514 adults aged 20 years or older, who participated in the National Health and Nutrition Examination Survey (1988–1994), were analyzed as the baseline examination. The participants were linked to mortality data from the survey date until December 31, 2015. The associations of RBC folate with all-cause and CVD mortality were examined using multivariable Cox regression models.

Results: During 297,708 person–years of follow-up (median of 19.2 years), 6,106 total deaths occurred, including 1,867 deaths from CVD, 1452 deaths from ischemic heart disease, and 415 deaths from stroke disease. The participants with the highest quartile of RBC folate had higher odds of diabetes (fully-adjusted odds ratio: 1.94 [95% CI: 1.53–2.48]). In Cox regression analyses, compared with the participants with the lowest quartile of RBC folate for diabetes, those from quartile 3 and quartile 4 had HRs (95% CIs) of 1.12 (0.87, 1.43) and 1.30 (1.04, 1.63) in all-cause mortality, respectively; in CVD mortality, the HRs were 1.73 (1.08, 2.76) and 1.47 (0.98, 2.22); in ischemic heart disease mortality, they were 2.01 (1.19, 3.39) and 1.62 (1.05, 2.50), respectively. However, high levels of RBC folate were negatively associated with all-cause mortality, CVD mortality and ischemic heart disease mortality in non-diabetes.

Conclusion: From the nationally representative data, increasing levels in RBC folate were independently associated with an increased risk of all-cause and CVD mortality among those diagnosed with diabetes, but high levels of RBC folate had a mild protective effect in non-diabetes. The underlying mechanism regarding folate and adverse outcomes in diabetes warrants further clarification.

Keywords: RBC folate, diabetes, cardiovascular disease, cohort study, national survey

INTRODUCTION

As one of the natural B vitamins, folate is widely found in staples, such as dark green vegetables, legumes, nuts, and fresh fruit (1, 2), or in the synthetic form used in supplements and food fortification programs (3). Folate is known to play an essential role in preventing neural tube defects (4). In 1998, the U.S. fully implemented the addition of folic acid to cereal grain (5). Subsequently, public health concerns have gradually shifted from inadequate intakes to potential adverse effects associated with excessive folate intakes (6).

Cardiovascular disease (CVD) is one of the most prominent global public health challenges in developed and developing countries in the 21st century (7). A meta-analysis suggested that folic acid contributes to lowering the risk of stroke and overall CVD events (8). However, some clinical research indicated that high-dose folic acid treatment provides no additional benefit on improving vascular function (9), and it does not alter markers of endothelial cell damage (10). A cohort study by Twum et al. (11) demonstrated that high folate levels are significantly associated with an increased risk of CVD among adults with hypertension. Recently, a cross-sectional study also concluded that elevated red blood cell (RBC) folate concentration is associated with increased odds of coronary heart disease in diabetes (12). The effect of high folate levels on CVD prevention among different metabolic populations has been disputed.

As one of the most common metabolic disorders in the world, diabetes has a great influence on macrovascular and microvascular systems, including CVD, diabetic kidney disease, retinopathy, and neuropathy (13). Current literature on the effect of folic levels on cardiovascular disease among patients with diabetes is limited. Thus, the data of diabetes from the National Health and Nutrition Examination Survey (NHANES) III, a nationally representative cohort, was analyzed in this study to examine the association of RBC folate with all-cause and CVD mortality.

METHODS

Study Population

This study used data from NHANES III (1988–1994), which was conducted by the National Center for Health Statistics (NCHS), who used a stratified, multistage design to obtain a nationally representative sample of the non-institutionalized US population.

Diagnosed diabetes was defined as self-report of diabetes diagnosis by a physician or other health professional. Undiagnosed diabetes was defined as having a fasting plasma glucose level of 126 mg/dL or more or HbA1c level of 6.5% or more among individuals without diagnosed diabetes. Diabetes included both diagnosed and undiagnosed diabetes (14). A total of 33,994 participants were recruited to NHANES III between 1988 and 1994. Those who were younger than 20 years ($n = 722$) and pregnant ($n = 288$) at baseline were excluded in this study. Those with missing data on RBC folate ($n = 760$) were further excluded from analyses (**Supplementary Figure 1**). Finally, 2,972 patients with diabetes and 12,542 diabetes-free adults were included. The NCHS's Institutional Review Board reviewed and approved NHANES, and all participants provided a written informed consent.

Assessment of RBC Folate

RBC folate reflects the folate turnover over the preceding 2–3 months and indicates tissue folate status; therefore, RBC folate is used rather than serum folate to assess long-term folate status in humans (15). Whole blood samples, which were frozen at or below −20°C and transported on dry ice, were measured to test RBC folate concentrations. The Centers for Disease Control and Prevention (CDC) used Quanta-phase I folate radioassay kit (Bio-Rad Laboratories) in NHANES III (1988–1991) and Quanta-phase II folate radioassay kit in NHANES III (1991–1994) to analyze RBC folate. Consistency of the two methods were confirmed by a series of quality control tests performed by CDC (16). The total participants were divided into four groups: RBC folate levels < 121 ng/mL (384.8 nmol/L) as lowest quartile; 122–161 ng/mL (388.0–512.0 nmol/L), 162–225 ng/mL (515.2–715.5nmol/L), and ≥ 226 ng/mL (718.7 nmol/L) as highest quartile.

Assessment of Outcomes

A total of 12 identified information, including social security number, sex, and date of birth, were used to link the NHANES III participants with the National Death Index to ascertain vital status and cause of death. Participants were lost to follow-up with insufficient information on these matching criteria. The NCHS introduced a specific matching methodology in 2013, and further details are available on this website (17). The number of person–years of follow-up from NHANES III (1988–1994) was followed up from the date of examination to December 31, 2015, or the date of death, whichever occurred first. The average follow-up time in this study is 19 years, with a maximum of 27 years. UCOD_113 was created to conduct mortality analyses that span across years by using ICD-9 and ICD-10 coding. Death was classified as having CVD with an UCOD_113 code of 001 or 005 (18). CVD mortality included acute rheumatic fever and chronic rheumatic heart diseases (I00–I09), hypertensive heart disease (I11), hypertensive heart and renal disease (I13), ischemic heart diseases (I20–I25), acute myocardial infarction (I21–I22), other acute ischemic heart diseases (I24), other forms of chronic ischemic heart disease (I20, I25), other heart diseases (I26–I51), subarachnoid hemorrhage (I60), intracerebral and other intracranial hemorrhage (I61–I62), cerebral infarction (I63), stroke, not specified as hemorrhage or infarction (I64), other cerebrovascular diseases and their sequelae (I67, I69) (19).

Covariates

Information on gender (male, female), age (continuous), and ethnicity (whites, blacks, Mexican Americans, or others), education (college or higher, high school, or less than high school), the ratio of family income to poverty (> 3.50, 1.31–3.50, or ≤ 1.30), alcohol consumption, and smoking status (non-smoker, past smoker, or current smoker) were collected from the participants by using standardized questionnaires. Current alcohol intake was categorized as non-drinker (0 g/day),

moderate drinker (< 28 g/day for men and < 14 g/day for women), and heavy drinker (≥ 28 g/day for men and ≥ 14 g/day for women) (20). For physical activity, inactive group was defined as those with reported no leisure-time physical activity. Active group was defined as those with self-reported leisure-time moderate activity of five or more times per week or leisure-time vigorous activity of three or more times per week. Those who were not inactive and did not meet the criteria for recommended levels of physical activity was defined as insufficiently active group (21). Healthy Eating Index−2010 (HEI−2010) reflected the sum total of 10 diet components (consumption of foods from the grain, fruit, vegetable, dairy, and meat food groups; intake of dietary fats, saturated fats, cholesterol, and sodium; and a variety score) and provided a measure of overall quality of an individual's diet. The score is from 0 to 100, with 100 being the best-quality diet (22). Total energy intake (TEI) was calculated using the U.S. Department of Agriculture Automated Multiple-pass Method. Body weight and height were used to calculate body mass index (BMI). Diabetes duration was categorized into < 1 year, from 1 to ≤ 4 years, from ≥ 5 to ≤ 9 years, and ≥10 years. Treatment of diabetes was further classified as no treatment, only oral hypoglycemic medication, only insulin treatment, and oral medication and insulin treatment. Fasting serum was obtained via venipuncture by a trained nurse. Blood biomarkers, including vitamin B12, glycated hemoglobinA1c (HbA1c), insulin, fasting glucose, and C-reactive protein (CRP), were also measured. Insulin resistance was assessed using homeostasis model assessment-insulin resistance (HOMA-IR; [fasting insulin × fasting glucose]/22.5). Dietary supplements of folic acids were provided by NHANES Dietary Supplements section. Estimated glomerular filtration rate (eGFR) details (23) were described in the **Supplementary Table 1**.

In the baseline interview, the participants were asked "Have you ever been told by a doctor that you had one or more of the following general medical illnesses: asthma, arthritis, cancer, chronic bronchitis, diabetes, hypertension, gout, lupus, stroke, heart disease, or thyroid disease?" Hypertension was defined as currently taking prescribed medication and/or systolic blood pressure level ≥ 140 mmHg and/or diastolic blood pressure level ≥ 90 mmHg. History of CVD and cancer was assessed from the answers to this question.

Statistical Analysis

Appropriate sampling weights were used to reconstitute data on a representative population level for the entire U.S. due to the complex sampling design adapted by NHANES (24). The means and proportions of baseline characteristics were compared by using linear regression for continuous variables and logistic regression for categorical variables. We examined the cross-sectional association between RBC folate levels and diabetes prevalence by logistic regression model (PROC SURVEYLOGISTIC). Models were successively adjusted for gender, age, ethnicity, education, family income level, smoking status, alcohol intake, physical activity, TEI, HEI-2010, BMI, history of hypertension, dyslipidemia, CVD, cancer, dietary folate intake and CRP.

We applied multivariable Cox regression models (PROC SURVEYPHREG) to further examine the temporal associations of RBC folate levels with all-cause and cause-specific mortality in diabetes and non-diabetes, respectively, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. We treated the lowest quartile of RBC folate (<121 ng/mL) as the reference group, and adjusted for succeeding confounders. Model 1 was adjusted for gender, age, ethnicity, education, family income level, smoking status, alcohol intake, physical activity TEI and HEI-2010. Model 2 was additionally adjusted for BMI, history of hypertension, dyslipidemia, CVD and cancer, diabetes medication use. Missing values of covariates were included in the model treated as dummy variables.

Stratified analyses were also conducted by age (< 60 or ≥ 60 years), sex (male or female), BMI (< 30.0 or ≥ 30.0 kg/m^2), race/ethnicity (White or non-White), educational level (less than high school, high school, college or higher), ratio of family income to poverty (≤ 1.30, 1.31–3.50 or > 3.50), smoking status (never/former or current smokers), alcohol intake (none/moderate or heavy), physical activity (inactive/insufficiently active or active), history of cardiovascular disease (yes or no), history of hypertension (yes or no), history of cancer (yes or no), diabetes duration (< 5 years or ≥ 5 years), and treatment for diabetes (none or yes). The joint test was used to obtain a *P* value for interaction to examine the statistical significance of the difference between subgroups. We performed several sensitivity analyses to examine the robustness of our results. First, we noted that diabetes medication use, hypertensive medication use and blood vitamin B12 were closed associated with the risk of CVD mortality, and RBC folate concentration was influenced by dietary folate intake. To prevent overadjustment, we adjusted these factors in sensitivity analyses instead of the main analyses. Second, homocysteine concentrations were reported to possibly have a strong relationship with folate levels (25), thus, further adjustment was made for homocysteine in diabetes [data were only available in NHANES (1991–1994), $n = 1244$]. Third, we performed a sensitivity to analysis the association between RBC folate and mortality in patients who used diabetes drugs. Forth, we also excluded patients with cancer diseases, and the analyses were rerun. Fifth, to minimize potential reverse causation bias, participants who died within 4 years of follow-up were excluded, and we reran the analyses.

Model assumptions were checked for all the analyses between January 12, 2021, and May 27, 2021. All statistical analyses were conducted using SAS version 9.4 (SAS Institute, USA), and a two-sided p value < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 15,514 participants (mean age of 45 ± 0.5 years, 7415 [47.8%] male) were included in the present analysis. **Table 1** showed the baseline characteristics between diabetes ($n = 2,972$) and non-diabetes ($n = 12,542$), and the baseline characteristics according to RBC folate quartiles were shown in **Supplementary Table 2**. The participants with diabetes were

TABLE 1 | Baseline characteristics of diabetes in comparison to non-diabetes aged 20+, NHANES III 1988–1994.

Characteristics	Baseline status of diabetes and non-diabetes			
	Diabetes (n = 2,972)		Non-diabetes (n = 12,542)	
	No. (%)	SE	No. (%)	SE
Age, mean, years	57.7	0.4	43.0	0.4
BMI, mean, kg/m^2	28.9	0.2	26.1	0.1
Sex, n (%)				
Male	1367 (45.1)	1.5	6048 (49.2)	0.5
Female	1605 (54.9)	1.5	6494 (50.8)	0.5
Race/ethnicity, n (%)				
Whites	1151 (74.0)	1.6	5368 (77.4)	1.3
Blacks	843 (12.9)	1.0	3370 (10.0)	0.6
Hispanics	867 (5.3)	0.5	3293 (4.9)	0.4
Others	111 (7.8)	0.9	511 (7.7)	0.9
Educational level, n (%)				
Less than high school	1129 (21.1)	1.4	2638 (10.0)	0.6
High school	1259 (48.2)	1.6	5984 (46.6)	1.1
College or higher	584 (30.6)	2.0	3920 (43.4)	1.2
Ratio of family income to poverty, n (%)				
≤1.30	1003 (21.1)	1.5	3531 (16.1)	1.0
1.31–3.50	1118 (40.0)	1.2	5140 (43.1)	1.1
>3.50	506 (29.8)	1.8	2744 (34.7)	1.3
Unknown	345 (9.0)	0.9	1127 (6.1)	0.4
Smoking status, n (%)				
Never	1329 (40.8)	1.4	6252 (46.2)	0.8
Former	1040 (37.8)	1.3	2859 (24.1)	0.7
Active	603 (21.4)	1.0	3430 (29.7)	0.9
Alcohol intake, n (%)				
None	2438 (79.9)	1.7	9419 (71.9)	1.3
Moderate	151 (5.2)	0.5	1093 (9.2)	0.6
Heavy	238 (10.7)	1.3	1639 (15.9)	0.8
Unknown	145 (4.1)	0.7	391 (3.0)	0.6
Physical activity, n (%)				
Inactive	879 (22.6)	1.2	2563 (13.7)	0.8
Insufficient	1175 (41.9)	1.8	5310 (45.0)	0.9
Sufficient	918 (35.4)	1.9	4669 (41.3)	1.1
HEI-2010, mean	65.1	0.4	63.5	0.3
Total energy intake, mean, Kcal	1830.4	24.4	2249.5	21.4
CVD, n (%)	395 (10.1)	0.6	544 (2.8)	0.2
Hypertension, n (%)	1615 (48.5)	1.3	3164 (19.9)	0.8
Cancer, n (%)	292 (12.9)	0.9	1164 (7.9)	0.4
Duration of diabetes, n (%)				
<1 year	1840 (66.7)	1.3	-	-
1–5 years	364 (11.6)	0.8	-	-
5–10 years	227 (7.3)	0.9	-	-
≥10 years	541 (14.3)	0.8	-	-
Treatment for diabetes, n (%)				
None	2057 (73.6)	1.4	-	-
Only Insulin	542 (15.6)	1.1	-	-
Only Pills	323 (9.6)	0.9	-	-
Pills and insulin	50 (1.3)	0.2	-	-
Dietary folate intake, mean, mcg	282.2	6.4	289.3	3.3
HbA1c, mean, %	6.6	0.1	5.2	0.04

(Continued)

TABLE 1 | Continued

Characteristics	Baseline status of diabetes and non-diabetes			
	Diabetes (n = 2,972)		Non-diabetes (n = 12,542)	
	No. (%)	SE	No. (%)	SE
GLU, mean, mg/dl	136.3	2.3	92.9	0.2
Insulin, mean, uU/mL	20.9	1.1	9.7	0.2
HOMA-IR, mean	8.6	0.9	2.3	0.04
Vitamin B12, mean, mcg	4.9	0.2	5.3	0.2
CRP, mean, mg/dL	0.6	0.01	0.4	0.03
eGFR, mean, mL/min per 1.73 m^2	66.0	0.6	77.7	0.4

Data were expressed as the mean (SD) or n (%).
BMI, body mass index; HEI, Healthy Eating Index; CVD, cardiovascular disease; HbA1c, glycosylated hemoglobin; GLU, fasting glucose; HOMA-IR, homeostasis model assessment-insulin resistance; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

TABLE 2 | Odds ratios of diabetes according to RBC folate status.

	Odds ratio (95%CI) of prevalence of diabetes				
	Q1, <121 ng/mL, n = 3,924	Q2, 122–161 ng/mL, n = 3,824	Q3, 162–225 ng/mL, n = 3877	Q4, ≥226 ng/mL, n = 3,889	P for trend
Model 1	1 (ref.)	1.55 (1.25, 1.92)	1.60 (1.31, 1.96)	1.89 (1.48, 2.41)	<0.001
Model 2	1 (ref.)	1.63 (1.30, 2.03)	1.73 (1.39, 2.15)	2.10 (1.63, 2.71)	<0.001
Model 3	1 (ref.)	1.62 (1.30, 2.01)	1.70 (1.38, 2.09)	2.04 (1.58, 2.63)	<0.001
Model 4	1 (ref.)	1.60 (1.29, 1.98)	1.65 (1.34, 2.03)	1.94 (1.53, 2.48)	<0.001

Data are presented as hazard ratio (95% confidence interval).
Model 1: adjusted for age, sex and race/ethnicity.
Model 2: model 1 + education, family income level, smoking status, alcohol intake, physical activity, TEI and HEI-2010.
Model 3: model 2 + BMI, history of hypertension, history of dyslipidemia, baseline CVD and baseline cancer, treatment for diabetes and duration of diabetes.
Model 4: model 3 + CRP+ dietary folate intake.
TEI, total energy intake; HEI, Healthy Eating Index; BMI, body mass index; CVD, cardiovascular disease; CRP, C-reactive protein.

more likely to be older, non-white, female, and less educated than those with no diabetes. They were also likely to have lower income, inactive physical activity, higher scores in HEI, and less TEI and less likely to be heavy drinkers and active smokers. In addition, they had a higher prevalence of CVD, hypertension, and cancer; more daily intake of folic acids from food; and higher HbA1c, insulin, HOMA-IR, fasting glucose, and CRP levels; and lower blood vitamin B12 and eGFR levels.

Association Between RBC Folate and Diabetes

RBC folate concentration was categorized as quartile, and its association with the prevalence of diabetes was examined (**Table 2**). In Model 1 adjusted for age, sex, and race, the odds ratios (ORs) for diabetes were 1.55 (95% CI: 1.25–1.92), 1.60 (95% CI: 1.31–1.96), and 1.89 (95% CI: 1.48–2.41) for quartiles 2–4, respectively, compared with the lowest quartile of RBC folate. Further adjusting for potential confounders, the association remained robust, and those with the highest quartile of RBC folate had higher odds of diabetes (fully adjusted OR: 1.94 [95% CI: 1.53–2.48], $P_{trend} < 0.001$).

Association of RBC Folate With All-Cause and Cause-Specific Mortality

During 297,708 person–years of follow-up (median of 19.2 years), 6,106 total deaths occurred, including 1,867 deaths from CVD, 1,452 deaths from ischemic heart disease, and 415 deaths from stroke disease. In Cox regression analyses, diabetes with moderate and high RBC folate quartiles showed an increased hazard in all-cause and CVD mortality, especially in ischemic heart disease mortality (Model 1, **Table 3**). These associations remained robust after stepwise adjustment for confounders. In the fully adjusted model, the HRs and 95% CIs from the lowest to the highest quartile of RBC folate for diabetes were 1.00 (reference), 1.10 (0.86, 1.42), 1.12 (0.87, 1.43), and 1.30 (1.04, 1.63) for all-cause mortality; 1.00 (reference), 1.19 (0.74, 1.92), 1.73 (1.08, 2.76), and 1.47 (0.98, 2.22) for CVD mortality; 1.00 (reference), 1.25 (0.71, 2.21), 2.01 (1.19, 3.39) and 1.62 (1.05, 2.50) for ischemic heart disease mortality; 1.00 (reference), 0.85 (0.32, 2.23), 0.81 (0.34, 1.89) and 0.93 (0.40, 2.17) for Stroke Mortality (Model 2, **Table 3**). However, for non-Diabetes, a Mild Protective Effect Was Found in all-Cause Mortality, Ischemic heart disease mortality. In multiplicative interaction analysis, a significant interaction between RBC folate level and diabetes status was observed for CVD mortality ($P_{interaction} = 0.0048$), and ischemic heart disease mortality ($P_{interaction} = 0.0022$) and

TABLE 3 | Association between RBC folate with all-cause and cause-specific mortality among 15,514 individuals.

	RBC folate, ng/mL									
	Diabetes					Non-diabetes				
	<121 ng/mL, *n* = 507	122–161 ng/mL, *n* = 667	162–225 ng/mL, *n* = 748	≥226 ng/mL, *n* = 748	Per 100 ng/mL increment	<121 ng/mL, *n* = 3,417	122–161 ng/mL, *n* = 3,157	162–225 ng/mL, *n* = 3,079	≥226 ng/mL, *n* = 2,889	Per 100 ng/mL increment
All-cause mortality										
Deaths/person years	317/8,066	405/11,266	533/12,667	729/14,391		909/71,446	898/65,690	968/62,101	1347/52,080	
Model 1	1 (ref.)	1.14 (0.89, 1.45)	1.37 (1.09, 1.71)	1.46 (1.17, 1.82)	1.10 (1.04, 1.16)	1 (ref.)	0.93 (0.83, 1.04)	0.89 (0.80, 0.999)	0.97 (0.86, 1.10)	1.04 (0.99, 1.09)
Model 2	1 (ref.)	1.12 (0.87, 1.43)	1.3 (1.04, 1.63)	1.31 (1.04, 1.67)	1.07 (1.02, 1.13)	1 (ref.)	0.94 (0.84, 1.04)	0.89 (0.80, 0.99)	0.97 (0.87, 1.09)	1.03 (0.99, 1.07)
P for interaction	0.057									
CVD mortality										
Deaths/person years	91/8,066	114/11,266	181/12,667	243/14,391		249/71,446	280/65,690	307/62,101	402/52,080	
Model 1	1 (ref.)	1.22 (0.80, 1.86)	1.83 (1.18, 2.84)	1.67 (1.14, 2.45)	1.08 (1.01, 1.15)	1 (ref.)	0.97 (0.74, 1.28)	0.97 (0.69, 1.35)	0.83 (0.62, 1.12)	0.98 (0.90, 1.05)
Model 2	1 (ref.)	1.19 (0.74, 1.92)	1.73 (1.08, 2.76)	1.47 (0.98, 2.22)	1.04 (0.97, 1.11)	1 (ref.)	0.98 (0.75, 1.29)	0.95 (0.69, 1.31)	0.82 (0.63, 1.08)	0.96 (0.90, 1.03)
P for interaction	0.0048									
Ischemic heart disease mortality										
Deaths/person years	67/8,066	83/11,266	148/12,667	199/14,391		202/71,446	210/65,690	228/62,101	315/52,080	
Model 1	1 (ref.)	1.30 (0.80, 2.12)	2.16 (1.33, 3.51)	1.87 (1.25, 2.79)	1.09 (1.01, 1.17)	1 (ref.)	0.86 (0.64, 1.16)	0.82 (0.55, 1.21)	0.73 (0.53, 0.996)	0.96 (0.88, 1.05)
Model 2	1 (ref.)	1.25 (0.71, 2.21)	2.01 (1.19, 3.39)	1.62 (1.05, 2.50)	1.06 (0.98, 1.14)	1 (ref.)	0.88 (0.65, 1.19)	0.81 (0.56, 1.17)	0.73 (0.54, 0.98)	0.95 (0.88, 1.03)
P for interaction	0.0022									
Stroke mortality										
Deaths/person years	24/8,066	31/11,266	33/12,667	44/14,391		47/71,446	70/65,690	79/62,101	87/52,080	
Model 1	1 (ref.)	0.87 (0.34, 2.23)	0.82 (0.35, 1.92)	1.02 (0.44, 2.34)	1.03 (0.86, 1.22)	1 (ref.)	1.70 (0.84, 3.42)	2.03 (1.14, 3.61)	1.55 (0.79, 3.04)	1.01 (0.88, 1.16)
Model 2	1 (ref.)	0.85 (0.32, 2.23)	0.81 (0.34, 1.89)	0.93 (0.40, 2.17)	0.99 (0.83, 1.18)	1 (ref.)	1.65 (0.84, 3.26)	1.92 (1.11, 3.31)	1.46 (0.78, 2.74)	0.99 (0.86, 1.14)
P for interaction	0.74									

Values are n or hazard ratio (95% confidence interval) and are weighted except No. of deaths/ person years.

Model 1: adjusted for age, sex and race/ethnicity, education, family income level, smoking status, alcohol intake, physical activity, TEI and HEI-2010.

Model 2: model1+ BMI, history of hypertension, history of dyslipidemia, baseline CVD and baseline cancer, diabetes medication use. P for interaction is only for model 2.

TEI, total energy intake; HEI, Healthy Eating Index; BMI, body mass index; CVD, cardiovascular disease.

null for all-cause mortality ($P_{interaction}$ = 0.057) and stroke mortality ($P_{interaction}$ = 0.74).

Sensitivity Analyses

In stratified analyses, the associations of RBC folate quartiles with all-cause and CVD mortality were robust across the strata of age, sex, BMI, race/ethnicity, educational level, ratio of family income to poverty, smoking status, alcohol intake, physical activity, CVD history, hypertension history, cancer history, diabetes duration, and diabetes treatment, and all the interaction terms were not significant ($P_{interaction}$ > 0.001, **Supplementary Table 3**). Further sensitivity analyses with additional adjustment of diabetes medication use, hypertensive medication use, dietary folate intake and blood vitamin B12 level produced similar results (**Supplementary Table 4**). The association between RBC folate quartiles and all-cause, CVD, and ischemic heart disease mortality remained the same with further adjustment of serum homocysteine (**Supplementary Table 5**). Further sensitivity analysis had been conducted with diabetes medication use (**Supplementary Table 6**) and cancer patients excluded (**Supplementary Table 7**), and the results remained robust.

In addition, participants who died within 4 years of follow-up were excluded, and the associations were re-examined. The results did not alter the significance of the associations between RBC folate quartiles and all-cause and cause-specific mortality (**Supplementary Table 8**).

DISCUSSION

In this nationwide prospective cohort study, high levels of RBC folate were found to be significantly associated with an elevated risk of CVD and all-cause mortality in patients diagnosed with diabetes. Conversely, high levels of RBC folate were negatively associated with all-cause and ischemic heart disease mortality in non-diabetes. The findings highlighted that the relationship between folate status and adverse outcomes may differ by baseline diabetes status.

Previous studies showed that folic acid may have CVD benefits for the general population. One cohort study suggested a 69% increased risk of mortality from coronary heart disease among participants with the lowest serum folate level category (< 3 ng/mL) compared with those with the highest category (> 6 ng/mL) (26). Compared with men whose serum folate concentrations were in the lowest tertile (< 8.4 nmol/L), those whose concentrations were in the highest tertile (>11.3 nmol/L) had a factor-adjusted relative risk of acute coronary events of 0.35 (95% CI: 0.17, 0.73) (27). A meta-analysis also found a 4% lower risk of overall CVD with folic acid supplementation (the dosage of folic acid in the intervention groups ranged from 0.5 to 15 mg/day) (8). However, the association between folate levels and CVD outcomes in different metabolic populations was inconsistent. In patients after acute myocardial infarction, a trend toward an increased risk of recurrent cardiovascular disease (relative risk of 1.22 [95% CI: 1.00–1.50]) was observed in the group with 0.8 mg of folic acid, 0.4 mg of vitamin B12, and 40 mg of vitamin B6 daily (28). Compared with placebo, active treatment (supplements combining folic acid and vitamins B6 and B12) did not significantly decrease the risk of death from cardiovascular causes in patients who had vascular disease or diabetes (relative risk of 0.96 [95% CI: 0.81–1.13]) (25). A limited study showed that the association between folate and mortality may differ by diabetes status (29). A study from the U.S. indicated higher RBC folate quartiles (≥ 634 nmol/L for men and ≥ 659 nmol/L for women) were significantly associated with the risk of death among patients with diabetes (HRs of 2.81 [95% CIs 1.48–5.32]) (30), consistent with the findings of the present study.

Limited literature exists on the biological mechanism underlying the association between high RBC folate and increased mortality from CVD among patients with diabetes. Endothelial dysfunction was found to be critical to the pathogenesis of microvascular and macrovascular complications of diabetes (31), and damaged endothelium may enhance thrombogenicity (7). Despite a related report showing that folic acid supplementation provided beneficial effects on endothelial functions (32), another report showed no evidence that folic acid could improve the markers of endothelial dysfunction or inflammation in patients with type 2 diabetes (33). High levels of RBC folate are cautiously suspected to possibly be detrimental to the endothelial function of patients with diabetes, thus warranting further investigation. The association between folate and accurate DNA synthesis and cell division under pathological conditions may help explain the observed results in the present study. In one-carbon metabolism, folate (as a critical cofactor) played an important function in biologic methylation and *de-novo* nucleotide synthesis pathways (34, 35). Some studies showed that adequate folate intake was crucial for accurate DNA synthesis and cell division for it could help sustain the normal patterns of DNA methylation and minimize DNA damage (35–37). However, a case-control study found that folate intake was inversely associated with promoter methylation of tumor suppressor and DNA repair genes in adenoma tissue specimens (38). Genetic polymorphisms of enzymes involved in one-carbon metabolism need to be considered when a complex picture of folate is being studied in the prevention of diseases (39). More observations should be conducted to investigate whether excessive folic acid may change promoter-specific methylation patterns in DNA or in histones among patients with diabetes.

Fortification of food with folic acid was introduced more than 20 years in North America to reduce the number of neural tube defects. Many countries are considering whether to adopt the same policy; however, little has been conducted at the population level to seriously trade-off the benefits to the few and the harm to some of the many exposed. Elderly people with high folate and low vitamin B-12 status had higher risk of cognitive impairment (OR: 5.1; 95% CI: 2.7, 9.5) and anemia (OR: 5.2; 95% CI: 2.5, 11.0) than those who had normal vitamin B-12 and normal folate concentrations (40). The Pune Maternal Nutrition Study showed that mothers with a combination of high blood folate and low vitamin B-12 concentrations during pregnancy had their children at greater risk of insulin resistance and higher blood folate concentration (41). Thus, whether high folate concentrations could impair normal folate function in different metabolic populations should be considered. The present study calls for more vigorous

assessment on the relationship between high folate levels and the conditions beyond neural tube defect, especially among populations with diabetes.

This study has several strengths. NHANES provides a large-scale nationally representative sample that allows delineation of the relation in a wide array of population. The follow-up of over 19 years is favorable in providing sufficient statistical power to estimate the long-term association between baseline folate status and mortality. Another feature of this study is that baseline information was collected prior to fortification of folic acid in foods, which avoids bias caused by fortification of food with folic acid. Nevertheless, several limitations of this study should be considered. First, RBC folate was only assessed once at baseline, thus precluding the observations of folate changes with mortality risk. Second, although the study population was selected from a nationally representative sample, it excluded institutionalized patients. Diabetes, CVD diseases, and other chronic diseases are more common among institutionalized elderly patients. Third, given the ethnic disparity in the metabolism and blood level of folate, directly generalizing the findings to other ethnicities should be given caution, and the results should be replicated in other ethnic and racial groups. Fourth, given the nature of the observational design, residual confounders and unmeasured bias may have existed, and causal inferences should be cautious.

CONCLUSION

In the nationally representative population of over 19 years of follow-up, increasing levels in RBC folate were independently associated with an increased risk of all-cause and CVD mortality among those diagnosed with diabetes, while high levels of RBC folate had a mild protective effect in non-diabetes. The findings highlighted that blood folate may play different roles in different disease conditions, thus requiring further investigation.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Center for Health Statistics's Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HX and XL analyzed, interpreted data, and drafted the first manuscript. HX conceived and designed the study. SC analyzed and interpreted data. PC and SG revised the manuscript. XH conceived, designed the study, and revised it critically for important intellectual content. YL conducted critical review and revision of the manuscript for important intellectual content. All authors meet authorship criteria and approved the final version of the manuscript to be published.

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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.2022.802247/full#supplementary-material

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Long-Term Exposures to Air Pollutants and Risk of Peripheral Arterial Occlusive Disease: A Nationwide Cohort Study in Taiwan

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Air pollution is one of the most alarming environmental issues which causes multiple health hazards. An association between air pollution and cardiovascular diseases has been established through many prior studies. In this study, we aimed to evaluate the risk of long-term exposure to air pollution ($PM_{2.5}$, CO, and NO_2) and its association with the risk of developing peripheral arterial occlusive disease (PAOD). PAOD is a condition involving impairment of perfusion of blood in the distal parts of the aorta due to narrowing of the arteries (arterial stenosis) and has been reported as a risk factor for developing cardiovascular diseases. Furthermore, the risk of PAOD increases with age, and hence is a serious public health issue and a cause for concern, especially for an aging society such as Taiwan. Two national-scale databases from Taiwan, the national health insurance database (NHIRD) and the Taiwan air quality-monitoring database (TAQMD), were linked to conduct this cohort study between 2003 and 2013. Cox proportional hazards regression with time-dependent modeling was used to evaluate the hazard ratio (HR) for PAOD with respect to daily exposure to air pollutants. The concentrations of each of the pollutants of interest ($PM_{2.5}$, NO_2, and CO) were categorized into four categories according to the daily average concentration of air pollutants for every quarter of the year, Q1 to Q4 (Q4 = highest). The cumulative incidence of PAOD was examined by Kaplan–Meier analysis with two-tailed log-rank test. A total of 1,598 PAOD cases were identified during the 10-year follow-up period, along with 98,540 non-PAOD controls. In the multivariate analysis, after adjusting for age, gender, urbanization level, residential area, baseline comorbidities, and medications, the adjusted HRs were $PM_{2.5}$ = 1.14 (95% CI 1.13–1.16), NO_2 = 1.03 (95% CI 1.02–1.04), and CO = 2.35 (95% CI 1.95–2.84). Kaplan–Meier analysis showed that CO ($P < 0.0001$) and $PM_{2.5}$ ($P < 0.0001$) concentrations were strongly and positively associated with the cumulative incidence of

PAOD during the follow-up period. Findings from this study established that prolonged exposure to air pollutants CO and $PM_{2.5}$ are significant factors that, among other well-known causes, may also play a potential role in PAOD pathogenesis.

Keywords: peripheral arterial occlusive disease (PAOD), $PM_{2.5}$, carbon monoxide (CO), nitrogen dioxide (NO_2), air pollutants, prolonged exposure

INTRODUCTION

Air pollution is one of the most alarming environmental issues which causes of multiple health hazards and ranks ninth among the modifiable disease risk factors, preceding other frequent factors such as low physical activity, a high-sodium diet, high cholesterol, and drug use. Air pollution is responsible for 3.1% of global disability-adjusted life years (DALY), an index that quantifies the time spent under reduced health conditions (1). Numerous studies on Caucasians, Black Americans, Europeans, and Asians have established that air pollutants, including gaseous compounds such as carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3), and particulate matter (PM) such as PM_{10} and $PM_{2.5}$, are associated with poorer cardiovascular (CV) conditions, and that exposure to air pollutants can increase the risk of CV events (1–3). The major cause for such CV morbidity is mainly due to coronary artery diseases that develop as an effect of continued exposure to air pollution. A multicity study from various countries (Europe, the United States, and Canada) has reported an increase of up to 0.6% in all-cause mortality with every 10 $\mu g/m^3$ increase in PM_{10} (4). Another project, the Air Pollution and Health: a European Approach (APHEA) study (5), conducted in 30 European cities, reported that a 10 $\mu g/m^3$ increase in NO_2 led to an up to 0.4% increase in total, CV, and respiratory mortality rates. Further, studies on European cohorts also demonstrated the effects of $PM_{2.5}$ on CV mortality for both outdoor and indoor subjects. Studies on the long-term effects of air pollution in the Asian countries are scarcely found. A study on the Taiwanese population reported a positive correlation of the risk of all-cause and cardiovascular disease (CVD) mortality with levels of SO_2, CO, NO_2, and PM_{10} especially for the elderly (age $>$ 65 years) (6). Air pollution in Taiwan, due to gaseous pollutants and $PM_{2.5}$ and PM_{10}, is categorized as either local pollution, the source being motor vehicles and power plants, or long-range transported pollution, the source being neighboring countries, and is an acute issue that has been a cause for great concern among laypersons and researchers alike (7).

Peripheral arterial occlusive disease (PAOD) is a condition involving impaired perfusion of blood in the distal parts of the aorta and/or the pelvic, femoral, and crural arteries due to narrowing of the arteries (arterial stenosis) or absolute arterial lumen blockage (occlusion). It has been reported as a risk factor for developing CVD (8, 9). PAOD has a rising incidence and currently affects ∼200 million people worldwide (estimated prevalence rose from 164 million in 2000 to 201 million in 2010) (8). In the Asia-Pacific region, PAOD prevalence ranges between 5.2 and 12.1% (10). A systematic review of 34 countries revealed that its prevalence in men in high-income countries was higher than that in low and middle-income countries; however, incidence in women was found to be uniform (11). Moreover, it was reported that the overall prevalence of PAOD has increased by 13.1% in high-income countries and by 28.7% in low- and middle-income countries. PAOD diminishes quality of life. Approximately 50% of PAOD patients are asymptomatic, therefore preventing an accurate estimation of its true prevalence (12). Furthermore, PAOD mostly occurs in people with atherosclerosis; therefore, it is an important indicator of atherosclerotic burden (13), and has been reported in multiple studies to have an association with an enhanced risk of mortality due to CV conditions (14, 15). As the risk of PAOD increases with age (9), it is a serious public health issue, and a cause for concern, especially for an aging society such as Taiwan (16). With the exception of a few studies on Asian cohorts, PAOD has not received enough attention compared with CVD, particularly in the context of risk of developing it as a result of prolonged exposure to pollution (17, 18). Therefore, this study focuses on evaluating the contribution of long-term exposure to air pollution ($PM_{2.5}$, CO, and NO_2) to the development of PAOD, using individual-level data from national databases in Taiwan.

MATERIALS AND METHODS

Databases

Two national-scale databases from Taiwan, the National Health Insurance Research Database (NHIRD) and the Taiwan Air Quality-Monitoring Database (TAQMD), were used to obtain individual clinical data and environmental data, respectively, between 2003 and 2013. NHIRD is a large-scale administrative healthcare database consisting of medical records, causes of death records, sorted registration files, and original claims data that are derived from the National Health Insurance (NHI) program by the NHI Bureau and is maintained by the National Health Research Institutes (NHRI). It houses information on 23 million residents of Taiwan covering 99% of the total population that is available to scientists in Taiwan for research purposes (19). TAQMD is a large-scale database that is set up by Taiwan's official Environmental Protection Administration (EPA) and Central Weather Bureau (CWB) to document and forecast air quality. The database consists of ∼260,000 samples that were retrieved in 2017 from 77 air monitoring stations (EPA) and 580 weather stations (CWB) in Taiwan, and the data are freely available for public access (20, 21). Both databases were utilized to obtain linked information for each of the individuals in this study.

Study Population

A total of 1,000,000 individuals from the longitudinal component of the NHIRD (19) were selected, among whom only those aged

40–80 years as on 1 January 2003, were included in this study. A total of 1,598 individuals were diagnosed with PAOD as per International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes 440-447, with first onset on or after 1 January 2003, and 98,540 without PAOD were retained for further analysis (**Figure 1**). Patients with a history of PAOD prior to the index date (1 January 2003) or those who were <40 years of age were excluded from further analysis. All patients were followed until 31 December 2013. The primary outcome was defined as the first PAOD event within the follow-up period of 10 years.

Clinical Variables

Gender, age, comorbidities, and medication usage information were included as clinical variables in this study (**Figure 1**). Four age groups (40–49, 50–59, 60–69, 70–80 years) were considered. Comorbidities were considered if individuals were diagnosed with any other related condition(s) within 1 year prior to the beginning of the study period. Comorbidities due to hypertension (ICD-9-CM: 401–405), diabetes mellitus (DM) (ICD-9-CM: 250.x), hyperlipidemia (ICD-9-CM: 272, A182), chronic obstructive pulmonary disease (COPD) (ICD-9-CM:490, 491, 492, 494, 496), congestive heart failure (CHF) (ICD-9-CM: 428), chronic kidney disease (CKD) (ICD-9-CM: 581–588, 403–404, 285.21), cerebral vascular disease (stroke) (ICD-9-CM: 430–438), ischemic heart disease (IHD) (ICD-9-CM: 411–414), and cancer (ICD-9-CM: 140–208) were included as variables in this study. Information on usage of medications, for each individual, was taken into consideration if particular medicines were used for more than a month during the study period. Medications such as statins, aspirin, clopidogrel, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), warfarin (anticoagulants), and steroids were included in the analysis.

Environmental Variables

Environmental variables such as urbanization level, residential areas, and level of air pollution were included. The NHRI has stratified all city districts and townships in Taiwan into seven urbanization levels (levels 1–7) on the basis of (1) population density (people/km^2), (2) the proportion of residents with higher education level, (3) occupation types (e.g., who work in agriculture), and (4) the number of physicians per 100,000 people in each area. Level 1 represents areas with a low population density and socioeconomic status and level 7 represents the highest socioeconomic status. As very few people reside in the rural areas that predominantly fall under classification 1–3, these areas were regrouped as level 1. Therefore, the urbanization level was reclassified into 5 levels. Air pollution levels were determined based on the level of exposure to pollutant, which was defined with respect to time period (T) and the concentration of pollutants to which an individual was exposed. The pollution data were collected every day of the year and the daily average was obtained over each year of the study period, which were linked with each of the individuals in this study. The concentrations of each of the pollutants of interest ($PM_{2.5}$, NO_2 and CO) were categorized into four groups based on the following thresholds: $PM_{2.5}$ (Q1 = <28.24 μg/m^3/day, Q2 = 28.24–31.46 μg/m^3/day, Q3 = 31.46–38.47 μg/m^3/day, and Q4 ≥ 38.47 μg/m^3/day); NO_2 (Q1 < 16.14 ppb/day, Q2 = 16.14–20.49 ppb, Q3 = 20.49–24.90 ppb/day, and Q4 ≥ 24.90 ppb/day); and CO (Q1 < 0.47 ppm/day, Q2 = 0.47–0.58 ppm, Q3 = 0.58–0.68 ppm/day, and Q4 ≥ 0.68 ppm/day). Residential areas for patients were broadly divided into six distinct regions, namely, (i) Northern, (ii) Taipei, (iii) Central, (iv) Southern, (v) Eastern, and (vi) Kao-Ping, based on the location where the most clinic and hospital visits were made for treating acute upper respiratory tract infections, as defined by ICD-9-CM code 460.

Statistical Analysis

All baseline variables, both clinical and environmental, were comparatively analyzed between individuals with PAOD and without PAOD, using chi-square tests or the means of independent samples *t*-test (22). First, univariate Cox proportional hazard regression analysis (23) was conducted with the PAOD event as the outcome. The crude hazard ratios (HRs) (univariate) for each of the air pollutants ($PM_{2.5}$, NO_2, and CO), gender, age group, urbanization level, residential area, comorbidities, and drug use were calculated. Then, three different Cox proportional hazards regression analyses with time-dependent covariates (24) were performed with the first PAOD event as the primary outcome for each of the pollutants ($PM_{2.5}$, NO_2, and CO), adjusted by the segmented time-dependent covariates, gender, age group, urbanization level, residential area, comorbidities, and drug use. This was done to avoid bias due to non-baseline variables that changed over the course of time in combination with time to event. Two-tailed log-rank tests were used to obtain *p*-values, and a threshold of $p < 0.05$ was used to define statistical significance. Further, the residential areas were divided into four categories according to the daily average concentration of air pollutants, Q1–Q4 (Q4 = highest) and were used to examine the cumulative incidence of PAOD, based on each of the pollutants under study ($PM_{2.5}$, CO, NO_2), through Kaplan–Meier analysis with a two-tailed log-rank test (25). Again, a *p*-value threshold of 0.05 was used to define statistical significance. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Clinical Characteristics and Linked Environmental Exposure Details

After exclusion due to prior PAOD diagnosis and age <40 years and >80 years, a total of 100,138 individuals were included in the final analysis, among whom 1,598 (1.6%) and 98,540 (98.4%) were with and without PAOD, respectively (**Figure 1**). **Table 1** gives a detailed account of all demographics, clinical variables, and environmental variables included for analysis in this study. Significant differences for all baseline variables were observed between individuals with and without PAOD. Men made up the majority of PAOD cases (53.32%), whereas women comprised the majority (54.79%) of those without PAOD. The mean age of individuals with PAOD was 63.22 years, the incidence being higher among older individuals. The highest

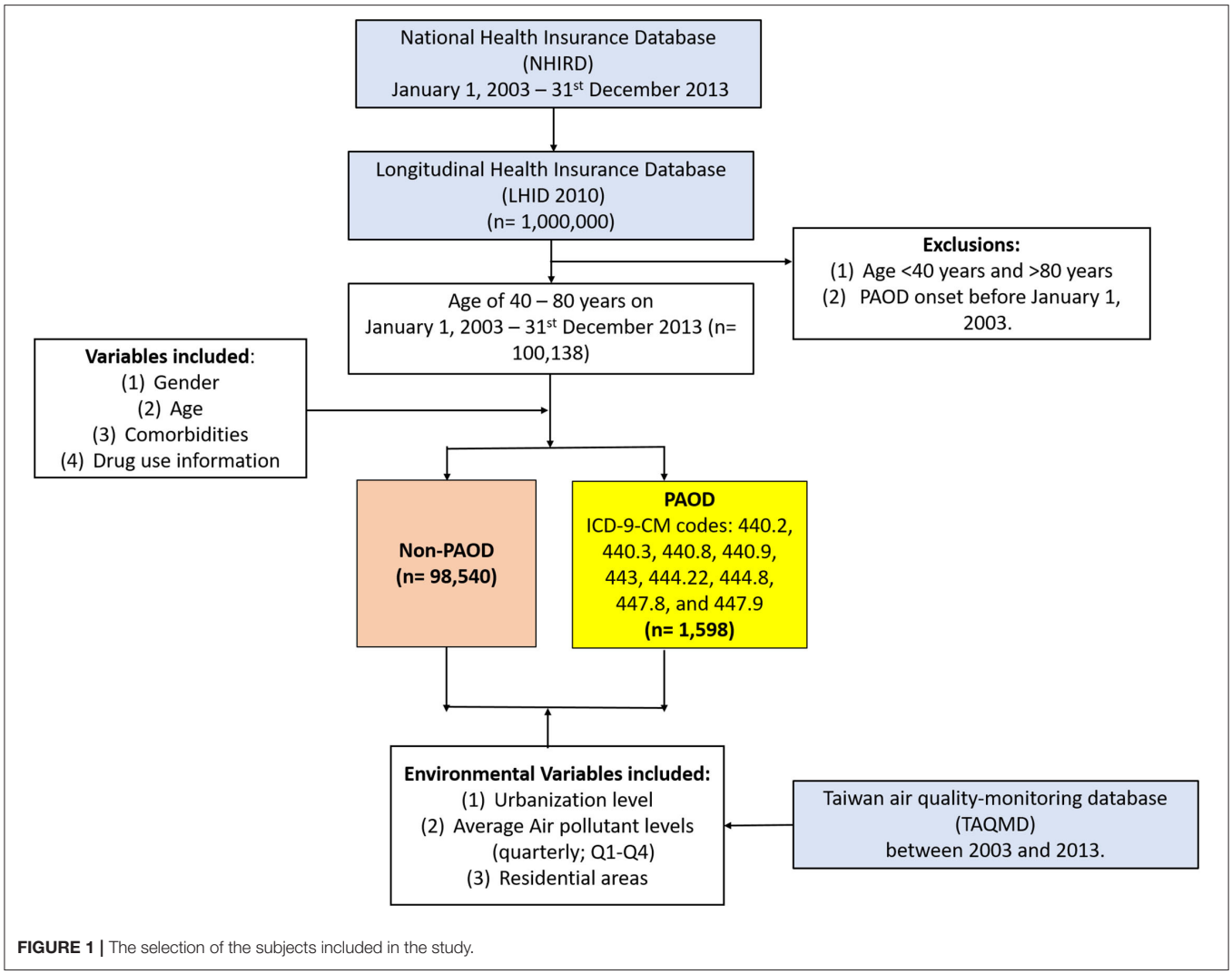

FIGURE 1 | The selection of the subjects included in the study.

number of events was observed in the age group 70–80 years (32.29%), followed by 60–69 years (32.1%), 50–59 years (21.84%), and 40–49 years (13.77%), while the trend was reverse for people with no PAOD. A majority of people with (64.14%) and without PAOD (66.84%) were from the top two urbanized zones (level 4 and level 5), and 69.33% of people with PAOD were from the northern areas of Taiwan, the highest number being from Taipei (37.17%). Comorbidities due to all related conditions were significantly different among people with and without event, with hypertension (42.74%) and DM (30.6%) being present in highest number of PAOD patients, and cancer (3.13%) and CHF (3%) in the lowest number of patients. A similar trend was observed for people without PAOD; however, the proportions of individuals with related conditions were significantly lower. Finally, drug usage was also found to be significantly different between individuals with and without PAOD. Most patients with PAOD were observed to be on drugs for reducing the risk of CVD, such as ARBs, aspirin, ACEIs, and statins. Therefore, to avoid bias due to time-dependent non-baseline variables (exposure to pollution) that changed along with time to event, the fitted models were adjusted by all baseline variables as covariates.

Survival Analysis

The crude HRs for both pollutants ($PM_{2.5}$, CO, NO_2) and baseline variables were obtained using univariate Cox proportional hazards regression models with PAOD event as the outcome. **Table 2** displays the results of the crude HRs and corresponding 95% confidence intervals (95% CIs); all variables were found to be statistically significant through log-rank tests, except certain urbanization levels and certain residential areas (Taipei was significant). Notably, individuals from higher urbanized regions (levels 1 and 2) were observed to be at a significantly higher risk of PAOD incidence in comparison to those from the least urbanized region (level 5) (**Table 2**). Next, each of the pollutants were entered into a multivariate model as a continuous, segmented time-dependent variable, adjusted by all baseline variables as covariates. The results displayed that each of the pollutants, $PM_{2.5}$ (HR = 1.14; 95% CI = 1.13–1.16;

TABLE 1 | Demographic characteristics of PAOD cases and non-PAOD subjects.

Variables	PAOD				*p*-value*
	No (n = 98,540, 98.40%)		Yes (n = 1,598, 1.60%)		
	N	%	*n*	%	
Gender					<0.0001
Female	53,986	54.79	746	46.68	
Male	44,554	45.21	852	53.32	
Age group					<0.0001
40–49	45,593	46.27	220	13.77	
50–59	27,017	27.42	349	21.84	
60–69	16,129	16.37	513	32.1	
70–80	9,801	9.95	516	32.29	
Mean ± SD (years)†	53.55 (10.31)		63.22 (10.34)		<0.0001
Urbanization level					<0.0001
1 (lowest)	6,769	6.87	152	9.51	
2	10,711	10.88	205	12.83	
3	15,179	15.41	216	13.52	
4	32,477	32.97	533	33.35	
5 (highest)	33,355	33.87	492	30.79	
Residential area					0.0022
Northern	14,996	15.22	269	16.83	
Taipei	37,960	38.52	594	37.17	
Central	14,960	15.18	245	15.33	
Southern	8,795	8.93	168	10.51	
Eastern	2,962	3.01	62	3.88	
Kao-Ping	18,867	19.15	260	16.27	
Comorbidities					
Hypertension	17,387	17.64	683	42.74	<0.0001
DM	7,506	7.62	489	30.6	<0.0001
Hyperlipidemia	6,547	6.64	221	13.83	<0.0001
COPD	5,152	5.23	178	11.14	<0.0001
CHF	850	0.86	48	3	<0.0001
CKD	1,920	1.95	117	7.32	<0.0001
Stroke	2,706	2.75	172	10.76	<0.0001
IHD	5,280	5.36	249	15.58	<0.0001
Cancer	2,208	2.24	50	3.13	0.0177
Drug use					
Statin	29,078	29.51	589	36.86	<0.0001
Aspirin	21,779	22.1	651	40.74	<0.0001
Clopidogrel	4,771	4.84	220	13.77	<0.0001
ACEI	22,942	23.28	640	40.05	<0.0001
ARB	30,617	31.07	718	44.93	<0.0001
Warfarin	1,826	1.85	74	4.63	<0.0001
Steroid	27,110	27.51	392	24.53	0.0081

ACEI, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin receptor blockers; CHF, Congestive heart failure; CKD, Chronic kidney disease; COPD, Chronic obstructive pulmonary disease; DM, Diabetes mellitus; IHD, Ischemic heart disease; PAOD, Peripheral artery occlusive disease; SD, Standard deviation.

†*Two-sample t-test.*

p-value < 0.0001), NO_2 (HR = 1.03, 95% CI = 1.02–1.04, p-value < 0.0001), and CO (HR = 2.35, 95% CI = 1.95–2.84, p-value < 0.0001) had a significant positive effect on the risk of PAOD (**Table 2**). Furthermore, for the purpose of illustration, **Figures 2**–**4** show the Kaplan–Meier plots wherein the broadly categorized residential areas were used for predicting PAOD incidence over a 10-year follow-up period. The Kaplan–Meier analysis showed that the most polluted area (Q4) had the highest cumulative incidence of PAOD with prolonged exposure to CO followed by areas Q2, Q3, and Q1 (**Figure 4**). Similarly, prolonged exposure to $PM_{2.5}$ led to the highest cumulative incidence of PAOD for area Q4 followed by Q3, Q2, and Q1

TABLE 2 | Cox model with hazard ratio and 95% confidence interval of PAOD in patients exposed to various daily average concentration of air pollutants.

Variables	Crude		Adjusted model 1		Adjusted model 2		Adjusted model 3	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
$PM_{2.5(}\mu g/m^3$), daily average	1.02 (1.02–1.03)	<0.0001	1.14 (1.13–1.16)	<0.0001	–	–	–	–
NO_2 (ppb), daily average	1.01 (1.00–1.02)	0.0029	–	–	1.03 (1.02–1.04)	<0.0001	–	–
CO (ppm), daily average	1.71 (1.44–2.03)	<0.0001	–	–	–	–	2.35 (1.95–2.84)	<0.0001
Gender								
Female	1.00		1.00		1.00		1.00	
Male	1.42 (1.29–1.57)	<0.0001	1.32 (1.19–1.47)	<0.0001	1.29 (1.17–1.42)	<0.0001	1.29 (1.17–1.43)	<0.0001
Age group								
40–49	1.00		1.00		1.00		1.00	
50–59	2.7 (2.28–3.2)	<0.0001	2.28 (1.9–2.72)	<0.0001	2.4 (2.03–2.85)	<0.0001	2.4 (2.02–2.84)	<0.0001
60–69	6.88 (5.88–8.06)	<0.0001	4.69 (3.93–5.59)	<0.0001	5.07 (4.29–6)	<0.0001	5.03 (4.25–5.95)	<0.0001
70–80	12.88 (11–15.09)	<0.0001	7.18 (5.96–8.64)	<0.0001	7.87 (6.61–9.38)	<0.0001	7.85 (6.59–9.36)	<0.0001
Urbanization level								
1 (lowest)	1.00		1.00		1.00		1.00	
2	1.12 (0.99–1.27)	0.0643	1.15 (0.99–1.33)	0.0598	1.16 (1.01–1.32)	0.038	1.15 (1.01–1.32)	0.0381
3	0.98 (0.83–1.15)	0.7714	0.99 (0.83–1.2)	0.953	1.04 (0.87–1.23)	0.6879	1.05 (0.89–1.25)	0.5497
4	1.32 (1.12–1.56)	0.0008	1.45 (1.19–1.76)	0.0002	1.28 (1.07–1.55)	0.0084	1.24 (1.03–1.49)	0.0209
5 (highest)	1.57 (1.31–1.89)	<0.0001	1.23 (0.99–1.52)	0.0564	1.39 (1.13–1.71)	0.0016	1.36 (1.11–1.67)	0.0032
Residential area								
Northern	1.00		1.00		1.00		1.00	
Taipei	0.87 (0.75–1)	0.0512	1.17 (0.99–1.39)	0.071	0.91 (0.77–1.06)	0.2197	0.88 (0.75–1.03)	0.1127
Central	0.92 (0.77–1.09)	0.3192	0.38 (0.31–0.47)	<0.0001	1.02 (0.86–1.22)	0.8152	1.06 (0.88–1.26)	0.5497
Southern	1.08 (0.89–1.31)	0.4416	0.23 (0.18–0.3)	<0.0001	1.13 (0.92–1.39)	0.2342	1.15 (0.94–1.41)	0.1729
Eastern	1.19 (0.9–1.57)	0.2213	5.16 (3.71–7.16)	<0.0001	1.4 (1.04–1.89)	0.0288	1.19 (0.89–1.59)	0.2328
Kao-Ping	0.78 (0.66–0.92)	0.0038	0.09 (0.07–0.13)	<0.0001	0.77 (0.65–0.92)	0.0031	0.8 (0.67–0.95)	0.0103
Comorbidities (ref = none)								
Hypertension	3.67 (3.32–4.05)	<0.0001	1.35 (1.19–1.55)	<0.0001	1.47 (1.3–1.67)	<0.0001	1.47 (1.3–1.66)	<0.0001
DM	5.75 (5.17–6.39)	<0.0001	2.85 (2.51–3.25)	<0.0001	3.16 (2.8–3.57)	<0.0001	3.17 (2.81–3.57)	<0.0001
Hyperlipidemia	2.26 (1.96–2.61)	<0.0001	1.04 (0.89–1.23)	0.6106	1.06 (0.91–1.24)	0.4352	1.07 (0.92–1.25)	0.4022
COPD	2.48 (2.13–2.9)	<0.0001	1.22 (1.02–1.46)	0.0332	1.36 (1.16–1.6)	0.0002	1.36 (1.16–1.6)	0.0002
CHF	4.43 (3.32–5.9)	<0.0001	1.44 (1.04–2.01)	0.0286	1.42 (1.06–1.91)	0.0207	1.42 (1.05–1.91)	0.0214
CKD	4.46 (3.69–5.38)	<0.0001	2.05 (1.66–2.54)	<0.0001	2.13 (1.76–2.58)	<0.0001	2.14 (1.76–2.59)	<0.0001
Stroke	4.93 (4.21–5.78)	<0.0001	1.51 (1.25–1.82)	<0.0001	1.6 (1.35–1.89)	<0.0001	1.58 (1.34–1.87)	<0.0001
IHD	3.47 (3.03–3.97)	<0.0001	1.1 (0.93–1.29)	0.2803	1.22 (1.05–1.42)	0.0084	1.21 (1.05–1.41)	0.0109
Cancer	1.69 (1.28–2.24)	0.0003	1.04 (0.76–1.41)	0.8184	1 (0.75–1.33)	0.9989	1 (0.76–1.33)	0.9851

(Continued)

TABLE 2 | Continued

Variables	Crude		Adjusted model 1		Adjusted model 2		Adjusted model 3	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Drug use (ref = none)								
Statin	1.35 (1.22–1.49)	<0.0001	0.87 (0.77–0.98)	0.0214	0.79 (0.71–0.89)	<0.0001	0.79 (0.71–0.89)	<0.0001
Aspirin	2.46 (2.23–2.72)	<0.0001	1.3 (1.16–1.47)	<0.0001	1.22 (1.09–1.36)	0.0005	1.22 (1.09–1.36)	0.0005
Clopidogrel	3.19 (2.76–3.67)	<0.0001	1.38 (1.17–1.62)	<0.0001	1.27 (1.09–1.48)	0.0027	1.26 (1.08–1.48)	0.003
ACEI	2.23 (2.02–2.47)	<0.0001	1.15 (1.01–1.3)	0.0314	1.08 (0.96–1.21)	0.2177	1.08 (0.96–1.21)	0.2235
ARB	1.78 (1.62–1.97)	<0.0001	0.85 (0.75–0.96)	0.009	0.72 (0.64–0.81)	<0.0001	0.72 (0.64–0.81)	<0.0001

HR, hazard ratio; CI, confidence interval; CEI, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin receptor blockers; CHF, Congestive heart failure; CKD, Chronic kidney disease; COPD, Chronic obstructive pulmonary disease; DM, Diabetes mellitus; IHD, Ischemic heart disease; PAOD, Peripheral artery occlusive disease; SD, Standard deviation; Model 1: adjusted for $PM_{2.5}$, age, gender, urbanization level, residential area, comorbidity, and drugs in Cox proportional hazards regression. Model 2: adjusted for NO_2, age, gender, urbanization level, residential area, comorbidity, and drugs in Cox proportional hazards regression. Model 3: adjusted for CO, age, gender, urbanization level, residential area, comorbidity, and drugs in Cox proportional hazards regression.

(**Figure 2**). However, for NO_2, the highest cumulative incidence of PAOD was not observed among residents from the worst polluted area but for Q2 residential area followed by Q4, Q3, and Q1 (**Figure 3**). The log-rank tests further confirmed that pollution level (Q1–Q4) was a significant predictor (CO: p-value < 0.0001, $PM_{2.5}$: p-value < 0.0001, and NO_2: p-value < 0.0001) of cumulative incidence of PAOD, and the findings concluded that CO and $PM_{2.5}$ concentrations were strongly positively associated with the cumulative incidence rate of PAOD during the 10-year follow-up period.

DISCUSSION

This study utilizes two national-scale databases from Taiwan and demonstrated that the risk of long-term exposure to $PM_{2.5}$ and CO has a significant positive association with the cumulative risk of incidence of PAOD, through a 10-year follow-up period. Higher risks of PAOD were reported for individuals residing in the most urbanized regions as opposed to those in lower urbanized areas (levels 1 and 2). Moreover, men were at a higher risk than women. Factors such as age, comorbidities, and usage of medications were also demonstrated to impose a higher risk of developing PAOD for individuals with long-term exposure to air pollutants. Hence, this is an important study that highlights the significant adverse effect that environmental factors have on the development of PAOD, a topic that has not received the attention that it warrants.

MESA air (Multi-Ethnic Study of Atherosclerosis and Air Pollution) studies were conducted extensively on participants from six US communities, utilizing high performance pollution exposure models to understand the association between environmental exposures and a wide range of CV-related outcomes. The series of studies provided knowledge about the impact of air pollutants on CV disease thereby offering a research paradigm for improving and developing environmental epidemiology (26–28). However, very few Asian studies exist in the literature that deals with the health effects of air pollution. One such study was conducted on national-level data from Korea; however, it reported that long exposure to SO_2 and NO_2 data was an independent risk factor for PAOD, whereas new incident cases were unaffected by exposure to particulate matter, such as $PM_{2.5}$ and PM_{10} (29). Another study reported a positive association of exposure to ozone (O_3) and CO (air pollution) with the number of emergency admissions for cerebrovascular disease among adults in Taipei (30). Despite its poor prognosis, PAOD is majorly underdiagnosed, underestimated, and undertreated. It has been claimed that there may be ethnic and region-based differences in the Asia-Pacific in terms of epidemiological resources, availability of diagnostic and therapeutic modalities, and patient treatment response. This is why the Asian Pacific Society of Atherosclerosis and Vascular Diseases (APSAVD) initiated an Asia-Pacific Consensus Statement (APCS) for managing and raising awareness for PAOD (10). This study may provide insights into the mechanisms for the development of PAOD, which may then lead to awareness in the general

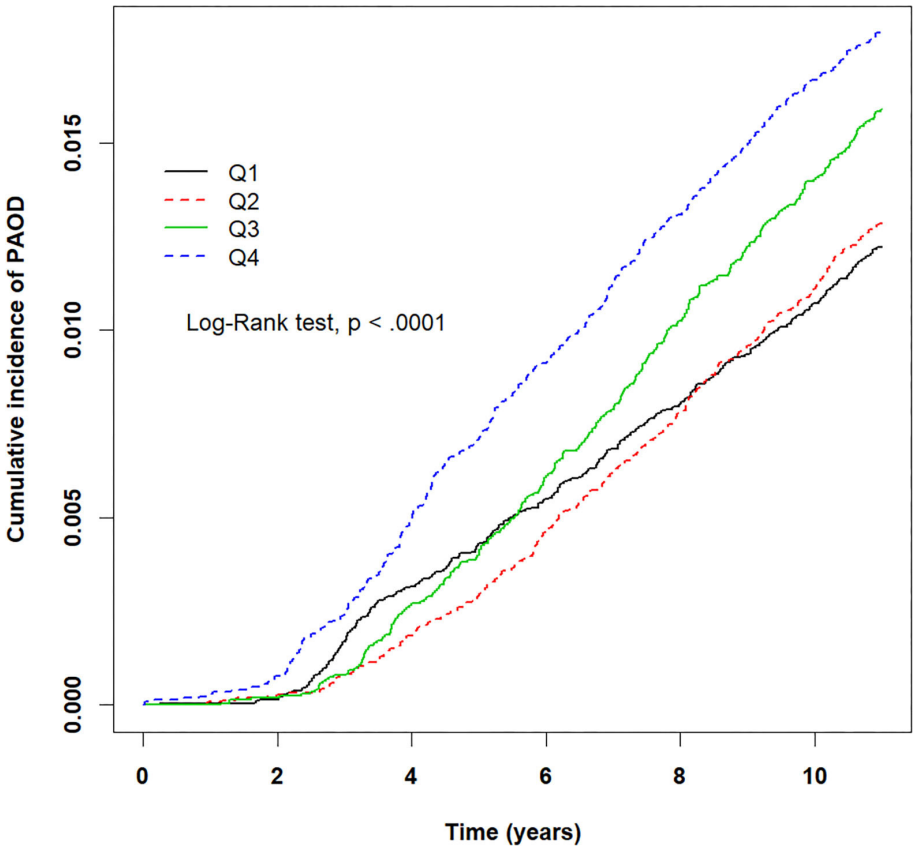

FIGURE 2 | Kaplan–Meier plots for different levels (Q1–Q4) of prolonged exposure to particulate matter $PM_{2.5}$ and its association with cumulative incidence rate of peripheral arterial occlusive disease (PAOD) during a 10-year follow-up period. X-axis displays the time in years. Y-axis displays the cumulative incidence of PAOD. Q1: $PM_{2.5} < 28.24\ \mu g/m^3$, Q2: $28.24 \leq PM_{2.5} < 31.46\ \mu g/m^3$, Q3: $31.46 \leq PM_{2.5} < 38.47\ \mu g/m^3$, and Q4: $PM_{2.5} \geq 38.47\ \mu g/m^3$. $p < 0.05$ is the threshold for significance.

population regarding one of the major risk factors for PAOD incidence among Asians.

CO exposure was found to have a positive and strong association with incident PAOD among the Taiwanese population. CO binds irreversibly to hemoglobin with a binding affinity 200–400 times to that of oxygen, thus leading to hypoxia and tissue damage. Moreover, long-term CO exposure could amount to an enhanced thickening of the carotid intima-media and elevated serum C-reactive protein, thereby conferring a higher risk for patients to develop CVD (31). Exposure to CO levels at urbanized regions has also been demonstrated to have an association with reduced myocardial perfusion reserve, implying coronary endothelial dysfunction (32). Furthermore, CO leads to atherogenic processes both in coronary arteries and in peripheral artery systems. Case reports on acute CO poisoning have been shown to result in subsequent arterial thrombosis in the intracardiac region, and there have been studies reporting hypercoagulative states in patients with CO exposure, leading to development of atherosclerotic or thrombotic diseases (33).

$PM_{2.5}$ exposure was also indicated as a risk factor for PAOD through our analysis. Oxidative stress, systemic inflammation, endothelial dysfunction, atherothrombosis, and arrhythmogenesis (18) are pathways that have been linked between $PM_{2.5}$ and CV mortality. $PM_{2.5}$ can lodge in the blood stream through the smallest airways and alveoli, penetrating the alveolar-capillary membrane, thereby leading to a high risk of weakening and rupture of vessels and to potential CV events.

Levels of CO and NO_2 are mainly attributed to emissions from vehicles, especially in urbanized places. Thermal power plants located in central Taiwan are responsible for particulate matter in the air; however, they are carried north, to Taipei, by the wind (34). Several other factors, such as the southwesterly monsoonal flow and northeasterly monsoonal flow, and geographical division, have an influence on the $PM_{2.5}$ concentrations. Taiwan is an island with a central mountain range running from north to south, and the complex topographical and geographical diversification leads to variations in $PM_{2.5}$. Furthermore, $PM_{2.5}$ is affected by the seasons, and its concentration starts to rise in autumn (September, October, and November) and winter (November, December, and January) until it peaks in spring (February, March, and April) and subsequently declines to a minimum during summer (May, June, and July). Hence, individuals' exposure to pollution was quantified using all the above factors through their residential areas along with the

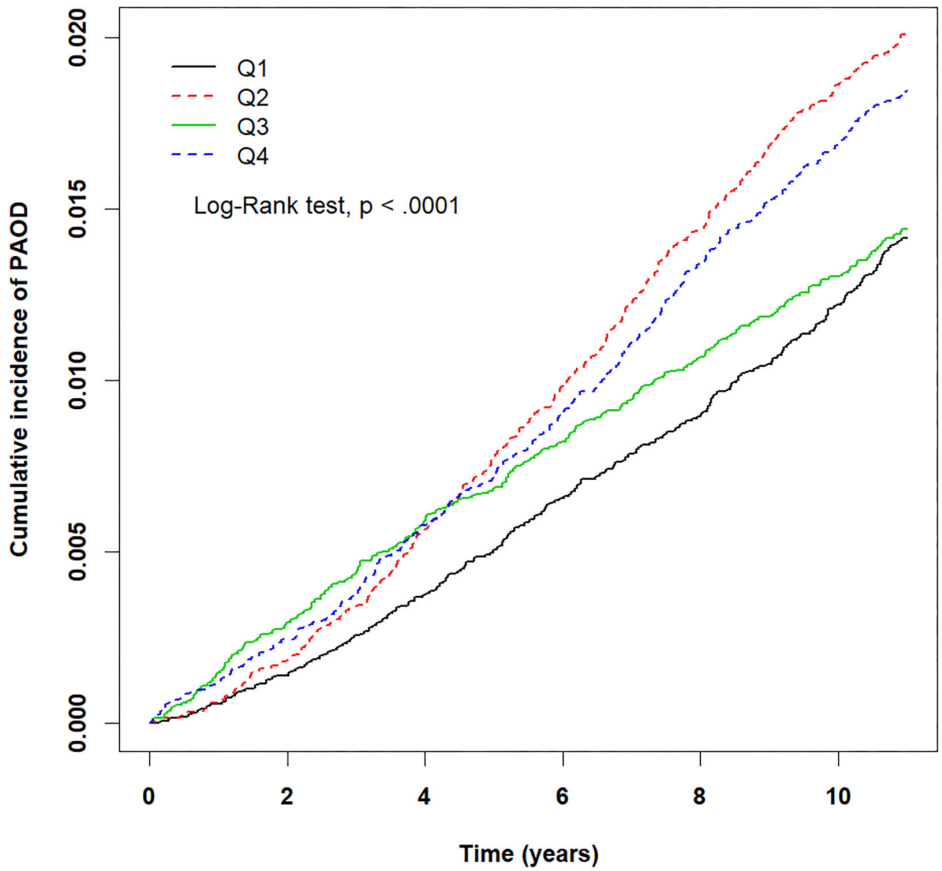

FIGURE 3 | Kaplan–Meier plots for different levels (Q1–Q4) of prolonged exposure to nitrogen dioxide (NO_2) and its association with cumulative incidence rate of peripheral arterial occlusive disease (PAOD) during a 10-year follow-up period. X-axis displays the time in years. Y-axis displays the cumulative incidence of PAOD. Q1: $NO_2 < 16.14$ ppb, Q2: $16.14 \leq NO_2 < 20.49$ ppb, Q3: $20.49 \leq NO_2 < 24.90$ ppb, and Q4: $NO_2 \geq 24.90$ ppb. $p < 0.05$ is the threshold for significance.

corresponding concentrations of NO_2, $PM_{2.5}$, and CO (Q1–Q4). This diversity in pollution among residential areas explains the differences in incident PAOD among geographical locations.

Therefore, based on the findings from this study, measures are required to minimize air pollution exposure and effective national pollution control plans need to be implemented in Taiwan. Physical activities of the elderly, children, and the general public should be strictly practiced in parks and gardens instead of primary traffic areas. Limiting outdoor activities during peak traffic hours, and wearing masks while outdoors, should be practiced at the individual level. Lastly, proper advice by health professionals regarding healthy living should be implemented. To have a comprehensive understanding of the far-reaching adverse effects of air pollution, follow-up studies will be conducted to fathom the effects of exposure to air pollutants on CVDs and other major health-related outcomes, for the Taiwanese population.

This study has several limitations. PAOD is based on the ICD-9-CM codes, which could be incorrect, hence there could be some misclassifications. However, prior studies on national cohorts have validated ICD-9-CM codes for diagnosis of chronic diseases (35–39). Moreover, the coverage of NHIRD is representative of the general population of Taiwan because of the universal reimbursement policy as operated by the government. The NHI Bureau of Taiwan also conducts extensive reviews of medical charts and imposes heavy penalties for any form of malpractice. In light of such strict measures, all ICD-9-CM codes for determination of PAOD are recorded as accurately as possible by the physicians after extensive assessments during the reimbursement process based on clinical and laboratory data. A second limitation of this study is the unavailability of some crucial CV-related parameters in the NHIRD, such as smoking status, body mass index (BMI), obesity, alcoholism, exercise, and dietary habits, which have been established as strong risk factors for PAOD (40). This was important, as ∼25% of the population in Taiwan is obese, and a similar proportion of people use tobacco products. Also, prior studies have established smoking to be a strong risk factor for PAOD (41). To try to account for the effect of BMI and obesity, we have adjusted our model using hypertension, DM, and hyperlipidemia in our analysis. Furthermore, to adjust for the effects of smoking, we have incorporated smoking-related disorders such as COPD and cerebral vascular disease (stroke) in our fitted model.

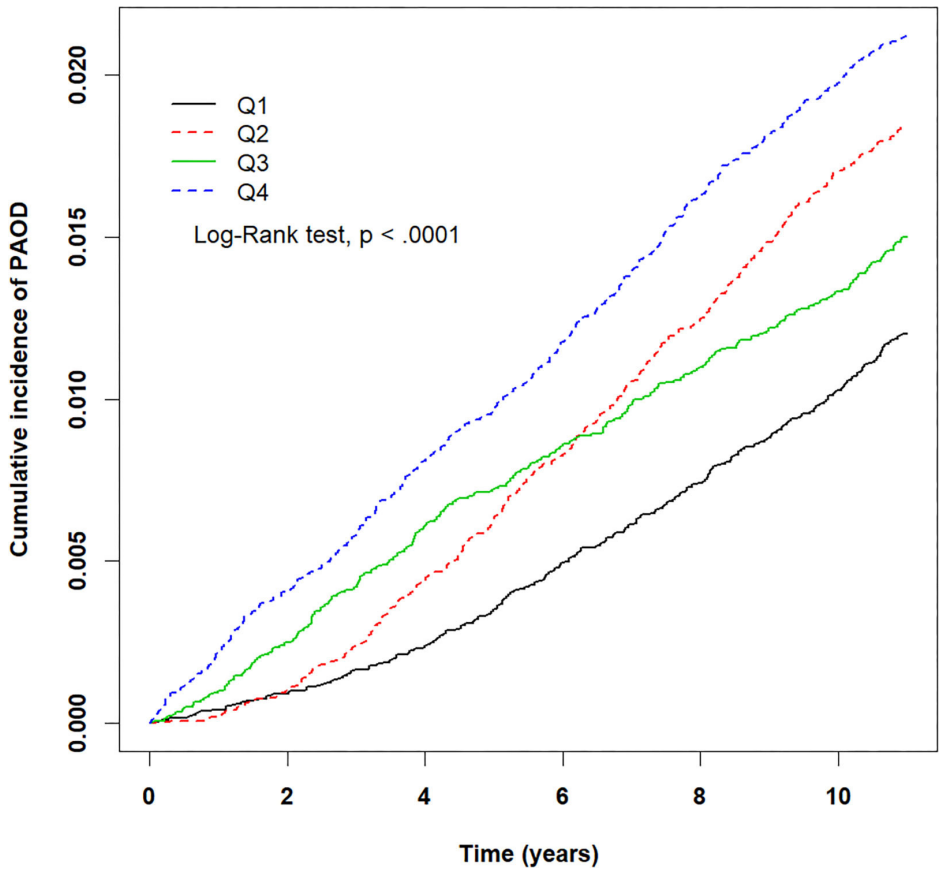

FIGURE 4 | Kaplan–Meier plots for different levels (Q1–Q4) of prolonged exposure to carbon monoxide (CO) and its association with cumulative incidence rate of peripheral arterial occlusive disease (PAOD) during a 10-year follow-up period. X-axis displays the time in years. Y-axis displays the cumulative incidence of PAOD. Q1: CO < 0.47 ppm, Q2: 0.47 ≤ CO < 0.58 ppm, Q3: 0.58 ≤ CO < 0.68 ppm, and Q4: CO ≥ 0.68 ppm. $p < 0.05$ is the threshold for significance.

CONCLUSION

This study showed that, in addition to well-known risk factors, PAOD exhibits a high risk of incidence in a 10-year follow-up period for subjects undergoing prolonged exposure to air pollutants such as $PM_{2.5}$ and CO. Findings from this study could be used to inform governmental institutions to develop effective pollution control plans to not only reduce the risk of overall incidence of PAOD but to also reduce the eventual risk of CV morbidity and mortality in an aging society such as Taiwan.

DATA AVAILABILITY STATEMENT

Data are available from the National Health Insurance Research Database (NHIRD) published by Taiwan National Health Insurance Administration (NHIA). Due to legal restrictions imposed by the government of Taiwan in relation to the "Personal Information Protection Act", data cannot be made publicly available. Requests for data can be sent as a formal proposal to the NHIRD (http://nhird.nhri.org.tw). The authors did not have any special access privileges that others would not have.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Taichung Veterans General Hospital, Taichung, Taiwan. 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

M-SH, S-HL, and C-SC: conceptualization and writing—original draft. S-HL and C-SC: formal analysis, investigation, and methodology. M-SH, L-HJ, S-YH, C-KH, and VH: project administration. C-SC: resources. M-SH, L-HJ, and C-SC: supervision. C-KH: validation. M-SH, L-HJ, C-KH, and VH: writing—review and editing. All authors have read and approved the manuscript for publication.

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