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Cardiorenal syndromes: From pathogenesis to clinical research

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Cardiorenal syndromes: From pathogenesis to clinical research

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The Association of Morning Hypertension With Target Organ Damage in Patients With Chronic Kidney Disease and Hypertension

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Objectives: To determine the association between morning hypertension and target organ damage (TOD) in patients with chronic kidney disease (CKD) and hypertension.

Methods: In this cross-sectional study, 447 patients with CKD and hypertension from two centers were enrolled. Ambulatory blood pressure monitoring was conducted in all patients. Linear regression and logistic regression analysis were used to determine the association between morning hypertension and TOD in patients with CKD and hypertension, including assessments of estimated glomerular filtration rate (eGFR), left ventricular mass index (LVMI), urine protein/creatinine ratio (UPCR), and left ventricular hypertrophy (LVH).

Results: Overall, 194 (43.4%) participants had morning hypertension. Morning hypertension was strongly correlated with LVH [odds ratio (OR), 2.14; 95% confidence interval (CI), 1.3–3.51; $p < 0.01$], lower level of eGFR ($\beta = -0.51$; 95%CI, −0.95–−0.08; $p < 0.05$), higher LVMI ($\beta = 0.06$; 95%CI, 0.04–0.08, $p < 0.001$), and UPCR ($\beta = 0.22$; 95%CI, 0.06–0.38, $p < 0.01$), independent of nocturnal hypertension and elevated morning blood pressure surge. As a continuous variable, both morning systolic blood pressure (SBP) and diastolic blood pressure (DBP) were found to be associated with LVH and higher level of UPCR and LVMI ($p < 0.05$), whereas only morning SBP was negatively correlated with eGFR ($p < 0.01$).

Conclusion: Morning hypertension was strongly correlated with cardiac damage and impaired kidney function in CKD patients with hypertension, independent of nocturnal hypertension and morning surge in blood pressure. Morning hypertension in CKD patients warrants further attention.

Keywords: morning hypertension, ambulatory blood pressure monitoring, chronic kidney disease, left ventricular mass index, urinary protein-creatinine ratio, estimated glomerular filtration rate

INTRODUCTION

Hypertension is very common in patients with chronic kidney disease (CKD), with the prevalence of 67–92% (1); because blood pressure (BP) measured using ambulatory blood pressure monitoring (ABPM) strongly associated cardiovascular (CV) events and renal outcomes, ABPM is considered the preferred metric of BP in both the general population and CKD patients (2). Furthermore, compared with office BP, ABPM is a better predictor of hypertension-mediated organ damage (3) and a more sensitive risk factor for CV events and mortality (4, 5). Therefore, according to current guidelines, ABPM is recommended for application in clinical practice (6).

Morning hypertension has been a recent research focus. It was shown to be associated with target organ damage (TOD) including left ventricular mass index (LVMI), urine albumin/creatinine ratio, maximum carotid intima media thickness (7, 8), and CV events (9, 10) in general or hypertensive patients. Experts from Asia suggested that the measurement and treatment of morning hypertension should be an indispensable part of treatment for hypertensive patients (11). However, studies on the association between morning hypertension and TOD in CKD patients are scarce. It is therefore imperative to investigate the prevalence and role of morning hypertension in CKD patients with TOD, given the high risk of progression to end-stage renal disease and CV damage in these patients.

Morning hypertension is consistently accompanied by nocturnal hypertension or elevated morning surge in blood pressure, which are also reported to be associated with TOD (12, 13). Therefore, some researchers suggested that the association between morning hypertension and TOD may be attributed to nocturnal hypertension or morning blood pressure surge (14, 15). In a recent cross-sectional study, Ye et al. demonstrated that the impact of morning hypertension on LVMI is dependent on the morning surge in normotensive patients (8). Additionally, a study conducted by Oh et al. found that only morning hypertension combined with elevated nocturnal hypertension was associated with vascular organ damage and high central BP (16).

We conducted the present cross-sectional study to further our understanding of the association between morning hypertension and TOD in patients with CKD and hypertension and to determine whether this association is independent of nocturnal hypertension and the morning surge in blood pressure.

MATERIALS AND METHODS

Participants

This cross-sectional study included patients from two centers (West China Hospital and Chengdu Seventh People's Hospital) in China. Adult patients were eligible if they (1) had CKD, (2) were diagnosed with hypertension, and (3) agreed to undergo 24 h ABPM. Exclusion criteria were patients (1) on dialysis (hemodialysis or peritoneal dialysis), (2) with history of malignancy, (3) with <70% valid records on 24 h ABPM, and (4) who were pregnant. Patients with albuminuria (albumin/creatinine ratio ≥30 mg/g) or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 or abnormalities of kidney structure for over 3 months were diagnosed with CKD (17). eGFR was determined from serum creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (18). The study protocol was approved by the ethics committee of the West China Hospital, Sichuan University, and was approved by the Institutional Review Board. Written informed consent was obtained from all patients.

A total of 447 CKD patients formed the cohort of this cross-sectional study. Baseline evaluation including medical history, physical examination, and laboratory tests were recorded at the time patients visited the outpatient clinic or were admitted to hospital. These data included sex, age, body mass index (BMI), current alcohol consumption, current smoking, CV history, antihypertensive drugs, diabetes mellitus (DM), biochemical parameters, urinary protein test, and two-dimensional echocardiogram.

Blood Pressure Measurements

Experienced nurses measured office BP when participants were admitted to our hospital or visited the outpatient clinic. After patients were allowed to rest quietly for 5–10 min, the mean value of three consecutive BP measurements at 5-min intervals using a mercury sphygmomanometer was recorded. The 24-h ABPM was performed using Space labs 90217 devices (Space labs Medical, Redmond, WA, USA) at West China Hospital and an ABPM 6100 (Welch Allyn, Chicago, IL, USA) at Chengdu Seventh People's Hospital, with BP readings set at 20-min intervals from 6:00 a.m. to 10:00 p.m., and 30- or 60-min intervals from 10:00 p.m. to 6:00 a.m. Day- and nighttime BPs were defined as mean BP during the period from 6:00 a.m. to 10:00 p.m. and 10:00 p.m. to 6:00 a.m., respectively. Patients were instructed to undergo their usual activities and take antihypertensive drugs as usual and were encouraged to sleep no later than 10:00 p.m. and to get up at approximately 6:00 a.m. ABPM was not conducted if the patient worked nightshifts. The time patients went to bed and woke up was recorded. A measurement with at least 70% of diurnal and nocturnal BP readings was regarded as a successful ABP. The ABP must be taken within 3 days after the measurement of office BP. Both office BP and ABP measurements were taken from the non-dominant arm with an appropriate cuff size based on arm circumference at the time of enrollment.

Cardiac Assessment

Echocardiography was performed by two experienced ultrasonologists according to standardized procedures, as previously reported (19). The linear method was applied to quantify LV mass (20): LVM (g) = $0.8 \times 1.04 \times$ [(interventricular septum + LV internal diameter + posterior wall thickness)^3 − LV internal diameter^3] + 0.6 g. Left ventricular hypertrophy (LVH) was defined according to the 2015 American Society of Echocardiography/European Association of Cardiovascular Imaging chamber quantification document, with LVMI per body surface area >95g/m^2 in women and 115 g/m^2 in men (19).

Definitions

Morning hypertension was defined as mean BP ≥135/85 within 2 h after waking (11). Morning surge in blood pressure was defined as SBP during the 2-h period immediately after waking minus the average of three SBP readings centered around the lowest nighttime SBP value (21). Patients were divided into the elevated morning surge group (≥15 mmHg) and low morning surge group (<15 mmHg) according to the third quantile. Dipping patterns of BP and heart rate (HR) were calculated according to the following formula: mean night/day ratio of SBP, DBP, and HR. Patients were diagnosed as dippers if the ratio was >0.8–0.9, non-dippers if the ratio was >0.9–1, or reverse dippers if the ratio was >1 (22). Patients were defined to have achieved the goal for ambulatory BP when 24-h, daytime, and nighttime BP was <130/80, <135/85, and <120/70 mmHg, respectively, and to have the goal for office BP when the BP was <140/90 (23). CV history was defined as history of heart disease, peripheral vascular disease, or cerebral vascular disease.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY, USA) and GraphPad Prism 8.0.1. Normally distributed data are presented as mean ± standard deviation, skewed data as median values with interquartile range, and categorical variables as numbers with percentage. Log-transformation or square root calculations were applied for normal transformation. Data were analyzed using the chi-square test or Fisher's exact test for categorical variables, the Student's *t*-test for normally distributed data, and the Wilcoxon rank-sum test for continuous skewed variables.

Receiver-operating characteristic (ROC) curve analysis was conducted, and the area under the curve (AUROC) was calculated to assess the ability of night blood pressure and morning blood pressure surge to predict morning hypertension. Binary logistic regression was applied to detect the factors associated with morning hypertension and to calculate the risk of LVH correlated with morning hypertension. Linear regression analysis was used to identify the association between morning hypertension and eGFR, UPCR, and LVMI. The regression β-coefficient represented the contribution of the independent variables to the dependent variables. To determine whether the correlation between morning hypertension and TOD was independent of night hypertension or morning surge, we added night hypertension and morning surge to the regression model in models 2 and 3, respectively. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, and a two-tailed $p < 0.05$ was considered statistically significant.

TABLE 1 | Demographic and clinical characteristics between morning hypertension and morning normotension groups.

Variables	Morning normotension (*n* = 253)	Morning hypertension (*n* = 194)	*P*
Age	72 (59, 79)	68 (56, 78)	NS
Gender (male, n, %)	112 (44.3)	88 (45.4)	NS
BMI	23.88 (22.23, 26.26)	24 (22.49, 26.72)	NS
Smoking	39 (15.4)	29 (14.9)	NS
Alcohol	36 (14.2)	12.4	NS
CV history	83 (32.8)	62 (32)	NS
DM	154 (60.9)	123 (63.4)	NS
CCB	137 (54.2)	146 (75.3)	<0.001
RAS	114 (45.1)	96 (49.5)	NS
Diuretic	30 (11.9)	29 (14.9)	NS
β blocker	72 (28.5)	72 (37.1)	NS
α blocker	6 (2.4)	20 (10.3)	<0.001
Numbers of drugs (>2)	41 (16.2)	50 (25.8)	0.013
Biomarkers			
Red blood cells	4.05 (0.70)	4.01 (0.90)	NS
Hemoglobin	120.03 (21.37)	116.85 (24.90)	NS
White blood cells	6.15 (4.74, 7.61)	6.69 (5.44, 8.42)	0.003
Neutrophil	0.65 (0.12)	0.68 (0.11)	0.007
Lymphocyte	0.24 (0.11)	0.22 (0.10)	0.049
Total protein	68.44 (6.99)	66.71 (9.91)	0.031
Albumin	39.50 (5.27)	37.62 (6.89)	0.001
Creatinine	111.8 (88.95, 143.15)	126.3 (95.95, 182.3)	0.001
Uric acid	344.36 (278, 435)	374 (311.75, 456.75)	0.005
Glucose	6.5 (5.2, 8.8)	6.49 (5.27, 9.44)	NS
Potassium	**4.07 (0.55)**	**4.08 (0.60)**	**NS**
Sodium	140.1 (137.7, 142.5)	140.20 (137.70, 142.60)	NS
Chlorine	105.93 (4.95)	105.92 (5.52)	NS
Calcium	2.30 (2.18, 2.43)	2.27 (2.12, 2.41)	0.036
Phosphorus	1.04 (0.92, 1.22)	1.14 (0.97, 1.29)	<0.001
Magnesium	0.86 (0.80, 0.93)	0.85 (0.80, 0.93)	NS
Cholesterol	4.31 (3.60, 5.18)	4.49 (3.70, 5.34)	NS
Triglyceride	1.49 (0.96, 2.23)	1.52 (1.08, 2.41)	NS
High density lipoprotein	1.18 (0.96, 1.44)	1.17 (0.92, 1.52)	NS
Low density lipoprotein	2.72 (2.02, 3.43)	2.60 (1.93, 3.43)	NS

BMI, body mass index; CV, cardiovascular; DM, diabetes mellitus; CCB, calcium channel blocker; RAS, renin–angiotensin system.

RESULT

Baseline Features

In total, 583 patients were enrolled. A total of 136 were excluded because of the following factors: complication with malignant tumors (5), incomplete ABPM data (50), and dialysis (81). Finally, 447 patients with CKD and hypertension were included in this study. Two hundred (44.7%) patients were male, and the mean age was 67 ± 14 years. A total of 194 (43.4%) participants had morning hypertension, among which 186 (95.9%) had nocturnal hypertension, and 97 (50%) had morning surge in blood pressure with ≥15 mmHg. A total of 320 (71.6%) participants had nocturnal hypertension, and the morning surge in 152 (34%) patients was ≥15 mmHg.

Baseline features of the participants grouped according to morning BP are shown in **Table 1**. No differences in age, sex, current smoking, current alcohol consumption, CV history,

TABLE 2 | The blood pressure characteristics from ABPM between morning hypertension and morning normotension groups.

Variables	Morning normotension ($n = 253$)	Morning hypertension ($n = 194$)	*p*
Office SBP	138 (124, 148)	145 (134, 160)	<0.001
Office DBP	78 (70, 85)	81.5 (73.5, 92)	<0.001
Office BP control	129 (51)	61 (31.4)	<0.001
24 h			
SBP	120.32 (11.72)	142.08 (13.69)	<0.001
DBP	65.53 (9.00)	76.41 (12.72)	<0.001
Heart rate	72 (10)	75 (11)	0.047
24 h BP control	195 (77.1)	24 (12.4)	<0.001
Day			
SBP	121.02 (12.08)	142.75 (13.60)	<0.001
DBP	66.47 (9.58)	77.20 (13.19)	<0.001
Heart rate	74 (10)	76.44 (11.98)	0.015
Day BP control	215 (85)	42 (21.6)	<0.001
Night			
SBP	118.74 (13.19)	140.79 (17.02)	<0.001
DBP	63.41 (9)	74.48 (12.83)	<0.001
Heart rate	65 (61, 73)	69 (62.75, 76)	0.01
Night BP control	119 (47)	8 (4.1)	<0.001
SBP dipping	0.98 (0.07)	0.99 (0.08)	NS
DBP dipping	0.96 (0.08)	0.97 (0.09)	NS
Heart rate dipping	0.92 (0.08)	0.92 (0.08)	NS
Morning surge in blood pressure	6.21 (12.38)	15.74 (14.98)	<0.001
Elevated morning surge in blood pressure	55 (21.7)	97 (50)	<0.001
ABP control	118 (46.6)	7 (3.6)	<0.001
Morning SBP	118.74 (10.48)	149.63 (14.64)	<0.001
Morning DBP	66.26 (9.49)	81.21 (14.50)	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; ABP, ambulatory blood pressure.

TABLE 3 | Binary logistic regression analysis for factors associated with morning hypertension.

Variables	OR (95% ci)	p
Night SBP	1.20 (1.11, 1.30)	<0.001
Night DBP	1.02 (0.92, 1.14)	NS
Day SBP	1.02 (0.95, 1.10)	NS
Day DBP	1.07 (0.95, 1.20)	NS
Morning surge in blood pressure	1,20 (1.15, 1.26)	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure.

or DM was found between the two groups. Compared with those with morning normotension, the proportion of use of calcium channel blockers, α blockers, and over two kinds of antihypertensive drugs was higher in patients with morning hypertension. Higher levels of white blood cells, neutrophils, creatinine, uric acid, and phosphorus were observed in the morning hypertension group, whereas the levels of lymphocytes, total protein, albumin, and calcium were lower in this group (**Table 1**). Regarding the BP parameters, control of 24 h, daytime, and nighttime BP and that measured at the clinic were all poorer in the morning hypertension group ($p < 0.001$). No differences were found in SBP, DBP, or HR dipping between the two groups (**Table 2**).

Association between nighttime blood pressure and morning surge in blood pressure with morning hypertension.

Among patients in the morning hypertension group, only 4.1% had normal nighttime BP, compared with 47% in the morning normotension group ($p < 0.001$). Moreover, 97 (50%) patients with morning hypertension had elevated morning surge, while the rate was 21.7% in patients with morning normotension ($p < 0.001$) (**Table 2**). According to the multivariate logistic regression model, nighttime SBP (OR, 1.2; 95%CI, 1.11–1.3) and morning surge (OR, 1.2; 95%CI, 1.15–1.26) were independently correlated with morning hypertension ($p < 0.001$) (**Table 3**).

To assess the ability to use nighttime BP and morning surge in blood pressure for predicting morning hypertension, ROC curve analysis was performed (**Figure 1**). Nighttime SBP could better distinguish morning hypertension (AUROC, 0.85; 95%CI, 0.81–0.88; $p < 0.001$), compared with nighttime DBP (AUROC, 0.75; 95%CI, 0.71–0.88; $p < 0.001$) and morning surge (AUROC, 0.69; 95%CI, 0.65–0.74; $p < 0.001$). The optimal cutoff points for morning hypertension were 130 mmHg for nighttime SBP (sensitivity, 73.2%; specificity, 83%) and 70 mmHg for nighttime DBP (sensitivity, 62%; specificity, 75%).

Association Between Morning Hypertension and Target Organ Damage

According to univariate analysis, both morning hypertension and nocturnal hypertension were shown to be associated with higher level of LVMI and UPCR, lower eGFR ($p < 0.01$), and LVH ($p < 0.001$), whereas only higher level of UPCR was found to correlate with elevated morning surge in blood pressure ($p = 0.026$) (**Supplementary Tables 1**–**3**).

According to multivariate linear and logistic regression analysis, morning hypertension correlated strongly with lower level of eGFR ($\beta = -0.51$; 95%CI, −0.95–−0.08; $p < 0.05$), higher level of LVMI ($\beta = 0.06$; 95%CI, 0.04–0.08; $p < 0.001$), UPCR ($\beta = 0.22$; 95%CI, 0.06–0.38; $p < 0.01$), and LVH (OR, 2.14; 95%CI, 1.3–3.51; $p < 0.01$) when nocturnal hypertension and elevated morning surge in blood pressure were considered (**Table 4**, model 3). After further adjustments of baseline features (age, sex, BMI, current alcohol consumption, current smoking, DM, and CV history), biochemical indices, and office BP control, the significance did not change. When morning hypertension was considered, TOD was not shown to be associated with nocturnal hypertension and elevated morning surge in blood pressure, except for UPCR, which was higher in patients with nocturnal hypertension (**Table 4**). However, the difference was not significant after adjustment of biochemical indices and office BP.

To further assess the correlation between morning BP and TOD, morning SBP and DBP were analyzed

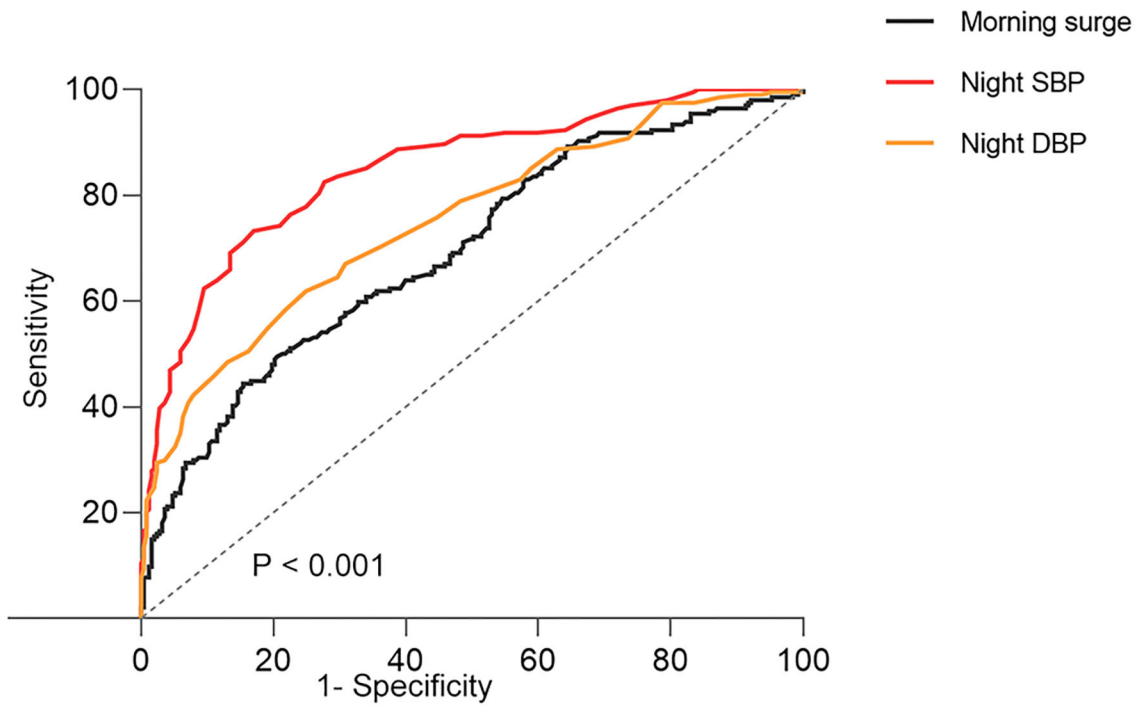

FIGURE 1 | The ROC curves of morning surge in blood pressure and night blood pressure about morning hypertension. SBP, systolic blood pressure; DBP, diastolic blood pressure; ROC, receiver-operating characteristic.

TABLE 4 | Multivariate linear regression and binary logistic regression analysis of BP indexes and target organ damage.

Variables	Model 1	Model 2	Model 3	Model 4	Model 5
$\sqrt{eGFR}$					
Morning hypertension	−0.58 (−0.94, −0.22)#	−0.44 (−0.85, −0.03)a	−0.51 (−0.95, −0.08)a	−0.52 (−0.95, −0.09)a	−0.47 (−0.83, −0.11)#
Nocturnal hypertension	–	−0.33 (−0.78, 0.12)	−0.29 (−0.75, 0.17)	−0.39 (−0.84, 0.07)	−0.16 (−0.54, 0.21)
Elevated morning surge in blood pressure	–	–	0.19 (−0.21, 0.59)	0.09 (−0.31, 0.49)	0.06 (−0.27, 0.39)
Lg LVMI					
Morning hypertension	0.06 (0.05, 0.08)*	0.05 (0.03, 0.07)*	0.06 (0.04, 0.08)*	0.06 (0.04, 0.08)*	0.05 (0.03, 0.07)*
Nocturnal hypertension	–	0.03 (0.002, 0.05)a	0.02 (−0.002, 0.05)	0.02 (−0.001, 0.05)	0.02 (−0.01, 0.04)
Elevated morning surge in blood pressure	–	–	−0.02 (−0.04, 0.004)	−0.02 (−0.04, 0.01)	−0.01 (−0.03, 0.01)
Lg UPCR					
Morning hypertension	0.35 (0.22, 0.48)*	0.26 (0.12, 0.41)*	0.22 (0.06, 0.38)#	0.22 (0.07, 0.38)#	0.15 (0.02, 0.29)a
Nocturnal hypertension	–	0.21 (0.04, 0.37)#	0.23 (0.07, 0.40)#	0.22 (0.06, 0.38)#	0.11 (−0.03, 0.25)
Elevated morning surge in blood pressure	–	–	0.11 (−0.04, 0.26)	0.10 (−0.05, 0.25)	0.08 (−0.05, 0.20)
LVH					
Morning hypertension	2.59 (1.71, 3.92)*	2.08 (1.31, 3.31)#	2.14 (1.30, 3.51)#	2.13 (1.26, 3.61)#	1.86 (1.08, 3.26)a
Nocturnal hypertension	–	1.74 (0.98, 3.10)	1.72 (0.96, 3.07)	1.97 (1.06, 3.65)a	1.73 (0.91, 3.26)
Elevated morning surge in blood pressure	–	–	0.93 (0.59, 1.48)	1.06 (0.65, 1.74)	1.20 (0.72, 1.99)

*$p < 0.001$; #$p < 0.01$; a$p < 0.05$. Model 1: morning hypertension; model 2: model 1 + nocturnal hypertension; Model 3: model 2 + morning surge in blood pressure; Model 4: model 3 + age, sex, BMI, alcohol, smoking, CV history, diabetes mellitus; Model 5: model 4 + hemoglobin, albumin, sodium, potassium, triglyceride, office BP control for eGFR; hemoglobin, albumin, sodium, potassium, creatinine, uric acid, triglyceride, office BP control for Lg LVMI, hemoglobin, albumin, glucose, p*ca, creatinine, cholesterol, high-density lipoprotein, low-density lipoprotein, office BP control for Lg PCR; hemoglobin, albumin, creatinine, sodium, potassium, triglyceride, high-density lipoprotein, low-density lipoprotein, office BP control for LVH. LVMI, left ventricular mass index; UPCR, urine protein–creatinine ratio; eGFR, estimated glomerular filtration rate.*

as continuous variables in the regression model. Both morning SBP and DBP were found to be associated with LVH, higher UPCR, and LVMI (**Figure 2**, **Supplemental Table 4**). Regarding eGFR, only morning SBP was shown to be negatively correlated with eGFR (all $p < 0.01$), and the correlation between morning

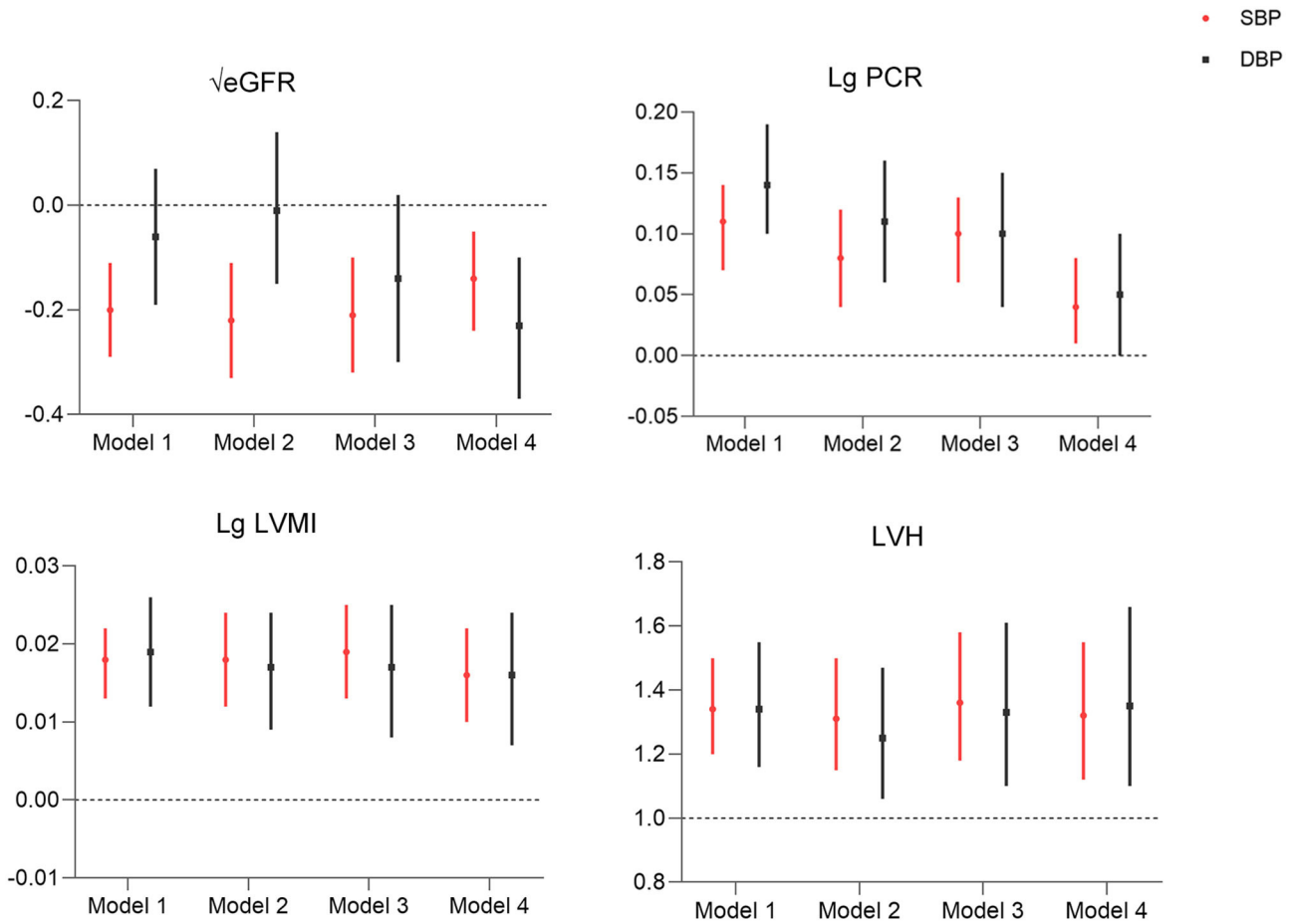

FIGURE 2 | Association of morning systolic blood pressure and diastolic blood pressure (per 10 mmHg) with target organ damage. Model 1: morning hypertension; model 2: model 1 + nocturnal hypertension + morning surge in blood pressure; model 3: model 2 + age, sex, BMI, alcohol, smoking, CV history, DM; model 4: model 3 + hemoglobin, albumin, Na, k, TG, office BP control for eGFR; hemoglobin, albumin, Na, K, creatinine, uric acid, TG, office BP control for Lg LVMI, hemoglobin, albumin, glucose, p*ca, creatinine, CHOL, HDL, LDL, office BP control for Lg PCR; hemoglobin, albumin, Creatinine, Na, K, TG, HDL, LDL, office BP control for LVH. LVMI, left ventricular mass index; PCR, protein–creatinine ratio; eGFR, estimated glomerular filtration rate; LVH, left ventricular hypertrophy.

DBP and eGFR was only significant in model 4 (**Figure 2**, **Supplementary Table 4**).

DISCUSSION

In this cross-sectional study, we found that the prevalence of morning hypertension in CKD patients was 43.4 and 95.9% of these patients also had nocturnal hypertension. Morning hypertension was primarily determined by nighttime BP and morning surge in blood pressure, and nighttime SBP distinguished morning hypertension effectively (AUROC, 0.85; 95%CI, 0.81–0.88; $p < 0.001$). Morning hypertension was strongly correlated with TOD in CKD patients, including cardiac damage (LVH, higher level of LVMI) and kidney function decline (higher UPCR and lower eGFR), independent of nocturnal hypertension and morning surge in blood pressure. As a continuous variable, both morning SBP and DBP correlated with UPCR, LVMI, and LVH, whereas only morning SBP was negatively associated with eGFR. These data suggest that morning hypertension plays an important role in TOD in patients with CKD and hypertension.

The rate of morning hypertension reached 43.4% in this study, and the prevalence varies substantially across countries and populations (from 15.9 to 60.7%) (24). Our study validated that morning hypertension can result from increased nocturnal BP and large morning surge in blood pressure. In this study, 95.9% of patients with morning hypertension also had nocturnal hypertension, and nighttime SBP could well distinguish morning hypertension. This indicated the strong association between nocturnal BP and morning BP in patients with CKD and hypertension.

Previous studies showed that morning hypertension is associated with higher risk of LVH (8), which is a strong predictor of poor CV and renal outcomes in both CKD and general patients (25, 26). This may be a factor explaining why morning hypertension was found to be associated with CV

events in some prospective studies (9, 10). However, studies on the association between morning hypertension and CV events in CKD patients are very limited. According to a recent cross-sectional study, only masked morning hypertension was demonstrated to be associated with increased prevalence of LVH in CKD patients (27). Our study further confirmed this observation. Both morning SBP and DBP correlated strongly with LVH and LVMI. The strong correlation may be supported by the observation that the high awaking BP was used to detect growth of LVM over time in CKD patients (28).

Our study showed that morning hypertension was associated with increased prevalence of kidney function decline, including higher UPCR and lower eGFR. Similar findings were reported in some previous studies (7, 14, 15). Hypertension may result in endothelial dysfunction, which subsequently contributes to increased arterial stiffness. This in turn can facilitate escape of albumin from renal glomeruli (29, 30). Interestingly, only morning SBP, not DBP, correlated with eGFR decline, which was consistent with previous studies (14, 15). However, the underlying mechanisms are not clearly defined. Previous studies suggested that the association between morning hypertension and TOD may be attributed to nocturnal hypertension or morning surge in blood pressure (14, 15). However, in our study, after adjustment of nocturnal hypertension and morning surge, morning hypertension showed an attenuated but nonetheless strong correlation with TOD, indicating that the correlation between morning hypertension and TOD was not only attributed to nocturnal hypertension or morning surge in blood pressure, but to some certain own factors. For example, morning hypertension may reflect inadequate antihypertensive treatment (7) or increased activities of neurohumoral factors in the morning, such as the sympathetic nervous system and renin–angiotensin system, which contribute to progression of arterial damage (31, 32).

Based on the results of our study and others, morning hypertension is believed to provide reliable information on BP control and high risk of TOD in CKD patients. Controlling morning BP should be considered an important measure for preventing CV and renal damage. However, current ABPM guidelines do not highlight the importance of morning BP, let alone treatment of morning hypertension (6, 33). Treatment of morning hypertension may not have attracted sufficient attention because of the absence of outcome trial evidence on its benefits. Only one interventional study demonstrated that morning BP control is associated with LVH resolution and can delay the progression of CKD (34). Additional prospective studies are necessary.

Our study had some strengths. First, to our knowledge, this is the first study to investigate the correlation between morning hypertension and TOD in CKD patients. Second, compared with previous studies that measured home morning BP (7, 9, 10, 14, 15), ABPM provided readings of nocturnal BP and morning surge in blood pressure, and additional readings of morning BP, and the influence of nocturnal hypertension and morning blood pressure surge on the association between morning hypertension and TOD was clarified. This study also had limitations. This was a cross-sectional study, and the causal association between morning hypertension and TOD could not be validated. Additionally, the sample size in this study (447) was relatively small. Finally, some important data including history of sleep apnea, and socioeconomic status were missing in this study; these data need to be complemented in the future research.

In conclusion, morning hypertension was strongly correlated with TOD, including LVH, higher UPCR, LVMI, and lower level of eGFR in CKD patients with hypertension, independent of nocturnal hypertension and morning surge in blood pressure. Morning hypertension in CKD patients warrants further attention. Proper management of morning BP may reduce cardiorenal injury in these 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 West China Hospital, Sichuan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL, FL, TZ, ZZ, HZ, AQ, YT, and WQ: conception, design, analysis, and interpretation of data. XL and FL: drafting the article or revising it. XL, YT, and WQ: providing intellectual content of critical importance to the work described. XL, FL, TZ, ZZ, HZ, AQ, YT, and WQ: final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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The Ratio of NT-proBNP to CysC$^{1.53}$ Predicts Heart Failure in Patients With Chronic Kidney Disease

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Background: The N-terminal pro B type natriuretic peptide (NT-proBNP) is important for prognosis of heart failure in patients with chronic kidney disease (CKD). However, the NT-proBNP level is easily affected by renal insufficiency, which limits its clinical use.

Methods: This study included 396 patients with CKD. Plasma levels of NT-proBNP and cystatin C (CysC) were measured during hospitalization. The echocardiographic parameters were also detected. Patients were divided into the heart failure group and control group according to the European Society of Cardiology Guideline on Chronic Heart Failure 2021. Multiple modeling analysis of the values of NT-proBNP and CysC, including NT-proBNP/Cysc^{n} and NT-proBNP/n^{CysC} was performed. The receiver operating characteristic (ROC) curve, combined with the cardiac function, was used to determine the formula with the best diagnostic efficiency. Then, the sensitivity and specificity of new predictors for cardiac insufficiency in CKD patients were calculated. Pearson correlation analysis was used to analyze the relationship between new predictors and the NT-proBNP level. The clinical data of CKD patients from another local hospital were used to validate the new predictors and the cut-off values.

Results: An elevated NT-proBNP/CysC$^{1.53}$ ratio was an independent risk factor for cardiac dysfunction in CKD and the best predictor derived from multiple modeling analysis. There was no correlation between the NT-proBNP/CysC$^{1.53}$ ratio and the NT-proBNP level ($r = 0.376$, $p = 6.909$). The area under the ROC curve for the NT-proBNP/CysC$^{1.53}$ ratio was 0.815 (95% confidence interval: 0.772–0.858), and for a cut-off point of 847.964, this ratio had a sensitivity of 78.24%, and a specificity of 69.44%.

When applied to the data of CKD patients from another local hospital, the NT-proBNP to CysC$^{1.53}$ ratio had a sensitivity of 70.27% and a specificity of 67.74%.

Conclusion: The NT-proBNP to CysC$^{1.53}$ ratio was superior to NT-proBNP alone for predicting cardiac dysfunction in patients with CKD.

Keywords: chronic kidney disease, heart failure, NT-proBNP, CysC, combined diagnostic index

INTRODUCTION

Chronic kidney disease (CKD) is diagnosed when the estimated glomerular filtration rate (eGFR) is <60 mL/min/1.73 m^2 for 3 consecutive months, or abnormal renal structure or function other than decreased eGFR for over 3 months (1). The main cause of death among patients with CKD is cardiovascular disease, including myocardial infarction and heart failure (HF) (2–4). When HF occurs in CKD patients, the retention of sodium and fluid leads to increases in vascular tension and cardiac preload (5). The increase in ventricular pressure induces the release of biomarkers, such as natriuretic peptide (6). Therefore, biomarkers of myocardial stretching are often used for the diagnosis and prognosis of HF (7). Brain natriuretic peptide (BNP) and N-terminal pro B type natriuretic peptide (NT-proBNP) are important indicators for the diagnosis, prediction, and treatment evaluation of HF, and NT-proBNP is superior to BNP in this prognostic assessment (8–11). Although they both are important indicators of HF (12), NT-proBNP is rarely used as a diagnostic biomarker for HF in patients with end-stage renal disease (ESRD) (13), because ESRD patients without HF have high levels of NT-proBNP due to decreased renal elimination, volume overload, hypertension, and increased left ventricular hypertrophy (14). NT-proBNP is mostly eliminated by glomerular filtration, which explains the strong influence of renal function on NT-proBNP levels (15).

When using NT-proBNP for the diagnosis of HF in patients with renal insufficiency, the diagnostic cut-off value must be adjusted according to the eGFR (16). The cut-off value of NT-proBNP for the diagnosis of acute HF (AHF) in patients with CKD stages 3–5 (eGFR <60 mL/min/1.73m^2) is higher than that in patients with CKD stages 1–2 (eGFR >60 mL/min/1.73m^2), ranging from 1,200–6,000 pg/mL, and both points are higher than the standard "Januzzi cut-off point." In general, the specificity and sensitivity of the NT-proBNP concentration for the diagnosis of AHF in patients with CKD stages 3–5 are low (7). To date, there have been no large-scale prospective clinical trials determining the diagnostic cut-off value of NT-proBNP for HF in CKD patients (17).

It is important to use a reliable serological indicator to predict HF in CKD patients with different eGFRs. Low molecular-weight proteins have been used to estimate the value of eGFR (18), and the most promising biomarker is cystatin C (CysC) (19). CysC is a non-glycosylated, 13-kDa basic protein that belongs to the cystatin superfamily of cysteine protease inhibitors. It is produced by all nucleated cells, unaffected by muscle mass (unlike creatinine) (20), and considered a replacement for serum creatinine (sCr) for the estimation of eGFR (21). CycC is also used for early diagnosis of renal damage (22).

In the present study, we investigated the prognostic potential of the ratio of NT-proBNP to CysC compared with NT-proBNP alone for cardiac dysfunction in Chinese CKD patients.

MATERIALS AND METHODS

Study Population

The clinical records of 938 adults who underwent serum CysC measurement and cardiac biomarker measurement simultaneously between September 2020 and August 2021 in Gezhouba Central Hospital of Sinopharm, China, were retrospectively reviewed. Individuals who were diagnosed with CKD, aged above 18 years, and had complete medical records were eligible for this study. This study was approved by the Hospital Ethics Committee, and informed consent was obtained from all patients. To minimize the confounding effects of circulating biomarkers, patients who met any of the following criteria were excluded: (1) diagnosed with acute myocardial infarction, acute HF, atrial fibrillation, or unstable angina within 1 month of enrollment; (2) diagnosed with malignant arrhythmia or hemodynamic changes; (3) diagnosed with primary liver disease complicated with HF; (4) diagnosed with an end-stage malignant tumor; (5) diagnosed with primary thyroid disease; (6) pregnant; or (7) diagnosed with blood system diseases. Finally, 396 patients were included in the study. Among them, there were 123 (31.06%) cases of diabetic nephropathy, 75 (18.94%) cases of chronic glomerulonephritis, 87 (21.97%) cases of hypertensive nephropathy, 41 (10.35%) cases of nephrotic syndrome, 39 (9.85%) cases of IgA nephropathy, 12 (3.03%) cases of polycystic kidney, 8 (2.02%) cases of after renal transplantation, and 11 (2.78%) cases of other types of kidney diseases. A total of 98 (24.74%) patients had been diagnosed with HF during previous hospitalization.

Abbreviations: NT-proBNP, N-terminal pro B type natriuretic peptide; CKD, chronic kidney disease; CysC, cystatin C; ESC, European Society of Cardiology; ROC, receiver operating characteristic; eGFR, estimated glomerular filtration rate; HF, heart failure; BNP, Brain natriuretic peptide; ESRD, end-stage renal disease; AHF, acute heart failure; sCr, serum creatinine; hs-cTnT, highly sensitive cardiac troponin T; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LMVI, left ventricular mass index; GAS, global area strain; GLS, global longitudinal strain; LAVI, left atrial volume index; FS, left ventricular fractional shortening; SV, stroke volume; E/e', the echocardiographic ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity; HFA, Heart Failure Association; PEFF, pre-test assessment; pre-test assessment; functional testing in case of uncertainty; final etiology; HFpEF, heart failure preserved ejection fraction; HFmrEF, heart failure with reduced ejection fraction; HFrEF, heart failure with reduced ejection fraction; AUC, area under the receiver operating characteristic curve; KDIGO, Kidney Disease: Improving Global Outcomes.

During hospitalization, all patients with CKD were given a low-salt (3–5 g/day), low-fat, high-quality, low-protein diet and medications to control blood pressure, blood glucose, and blood lipids. All patients were given 3–5 compound ketoic acid tablets orally (three times per day). Patients with proteinuria ($n = 156$) were given huangkui capsule (three times per day). Seventy-two patients received hormone and immunosuppressant therapy. Spironolactone (20 mg, once daily) was routinely given to CKD patients with previously diagnosed HF. Beta-blockers (12.5–25 mg, twice daily) and angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB) drugs were used in antihypertensive treatment for patients with eGFR >30%. Calcium channel blocker drugs were used in antihypertensive treatment for patients with eGFR <30%. All enrolled patients were treated with cardiac intensification and diuretic therapy for acute HF during the observation period. Serum potassium was regularly monitored in all patients treated with concomitant spironolactone and angiotensin converting enzyme inhibitors or angiotensin receptor blocker.

During the follow-up period, 57 patients showed clinical symptoms of HF, including 25 patients with newly diagnosed HF and 32 patients diagnosed with HF during previous hospitalization. Thirty-nine patients required hospitalization, while the other 18 were in stable condition after outpatient cardiotonic and diuretic treatment. During the follow-up period, all patients with HF clinical symptoms were continually followed after their condition became stable, and HF was treated according to aforementioned regimes. Serum potassium was routinely monitored in all patients treated with concomitant spironolactone and ACEI/ARB.

Biomarker Assessment

The levels of highly sensitive cardiac troponin T (hs-cTnT) and NT-proBNP were measured by the Cobas 601 automatic chemiluminescence analyzer (Roche, Inc.). The levels of CysC, serum urea and sCr were measured using the 7600 automatic biochemical analyzer (Hitachi, Inc.). The levels of hemoglobin (Hb) were measured using the XN-1000 automatic blood cell analyzer (Sysmex, Inc.).

Echocardiography

On the day that blood tests were performed, echocardiography also was performed using the GE Vivid E90 Ultrasound System (M5S probe, 1.7∼3.3 MHz; GE Healthcare) with participants laying on their left side and staying calm during the test. The following parameters were measured: left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), left ventricular ejection fraction (LVEF), left atrial volume index (LAVI), left ventricular mass index (LMVI), global area strain (GAS), global longitudinal strain (GLS), left ventricular fractional shortening (FS), stroke volume (SV), and the echocardiographic ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity (E/e').

Diagnostic Criteria for HF

HF was diagnosed based on the symptoms and/or signs of HF, echocardiography report, and the Heart Failure Association (HFA)-PEFF (P, pre-test assessment; E, echocardiography and natriuretic peptide score; F1, functional testing; F2, final etiology) diagnostic algorithm (23). The algorithm consists of three domains: functional, morphological, and biomarker domains. For each domain, 2 points are scored when the main criteria are met, while 1 point is scored when minor criteria are met. Points from different domains are then summed. A total score of ≥5 is considered a diagnosis of HF preserved ejection fraction (HFpEF); while a score of ≤1 indicates an unlikely diagnosis of HFpEF (24). The diagnosis of HF with mildly reduced ejection fraction (HFmrEF) requires the presence of symptoms and/or signs of HF, the LVEF between 40 and 50%, elevated levels of natriuretic peptides (BNP ≥35 pg/mL or NT-proBNP ≥125 pg/mL), and other evidence of structural heart disease (25). The diagnosis of HF with reduced ejection fraction (HFrEF) requires the presence of symptoms and/or signs of HF and a reduced ejection fraction (LVEF ≤40%) (25).

Statistical Analysis

SPSS 22.0 software (SPSS, Inc.) was used for data analysis. The levels of NT-proBNP were log-transformed, and then statistical analysis was performed to eliminate the influence of extreme values. Normally distributed data are expressed as $\bar{x} \pm s$. Data that were not normally distributed [i.e., NT-proBNP levels, CysC levels, sCr, eGFR, and the ratio of NT-proBNP/CysC$^{1.53}$ (new predictors)] are expressed as median (P25∼P75) values. An independent sample *t*-test was used to compare the results between two groups. Multiple modeling analysis of the values of NT-proBNP and CysC, including NT-proBNP/CysC^{n} and NT-proBNP/n^{CysC}, was performed. The ROC curve, combined with the cardiac function, was used to determine formula with the best diagnostic efficiency. Then, the sensitivity and specificity of new predictors for cardiac insufficiency in CKD patients were calculated. ROC curve analysis was used to analyze echocardiography indicators with statistical significance. The area under the ROC curve (AUC) was calculated to evaluate the ability of these factors to predict HF in patients with CKD. The optimal diagnostic cut-off point was determined by the "Youden index" (sensitivity + specificity−1). The significance level was $\alpha = 0.05$, and $p < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of 396 hospitalized patients with CKD are summarized in **Table 1**. Patients were divided into two groups according to their cardiac function assessed following the European Society of Cardiology (ESC) Guidelines for Chronic Heart Failure 2021: the HF group ($n = 216$) and the control group ($n = 180$).

The ROC curve showed that the cut-off value of the NT-proBNP/CysC$^{1.53}$ ratio was 847.964.

Pearson correlation analysis showed that the level of NT-proBNP was not correlated with the NT-proBNP/CysC$^{1.53}$ ratio ($r = 0.376$, $p = 6.909$; **Figure 1**).

The E/e', GAS, GLS, LAVI, LVMI, serum urea, sCr, hs-cTnT, NT-proBNP, and NT-proBNP/CysC$^{1.53}$ ratio in the HF group were significantly higher than those in the control group, while the LVEF was significantly lower than that in the control group

TABLE 1 | Clinical characteristics of CKD patients at baseline.

Variables	HF group (n = 216)	Control group (n = 180)	*t-value*	*p*-value
Age (years)	74.296 ± 8.938	72.872 ± 9.777	1.513	0.291
Male [cases (%)]	132 (61.11)	115 (63.89)	0.323	0.322
BMI (kg/m^2)	25.195 ± 3.088	24.442 ± 2.742	2.540	0.186
LVDd (mm)	51.621 ± 9.033	50.204 ± 8.028	1.634	0.497
LVDs (mm)	34.726 ± 10.030	32.334 ± 8.871	2.488	0.131
LVEF (%)	56.661 ± 11.766	62.446 ± 8.347	5.537	0.000
SV	73.819 ± 24.478	75.2905 ± 24.151	0.599	0.489
FS	32.257 ± 8.313	35.233 ± 7.985	3.311	0.279
E/e'	18.728 ± 4.690	14.902 ± 2.060	10.157	0.000
GAS (%)	−25.911 ± 3.118	−26.672 ± 2.731	2.560	0.016
GLS (%)	−14.958 ± 2.589	−15.258 ± 2.337	1.200	0.019
LAVI (mL/m^2)	38.248 ± 3.757	33.175 ± 3.766	12.310	0.003
LVMI (g/m)	110.558 ± 11.946	108.465 ± 13.760	1.600	0.019
Hb (g/L)	102.944 ± 24.874	105.633 ± 25.148	1.066	0.549
serum urea (mmol/L)	14.602 ± 6.787	14.100 ± 5.281	0.808	0.013
sCr (μmol/L)	257.00 (176.25, 409.00)	202.25 (279.00, 390.00)	0.507	0.010
CysC (mg/L)	3.160 (2.183, 4.703)	3.295 (2.183, 4.290)	0.651	0.057
eGFR (mL/min/1.73 m^2)	15.442 (9.341, 25.444)	15.534 (10.595, 26.007)	0.164	0.814
hs-cTnT (μg/L)	0.018 ± 0.007	0.015 ± 0.007	4.087	0.001
NT-proBNP (pg/mL)	8,505 (4553, 16128)	4,271 (2203, 6878)	6.728	0.000
NT-proBNP/CysC$^{1.53}$	1282.764 (843.440, 2117.184)	636.921 (307.293, 1037.159)	8.065	0.000

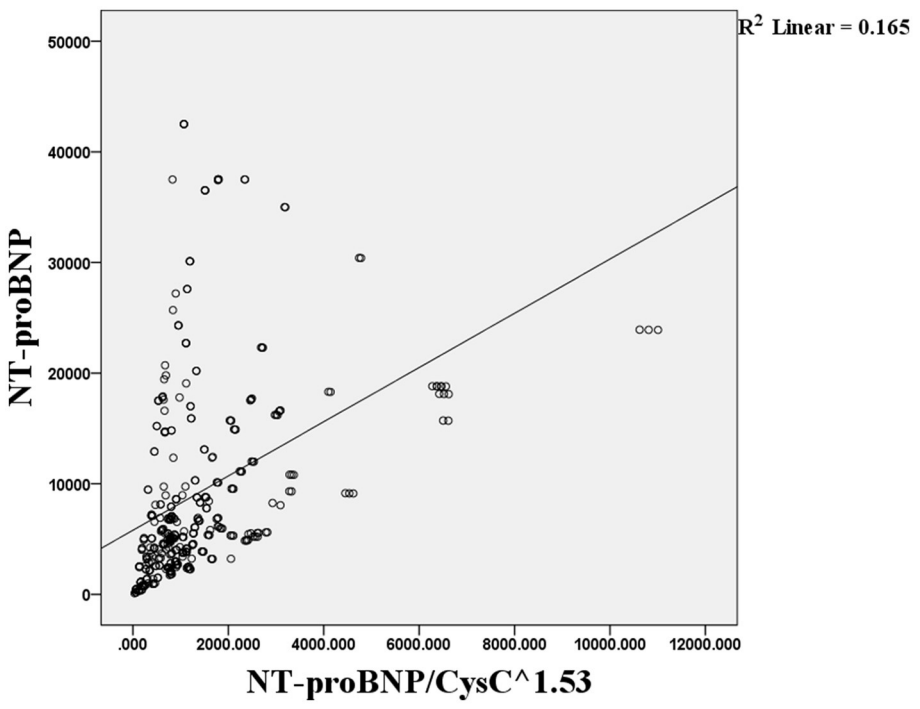

FIGURE 1 | Pearson correlation analysis of the association between the NT-proBNP level and NT-proBNP/CysC$^{1.53}$ ratio.

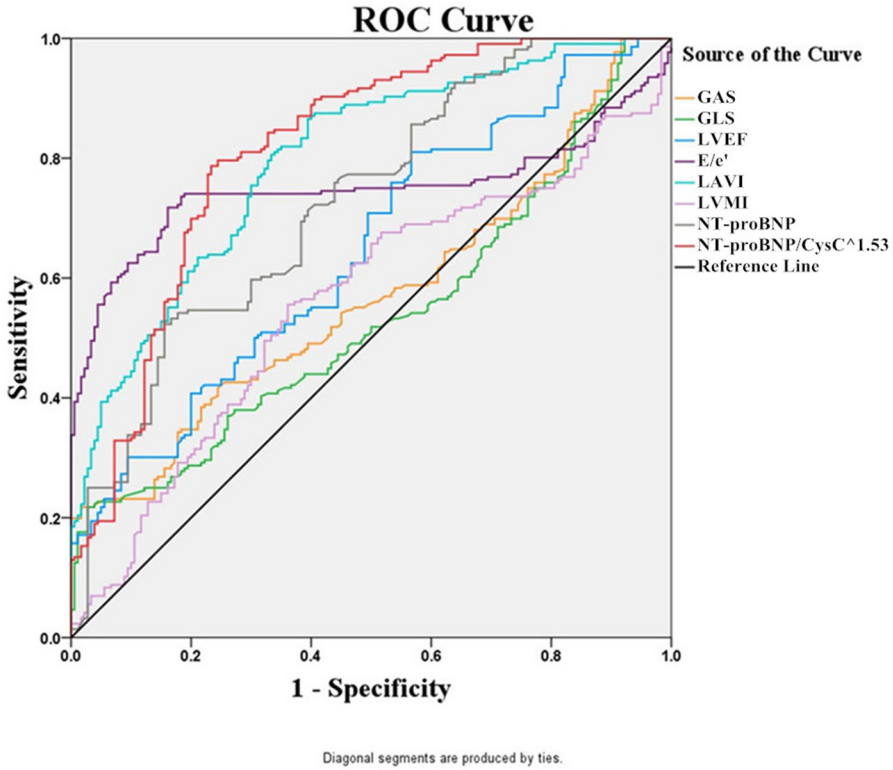

FIGURE 2 | The ROC curves of NT-proBNP/CysC$^{1.53}$, NT-proBNP, GAS, GLS, LVMI, LAVI, E/e', and LVEF for the diagnosis of HF in patients with CKD.

($p < 0.05$). No significant differences in age, CysC, LVDd, and LVDS were observed between the two groups ($p > 0.05$).

Then, we used ROC curve analysis to identify the predictors that were statistically different between the two groups. The ROC curves for eight potentially predictive factors (NT-proBNP/CysC$^{1.53}$, NT-proBNP, GAS, GLS, LVMI, LAVI, E/e', and LVEF) were plotted. The following AUC values for the eight factors were calculated: AUC (NT-proBNP/CysC$^{1.53}$) = 0.815, AUC (LAVI) = 0.798, AUC (E/e') = 0.747, AUC (NT-proBNP) = 0.726, AUC (LVEF) = 0.646, AUC (GAS) = 0.570, AUC (LVMI) = 0.561, and AUC (GLS) = 0.535. The AUC for the NT-proBNP/CysC$^{1.53}$ ratio was greater than those for NT-proBNP and LVEF. The diagnostic cut-off value for the NT-proBNP/CysC$^{1.53}$ ratio was 847.964, with a sensitivity of 78.24% and a specificity of 69.44%. The diagnostic cut-off value for NT-proBNP was 8,198 pg/mL, with a sensitivity of 52.31% and a specificity of 84.44% (**Figure 2**).

The clinical data of CKD patients from another local hospital were used to validate the new predictors and the truncation value. The baseline characteristics of 68 hospitalized patients with CKD are summarized in **Table 2**. The results showed a sensitivity of 70.27% and a specificity of 67.74%.

DISCUSSION

Renal function and cardiac function are interdependent in patients with CKD. HF has been identified as an independent risk factor for all-cause mortality in hospitalized CKD patients. The morbidity and mortality rates are high in CKD complicated with cardiac insufficiency and have been increasing in recent years (26). Thus, early diagnosis of cardiac insufficiency may improve the prognosis of patients with CKD.

HF can be divided into HFrEF, HFmrEF, and HFpEF. It has been reported that HFpEF accounts for more than half of all hospital admissions for HF (27). Early diagnosis of HFpEF is more difficult than that of HFrEF (LVEF ≤40%) and HFmrEF. The widely used New York Heart Association Classification System classifies HF based on subjective feelings of patients; therefore, the results may not be accurate. Even when the objective HFA-PEFF diagnostic algorithm is used, a cardiovascular ultrasound system (e.g., GE Vivid E90 Ultrasound System designed by GE Healthcare) is always needed for the classification of HF. However, in China, most primary and intermediate hospitals do not have such equipment. NT-proBNP is a diagnostic marker for HF, and its serum levels are closely associated with renal insufficiency and age. Therefore, the diagnostic cut-off value of NT-proBNP for HF should be adjusted based on the level of eGFR. However, no large-scale prospective clinical trials have provided an accurate cut-off value of NT-porBNP for the diagnosis of HF in patients with CKD (17).

By comparing the baseline characteristics of the HF and control groups, we found that the NT-proBNP/CysC$^{1.53}$ ratio differed significantly between the two groups. Patients with HF showed a significantly higher NT-proBNP/CysC$^{1.53}$ ratio than

TABLE 2 | Baseline clinical characteristics of CKD patients from another local hospital.

Variables	HF group (n = 37)	Control group (n = 31)	*t-value*	*p*-value
Age (years)	69.459 ± 8.878	70.613 ± 10.990	0.479	0.223
Male [cases (%)]	23 (62.16)	18 (58.06)	0.118	0.462
BMI (kg/m^2)	26.076 ± 2.838	25.419 ± 2.148	1.058	0.150
LVEF (%)	54.702 ± 12.168	60.111 ± 8.814	2.032	0.054
E/e'	19.232 ± 5.481	15.302 ± 1.995	3.501	0.000
GAS (%)	−24.687 ± 2.832	−26.786 ± 1.692	4.054	0.024
GLS (%)	−13.774 ± 2.011	−15.191 ± 2.261	3.598	0.033
LAVI (mL/m^2)	40.070 ± 4.158	32.228 ± 4.032	3.357	0.071
LVMI (g/m)	113.238 ± 11.206	109.310 ± 10.619	1.474	0.377
Hb (g/L)	101.919 ± 25.537	112.871 ± 26.331	1.737	0.684
Urea (mmol/L)	16.132 ± 8.031	11.158 ± 4.599	2.542	0.012
sCr (μmol/L)	219.00 (127.00, 535.50)	232.50 (113.00, 301.50)	1.686	0.007
CysC (mg/L)	2.920 (1.820, 5.450)	3.010 (1.970, 3.620)	1.209	0.074
eGFR (mL/min/1.73 m^2)	16.296 (7.802, 31.186)	17.076 (13.115, 31.560)	0.525	0.408
hs-cTnT (μg/L)	0.019 ± 0.007	0.016 ± 0.008	1.336	0.335
NT-proBNP (pg/mL)	9,301 (4,122, 13,005)	3,697 (936, 7059)	3.332	0.057
NT-proBNP/CysC$^{1.53}$	1198.345 (736.270, 1937.597)	782.908 (242.424, 1255.494)	2.736	0.025

the control group, suggesting that a high NT-proBNP/CysC$^{1.53}$ may be related to the occurrence of cardiac dysfunction in CKD. Our results also demonstrated that the NT-proBNP/CysC$^{1.53}$ ratio was a more reliable predictor of HF than NT-proBNP, GAS, GLS, LVMI, LAVI, E/e', and LVEF in patients with CKD. According to the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 clinical practice guideline for the evaluation and management of CKD (1), CysC is the best indicator of eGFR and has been used for eGFR calculation. Therefore, in this study, we used both NT-proBNP and CysC to evaluate the cardiac function of patients with CKD. To our knowledge, this is the first study to explore the potential of NT-proBNP combined with CysC as a predictor of HF in CKD patients.

A previous study (28) proposed that LVEF is an indicator of cardiac insufficiency in patients with early diagnosed CDK. However, the use of LVEF as a diagnostic marker cannot rule out cases with HFpEF. In the present study, patients with HFpEF, HFmrEF, and HFrEF were included in the HF group for the analysis of cardiac function in CKD patients, and thus, this study may provide more comprehensive results.

Gao et al. (29) proposed that the level of NT-proBNP can indicate HF in different eGFR intervals. However, whether the same cut-off value of NT-proBNP can be used for the diagnosis of HF over a wide range of eGFR values remains unknown. Moreover, they only included patients with HFrEF (LVEF ≤40%). In our study, both NT-proBNP and CysC levels were used to predict cardiac insufficiency, which may better reflect individual differences.

We further validated the results using the clinical data of patients from another local hospital. The NT-proBNP/CysC$^{1.53}$ ratio accurately determined the cardiac function of these patients.

It should be noted that the diagnostic criteria that we have derived in this study were simpler than those recently published in ESC Chronic Heart Failure Guidelines 2021 and require fewer tests. In addition, laboratory test results have less interference and are more accurate than the results of ultrasound scans, and therefore, may have a wider range of applications.

The present study has some limitations. There were only 9 (2.27%) cases with stage 1–2 CKD. Due to compliance issues, patients with symptoms or signs of HF and with a HFA score between 2 and 4 did not undergo a diastolic pressure test or invasive hemodynamic measurement. Because there were only 12 (3.03%) cases with symptoms or signs of HF and with a HFA score between 2 and 4, it might not significantly affect the overall results. We only recruited a small number of Chinese subjects from another local hospital to validate the results, which may not represent the population in other areas. Taking into account the differences in regions, populations, and examinations (e.g., altitude, ethnicity, equipment, reagent), it is recommended that the current findings be validated in a local population. In summary, our study showed that the NT-proBNP/CysC$^{1.53}$ ratio was a predictor of cardiac insufficiency in patients with CKD and might be used for the early detection of HF in this population in clinical settings.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

SW, XW, and JL conceived and designed research. ML, CL, XY, HA, and TY collected data and conducted research. SW and YZ analyzed and interpreted data. YH and ML wrote the initial paper. SW, ML, YZ, and XL revised the paper. SW had primary responsibility for final content. All authors read and approved the final manuscript.

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Revascularization vs. Conservative Medical Treatment in Patients With Chronic Kidney Disease and Coronary Artery Disease: A Meta-Analysis

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Background: As a strong risk factor for coronary artery disease (CAD), chronic kidney disease (CKD) indicates higher mortality in patients with CAD. However, the optimal treatment for the patients with two coexisting diseases is still not well defined.

Methods: To conduct a meta-analysis, PubMed, Embase, and the Cochrane database were searched for studies comparing medical treatment (MT) and revascularization [percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG)] in adults with CAD and CKD. Long-term all-cause mortality was evaluated, and subgroup analyses were performed.

Results: A total of 13 trials met our selection criteria. Long-term (with at least a 1-year follow-up) mortality was significantly lower in the revascularization arm [relative risk (RR) = 0.66; 95% CI = 0.60–0.72] by either PCI (RR = 0.61; 95% CI = 0.55–0.68) or CABG (RR = 0.62; 95% CI = 0.46–0.84). The results were consistent in dialysis patients (RR = 0.68; 95% CI = 0.59–0.79), patients with stable CAD (RR = 0.75; 95% CI = 0.61–0.92), patients with acute coronary syndrome (RR = 0.62; 95% CI = 0.58–0.66), and geriatric patients (RR = 0.57; 95% CI = 0.54–0.61).

Conclusion: In patients with CKD and CAD, revascularization is more effective in reducing mortality than MT alone. This observed benefit is consistent in patients with stable CAD and elderly patients. However, future randomized controlled trials (RCTs) are required to confirm these findings.

Keywords: meta-analysis, revascularization, conservative medical treatment, coronary artery disease, chronic kidney disease

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INTRODUCTION

As one of the major cardiovascular diseases affecting the global human population, coronary artery disease (CAD) is the major cause of death in both developed and developing countries (1). Chronic kidney disease (CKD), an independent and strong CAD risk factor, exerts great coronary artery implications and indicates higher mortality (2). Therefore, many patients with CKD and CAD require cardiovascular optimization. However, the optimal treatment of these patients is still not well defined, and a fundamental issue is whether they will fare better with myocardial

revascularization or medical therapy. Patients with CKD were excluded from most trials, and only 10 to 40% of patients with CKD and CAD undergo revascularization in clinical practice owing to concerns about acute renal injury and major bleeding events after revascularization (3). Consequently, this population [especially regarding advanced CKD and/or end-stage kidney disease (ESKD)] is underrepresented and management is still mainly extrapolated from non-CKD cohorts (4, 5). Several investigations, mainly observational investigations, have provided varied opinions on this controversial issue, and the majority of them supported revascularization. However, according to the International Study of Comparative Health Effectiveness with Medical and Invasive Approaches-Chronic Kidney Disease (ISCHEMIA-CKD), a well-known large randomized controlled trial (RCT), there was no incremental benefit of an invasive strategy in patients with stable CAD and advanced CKD. Up to now, no agreement has been achieved on the optimal treatment in this population.

Therefore, for the purpose of providing more evidence-based ideas on the treatment of patients with CAD and CKD, eligible studies were identified and included to conduct a meta-analysis investigating the long-term effects of revascularization and medical treatment (MT) in patients with CAD and CKD or ESKD. We present the following article in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist.

METHODS

Study Selection and Data Extraction

We searched the PubMed, Embase, and the Cochrane Library databases from inception to May 3, 2021, using search terms such as "revascularization, percutaneous coronary intervention, coronary revascularization, PCI, coronary artery bypass grafting, CABG," "drug therapy, conservative treatment, optimal medication therapy, OMT," and "chronic kidney disease, renal failure, renal disease, kidney disease, CKD" (**Supplemental Material**-words search strategy). In addition, all the references of key reviews and included articles were hand-searched for potentially missed eligible studies following a snowball procedure. Discrepancies were resolved by consensus with the addition of a third reviewer. Eligible studies met

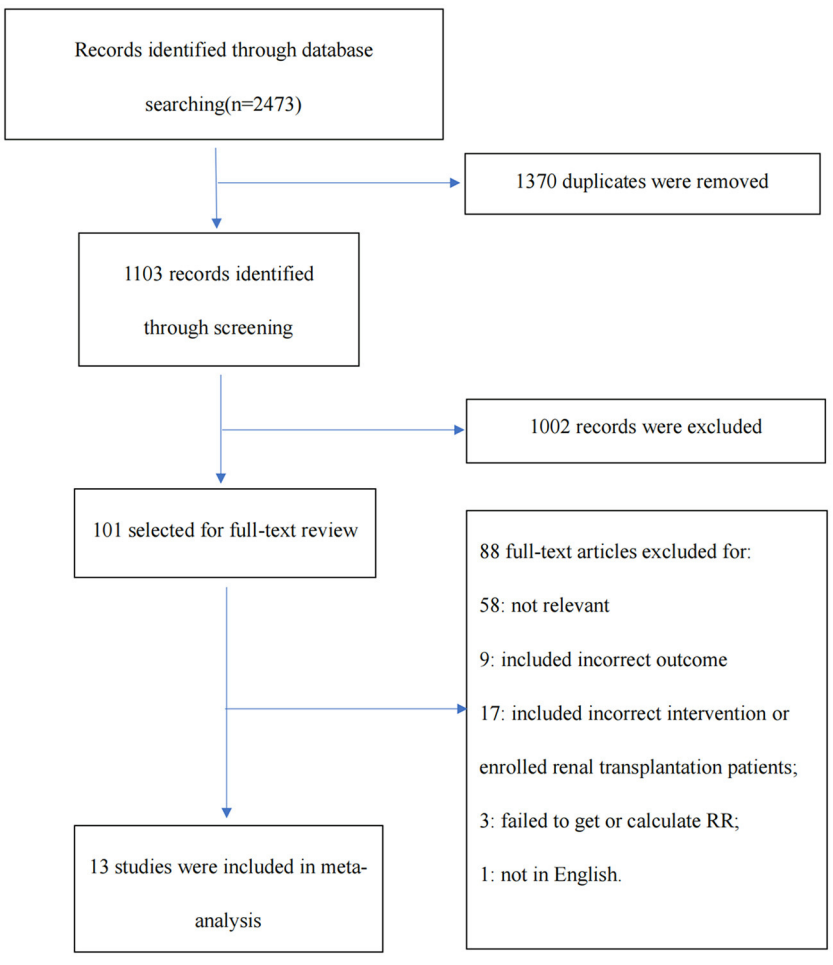


FIGURE 1 | The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram illustrating the study selection process.

TABLE 1 | Baseline characteristics of included studies.

Study	Follow-up months	Types of Re	CKD stages	N	Age, mean or median	Male, %	Stable CAD, %	MVD or LAD, %	Dialysis, %	Diabetes, %
Chertow 2000 (6)	12	PCI and CABG	Moderate	0	/	/	/	/	/	/
			Advanced	640	NA	59.0	0.0	NA	100.0	52.0
Keeley 2003 (7)	60	PCI and CABG	Moderate	1,159	65.6	52.0	0.0	NA	0.0	40.1
			Advanced	495	64.9	50.5	0.0	NA	19.0	
ICTUS 2005 (8)	12	PCI	Moderate	109	72.0	55.0	0.0	NA	0	24.1
			Advanced	8	74.9	75.0	0.0	NA	NA	37.5
Yasuda 2006 (9)	39	PCI	Moderate	0	/	/	/	/	/	/
			Advanced	134	63.3	64.2	64.9	66.4	100.0	57.4
COURAGE 2009 (10)	36	PCI	Moderate	304	68.0	77.0	100.0	NA	0.0	42.4
			Advanced	16			100.0	NA	0.0	
Eisenstein 2009 (11)	36	PCI	Moderate	1,459	74.8	48.4	NA	>70	0.0	29.3
			Advanced	0	/	/	/	/	/	/
Sakakibara 2011 (12)	44	PCI	Moderate	0	/	/	/	/	/	/
			Advanced	391	NA	NA	NA	0.0	NA	NA
Hawranek 2017 (13)	12	PCI and CABG	Moderate	5768	74.6	47.1	0.0	NA	0.0	36.7
			Advanced	1,183	76.3	40.0	0.0	NA	24.1	43.2
Kim 2018 (14)	60	PCI and CABG	NA	331	68.2	68.9	NA	>80	NA	65.0
APPROACH 2018 (15)	120	PCI and CABG	Moderate	2,157	71.8	72.8	100.0	>80	0.0	32.3
			Advanced	333	68.3	73.2	100.0	>80	35.4	56.8
Eduardo 2018 (16)	120	PCI and CABG	Moderate	150	67.0	67.3	100.0	100.0	0.0	60.0
			Advanced	0	/	/	/	/	/	/
ISCHEMIA-CKD 2020 (17)	36	PCI and CABG	Moderate	0	/	/	/	/	/	/
			Advanced	777	63.0	68.9	100.0	NA	53.4	57.1
SWEDEHEART 2009 (18)	12	PCI and CABG	Moderate	4,517	72.0	55.2	0.0	NA	0.0	31.6
			Advanced	757	71.2	57.6	0.0	NA	31.8	49.9
Total number	/	/	/	20,688	/	/	/	/	/	/

CKD, chronic kidney disease; CAD, coronary artery disease; Re, revascularization; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; NA, not available; MVD, multivessel disease; LAD, left artery disease; N, number.

the following PICOS criteria: (1) Population: adult patients with clinical diagnoses of CKD (defined as eGFR or Ccr < 60 ml/min/1.73 m^2 or dialysis dependence) and CAD [had lesions with $\geq 50\%$ diameter stenosis or had acute coronary syndrome (ACS)]; (2) Intervention: invasive strategies including PCI or CABG; (3) Comparative intervention: conservative medical therapy referred to patients whose initial treatment strategy did not include PCI or CABG but only drug therapy; (4) Outcome: long-term (with at least a 1-year follow-up) all-cause mortality; and (5) Study design: non-RCTs and RCTs. The exclusion criteria were as follows: (1) studies in which most patients underwent renal transplantation or were formally placed on the transplant waiting list; (2) studies not written in English; and (3) registries with overlapping populations. Two reviewers (Liao G and Li Y) screened each study by title and abstract for inclusion, reviewed the full texts of studies that qualified, and then extracted the data independently. All disagreements were resolved by discussion. The characteristics extracted from each study were the year of publication, follow-up duration, number of patients enrolled, and type of invasive therapy. The variables of patients we collected included mean or median age; sex; the proportion of the patients with complicated lesions (multivessel disease or left main artery disease), dialysis dependence, stable CAD, and diabetes. A small portion of outcome data was collected from the previous meta-analysis (3). Non-RCT and RCT quality was assessed using the Newcastle–Ottawa Scale and Cochrane Collaboration's tool, respectively.

Data Analysis

We chose Stata MP software version 15.0 to pool relative risk (RR) with a 95% CI for the endpoint, utilizing the Mentel-Haenszel method. Between-study heterogeneity was assessed by estimating I^2, and a random-effects model was used to obtain the combined RRs when the I^2 statistic was over 50%. In addition, a sensitivity analysis method was applied to explain the cause of heterogeneity with a "leave-one-out" approach. Publication bias was assessed visually by inspecting funnel plots.

Subgroup Analysis

We performed subgroup analyses of patients with dialysis dependence, stable CAD, ACS, and relatively advanced age. We also compared MT with PCI and CABG.

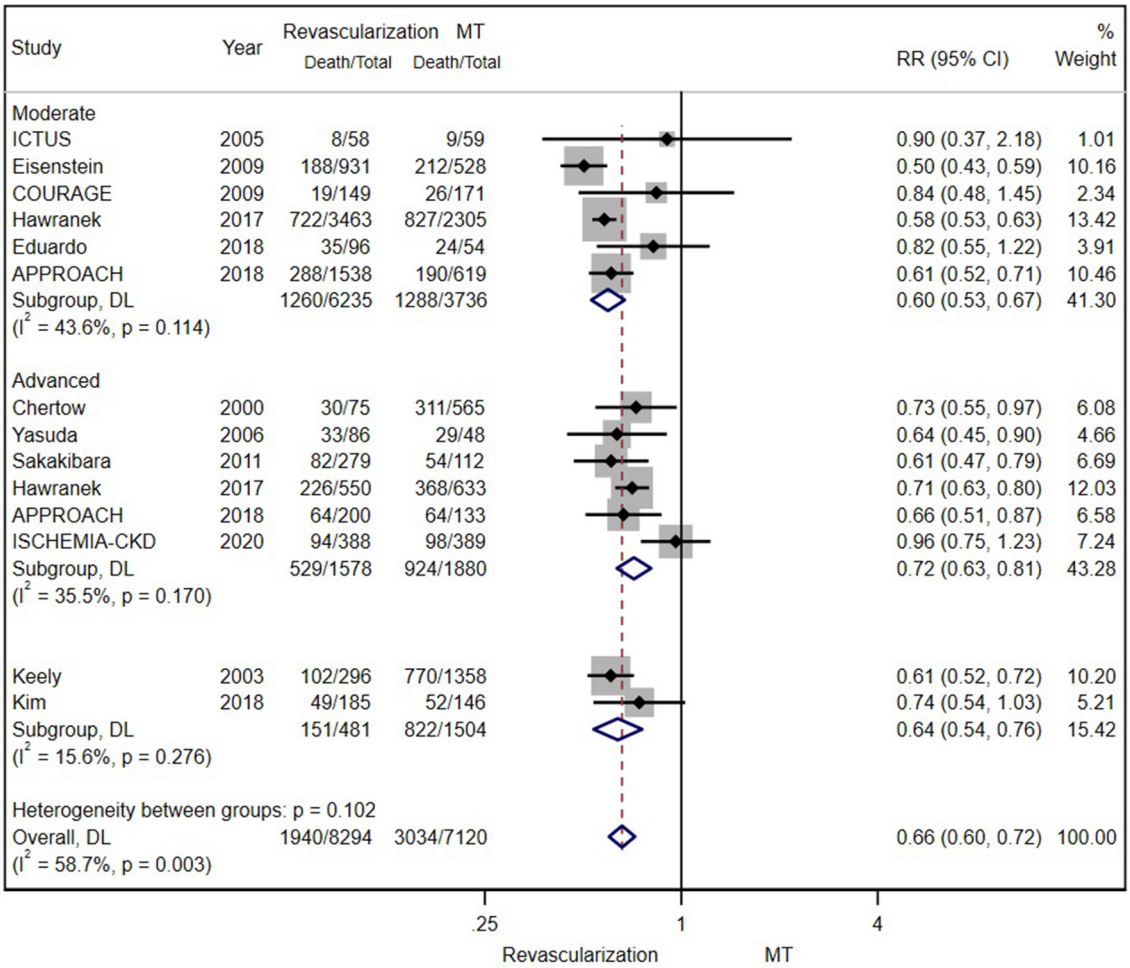


FIGURE 2 | The pooled effect of revascularization and medical treatment (MT) alone on the long-term mortality of patients with coronary artery disease (CAD) and chronic kidney disease (CKD). The pooled estimate of the meta-analysis was represented with a diamond. Revascularization was more effective than MT in reducing mortality in patients with CKD. Weights and between-subgroup heterogeneity test are from random-effects model.

RESULTS

Study Selection and Patient Characteristics

Our search strategy identified 2,473 records. Once duplicates had been removed, 1,103 unique records were screened, of which 101 full texts were assessed for eligibility. This process finally yielded 13 studies including two randomized controlled trials, as summarized in the PRISMA chart (**Figure 1**) (6–18). One trial that enrolled patients with serum creatinine (Scr) > 5 mg/dl was also included (7). The overall risk of bias was considered low in two RCTs, and the quality evaluation of non-RCTs based on the Newcastle–Ottawa scale found that all scores were ≥ 6 (**Supplementary Table S2** in the supplemental material).

Study characteristics and patient demographics are summarized in **Table 1**. A total of 20,688 patients were included in this meta-analysis. As **Table 1** shows, most of them were old (≥ 70 years old) and male patients. Diabetes is a common comorbidity, and coronary artery lesions are frequently complicated. The medications that most patients took included antiplatelet agents, β blockers, ACEIs/ARBs, and statins. In the early years, fewer patients enrolled in the trials took statins, the benefits of which have been increasingly stressed by many researchers in recent decades (19).

Outcome: Long-Term All-Cause Mortality

Revascularization vs. MT

According to the original data of all-cause mortality from 12 trials (excluding SWEDEHEART 2009), we found that invasive therapy (PCI or CABG) was associated with lower long-term mortality (RR = 0.66; 95% CI = 0.60–0.72) than conservative MT (**Figure 2**). Subgroup analyses based on renal function categories, the moderate CKD group (RR = 0.60; 95% CI = 0.53–0.67), the advanced CKD group (RR = 0.72; 95% CI = 0.63–0.81) and even dialysis group (or with eGFR ≤ 15 ml/min/1.73 m^2) (RR =

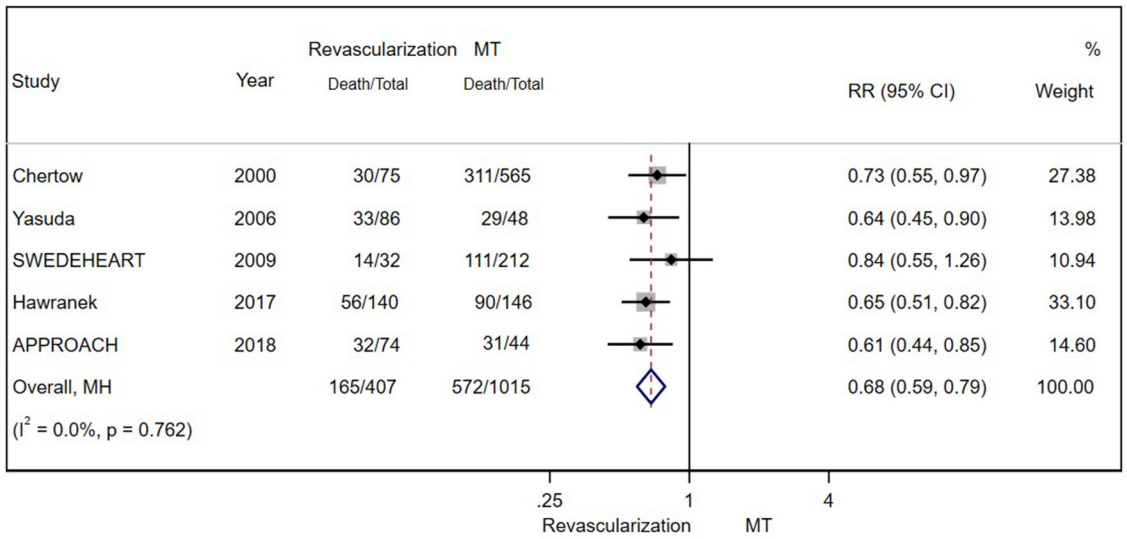


FIGURE 3 | The pooled effect of revascularization and MT alone on the long-term mortality of patients with CAD and estimated glomerular filtration rate (eGFR) ≤ 15 ml/min/1.73 m^2 or receiving dialysis treatment. Revascularization was more helpful than MT in reducing mortality. Weights and between-subgroup heterogeneity test are from Mantel-Haenszel model.

0.68; 95% CI = 0.59–0.79) (**Figure 3**), also revealed the consistent survival benefit of revascularization.

Subgroup Analyses

Percutaneous Coronary Intervention vs. MT and CABG vs. MT

There were eight and four studies comparing PCI and CABG with MT in patients with CAD and CKD, respectively. The results showed that PCI (RR = 0.61; 95% CI = 0.55–0.68) (**Figure 4A**) and CABG (RR = 0.62; 95% CI = 0.46–0.84) (**Figure 4B**) were both associated with lower mortality.

Stable CAD

A total of 3,737 patients with stable CAD were separately examined. There was still a significant difference in the risk for all-cause mortality between moderate patients with CKD who underwent revascularization and those who received MT alone (RR = 0.75; 95% CI = 0.61–0.92) (**Figure 5A**).

Acute Coronary Syndrome

A total of 9,362 patients with ACS in 4 studies were enrolled and studied (after excluding SWEDEHEART 2009). Regardless of the severity of renal insufficiency, the results showed the long-term benefit of invasive therapy (RR = 0.62; 95% CI = 0.58–0.66) (**Figure 5B**). Further analysis of long-term outcomes in patients with different ACS types (unstable angina/NSTEMI and STEMI) was not conducted because of the insufficient number of related studies.

Elderly Patients

The mean/median age was over 70 years in four trials (after excluding SWEDHEART 2009), so we performed a subgroup analysis of these enrolled patients. As the figure shows, revascularization was associated with a reduction in long-term mortality (RR = 0.57; 95% CI = 0.54–0.61) (**Figure 6**).

Sensitivity Analysis and Publication Bias

Applying a "leave-one-out" approach, we found that excluding anyone did not exert a significant impact on the result (**Figure 7**). However, the exclusion of SWEDEHEART 2009, a study with high weight, yielded a significantly lower I^2 value (**Supplementary Table S1** in the supplemental material). Consistently, the SWEDEHEART 2009 study also had a similar influence on I^2 in two subgroup studies of ACS and geriatric patients (**Supplementary Table S1** in the supplemental material). Given, the excessively significant heterogeneity, we finally excluded the SWEDEHEART 2009 study from analyses of all eligible patients, patients with ACS, and geriatric patients.

As **Figure 8** shows, the funnel plot suggested that there may be publication bias in our meta-analysis.

DISCUSSION

The principal findings of our meta-analysis are as follows: (1) Compared with drug therapy alone, revascularization (by either PCI or CABG) decreased the long-term risk for all-cause mortality in patients with CAD and CKD despite the severity of renal impairment; (2) Invasive therapy also yielded a consistent survival benefit in the subgroups with a mean age of over 70 years. Nearly, all of them were patients with moderate CKD; and (3) A lower mortality associated with revascularization was observed in the stable CAD group.

Some previous meta-analyses also compared the effects of revascularization with those of MT in patients with CAD and CKD/ESKD (3, 20). However, there were some limitations in these reviews: (1) they did not exclude patients who

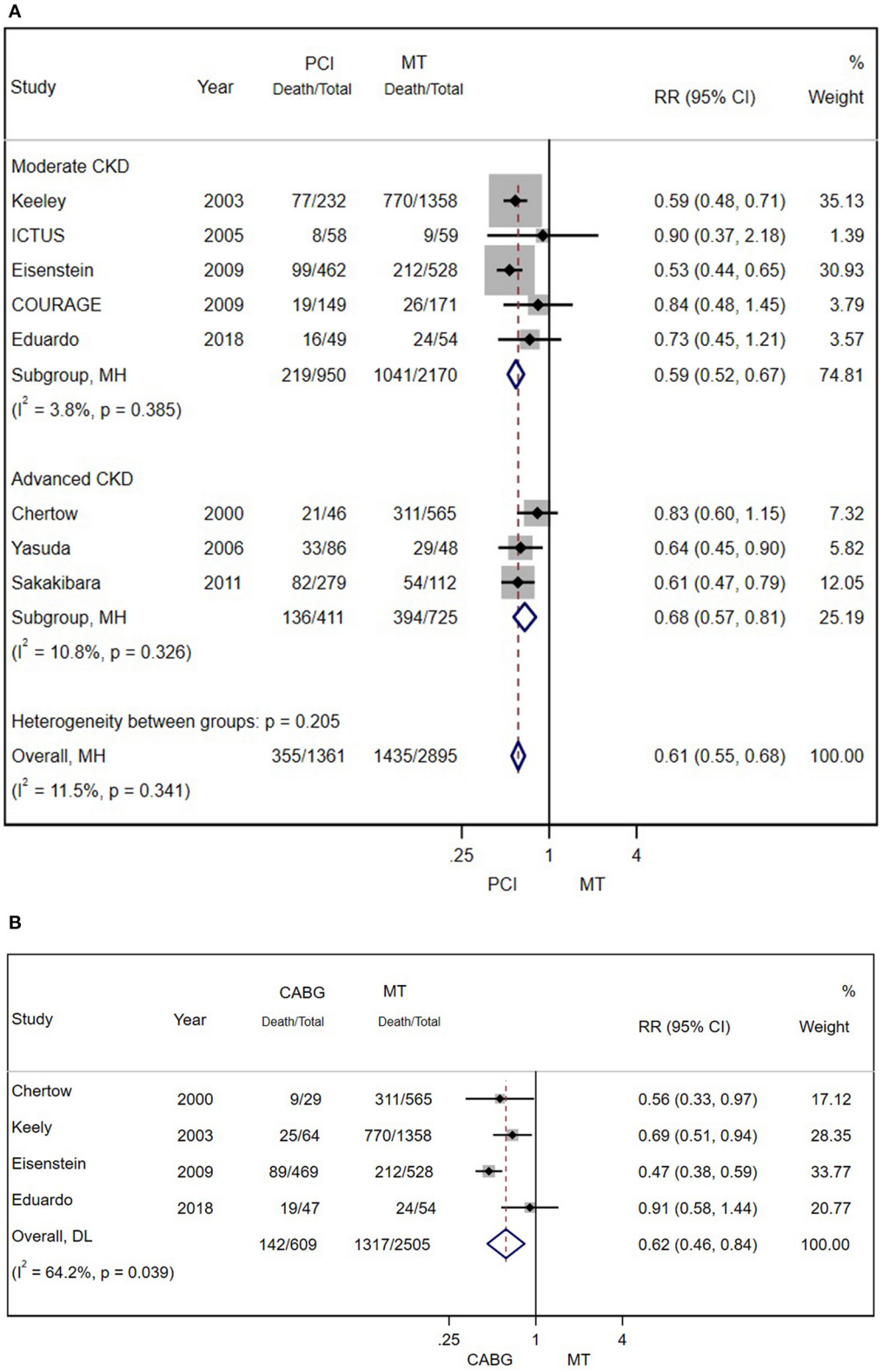
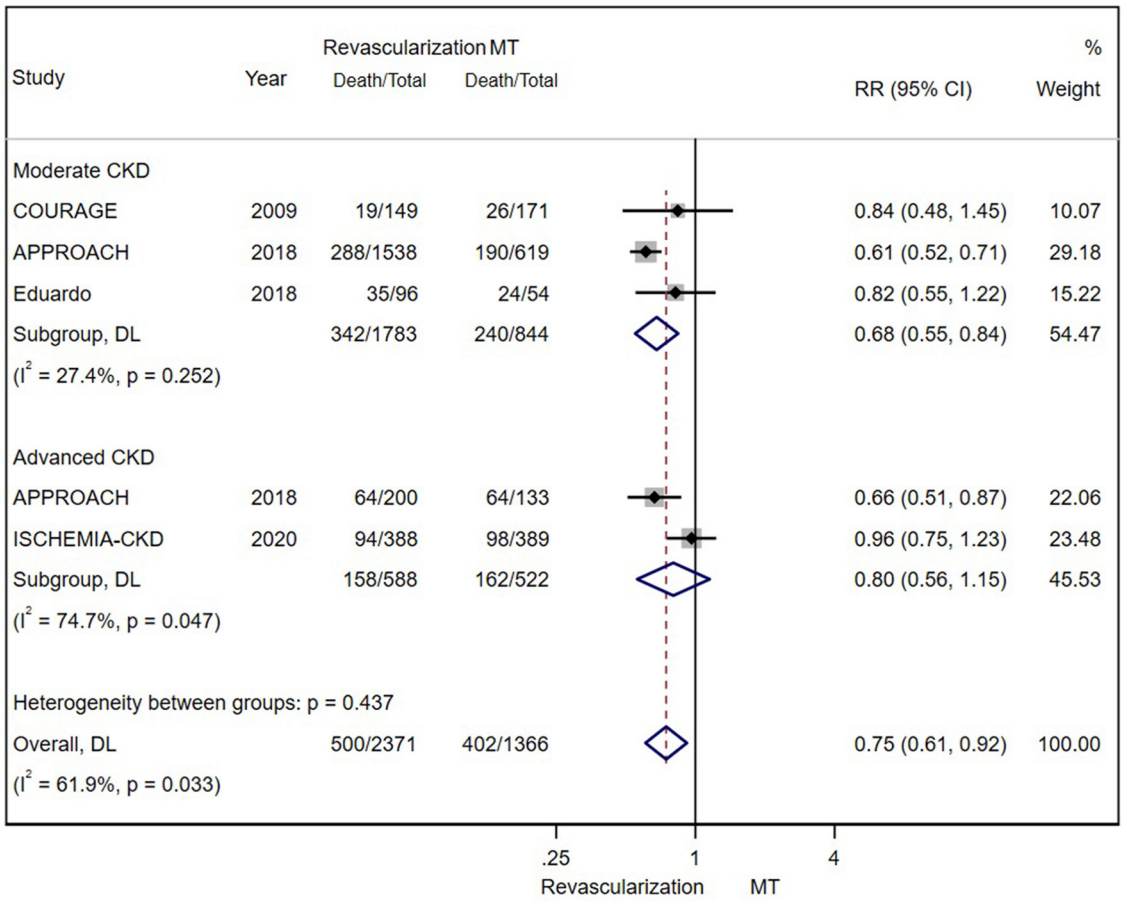


FIGURE 4 | (A) Pooled effect of percutaneous coronary intervention (PCI) and MT alone on the long-term mortality of patients with CAD and CKD. Compared with MT alone, PCI was more effective in reducing mortality. Weights and between-subgroup heterogeneity test are from Mantel-Haenszel model. **(B)** The pooled effect of CABG and MT alone on the long-term mortality of patients with CAD and CKD. Compared with MT alone, PCI was more effective in reducing mortality. Weights and between-subgroup heterogeneity test are from random-effects model.



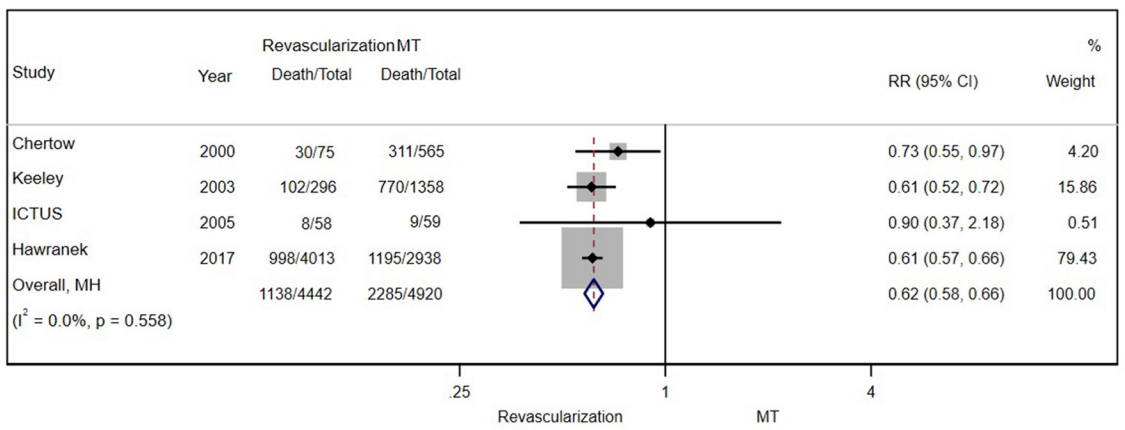


FIGURE 5 | (A) The pooled effect of revascularization and MT alone on the long-term mortality of stable patients with CAD and CKD. Compared with MT alone, revascularization was more effective in decreasing mortality. Weights and between-subgroup heterogeneity test are from random-effects model. **(B)** The pooled effect of revascularization and MT alone on the long-term mortality of patients with ACS and CKD. Compared with MT alone, revascularization was more effective in reducing mortality. Weights and between-subgroup heterogeneity test are from Mantel-Haenszel model.

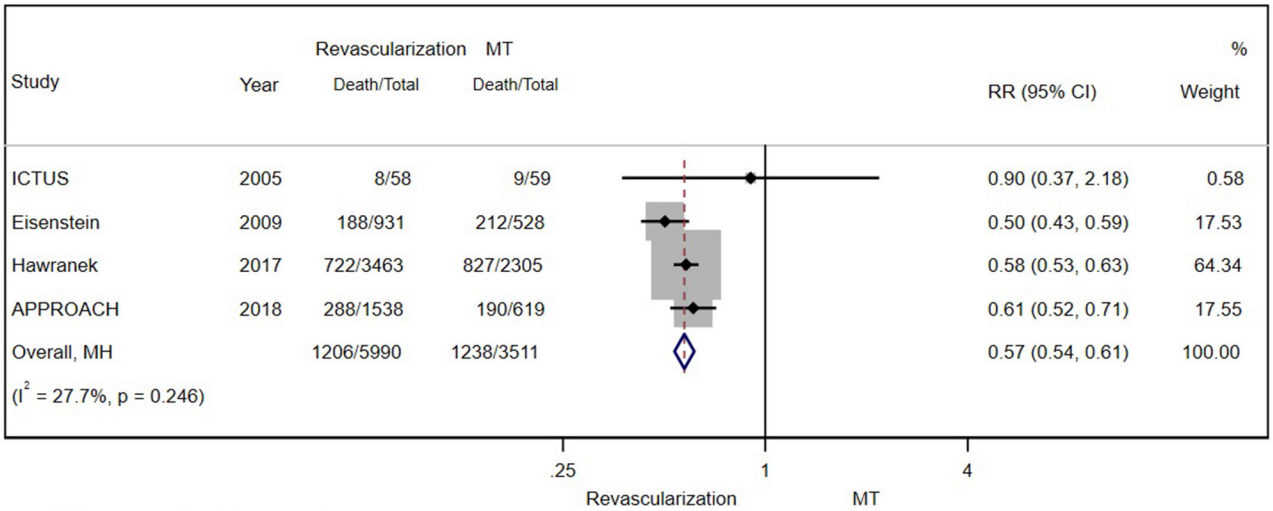


FIGURE 6 | The pooled effect of revascularization and MT alone on the long-term mortality of elderly patients with CAD and moderate CKD. Compared with MT alone, revascularization was more effective in reducing mortality. Weights and between-subgroup heterogeneity test are from Mantel-Haenszel model.

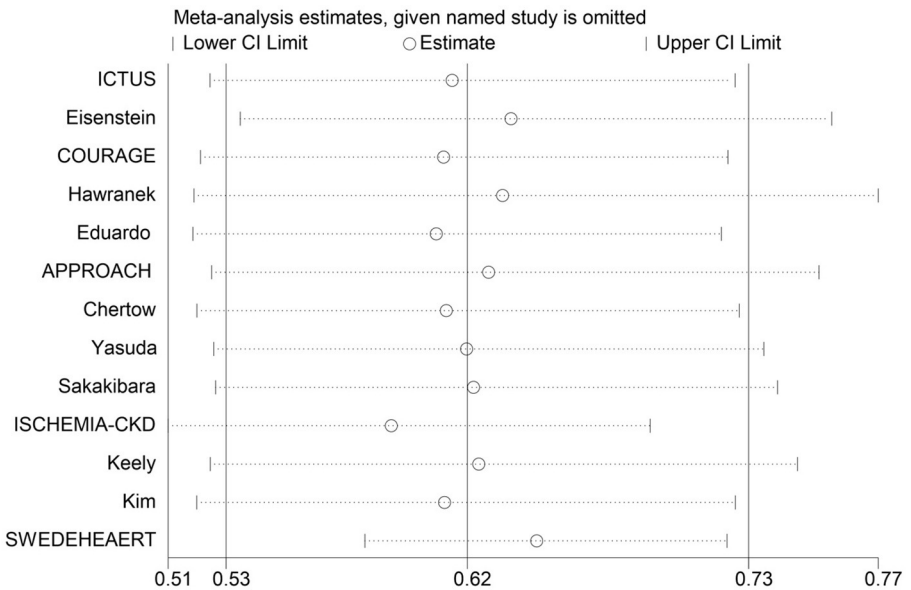


FIGURE 7 | Sensitivity analysis examining the influence of individual studies on relative risk. The sequential exclusion of each study had no obvious effect on the result, indicating the robustness of our result.

underwent renal transplantation or were formally placed on the waiting list, although their prognosis was different; (2) some subgroup analyses were omitted; and (3) the heterogeneity was excessively high. To reduce heterogeneity, the present meta-analysis excluded studies with most patients receiving renal transplantation or on the waiting list of renal transplantation and conducted a series of subgroup analyses. In addition, we analyzed the potential causes and mechanisms leading to our results. Consistent with the conclusions of a previous meta-analysis, our findings further supported invasive therapy. In addition, our subgroup analyses may provide new ideas for the researchers and physicians in the future.

In the revascularization era, the benefits of invasive therapy in improving patient prognosis have been accepted widely (21, 22). However, the benefits were not clear among patients with CKD (especially ESKD) because of the absence of dedicated clinical trials. Our meta-analysis suggested that positive invasive therapy regardless of PCI or CABG may predict lower long-term mortality, which can be explained by the unique characteristics of patients with CKD. On the one hand, patients with CKD

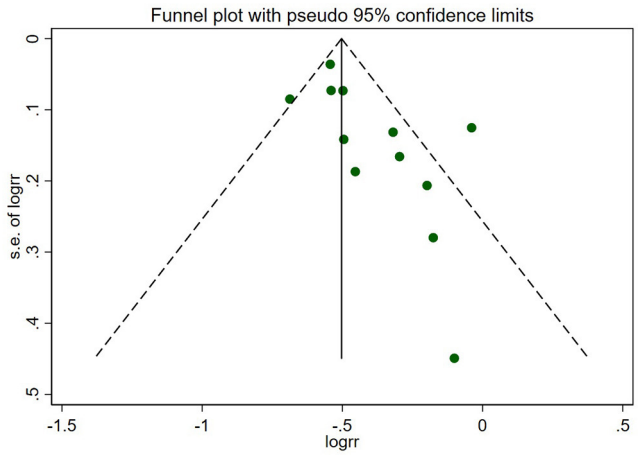


FIGURE 8 | Funnel plot of the primary outcome. The circles represent the included studies. The plot suggested that there may be publication bias in this review.

were characterized by more frequently having diabetes and three-vessel CAD or left artery disease, the indications to perform revascularization (21, 23). On the other hand, a higher stage of CKD is associated with more complex coronary lesions: larger plaque burden and necrotic cores but thin less fibrous caps, which is a symbol of vulnerable plaque morphologies predisposed to plaque rupture (24–26). They all significantly increase the risk of death and other major adverse cardiovascular events. As such, these high-risk individuals with CKD may benefit from revascularization. In addition, positive intervention may also attenuate the risk of sudden death by increasing the myocyte reserve in patients with CAD to handle fluxes in fluid/electrolytes or transient changes in sympathetic tone that can otherwise result in potentially lethal arrhythmic events in patients with CKD (15, 27).

Interestingly, in contrast to the ISCHEMIA-CKD study and other studies comparing revascularization and MT in stable patients with CAD (with or without CKD) (28, 29), our results showed an association between reduced mortality and revascularization in those with CKD. However, some issues should be considered seriously in interpreting this result. In addition to the significant I^2 statistic shown in **Figure 5A**, we noted that a negative result was yielded after the exclusion of the APPROACH study. Unlike the APPROACH study, the ISCHEMIA-CKD trial and other eligible observational studies in this subgroup analysis revealed the failure of revascularization to reduce mortality in stable patients with CAD and CKD. This difference could be explained by the considerable discrepancy in patient selection and follow-up duration. Compared with other studies, the APPROACH study enrolled a higher risk patient profile—individuals with $\geq$ 70% stenosis and surgical coronary disease—which was associated with the requirement of revascularization. Moreover, up to 10 years of follow-up possibly suggested late gains of revascularization. In summary, owing to the significant inconsistency in outcomes caused by various reasons, our results should be explained cautiously.

The other subgroup studies might also alter a few traditional views. It is known that geriatric patients carry a greater risk of in-hospital death and bleeding events after revascularization than younger patients (30), which leads to the underuse of revascularization in this high-risk group, let alone those with CKD. However, the survival benefit of invasive therapy was presented by our analysis in elderly individuals with CKD, which was consistent with the findings of previous studies in patients with geriatric CAD (with or without CKD) (31–33). Therefore, our results might further reveal the potential benefit of revascularization in improving the prognosis in elderly patients. However, the enrolled patients were not all aged over 70 years, which limited the representativeness of our findings in geriatric patients. The effect of invasive therapy in elderly patients with CKD should be defined according to evidence from more related trials.

For maintenance hemodialysis patients or those with eGFR $\leq$ 15 ml/min/1.73 m^2, PCI and CABG are frequently not favorable options in the clinical practice, as they are usually complicated with dyslipidemia, anemia, electrolyte disorders, and arterial stiffness, increasing the risk of major bleeding and mortality during or after revascularization. Our result of the hemodialysis group was consistent with the guidelines, in which revascularization was deemed appropriate for patients with MI, including NSTEMI patients with chronic nephrosis (34, 35). However, in NSTEMI patients with ESKD, especially maintenance dialysis, whether late gains can also offset early high risks after revascularization is still not clear. As a result of scarce information, we failed to perform analysis on non-STEMI patients, indicating the great significance of performing related studies on this issue.

LIMITATIONS

We must admit that there were a few limitations in this meta-analysis: (1) Ultimately, only 13 studies were identified to perform the meta-analysis, and they were mostly non-RCTs, in which more selection and confounding biases existed. It was difficult to control the diversities in baseline characteristics between the two arms. Since the comparisons between revascularization and MT alone compose the subgroup studies in some eligible studies, the absence of original data, especially several important baseline information such as the proportion of diabetes patients and the drug use in two arms, was a significant problem; (2) The heterogeneity of renal function in patients enrolled in different studies could not be ignored. For instance, patients in the COURAGE study were characterized by moderate impairment of renal function, while the ISCHEMIA-CKD trial only enrolled patients with advanced CKD. Therefore, it may not be appropriate to include them both to conduct the subgroup analysis of the stable CAD group, although the results of the advanced CKD/dialysis groups remained consistent; (3) Several studies calculated hazard ratios (HRs) and conducted survival analyses. In our meta-analysis, the RR was pooled to compare revascularization with MT, which consequently yielded a different result. For instance, according to the SWEDEHEART

2009 study, early revascularization improved 1-year survival in patients with NSTEMI and mild–moderate renal insufficiency (30 ml/min/1.73 m^2 <eGFR≤90 ml/min/1.73 m^2), but the observed benefit declined with the lower renal function, and there was a trend toward harm in those with end-stage renal disease or on dialysis. The result was different when we used RR to estimate the benefit of revascularization, suggesting a strong association between invasive therapy and lower mortality even in ESKD individuals. Therefore, the instability of the results showed that we must seriously explain the finding; and (4) The funnel plot (**Figure 8**) showed publication bias in this review, which questioned the reliability of our results.

CONCLUSION

In aggregate, the current evidence indicates that revascularization (PCI or CABG) is associated with a lower risk of long-term death than MT alone in patients with CAD and CKD. This long-term benefit was also observed in the geriatric and stable CAD groups. However, more randomized trials are urgently necessary to confirm these findings. Meanwhile, future studies should focus on renal-protective strategies to better manage these high-risk 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.

AUTHOR CONTRIBUTIONS

G-zL conceptualized and designed the study, collected and organized the data, conducted the analyses, drafted the initial manuscript and revised it. Y-mL conducted the analyses, reviewed the included articles, and reviewed and revised the manuscript. LB and Y-yY collected and organized the data and reviewed the included articles. YP conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed and revised the manuscript. All the authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.818958/full#supplementary-material

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A Novel Nomogram Based on a Competing Risk Model Predicting Cardiovascular Death Risk in Patients With Chronic Kidney Disease

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Objective: Chronic kidney disease (CKD) patients are more likely to die from cardiovascular disease (CVD) than develop renal failure. This study aimed to develop a new nomogram for predicting the risk of cardiovascular death in CKD patients.

Methods: This study enrolled 1656 CKD patients from NHANES 2003 to 2006 survey. Data sets from 2005 to 2006 survey population were used to build a nomogram for predicting the risk of cardiovascular death, and the nomogram was validated using data from 2003 to 2004 survey population. To identify the main determinants of cardiovascular death, we performed univariate analysis and backward-stepwise regression to select the key factors. The probability of cardiovascular death for each patient in 5, 7, and 9 years was calculated using a nomogram based on the predictors. To assess the nomogram's performance, the area under receiver operating characteristic curve (AUC) and the calibration curve with 1,000 bootstraps resamples were utilized. The prediction model's discrimination was examined using cumulative incidence function (CIF).

Results: Age, homocysteine, potassium levels, CKD stage, and anemia were included in the nomogram after screening risk factors using univariate analysis and backward-stepwise regression. Internal validation revealed that this nomogram possesses high discrimination and calibration (AUC values of 5–, 7–, and 9-years were 0.79, 0.81, and 0.81, respectively). External validation confirmed the same findings (AUC values of 5–, 7– and 9-years were 0.76, 0.73, and 0.73, respectively). According to CIF, the established nomogram effectively differentiates patients at a high risk of cardiovascular death from those at low risk.

Conclusion: This work develops a novel nomogram that integrates age, homocysteine, potassium levels, CKD stage, and anemia and can be used to more easily predict cardiovascular death in CKD patients, highlighting its potential value in clinical application.

Keywords: nomogram, chronic kidney disease, competing risk model, cardiovascular death, prediction model

INTRODUCTION

Chronic kidney disease (CKD) has developed into a global health crisis, lowering patients' quality of life declining and resulting in disability (1). At present, there are a large number of CKD patients worldwide. According to statistics, 42 million of Americans suffer from CKD (2), increasing to 700 million people globally (3). CKD has a slowly progressive clinical course and results in irreversible deterioration in renal function. Along with kidney function deterioration, the risk of cardiovascular (CV) complications also increases. According to the 2016 ESC/EAS guidelines (4), patients with stages 3–5 CKD are at high risk of experiencing CV adverse events. Additionally, the 2016 USRDS annual data report (2) indicates that CKD patients are more likely to experience CV events than undergoing dialysis. Cardiovascular disease (CVD) is also a leading cause of death in CKD patients. About 45.1% of people who reach stage 3 CKD will die of CV causes, and the mortality rate increases to 58% at stage 5 (5, 6). Given the high incidence and mortality of CV events, global attention has shifted to improving the prediction of cardiovascular outcomes in CKD patients.

Although adverse cardiovascular outcomes are common in CKD patients, there is currently no reliable tool to predict them (7). Constructing an accurate prediction model for cardiovascular outcomes is critical for clinical decision-making. It can not only identify high-risk groups and improve their prognosis but also reduce excessive medical expenses for low-risk patients. Currently, nomograms have emerged as a promising method for predicting adverse outcomes in kidney diseases. It takes into account a variety of factors, including demographics, biomarkers, health status, and behaviors, and can classify patients into different clinically distinguishable clusters linked to different outcomes, such as CKD progression, CVD, and death. It is a good way to group patients into different subtypes based on their baseline features, which is more helpful for the realization of precision medicine and individualized medicine. Therefore, this study aims to establish a visualized prediction model, scoring cardiovascular death risk in CKD patients using a nomogram.

MATERIALS AND METHODS

Patients and Predictors

In this study, the data of enrolled patients were obtained from the 2003 to 2006 National Health and Nutrition Examination Survey (NHANES). The population from 2005 to 2006 was used to construct the model, whereas the population in 2003–2004 served as an external validation cohort. The inclusion criteria include the following: (1) patients with CKD [defined as estimated glomerular filtration rate (eGFR; eGFR calculation was based on CKD-EPI formula) $<$60 mL/min per 1.73 m^2 or urinary albumin-to-creatinine ratio (UACR) $>$30 mg/g]; (2) patients with 18–75 years old. Patients with incomplete clinical data or lost contact during the follow-up period were excluded. Based on our clinical experience and literature review, we selected some risk factors that might affect cardiovascular prognosis, including diabetes, hypertension, gender, age, glycosylated hemoglobin (HbA1c), blood urea nitrogen (BUN), serum phosphorus (P), serum potassium, serum uric acid (UA), UACR, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total cholesterol (TC), serum total triglycerides (TG), calcium (Ca), Body Mass index (BMI), albumin (Alb), C-reactive protein (CRP), homocysteine, CKD stage (eGFR $<$ 60 mL/min per 1.73 m^2 or eGFR $\geq$ 60 mL/min per 1.73 m^2), and anemia (defined as hemoglobin $<$13 g/dL for men and $<$12 g/dL for women). The information of questionnaire was used to determine the presence of complications.

Prespecified Outcomes

Cardiovascular death was the main prespecified outcome. Since the occurrence of cardiovascular events in CKD patients may be a long-term process, we measured the probability of cardiovascular deaths at three different time points (5–, 7–, and 9-year). The situation of cardiovascular death and the follow-up time were extracted from the public-use linked mortality file from the National Center for Health Statistics (NCHS) and matched with patients' ID from NHANES database. The follow-up time is defined as the interval between the interview date and the death date.

Statistical Analysis

The three-knot cubic spline (10, 50, and 90%) was employed to account for potential non-linear effects of continuous data. Shapiro-Wilk method was utilized to examine the normal distribution of continuous data. The continuous variables conformed to normal distribution were compared using independent samples *t*-test and are presented as mean ± standard deviation (SD). Mann-Whitney *U*-test was used to determine the non-normally distributed variables, and they were presented as median (1st–3rd quartile). Chi-square tests were performed to compare categorical variables. If the theoretical frequency was less than 10, Fisher's exact test was preferred. Given the possibility of competitive risk, relying solely on one endpoint-analysis approach can lead to an inaccurate assessment of the probability of adverse events. Compared with Cox proportional hazards model, the competitive risk model can provide a more accurate estimate of the cumulative incidence of multiple outcomes (8). Therefore, in this study, we use a competing risk model for analysis, defining cardiovascular mortality as the primary outcome and other causes of death as competing events. First, fine and gray regression models were used for univariable analysis (9); variables with a statistical significance of the estimated regression coefficients of $P \geq 0.2$ were removed. Second, significant variables were selected using backward-stepwise regression (10), and the model with the lowest akaike information criterion (AIC) value was used to construct the competing risk nomogram (11). To evaluate the relative risk of each model, sub-distribution hazard ratios (SHRs) and 95% confidence intervals (95% CIs) were determined. The receiver operating characteristic (ROC) curves (12) were deployed to evaluate the sensitivity and specialty of the established model. The model's discrimination ability is determined using the area under the curve (AUC) at different time points. Larger AUC values indicate better overall discrimination. The nomogram's accuracy was measured using a calibration curve with 1,000 bootstrap resamples (13). Decision curve analysis (DCA) was conducted

to determine the nomogram's clinical utility by quantifying the net benefits at different threshold probabilities (14). For external validation, we calculated the total point of each patient according to an established nomogram and used it as a factor in the competing risk model. AUC and calibration curve were performed to verify the model's external applicability. To better assess the model's discrimination ability, we calculated the total score for each patient and divided them into high- and low-risk groups based on their median score. Then, CIF curves (cumulative incidence function) were constructed using Gray's test (15) to explore the risk differences between high- and low-risk groups. In all analyses, $P < 0.05$ (double) was considered statistically significant.

All statistical analyses were performed using R software version 4.05.[1] R software was used to implement the three-knot cubic spline (survminer package), competing risk model (mstate, riskRegression package), nomogram (survival package), CIF (cmprsk Package), ROC curve (timeROC), AUC, and calibration plot.

RESULTS

Baseline Characteristics

According to our prespecified inclusion and exclusion criteria, this study enrolled 1656 CKD patients. Among them, 10.5 and 15.4% died from CVD in training and validation cohorts, accounting for 33 and 35% of the total deaths, respectively. The median ages in training and validation cohorts were 66 and 70 years, respectively. The median follow-up times were 121 and 142 months in training and validation cohorts, respectively. In the training cohort, 53.3% of participants were males, 22.41% had diabetes, and 56.23% had hypertension. In the validation cohort, 53.71% were males, 25.42% had diabetes, and 59.18% had hypertension. Patients with stage 3–5 CKD account for 38.74% in training cohort and 37.82% in validation cohort. According to the observation of clinical characteristics, age, potassium, AST, homocysteine, and sodium levels were significantly different between the two cohorts ($P < 0.05$). **Table 1** summarizes laboratory and clinical characteristics of patients.

Cardiovascular Death Risk Prediction Models

Following univariate analyses of all variables using fine and gray regression models, ten significant variables in univariate analysis ($P < 0.2$) were entered into multivariate competing risk model (**Table 2**). Five variables (age: SHR 1.09, [95% CI: 1.06–1.11], homocysteine: SHR 1.83, [95% CI: 1.12–2.99], CKD stage: SHR 0.61, [95% CI: 0.39–0.94], anemia: SHR 1.67, [95% CI: 1.08–2.58], and potassium: middle/low: SHR 0.57, [95% CI: 0.28–1.14], high/low: SHR 0.40, [95% CI: 0.18–0.90]) were retained after backward-stepwise selection with the lowest AIC. We randomly selected a patient to calculate the probability of cardiovascular death. By summing up the scores of various risk factors, we can calculate that the cardiovascular death

[1]https://www.rproject.org/

TABLE 1 | Baseline characteristics of the training cohort and validation cohort.

Variables	Training cohort (n = 955)	Validation cohort (n = 661)	P-value
Age (years)	66.00 [50.00–77.00][a]	70.00 [54.00–80.00]	**0.002**
Gender (n, %)			0.872
Female	446 (46.70%)[b]	306 (46.29%)	
Male	509 (53.30%)	355 (53.71%)	
BMI (n, %)			0.68
<24 Kg/m^2	243 (25.45%)	180 (27.23%)	
24–27 Kg/m^2	199 (20.84%)	139 (21.03%)	
≥28 Kg/m^2	513 (53.72%)	342 (51.74%)	
Hypertension (n, %)			0.239
Yes	537 (56.23%)	392 (59.18%)	
No	418 (43.77%)	269 (40.82%)	
Diabetes (n, %)			0.162
Yes	214 (22.41%)	168 (25.42%)	
No	741 (77.59%)	493 (74.58%)	
Anemia (n, %)			0.225
No	801 (83.87%)	569 (86.08%)	
Yes	154 (16.13%)	92 (13.92%)	
AST (U/L)	24.00 [20.00–28.00]	23.00 [20.00–27.00]	**0.028**
TC (mmol/L)	5.00 [4.00–6.00]	5.00 [4.00–6.00]	0.119
Homocysteine (n, %)			**<0.001**
≥15.3 umol/L	852 (89.21%)	529 (80.03%)	
<15.3 umol/L	103 [10.79%]	132 (19.97%)	
CRP(n, %)			0.389
<1.8 mg/dL	830 (86.91%)	584 (88.35%)	
≥1.8 mg/dL	125 (13.09%)	77 (11.65%)	
HbA1c (n, %)			0.447
<6.0%	649 (67.96%)	461 (69.74%)	
≥6.0%	306 (32.04%)	200 (30.26%)	
Albumin (n, %)			0.472
<40 g/L	308 (32.25%)	202 (30.56%)	
≥40 g/L	647 (67.75%)	459 (69.44%)	
ALT(n, %)			0.747
<35 U/L	849 (88.90%)	591 (89.41%)	
≥35 U/L	106 (11.10%)	70 (10.59%)	
BUN(n, %)			0.709
<8.6 mmol/L	824 (86.28%)	566 (85.63%)	
≥8.6 mmol/L	131 (13.72%)	95 (14.37%)	
Phosphorous(n, %)			0.269
<1.5 mmol/L	476 (49.84%)	311 (47.05%)	
≥1.5 mmol/L	479 (50.16%)	350 (52.95%)	
TG (n, %)			0.785
<1.3 mmol/L	362 (37.91%)	255 (38.58%)	
≥1.3 mmol/L	593 (62.09%)	406 (61.42%)	
Sodium (n, %)			**0.028**
<140 mmol/L	527 (55.18%)	328 (49.62%)	
≥140 mmol/L	428 (44.82%)	333 (50.38%)	
Potassium (n, %)			**<0.001**
<3.5 mmol/L	44 (4.61%)	42 (6.35%)	
3.5–5.0 mmol/L	777 (81.36%)	599 (90.62%)	
≥5.0 mmol/L	134 (14.03%)	20 (3.03%)	
UACR (n, %)			0.991
≤30 mg/g	448 (46.91%)	309 (46.75%)	
31–300 mg/g	425 (44.50%)	294 (44.48%)	
>300 mg/g	82 (8.59%)	58 (8.77%)	
CKD. Stage (n, %)			0.708

(Continued)

TABLE 1 | (Continued)

Variables	Training cohort (*n* = 955)	Validation cohort (*n* = 661)	*P*-value
eGFR < 60 mL/min per 1.73 m^2	370 (38.74%)	250 (37.82%)	
eGFR ≥ 60 mL/min per 1.73 m^2	585 (61.26%)	411 (62.18%)	
CVD. Death (*n*, %)			**0.003**
No	855 (89.53%)	559 (84.57%)	
Yes	100 (10.47%)	102 (15.43%)	

[a]*Weighted median [95% CI for median].*
[b]*Actual frequency (weighted percentage).*
eGFR, estimated glomerular filtration rate; BMI, body mass index; CRP, C-reactive protein, HbA1c: glycosylated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; TG, serum total triglycerides; TC, serum total cholesterol; CVD, cardiovascular disease.
Bold values indicate the results were statistical significance.

probabilities of patients in 5, 7,and 9 years are 3.63, 5.94, and 8.01% respectively.

Development and Assessment of Predictive Nomogram

Internal Validation

A predictive nomogram containing age, homocysteine, CKD stage, potassium levels, and anemia has been established after univariate and backward-stepwise regression (**Figure 1**). All variables were statistically significant. AUC values of 5–, 7– and 9-years were 0.79 (95% CI: 0.73–0.85), 0.81 (95% CI: 0.77–0.86), and 0.81 (95% CI: 0.77–0.87), respectively (**Figure 2**). The calibration curves after 1,000 times of bootstraps revealed a good agreement between actual and predicted values (**Figure 3**). To better reflect the established model's discrimination. We calculated the total points of each patient according to the established nomogram and divided patients into high- and low-risk CVD death groups. CIF (**Figure 4**) showed that this nomogram has good discrimination, with a remarkable difference between the two groups (Gray'test: $P < 0.05$). DCA curves indicated that the model provided a net benefit across approximately 60, 70, and 75% of the risk threshold range in 5–, 7–, and 9-years, respectively (**Figure 5**).

External Validation

The external validation also indicated that this nomogram has a good predictive performance. The calibration curve demonstrated that the predicted value basically coincides with the actual value (**Figure 3**). AUC values of 5, 7 and 9-year OS were 0.76 (95% CI: 0.69–0.84), 0.73 (95% CI: 0.66–0.81), and 0.73 (95% CI: 0.68–0.78), respectively (**Figure 2**). CIF showed good discrimination between high- and low-risk CVD death groups (**Figure 4**). DCA curves showed a net benefit across approximately 50, 60, and 70% of the risk threshold range in 5–, 7–, and 9-years, respectively (**Figure 6**).

DISCUSSION

Cardiovascular disease is recognized as one of the most prevalent complications of CKD. CVD is also a leading cause of death in

TABLE 2 | Univariate and multivariate fine and gray competing risk regression analyses.

Variables	Univariate analysis		Multivariate analysis (Stepwise model)	
	SHR (95% CI)	*P*-value	SHR (95% CI)	*P*-value
Age (years)	1.08 (1.06–1.10)	<0.01	1.09 (1.06–1.11)	**<0.01**
Gender				
Female	Ref	–		
Male	0.73 (0.49–1.08)	0.12		
BMI				
<24 Kg/m^2	Ref	–		
≥24 Kg/m^2	0.83 (0.66–1.04)	0.11		
Hypertension				
No	Ref	–		
Yes	0.65 (0.43–0.98)	0.04		
Diabetes				
No	Ref	–		
Yes	1.08 (0.67–1.75)	0.75		
Anemia				
No	Ref	–	Ref	**–**
Yes	2.24 (1.45–3.43)	<0.01	1.67 (1.08–2.58)	**0.02**
AST (U/L)	1.01 (1.00–1.02)	0.11		
TC (mmol/L)	0.89 (0.77–1.04)	0.15		
Homocysteine				
<15.3 umol/L	Ref	–	Ref	–
≥15.3 umol/L	2.67 (1.69–4.25)	<0.01	1.83 (1.12–2.99)	**0.02**
CRP				
<1.8 mg/dL	Ref	–		
≥1.8 mg/dL	0.91 (0.50–1.67)	0.76		
HbA1c				
<6.0%	Ref	–		
≥6.0%	1.05 (0.69–1.58)	0.83		
Albumin				
<40 g/L	Ref	–		
≥40 g/L	0.87 (0.57–1.31)	0.49		
ALT				
<35 U/L	Ref	–		
≥35 U/L	0.99 (0.53–1.85)	0.98		
BUN				
<8.6 mmol/L	Ref	–		
≥8.6 mmol/L	1.50 (0.91–2.46)	0.11		
Phosphorous				
<1.5 mmol/L	Ref	–		
≥1.5 mmol/L	1.08 (0.73–1.60)	0.70		
Sodium				
<140 mmol/L	Ref	–		
≥140 mmol/L	1.48 (1.00–2.19)	0.05		
Potassium				
<3.5 mmol/L	Ref	–	Ref	–
3.5–5.0 mmol/L	0.51 (0.25–1.04)	0.07	0.57 (0.28–1.14)	0.11
≥5.0 mmol/L	0.73 (0.32–1.66)	0.45	0.40 (0.18–0.90)	**0.03**
TG				
<1.3 mmol/L	Ref	–		
≥1.3 mmol/L	1.05 (0.70–1.57)	0.82		
CKD. Stage				
eGFR < 60 mL/min/1.73 m^2	Ref	–	Ref	–
eGFR ≥ 60 mL/min/1.73 m^2	1.35 (0.89–2.06)	0.16	0.61 (0.39–0.94)	**0.03**
UACR				
≤30 mg/g	Ref	–		
31–300 mg/g	1.13 (0.74–1.72)	0.57		
>300 mg/g	1.77 (0.95–3.31)	0.07		

eGFR, estimated glomerular filtration rate; BMI, body mass index; CRP, C-reactive protein; HbA1c, glycosylated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; TG, serum total triglycerides; TC, serum total cholesterol; CVD, cardiovascular disease.
Bold values indicate the results were statistical significance.

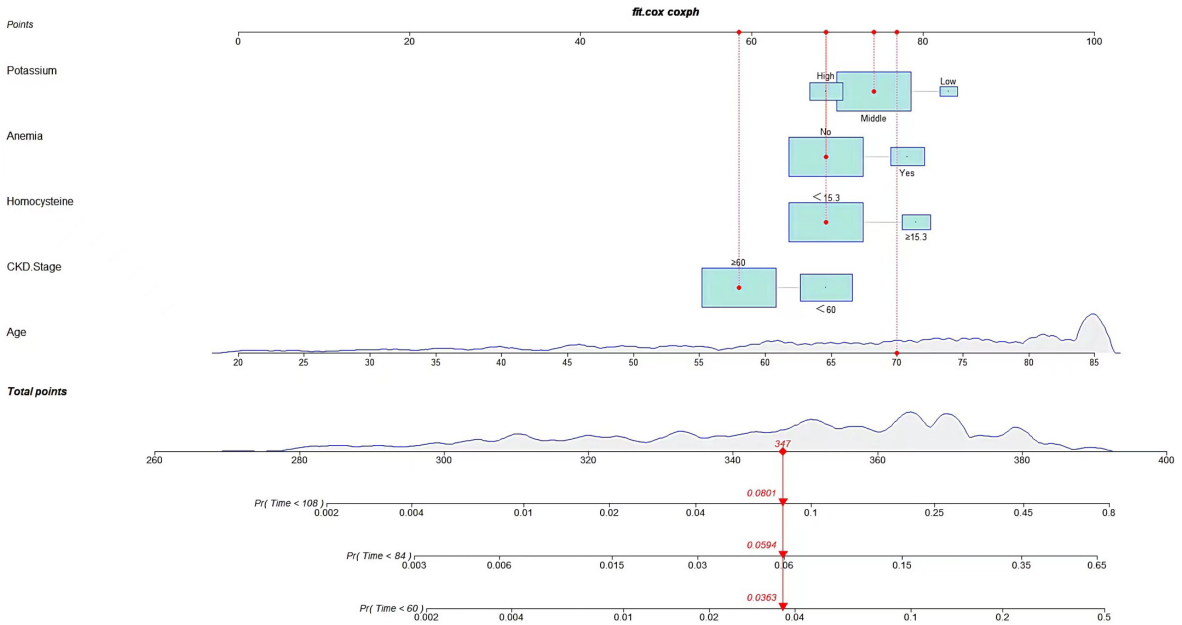

FIGURE 1 | Nomogram to predict the probability of cardiovascular death in CKD patients.

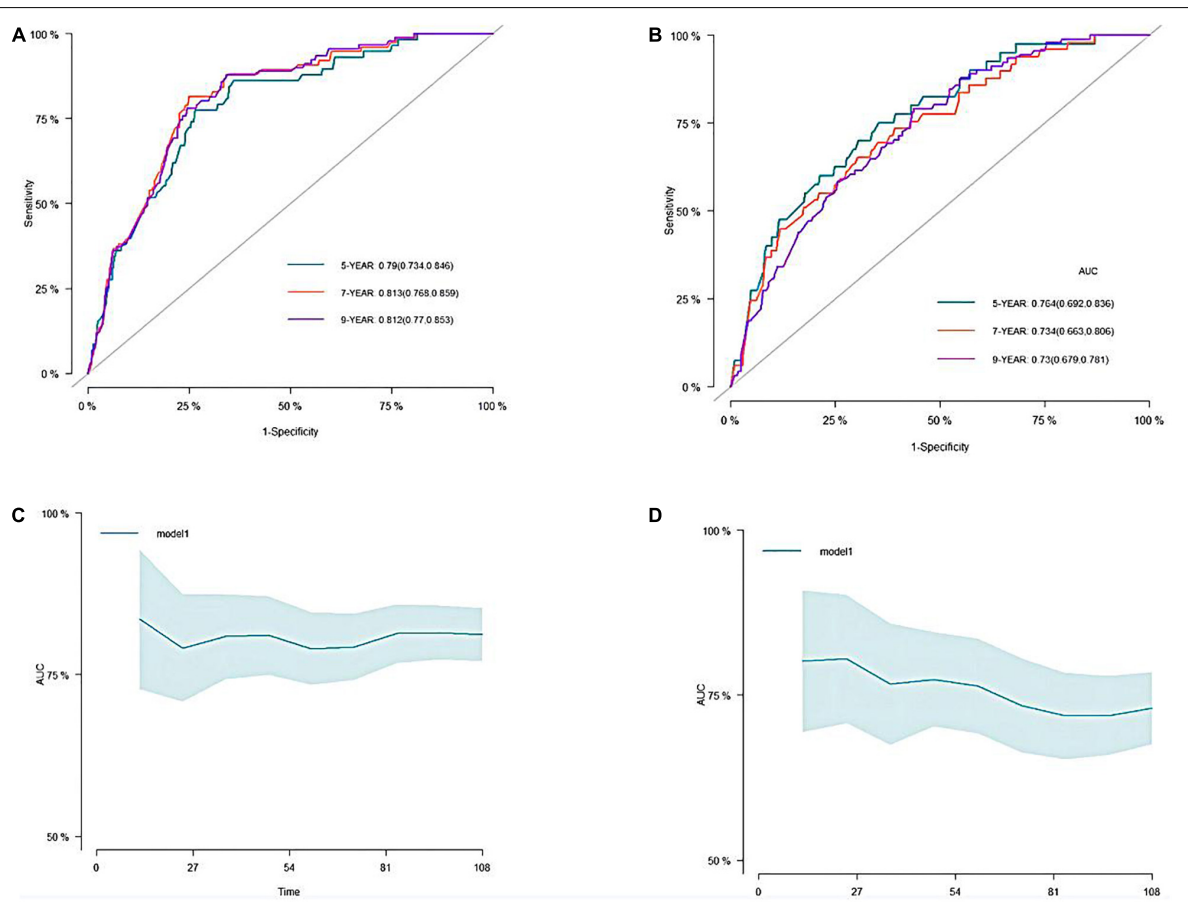

FIGURE 2 | The receiver operating characteristic (ROC) and the area under the curve (AUC) of the nomogram. **(A)** ROC curve of nomogram in the training cohort. **(B)** ROC curve of nomogram in the validation cohort. **(C)** The AUC of nomogram in the training cohort. **(D)** The AUC of nomogram in the validation cohort.

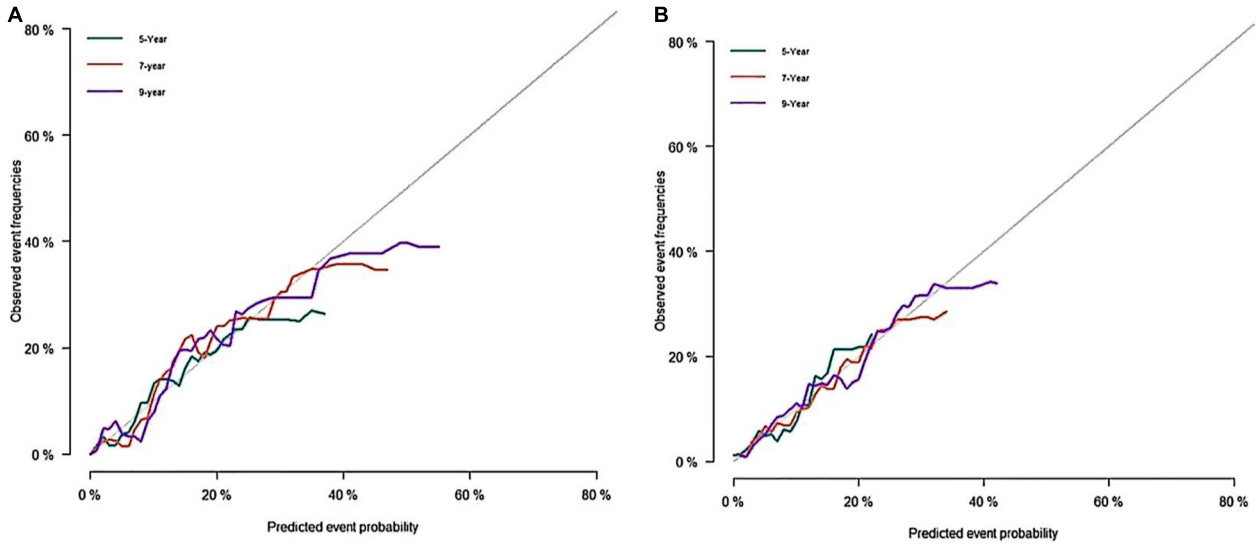

FIGURE 3 | Calibration curves of the nomogram. **(A)** Calibration curve of nomogram in the training cohort; **(B)** calibration curve of nomogram in the validation cohort.

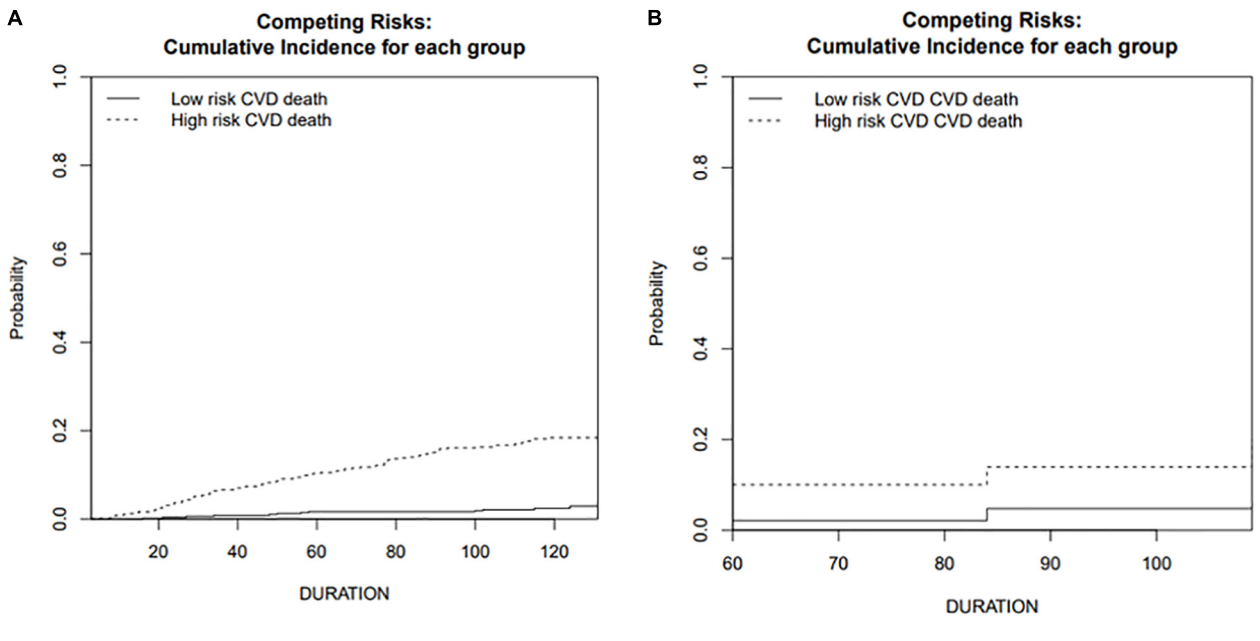

FIGURE 4 | Cumulative incidence function curve of cardiovascular death in training and validation cohorts. **(A)** Training cohort; **(B)** validation cohort.

CKD patients. The need for optimal risk prediction for CVD prevention is clinically significant. It can distinguish patients based on their different baseline features, identify patients with poor prognosis, and employ medical resources in an efficient manner. This study constructed a novel nomogram based on a competing risk model that included age, homocysteine, CKD stage, anemia, and potassium levels to predict long-term cardiovascular death in CKD patients. To our knowledge, this is the first nomogram to predict cardiovascular death risk in CKD patients. Internal and external validations of the nomogram revealed good predictive abilities, implying that the established nomogram exhibits good external applicability. In this work, we replaced the traditional Cox regression with a competing risk model. Compared with the traditional survival analysis, the competitive event model ensures that the chosen influencing factors are most directly associated with cardiovascular death prognosis (16). CIF demonstrated that our established model could effectively distinguish patients at high risk of CVD death from those at low risk. This is advantageous for identifying patients at high risk of CVD death and then providing appropriate intervention to improve their cardiovascular outcomes.

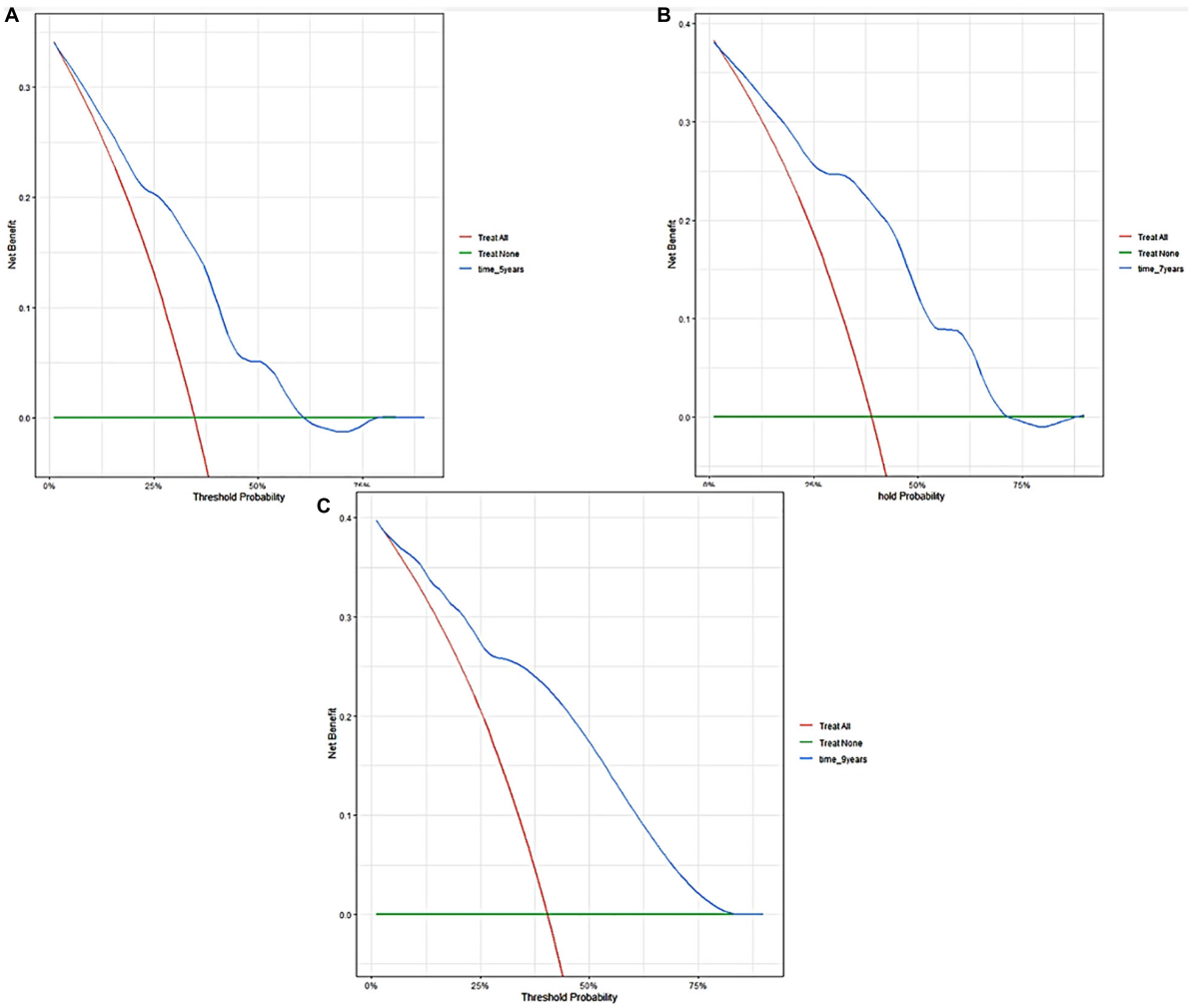

FIGURE 5 | Decision curve analyses of the nomogram in training cohort. Decision curve of the nomogram to predict 5-year **(A)**, 7-year **(B)**, and 9-year **(C)** in training cohort.

According to our descriptive statistics for the study population, CVD death accounted for one-third of all deaths, ranking as the first most common cause of death for CKD patients. Previous studies have revealed that mild-to-moderate CKD patients (stages 3A and 3B) have a considerably higher risk of cardiovascular mortality than patients with kidney failure (17). These findings indicate that core issues that clinicians should prioritize the risk of cardiovascular diseases over the risk of reaching kidney failure requiring renal replacement therapy. As for the key factors to consider after selection, our study revealed that age is the main factor causing CVD death in CKD patients since it accounted for most points in established nomograms. According to the 2013 global burden disease (18), the risk of cardiovascular death due to population aging has increased by 40.8% over the last two decades. Among them, death caused by arrhythmia and hypertension mostly increased. The assessment of CVD risk in the elderly is also important. The 2021 ESC guidelines (19) advocated routine cardiovascular risk assessment in men over 40 and women over 50. Our study revealed that, besides age, potassium levels also play a major role in determining cardiovascular outcomes. Interestingly, hypokalemia appears to be more likely to contribute to cardiovascular mortality. This could be due to the lower number of patients with higher blood potassium levels in the enrolled population, whereas patients with mild elevations in blood potassium levels have a correspondingly high potassium tolerance. SCREAM trial (20) enrolled 78,997 patients with stages 3–5 CKD and indicated that patients with poor kidney function seem to have better potassium tolerance. This may explain why our study found that hyperkalemia did not significantly increase the risk of cardiovascular outcomes. Anemia is considered as one of the predictors of cardiovascular events in CKD patients. Some scholars have proposed the concept of "cardio-renal-anemia syndrome" (21, 22), which aims to emphasize the critical function of anemia in the cardiorenal axis. A large cohort study in the United States discovered that the risk of developing various cardiovascular diseases increased when hemoglobin levels decreased in CKD patients (23). An epidemiological

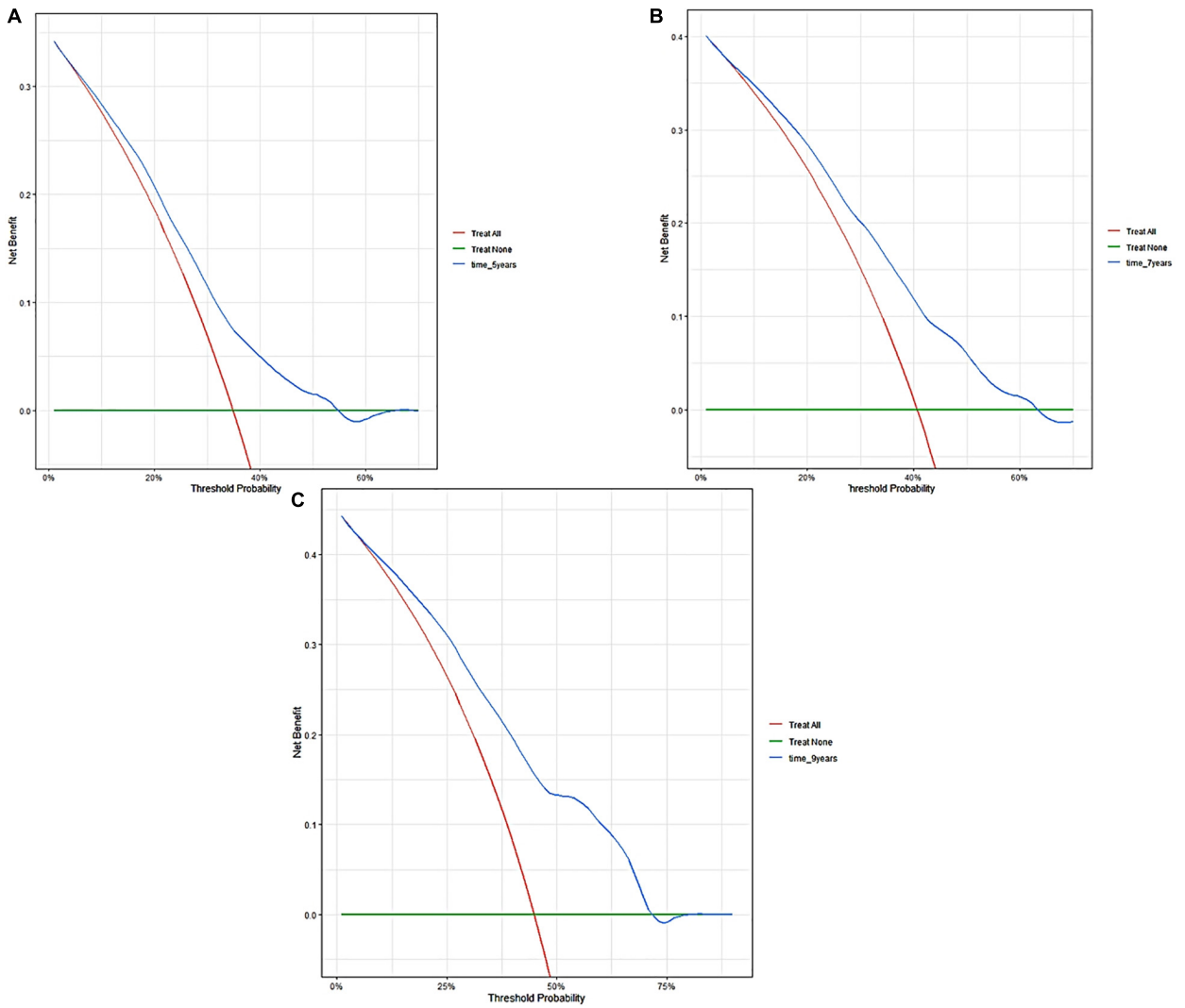

FIGURE 6 | Decision curve analyses of the nomogram in validation cohort. Decision curve of the nomogram to predict 5-year **(A)**, 7-year **(B)**, and 9-year **(C)** in validation cohort.

study found that after 3 years of observation of patients with stage 3 CKD, those with anemia had a twice greater incidence of cardiovascular events than those who were not anemic (24). CKD stage is known to be a direct indicator of cardiovascular events. In our study, cardiovascular death risk increases significantly from stage 3 CKD. This finding is consistent with previous studies, demonstrating that cardiovascular death risk was approximately twice as high in patients with stage 3 CKD (eGFR 30–59 mL/min per 1.73 m^2) and three times higher in stage 4 (15–29 mL/min per 1.73 m^2) than in individuals with normal kidney function (25, 26). Homocysteine is generally considered to be linked to cardiovascular and cerebrovascular prognosis. According to a meta-analysis, elevated homocysteine levels are an independent predictor of cardiovascular mortality (27). Together with our findings, we recommended that homocysteine should be routinely measured in CKD patients to detect their prognosis of cardiovascular outcomes.

It is undeniable that our prediction model has some limitations. First, all data of our enrolled patients were obtained from NHANES database, although we validated using data from many time points. Additionally, multicenter clinical validation is required to assess the nomogram's external applicability. Second, due to limited data of NHANES database, some crucial indicators of cardiovascular prognoses, such as brain natriuretic peptide or cardiac troponin, were not investigated. Third, our study included a substantial number of non-dialysis patients. The prediction efficacy of the developed nomogram for dialysis patients should be further validated.

CONCLUSION

Through a competing risk model, we established a novel nomogram incorporating age, homocysteine, CKD stage, anemia,

and potassium levels. The internal and external validations revealed the good predictive performance of this nomogram. Additional data on CKD patients should be used in the future to confirm the nomogram's prediction ability.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

NL and LZ contributed to the concept and design of this study. NL, YX, JZ, GZ, and YZ were responsible for statistical analysis and writing of the report. WH assisted in statistical analysis. MY, LX, EZ, and WS reviewed the article and provided critical feedback to improve and structure the report. All authors read and approved the final manuscript.

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A Novel Risk Prediction Model for Severe Acute Kidney Injury in Intensive Care Unit Patients Receiving Fluid Resuscitation

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Background: To develop a risk prediction model for the occurrence of severe acute kidney injury (AKI) in intensive care unit (ICU) patients receiving fluid resuscitation.

Methods: We conducted a secondary analysis of the Crystalloid vs. Hydroxyethyl Starch Trial (CHEST) trial, a blinded randomized controlled trial that enrolled ICU patients who received intravenous fluid resuscitation. The primary outcome was the first event in a composite outcome of doubling of serum creatinine and/or treatment with renal replacement treatment (RRT) within 28 days of randomization. The final model developed using multivariable logistic regression with backwards elimination was validated internally and then translated into a predictive equation.

Results: Six thousand seven hundred twenty-seven ICU participants were studied, among whom 745 developed the study outcome. The final model having six variables, including admission diagnosis of sepsis, illness severity score, mechanical ventilation, tachycardia, baseline estimated glomerular filtration rate and emergency admission. The model had good discrimination (c-statistic = 0.72, 95% confidence interval 0.697–0.736) and calibration (Hosmer-Lemeshow test, $\chi^2 = 14.4$, $p = 0.07$) for the composite outcome, with a c-statistic after internal bootstrapping validation of 0.72, which revealed a low degree of over-fitting. The positive predictive value and negative predictive value were 58.8 and 89.1%, respectively. The decision curve analysis indicates a net benefit in prediction of severe AKI using the model across a range of threshold probabilities between 5 and 35%.

Conclusions: Our model, using readily available clinical variables to identify ICU patients at high risk of severe AKI achieved good predictive performance in a clinically relevant population.

Keywords: acute kidney injury, risk prediction, model, ICU, fluids resuscitation

BACKGROUND

Acute kidney injury (AKI) is common in the intensive care unit (ICU), with a reported incidence of 7–25% for adults (1, 2). The close association between AKI and a range of adverse outcomes, including death, is well accepted, but there are few validated tools to identify ICU patients most at risk of these outcomes (3–5).

The development of consensus definitions for AKI has allowed more consistent diagnosis and comparison between different populations. There are three main AKI definitions in widespread use (6–8) which are broadly similar, using combinations of reductions in urine output, increases in serum creatinine and/or treatment with renal replacement therapy (RRT), to classify the severity of kidney injury. These definitions categorize AKI into stages of increasing severity that correlate with adverse outcomes such as increased mortality and prolonged length of hospital stay (9).

To facilitate early diagnosis and treatment, several models to predict AKI in ICU patients have been proposed (10). However, these models have examined a mixture of approaches (11–15), with data from varying numbers of ICUs and including both randomized trial cohorts and observational cohorts, some of which include specific biomarkers that are not in routine clinical use yet (12–15). In addition, the studied populations are often selected and this may affect the predictive ability and applicability to broader ICU populations of the resultant models. Patients receiving intravenous fluid resuscitation in ICU are common, readily identifiable, and may represent an enriched population at increased risk of severe AKI.

The Crystalloid vs. Hydroxyethyl Starch Trial (CHEST) was a multicenter, prospective, randomized-controlled, clinical trial (RCT) that compared the efficacy and safety of 6% hydroxyethyl starch (HES) (130/0.4) and 0.9% sodium chloride (normal saline) for intravenous fluid resuscitation in patients treated in ICU (16). A key secondary outcome was the incidence and severity of AKI using the Risk, Injury, Failure, Loss and End-stage kidney injury (RIFLE) criteria (7) and the incidence and duration of associated RRT. Using this trial database, we developed a prediction model to determine the risk of developing severe AKI for ICU patients receiving fluid resuscitation.

METHODS

Study Design

In brief, CHEST was a multicenter, prospective, randomized-controlled, clinical trial (RCT) that compared the use of HES and saline for intravenous fluid resuscitation in patients in 32 ICUs in Australia and New Zealand (Clinicaltrials.gov identifier#: NCT00935168). Detailed descriptions of the study protocol, statistical analysis plan and results have been published previously (16, 17). The CHEST study was approved by the human research Ethics Committee of Northern Sydney Central Coast Health (AU RED Ref: HREC/09/HARBR/14), and by each participating institution. The present study is a *post-hoc* analysis of the CHEST database with the objective of developing a model for the prediction of severe AKI in ICU patients receiving fluid resuscitation.

Study Outcomes

The primary outcome of the model is severe AKI, defined as the first event in a composite outcome incorporating doubling of serum creatinine (from the pre-randomization value) and/or treatment with RRT within 28 days of randomization. It is important to note that this outcome differs from the reported AKI outcome in the primary CHEST article, which used the RIFLE criteria (17) defined by changes in either urine output or serum creatinine from randomization.

Demographic and Clinical Variables

In order to minimize selection bias, our analysis included all patients from the original study population for whom consent to use of their data was obtained and were not lost to follow-up. Demographic and clinical variables were collected as described previously (16), and variables collected immediately before randomization were deemed as baseline variables. We examined 17 candidate baseline variables that were prospectively considered by the authors to be potential indicators of AKI risk: age, sex, weight, heart rate (HR), central venous pressure (CVP), mean arterial pressure (MAP), urine output during 6 h prior to randomization, serum creatinine (serum Cr), estimated glomerular filtration rate (eGFR), serum lactate concentration, Acute Physiology and Chronic Health Evaluation (APACHE) II score (18), Sequential Organ Failure Assessment (SOFA) score for the cardiovascular system (19), the presence of sepsis (20), the presence of trauma, source of admission, treatment with mechanical ventilation, and the nature of ICU admission (surgical or nonsurgical). As the allocated study treatment (HES or saline) was assigned at randomization, this variable was not considered as a baseline variable and excluded from multivariate regression models.

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (21). The source of admission was defined as the place from where the patients had been transferred to ICU and was classified as: hospital floor, emergency department, operating room following elective surgery, operating room following emergency surgery, and other hospitals. Mechanical ventilation included either invasive ventilation via an endotracheal tube or non-invasive respiratory support via a mask or other non-invasive interface.

Statistical Analysis

As the analysis focused on the baseline clinical variables, all patients in the baseline analysis of the original study publication with complete demographic data and information about the study outcome were included. Continuous variables were presented as median (interquartile range) or mean ± standard deviation (SD), categorical variables were presented as number (percentage). APACHE II score was considered as a continuous variable. Univariable regression was used to examine the relationship between candidate predictors and the study outcome. Multivariable logistic regression model was used to identify independent risk factors for the study outcome.

For modeling, we followed a three-step procedure. First, we developed a primary model by multivariable logistic regression using a backward elimination approach (threshold $p < 0.05$)

to select variables (22) after excluding variables that would potentially have high risk of in the development population. Possible first-order interactions were also explored unavailability in real-world clinical practice based on their proportions of missing data and kept in the model if statistically significant ($p < 0.05$). Second, we developed a secondary model by removing interaction terms in the primary model and compared the performance of these two models using c-statistics (equal to the area under the receiver-operating characteristic curve). If no difference between these primary and secondary models was found, the secondary model was preferred due to its greater simplicity and avoidance of over-fitting, consistent with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statements (23).Third, we simplified the model from step two by removing variables that were not statistically significant and tested the performance of the final parsimonious model. The three models were compared, and the final model was chosen as the best balance between calibration, discrimination and clinical practicability. To test the performance of the model, discrimination was assessed by c-statistics (24), and calibration was assessed by Hosmer-Lemeshow test (25) and calibration plot.

In addition we performed two sensitivity analyses. The first tested whether forcing the randomization treatment (HES or normal saline) into the multivariable regression model, altered the model's predictive performance, and the second examined whether the timing of the primary outcome, at 7 days rather than 28 days from randomization altered model performance to be consistent with current diagnostic criteria of AKI.

For validation, we randomly sampled from the study dataset using the bootstrapping method (26) ($n = 10{,}000$ replications) to evaluate the over-optimism inherent in the final model. Confidence intervals of the over-fitting in c-statistics for the bootstrap corrections were computed based on the assumption of normal distributions. The bootstrap-adjusted performance was calculated and the final risk prediction model was translated

TABLE 1 | Baseline characteristics and the study outcome of the study population.

Variables		Summary values*	Number of values in dataset **
Demographic			
Age (years)		63.1 ± 16.9 (6,727)	6,727
Male		4,060 (60.4%)	6,726
Renal parameters			
serum Cr (μmol/L)		100.5 ± 57.2	6,639
eGFR (ml/min/1.73 m^2)		73.8 ± 30.8	6,637
Urine output 6 h before randomization (ml)		440.2 ± 420.7	2,802
Other clinical parameters			
HR (beats per minute)		89.0 ± 23.4	6,691
Weight (kg)		78.9 ± 20.9	6,727
CVP (mmH_2O)		9.2 ± 5.3	2,300
MAP (mmHg)		73.8 ± 14.8	6,687
Lactate (mmol/L)		2.0 ± 1.76	5,555
APACHE II score		17.9 ± 7.6	6,688
Cardiovascular SOFA score	0	1,172 (17.5%)	6,698
	1	2,415 (36.1%)	6,698
	2	40 (0.6%)	6,698
	3	2,161 (32.3%)	6,698
	4	910 (13.6%)	6698
Presence of sepsis		1,936 (28.8%)	6,724
Presence of Trauma		528 (7.9%)	6,727
Presence of nonsurgical diseases		3,844 (57.2%)	6,716
Admission Source to ICU	Hospital floor	1,323 (19.7%)	6,723
	Emergency department	1,857 (27.6%)	6,723
	OR following elective surgery	1,574 (23.4%)	6,723
	OR following emergency surgery	1,254 (18.7%)	6,723
	Other hospitals (ICU or non-ICU%)	715 (10.6%)	6,723
Mechanical ventilation		4,307 (64.5%)	6,679

APACHE II, Acute Physiology and Chronic Health Evaluation II; CVP, central venous pressure; eGFR, estimated glomerular filtration rate; HES, hydroxyethyl starch; MAP, mean arterial pressure; OR, operation room; RRT, renal replacement therapy, serum Cr, serum creatinine; SD, standard deviation; SOFA, Sequential Organ Failure Assessment.

**Values were expressed as mean ± standard deviation for continuous variables or number (percentage) for categorical variables.*

***Some numbers were less than the total number (n = 6,727) of the study population due to missing data.*

into a predictive equation. Positive predictive value (PPV) and negative predictive value (NPV) were calculated, and a decision curve analysis was performed to evaluate the net benefit of using the model in predicting the primary outcome across a range of threshold probabilities.

The reporting of this prognostic model study followed the TRIPOD statement (23). The risk of bias of the final model was assessed using the Prediction Model Risk of Bias Assessment Tool (PROBAST) (27). In addition, we used the final model and REDCap electronic data capture tools (28) securely hosted at Sichuan Provincial People's Hospital to develop a web-based risk assessment tool accessible to readers.

All analyses were performed using SAS/STAT software v.9.1 (SAS Institute Inc., Cary, NC, USA) with statistical significance set at $P < 0.05$.

RESULTS

Baseline Characteristics and Study Outcome

Baseline variables and the study outcome in the study population are listed in **Table 1**. Of the 6,742 patients reported in the original publication, 15 patients were excluded because of missing RRT follow up data, leaving 6,727 patients for analysis. The majority of the 6,727 patients entered ICU from the operating room or emergency department. Data for urine output during 6 h prior to randomization, baseline CVP and baseline serum lactate were available for only 2,802, 2,300 and 5,555 patients respectively, so these three variables were excluded from modeling (see **Table 1**). The proportion of individuals with at least one variable with missing value was 2.79% (188/6727).

Within 28 days after randomization, 514 (7.6%) participants experienced a doubling of serum Cr, 427 (6.4%) were treated with RRT and 196 (2.9%) experienced both events. In total 745 (11.1%) participants developed the study outcome (**Figure 1**).

Univariate Analysis of Candidate Predictors

The univariate relationships of all candidate model variables with the study outcome are presented in **Table 2**. The majority of these variables were associated with the study outcome, but sex, MAP, admission following emergency surgery (compared to admission from emergency department) and mechanical ventilation were not.

Prediction Model

Evaluated variables in each model were examined and compared (**Supplementary Table S1**). In addition to stand-alone significant variables including age, baseline eGFR, heart rate, APACHE II score, sepsis, mechanical ventilation at admission, and admission source, the primary model (Model A) also included eight interactive terms as significant factors. However, the performance of the secondary model (Model B) after removing interactive terms did not materially differ from the primary model (**Figure 2**). After removing these interactive terms, seven variables became non-significant, resulting in the final model (Model C) including six significant predictors (**Table 3**). The comparison of all three models indicated no meaningful difference in C-statistics for the primary, secondary and final models (**Figure 2**).

The sensitivity analysis of including randomization treatment in the final model showed no change in the effect of the six predictors or the C-statistic (**Table 4**). The sensitivity analysis of the ability of the final model to predict doubling of serum creatinine and RRT within 7 days after randomization again showed no change in the effect of the model variables indicated all predictors remained significant except for mechanical ventilation at admission (**Supplementary Table S2**).

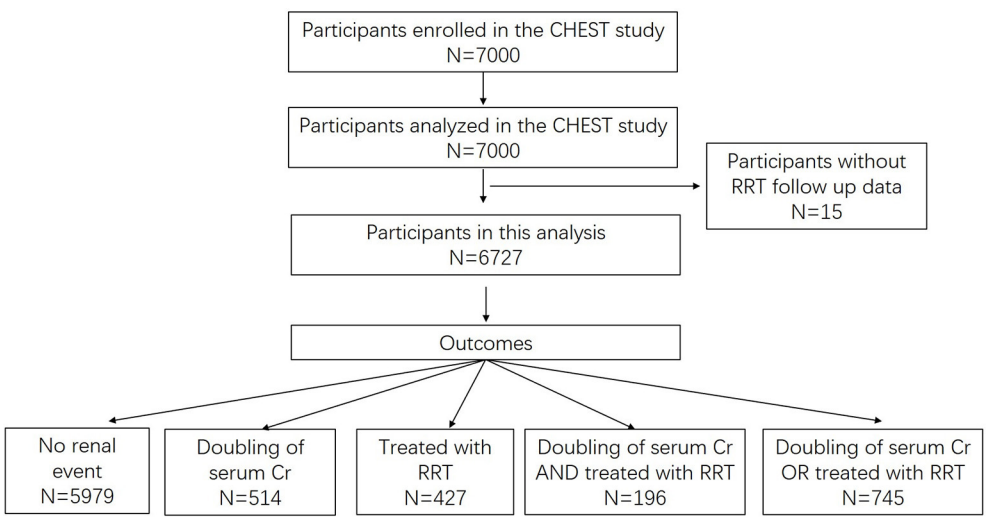

FIGURE 1 | Participant flow diagram. Cr, creatinine; RRT, renal replacement treatment.

TABLE 2 | Univariate odds ratios of candidate predictive variables for the study outcome.

Variables		Odds ratios	95% CI	*P*-value
Male[†]		0.95	0.82–1.11	0.5375
Age per 5 years increase		1.03	1.01–1.06	0.0084*
Baseline eGFR per 5 ml/min/1.73 m^2 decrease		1.08	1.06–1.09	0.0000*
HR per 5 bpm increase		1.11	1.09–1.13	0.0000*
Weight per 5 kg increase		1.02	1.01–1.04	0.0080*
MAP per 10 mmHg increase		1.02	0.97–1.08	0.4367
APACHE II score		1.06	1.05–1.07	0.0000*
Cardiovascular SOFA score		0.83	0.71–0.97	0.0172*
Presence of sepsis		2.42	2.07–2.83	0.0000*
Presence of Trauma		0.53	0.37–0.76	0.0005*
Presence of nonsurgical diseases		2.09	1.31–3.33	0.0019*
Admission source[#]	Hospital floor	1.55	1.25–1.91	0.0000*
	OR after elective surgery	0.55	0.42–0.70	0.0000*
	OR after emergency surgery	1.04	0.83–1.31	0.7086
	Other hospital	1.46	1.13–1.88	0.0038*
Mechanical ventilation at admission		1.16	0.99–1.37	0.0727

Serum Cr was not included in the analysis due to its high correlation with eGFR. Continuous APACHE II score and Cardiovascular SOFA score were used.

APACHE II, Acute Physiology and Chronic Health Evaluation II; bpm, beats per minutes; CVP, central venous pressure; eGFR, estimated glomerular filtration rate; HES, hydroxyethyl starch; HR heart rate, -MAP, mean arterial pressure; OR, operating room; RRT, renal replacement therapy, serum Cr, serum creatinine; SOFA, Sequential Organ Failure Assessment.

[†] *vs. female.*

[#] *vs. admission from the emergency department.*

[*] *Statistical significance.*

Final Model Performance and Internal Validation

The observed and predicted risk of the composite primary outcome based on the final model were similar, with a test of goodness of fit indicating good calibration (modified Hosmer-Lemeshow test, $\chi^2 = 14.4$, $p = 0.07$) (**Figure 3**). Internal validation using the bootstrap method revealed the degree of over-optimism on c-statistics of the final prediction model was 0.0055 (95% CI, −0.0129 to 0.0240), resulting in an equivalent c-statistic after bootstrap validation of 0.711. The PPV and NPV of the final model to predict severe AKI in ICU patients receiving fluid resuscitation within 28 days from admission were 58.8 and 89.1%, respectively. The decision curve analysis indicates a net benefit in prediction of severe AKI using the model across a range of threshold probabilities between 5 and 35% (**Figure 4**), illustrating the additional benefit of the model in predicting severe AKI over and above the approaches of "intervention for all" and "intervention for none".

Risk of Bias Assessment of the Final Model

According to the PROBAST assessment, 19 of 20 signaling questions were rated as "Yes," and 1 was rated as "Probably Yes," thus the overall risk of bias of the final model was rated as low risk of bias. Detailed rationales of answers were described in **Supplementary Table S3**.

Risk Predictive Equation for the Study Outcome

From the above computation, the following risk prediction equation was derived:

$$P_{outcome} = 1/(1 + \exp(-A)) \quad (1)$$

where $P_{outcome}$ indicates the probability for the study outcome occurring, and A = −0.0101 * eGFR + 0.0161 * Heart Rate + 0.0378*APACHE II score + 0.4577* (if sepsis) + 0.3747* (if admitted from hospital floor) + 0.2080* (if admitted from OR after elective surgery) + 0.2577* (if admitted from OR after emergency surgery) + 0.3699* (if admitted from other hospitals) + 0.2154* (if mechanical ventilation on admission) – 4.1529.

The prediction model was translated using REDCap to an online calculator for readers' convenience (to visit, please scan the QR code in **Supplementary Figure S1** using mobile devices or visit: http://redcap.scrds.net/surveys/ and enter code: 7KMH8HN7N). The probability would be shown automatically at the completion of all predictors. Readers are encouraged to submit their data that can be collected for further improvement of the model.

DISCUSSION

We report the development of a model for predicting severe AKI risk in a population of ICU patients who received fluid resuscitation, using data from a large, multi-center randomized trial. The final model includes six significant clinical predictors: admission diagnosis of sepsis, illness severity score, mechanical ventilation, tachycardia, baseline estimated glomerular filtration rate and emergency admission; all of which are readily evaluable close to the point of ICU admission and, together, demonstrate robust predictive ability, discrimination and calibration.

Our findings broadly accord with those of other similar recent studies (12, 15, 29, 30), but also highlight some important challenges in this field. Flechet et al.'s model (12) is the closest in design to our analysis, being a randomized trial dataset collected across 7 centers (the Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically Ill Patients Study, EPaNIC), and both models share a number of predictive variables such as baseline renal function, requirement for emergency surgery and clinical suspicion of sepsis at baseline. Flechet's report included several models defined by different time points in patients' hospital stay, with their "admission model" the most analogous to our final model. Its discriminative ability for AKI within the first week of ICU stay was similar to our finding (c statistic of 0.75), and improved when additional variables from the first 24 h following admission to ICU were included (c statistic rising to 0.82). The underlying populations had some important differences, with the EPaNIC study having a higher proportion of patients entering ICU following elective cardiac surgery and a 30% lower mortality rate, but similar rates of renal replacement therapy use, compared to the CHEST population (16, 31).

Malhotra and colleagues used prospective data from a single center to derive a model and validated it using data from another US facility (29). This model included several chronic

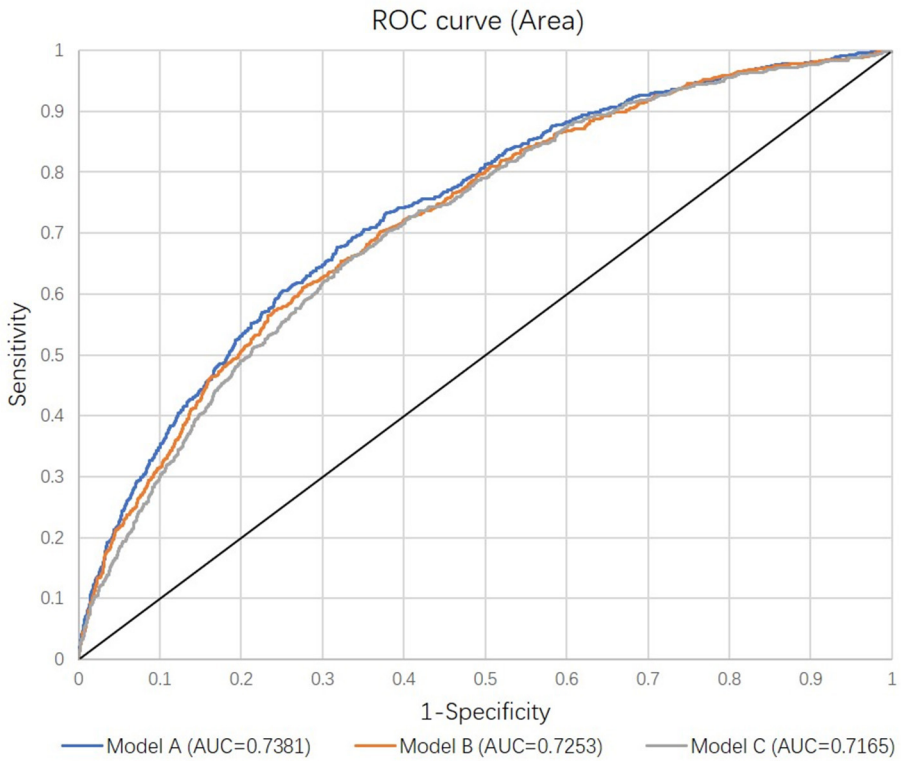

FIGURE 2 | Receiver Operating Curves comparing the discrimination of the three models. Each model was adjusted for randomly assigned treatments. See details in Methods.

TABLE 3 | Odds ratios of independently significant predictors in the final model.

Variables		Doubling of serum Cr or RRT within 28 days after randomization		
		Odds ratios	**95% CI**	***P*-value**
Baseline eGFR per 5 ml/min/1.73 m^2 decrease		1.052	1.037–1.067	<0.0001
HR per 5 bpm increase		1.084	1.065–1.103	<0.0001
APACHE II score		1.039	1.027–1.052	<0.0001
Presence of sepsis		1.580	1.325–1.885	<0.0001
MV at admission		1.242	1.032–1.491	0.02
Admission source*	Hospital floor	1.455	1.166–1.814	0.009
	OR after elective surgery	1.231	0.922–1.644	
	OR after emergency surgery	1.294	1.009–1.659	
	Other hospitals	1.448	1.103–1.900	

Continuous APACHE II score was used.

APACHE II, Acute Physiology and Chronic Health Evaluation II; bpm, beats per minutes; eGFR, estimated glomerular filtration rate; HR, heart rate; MV, mechanical ventilation; OR, operating room; RRT, renal replacement therapy.

**vs. admission from the emergency department.*

disease conditions along with acute risk factors such as acidosis, treatment with mechanic ventilation and the presence of sepsis. The discrimination of this model was high in both development and validation cohorts, but the inclusion of data from the first 48 h of participants ICU stay likely played a part in this, as Flechet et al. also saw increases in the c statistic as data from later in the patient journey was added to their admission model (12). Koyner et al.'s model, which included both general hospital and ICU patients, using extensive electronic health record data, had a better discrimination compared with our model, which may be in part due to greater number of variables and the use of data from after the patients' admission (30).

Our model benefits from the prospective nature of the CHEST study, which examined a large, clearly defined cohort of general

TABLE 4 | Sensitivity analysis of including randomization treatment in the multivariable regression model.

Variables		Doubling of serum Cr or RRT within 28 days after randomization					
		Final model			Model with treatment forced in		
		Odds ratios	95% CI	*P*-value	Odds ratios	95% CI	*P*-value
Randomization treatment (HES) *				1.177	1.002–1.382	0.047	
Baseline eGFR per 5 ml/min/1.73 m^2 decrease	1.052	1.037–1.067	<0.0001	1.052	1.037–1.067	<0.0001	
HR per 5 bpm increase	1.084	1.065–1.103	<0.0001	1.084	1.065–1.103	<0.0001	
APACHE II score	1.039	1.027–1.052	<0.0001	1.039	1.027–1.052	<0.0001	
Presence of sepsis	1.580	1.325–1.885	<0.0001	1.580	1.325–1.885	<0.0001	
MV at admission	1.242	1.032–1.491	0.020	1.242	1.033–1.493	0.021	
Admission source†	Hospital floor	1.455	1.166–1.814	0.009	1.456	1.167–1.815	0.094
	OR after elective surgery	1.231	0.922–1.644		1.233	0.923–1.647	
	OR after emergency surgery	1.294	1.009–1.659		1.292	1.008–1.656	
	Other hospitals	1.448	1.103–1.900		1.442	1.099–1.892	
	AUC (95% CI)		0.717 (0.697, 0.736)			0.715 (0.696, 0.735)	

**vs. saline.*

†vs. admission from the emergency department.

APACHE II, Acute Physiology and Chronic Health Evaluation II; bpm, beats per minutes; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, heart rate; MV, mechanical ventilation; OR, operating room; RRT, renal replacement therapy.

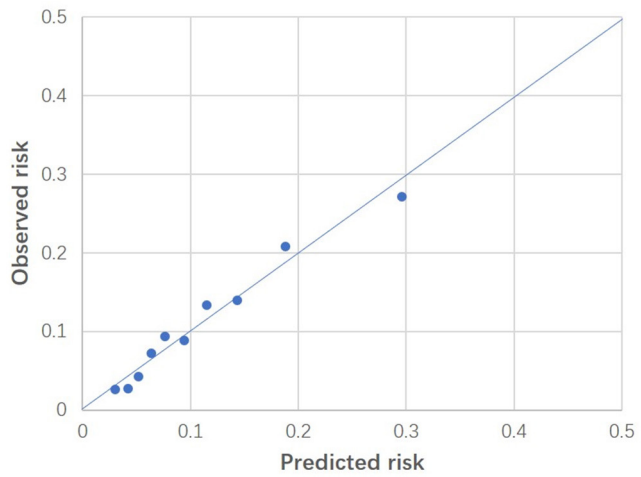

FIGURE 3 | Calibration plot of the final model. Predicted risk was indicated on horizontal axis, whereas observed risk was indicated on vertical axis. These results were based on patients grouped into deciles of predicted risk. Modified Hosmer-Lemeshow test indicated good fitness ($p = 0.07$).

ICU patients, was successful in recruiting patients within a median of 12 h of ICU admission, and uses data from a large number of centers. The resultant final model is relatively simple, enhancing usability and reducing the risk of model over-fitting, and estimates a probability for more severe AKI in the 28 days after baseline. For example, a septic patient with a baseline eGFR of 30 ml/min/1.73 m^2, heart rate of 90, APACHE II score of 18 who has been transferred to ICU from the hospital floor on mechanical ventilation, the risk of developing the composite event (doubling of serum Cr or treatment with RRT) in the following 28 days would be 21.8%. However, external validation of these findings is an important next step.

Our modeling is the first in this area to use decision curve analysis, first described in 2006 (32), as a tool to understand the "net benefit" of a predictive model compared to strategies that clinically act upon all or none of defined patient groups. A challenge in interpreting these results is understanding the nature of clinical responses to a severe AKI diagnosis, where there is an absence of effective treatments proven to reduce the occurrence, or outcomes, of severe AKI. However, identification of populations at high risk of severe AKI may assist in the prognostic enrichment of clinical trials that test treatments or strategies to prevent AKI, and could be used to assess baseline balance in risk of developing AKI in clinical trials where AKI is a trial endpoint. Additionally, our model may also have value in smaller intensive care units, where RRT support is limited, by aiding timely decisions to transfer patients at higher risk to centers with greater capacity to treat AKI. As such, the low risk of harms from applying the model and responding to a diagnosis of severe AKI, would suggest the threshold probability for its use is at the lower end of the presented range, where the net benefit is most pronounced.

Our study has limitations. First, the study population was derived from an existing clinical trial dataset and, whilst the inclusion criteria for the CHEST study were broad, it excluded important patient groups such as those with advanced AKI at baseline and children. Second, a relatively small proportion of the patients developed the study outcome, accounting for 11.1% of the original population, which was lower than the average reported prevalence of AKI in general ICU population (1, 2), and was likely a function of study entry criteria. Third, the prediction model did not include urine output, which is a part of most definitions for AKI, as the data were incomplete for a large proportion of the study population; an issue also seen in other published models (10, 29). Fourth, the PPV is relatively low, indicating that we are less confident in predicting a patient who would develop AKI in 28 days than in ruling out a patient

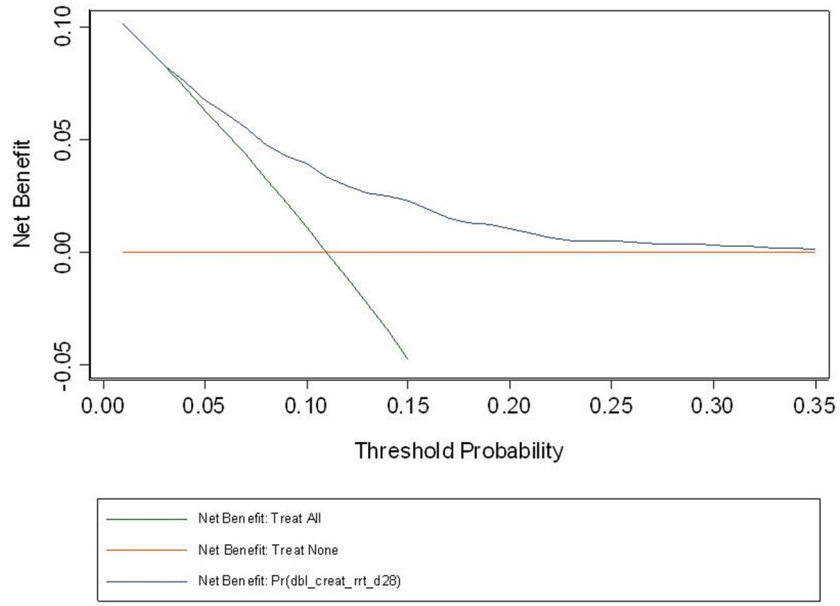

FIGURE 4 | Decision curve based on the final model. Decision curve analysis is a relatively recent approach, seeking to overcome the limitation of the usual model assessment tools such as calibration and discrimination in their clinical application. If one doesn't use the model, then any intervention could be applied to everyone (intervention for all) or no-one (intervention for none). Between these two interventions sits the impact of the model, and the fact that our curve sits above the intersection of both curves, across the range of probabilities for AKI in our study population, suggest that the model will be of net benefit in this population. At a population level, the net benefit of the intervention treatment will be realized across a larger number of patients across the spectrum of risk.

who would not develop the same outcomes. Fifth, it needs to be borne in mind that the dataset underlying this model includes 620 deaths that did not experience the study outcome, which mostly occurred within the first 10 days of the study. Finally, external validation is central to fully understanding the value of our model.

CONCLUSIONS

We have developed a novel risk prediction model for severe acute kidney injury in a population of general ICU patients receiving fluid resuscitation. The final model includes six significant predictors and has good discrimination and calibration for the composite outcome of doubling of serum creatinine or treatment with RRT within 28 days. External validation is needed to explore its generalizability.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by human research Ethics Committee of Northern Sydney Central Coast Health. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM, SF, MJ, VP, AW, and MG conceived the study. QL, YF, and MJ did the data analyses. The article was written by YF, with input from all co-authors. All authors interpreted the results, contributed to the critical revising of the manuscript, and have approved the final 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.2022.840611/full#supplementary-material

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Creatine Kinase and Mortality in Peritoneal Dialysis

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Background: The association between serum creatine kinase and mortality in patients with peritoneal dialysis (PD) remained unknown.

Methods: We retrospectively collected data on 3,446 incident patients with from five PD centers in China between 1 January 2005 and 31 May 2020. Creatine kinase was collected 1 week before the start of PD. We examined the association between creatine kinase and mortality using Cox proportional hazards model.

Results: The median creatine kinase was 113 (range, 1.22–4,574) IU/L. With a median follow-up of 39.5 (range, 3.1–181.5) months, 763 (22.1%) all-cause deaths occurred, including 384 (11.1%) cardiovascular deaths. As compared with a creatine kinase of 111–179 IU/L (reference range), a higher creatine kinase (>179 IU/L) was associated with increased risks of all-cause mortality [hazards ratio (HR), 1.72; 95% CI, 1.35–2.00; *E*-value = 2.83] and cardiovascular mortality (HR, 1.44; 95% CI, 1.05–1.98; *E*-value = 2.24). As compared with the reference range, a lower creatine kinase (<111 IU/L) was associated with increased risks of all-cause mortality (HR, 1.40; 95% CI, 1.12–1.76; *E*-value = 2.15) and cardiovascular mortality (HR, 1.45; 95% CI, 1.08–1.94; *E*-value = 2.26). Interaction between creatine kinase and no hyperlipidemia (p = 0.034 for interaction) was observed.

Conclusion: A creatine kinase before the start of PD between 111 and 179 IU/L was associated with a lower risk of death than a higher or lower creatine kinase, resulting in a U-shaped association curve.

Keywords: creatine kinase, mortality, cardiovascular mortality, peritoneal dialysis, prognosis

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INTRODUCTION

Creatine kinase is an important enzyme that consumes adenosine triphosphate rapidly (1). Serum creatine kinase levels are higher in healthy men and associated with muscle mass and body mass index (2–4). Serum creatine kinase activity is detected in myocardial infarction, rhabdomyolysis, myositis, and muscle dystrophy (5). Several previous studies reported that creatine kinase was positively associated with blood pressure (6, 7) and was associated with the failure of antihypertensive therapy in the general population (8).

A 12-year population-based cohort study in Japan showed that elevated serum creatine kinase levels were associated with a moderately increased risk for myocardial infarction (9). More importantly, previous studies reported that high serum creatine kinase levels were associated with increased mortality in patients with rhabdomyolysis, traumatic injuries, hantaviruses, and genetic myopathies (10–13). Notably, there to date was no study regarding the association between serum creatine kinase and mortality in patients with peritoneal dialysis (PD).

A recent study showed that a low serum creatine kinase level was associated with an increased risk of death in the non-dialysis chronic kidney disease (CKD) population (5), which was inconsistent with those findings above. Early study found that elevated creatine kinase had been reported in dialysis patients compared with the general population (14). Thus, based on these findings earlier, we wondered whether elevated or lowered levels of creatine kinase were associated with an increased risk of mortality in patients with dialysis. In this study, we examined the association between creatine kinase and mortality in patients on continuous ambulatory peritoneal dialysis (CAPD).

MATERIALS AND METHODS

Study Design and Participants

We conducted a retrospective study that included 3,566 incident patients with CAPD from five PD centers in China between 1 January 2005 and 31 May 2020. To evaluate the association between creatine kinase 1 week before the start of PD and mortality in the real-world setting, we only excluded patients aged <18 years and those with <3 months of the follow-up. Plus, although no vigorous exercise was recorded in medical records, patients with creatine kinase ≥5,000 IU/L were excluded according to rhabdomyolysis, which is defined as levels of five times above the upper limit of normal serum creatine kinase (1,000 IU/L) (15). Baseline data were collected 1 week before the start of PD, representing a severe uremic status. Therefore, it was challenging for patients to perform excessive physical activity, and we did not evaluate physical activity in our study. The data were anonymous, and the need for informed consent was waived. The study protocol complied with the Declaration of Helsinki and had full approval from each Clinical Research Ethics Committee.

Data Collection and Follow-Up

We respectively collected demographic data, comorbidities, medication use, and laboratory data 1 week (5.3 ± 1.2 days) before the start of PD, including age at study entry, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, diastolic blood pressure, comorbidities [diabetes mellitus, hypertension, prior cardiovascular disease, hyperlipidemia, chronic obstructive pulmonary disease (COPD), and gastrointestinal bleeding], medication use [calcium antagonist, beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (ACEI or ARBs), diuretics, and statins], and laboratory measurements [serum creatine kinase, hemoglobin, albumin, estimated glomerular filtration rate (eGFR), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and serum sodium].

Our primary outcome measure was all-cause and cardiovascular mortality. Details for the CAPD follow-up were previously described elsewhere (16). The follow-up period was from the start of PD to the date of death, transfer to hemodialysis, receiving renal transplantation, transfer to other dialysis centers, loss of follow-up, or 31 May 2020. Patients who were lost to follow-up were censored at the date of the last examination.

Statistical Analysis

Differences in the baseline characteristics among the study population in the different categories of creatine kinase were compared using the chi-square test for categorical variables and ANOVA for continuous variables. We used restricted-cubic-spline plots to explore the shape of the association between creatine kinase and mortality, fitting a restricted-cubic-spline function with four knots (at the 25th, 50th, 75th, and 95th percentiles) (17).

Based on our restricted-cubic-spline plots for the primary outcome, we selected a level of 111–179 IU/L as the reference category for creatine kinase. Cumulative all-cause and cardiovascular mortality were analyzed using the Kaplan–Meier failure function. We performed a multivariable Cox proportional hazards model in order to determine the association between creatine kinase and all-cause and cardiovascular mortality, using four sequential models. Model 1 was adjusted for age, sex, body mass index, current smoker (yes or no), current alcohol use (yes or no), and systolic blood pressure. Model 2 included model 1 and diabetes mellitus (yes or no), hypertension (yes or no), prior cardiovascular events (yes or no), COPD, gastrointestinal bleeding, and hyperlipidemia. Model 3 included model 2 and taking calcium antagonist (yes or no), beta-blocker (yes or no), ACE inhibitor or ARB (yes or no), diuretics (yes or no), and statins (yes or no). Model 4 included model 3 and hemoglobin, albumin, eGFR, HDL, LDL, and serum sodium.

We tested for interactions of age, sex, diabetes mellitus, hypertension, prior cardiovascular disease, and hyperlipidemia. We explored the potential influence of unmeasured confounders on our risk estimates using *E*-value analysis to determine how solid and imbalanced a confounding effect would need to be to alter the direction of findings (18). To minimize the potential for reverse causation, we conducted analyses that excluded patients with prior cardiovascular disease or those deaths in the first 2 years of follow-up. When considering a transfer to hemodialysis, receiving renal transplantation, transfer to other centers, and loss of follow-up as competing risks for all-cause mortality, we further analyzed the association between creatine kinase and all-cause mortality using the Gray test. Missing data for serum creatine kinase (n = 97) or any other explanatory variables (n = 143) at the start of PD were replaced by the most recent available values by checking patients' medical records of receiving the first PD procedure. All the analyses were conducted with Stata 15.1. statistical software (StataCorp, College Station, TX, United States).

RESULTS

Baseline Characteristics

We excluded 55 patients aged <18 years, 62 patients with less than 3 months of follow-up, and three patients with creatine kinase ≥5,000 IU/L. Thus, 3,446 eligible patients were finally included in this study.

Of 3,446 patients with a mean age of 49.6 years, 1,796 (52.1%) were male sex, 662 (19.2%) had diabetes mellitus, 2,415 (70.1%) had hypertension, 368 (10.7%) had a history of cardiovascular disease, and 597 (17.3%) had hyperlipidemia. The median creatine kinase was 113 (range, 1.22–4,574) IU/L. Based on our restricted-cubic-spline plots for the primary outcome, we selected a level of 111–179 IU/L as the reference category for creatine kinase (**Figure 1**). The baseline characteristics of patients according to creatine kinase were shown in **Table 1**. Compared with the moderate group, the high group was more likely to be female and diabetes mellitus, but less likely to be current smoker and alcohol use. In contrast, the low group was more likely male gender and current alcohol use but less likely to be taking ACEI or ARB.

Creatine Kinase and Mortality

During the median of 39.5 (range, 3.1–181.5) months of follow-up, 763 (22.1%) patients died, 466 (13.5%) patients transferred to hemodialysis, 229 (6.6%) patients received renal transplantation, 434 (12.6%) patients transferred to other dialysis centers, and 58 (1.7%) patients had been the loss of follow-up. Of 763 deaths, 384 (50.3%) deaths were due to cardiovascular disease, 135 (17.8%) deaths due to infectious disease, 72 (9.4%) deaths due to gastrointestinal bleeding, 14 (1.8%) deaths due to malignancy, 66 (8.7%) deaths due to other reasons, and 92 (12.1%) deaths due to unknown reasons. Deaths occurred in 370 (56.5/1,000 person-years), 134 (40.7/1,000 person-years), and 259 (63.2/1,000 person-years) patients in those <111, 111–179, and >179 IU/L patients, respectively (**Table 2**).

Survival analyses showed that a creatine kinase of 111–179 IU/L had the lowest cumulative all-cause and cardiovascular mortality (**Figure 2**). As compared with a creatine kinase of 111–179 IU/L (the reference category), a creatine kinase of >179 IU/L was associated with increased risks of all-cause mortality [hazards ratio (HR), 1.72; 95% CI, 1.35–2.00; *E*-value = 2.83] and cardiovascular mortality (HR, 1.44; 95% CI, 1.05–1.98; *E*-value = 2.24) on multivariable analysis (**Tables 3**, **4** and **Figure 1**). Plus, as compared with the reference range, a lower serum creatine kinase (<111 IU/L) was also associated with increased risks of all-cause mortality (HR, 1.40; 95% CI, 1.12–1.76; *E*-value = 2.15) and cardiovascular mortality (HR, 1.45; 95% CI, 1.08 to 1.94; *E*-value = 2.26) on multivariable analysis (**Tables 3**, **4** and **Figure 1**).

Subgroup Analysis

No hyperlipidemia modified the association between a low creatine kinase and all-cause mortality ($p = 0.034$ for interaction, **Table 5**). In further analysis, significantly increased risk was observed in patients with no hyperlipidemia and a creatine kinase of <111 IU/L (all-cause mortality: HR, 1.56; 95% CI, 1.22–2.01), whereas there was no significant association among those with hyperlipidemia. Plus, no hyperlipidemia also modified the association between a low creatine kinase and cardiovascular mortality ($p = 0.023$ for interaction, **Table 6**). There were no other significant subgroup interactions (**Tables 5**, **6**).

Sensitivity Analysis

The exclusion of patients with prior cardiovascular disease or those who died in the first 2 years of follow-up did not materially affect the results from the creatine kinase analyses (**Tables 3**, **4**).

When considering a transfer to hemodialysis, receiving renal transplantation, transfer to other centers, and also loss of follow-up as competing risks for all-cause mortality, we found that compared with the moderate group, the high and low groups were associated with 1.32 (95% CI, 1.05–1.68; *E*-value = 1.97) and 1.54 (95% CI, 1.09–2.19; *E*-value = 2.45) times of risk of all-cause mortality, respectively (**Supplementary Table 1**).

DISCUSSION

In this large, multi-center, retrospective cohort study, we investigated the association between serum creatine kinase and mortality in patients with PD. The lowest all-cause and cardiovascular mortality risks were seen among patients with serum creatine between 111 and 179 IU/L. Higher and lower creatine kinase levels were associated with increased risks, resulting in a U-shaped association curve. Although our findings were inconsistent with those previous findings (10–13), the association between creatine kinase and mortality in our study may be more plausible in clinical practice.

More excellent creatine kinase activity is thought to promote vascular contractility and retain sodium (6, 19). Individuals with high creatine kinase activity are at a greater risk of developing hypertension, with more excellent resistance against blood pressure-lowering therapy (6). Previous studies showed elevated serum creatine kinase levels were associated with increased mortality in patients with different comorbid conditions (10, 12, 13). A previous study reported high creatine kinase levels in patients on dialysis compared with the healthy controls, and hemodialysis did not seem to contribute to the creatine kinase elevation. Plus, post-dialysis creatine kinase values were lower (albeit not significant) when compared with pre-dialysis values (14). The fall of post-dialysis creatine kinase values was possibly due to a change of blood PH during dialysis and the use of a dialysis bath containing a significant quantity of sodium acetate, which can inhibit the activity of creatine kinase (20). Therefore, to eliminate the effect of dialysis on creatine kinase, we examined the association between creatine kinase before the first PD procedure and death during the follow-up period. We found a U-shaped association between creatine kinase and mortality. Even after adjusting for confounding factors or sensitivity analyses, the results remained robust. Additional subgroup analyses found an interaction between

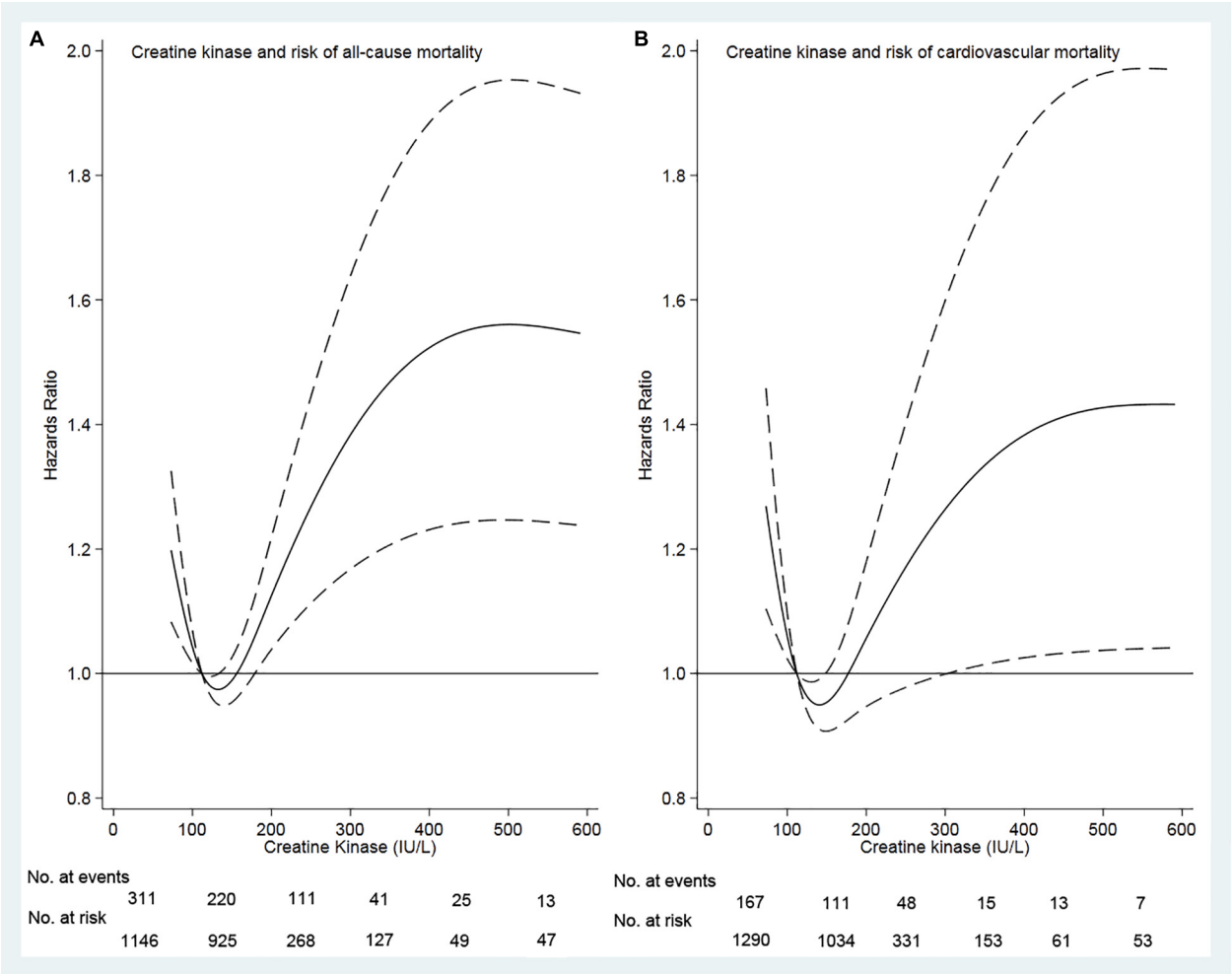

FIGURE 1 | Association of serum creatine kinase with risk of mortality. Panel **(A)** showed a restricted-cubic-spline plot of the association between creatine kinase and the all-cause mortality. Panel **(B)** showed a restricted cubic–spline plot of the association of creatine kinase and cardiovascular mortality. All the plots were adjusted for age, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, comorbidities, medication use, and lab measurements. Dashed lines indicate 95% CIs. The median creatine kinase (113 IU/L) was the reference standard, indicated by the red line.

no hyperlipidemia and serum creatine kinase. In patients with hyperlipidemia, low levels of creatine kinase were associated with a high risk of all-cause and cardiovascular mortality, but no significant association in those with hyperlipidemia. Medication for hyperlipidemia that may lead to serum creatine kinase elevation (21, 22). Nonetheless, how no hyperlipidemia modified the association between low creatine kinase levels and mortality is inconclusive.

Although the previous study reported elevated creatine kinase in dialysis patients (14), the association between creatine kinase and prognosis of patients on dialysis remained unknown. Due to the high presence of comorbidities, such as diabetes mellitus, hypertension, prior cardiovascular disease, and hyperlipidemia, which may influence the association between creatine kinase and mortality in dialysis patients, it is difficult to speculate on the association. In our study, we found a specific association in CAPD patients. Data analyzed that lower or higher serum creatine kinase levels were associated with increased all-cause and cardiovascular mortality risks. A previous study found that lower serum creatine kinase levels may reflect poor nutritional status (5). As we all know, poor nutritional status was associated with an increased risk of all-cause mortality (23). Early publications estimated that 40–66 percent of patients with PD in the United States are malnourished (24–26). Meanwhile, individuals with higher serum creatine kinase levels were at greater risk of developing hypertension, with more excellent resistance against blood pressure-lowering therapy (6, 8). Therefore, based on these aforementioned findings, our findings may be more plausible in the clinical practice setting.

To date, there was one study reporting the association between serum creatine kinase and mortality in non-dialysis CKD patients, which reported that a low level of serum creatine kinase was associated with an increased risk of death (5).

TABLE 1 | Baseline characteristics of the study patients, according to creatine kinase.

	Creatine kinase				
Characteristics	**All levels**	**Low (<111 IU/L)**	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**	***P*-value**
Proportion of participants, n	3,446	1,679	800	967	
Creatine kinase (IU/L)	113 (73–195)	72 (57–90)	136 (123–153)	292 (225–478)	
Age (years)	49.6 ± 14.4	50.0 ± 14.5	48.7 ± 14.2	49.7 ± 14.6	0.136
Male sex, n (%)	1,796 (52.1%)	1,171 (69.7%)	437 (54.6%)	188 (19.4%)	<0.001
Body mass index (kg/m^2)	22.4 ± 3.3	22.3 ± 3.3	22.3 ± 3.2	22.4 ± 3.3	0.71
Current smoker, n (%)	349 (10.1%)	233 (13.9%)	99 (12.4%)	17 (1.8%)	<0.001
Current alcohol use, n (%)	128 (3.7%)	85 (5.1%)	37 (4.6%)	6 (0.6%)	<0.001
Systolic blood pressure (mmHg)	147.6 ± 22.8	146.8 ± 22.9	148.3 ± 22.1	148.4 ± 23.0	0.134
Diastolic blood pressure (mmHg)	87.1 ± 14.0	86.6 ± 13.5	87.7 ± 14.1	87.6 ± 14.8	0.087
Comorbidities, n (%)					
Diabetes mellitus	662 (19.2%)	296 (17.6%)	142 (17.8%)	224 (23.2%)	0.001
Hypertension	2,415 (70.1%)	1,193 (71.1%)	537 (67.1%)	685 (70.8%)	0.113
Prior cardiovascular disease	368 (10.7%)	200 (11.9%)	71 (8.9%)	97 (10.0%)	0.054
COPD	30 (0.9%)	14 (0.8%)	6 (0.8%)	10 (1.0%)	0.794
Gastrointestinal bleeding	95 (2.8%)	51 (3.0%)	20 (2.5%)	24 (2.5%)	0.618
Hyperlipidemia	597 (17.3%)	298 (17.7%)	133 (16.6%)	166 (17.2%)	0.778
Medication use, n (%)					
Calcium antagonist	2,202 (63.9%)	1,062 (63.3%)	506 (63.3%)	634 (65.6%)	0.441
Beta-blocker	1,305 (37.9%)	637 (37.9%)	291 (36.4%)	377 (39.0%)	0.528
ACE inhibitor or ARB	946 (27.5%)	418 (24.9%)	233 (29.1%)	295 (30.5%)	0.004
Diuretics	547 (15.9%)	274 (16.3%)	137 (17.1%)	136 (14.1%)	0.169
Statins	510 (14.8%)	261 (15.5%)	105 (13.1%)	144 (14.9%)	0.283
Laboratory measurements					
Hemoglobin (g/L)	87.7 ± 19.8	88.0 ± 19.7	88.2 ± 19.4	86.7 ± 20.3	0.203
Albumin (g/L)	34.5 ± 5.3	34.7 ± 5.2	34.6 ± 5.1	34.1 ± 5.5	0.014
eGFR (mL/min × 1.73 m^2)	7.1 ± 3.8	7.7 ± 3.9	6.9 ± 3.4	6.5 ± 3.7	<0.001
HDL (mEq/L)	1.14 ± 0.39	1.09 ± 0.36	1.16 ± 0.40	1.19 ± 0.43	<0.001
LDL (mEq/L)	2.56 ± 0.89	2.48 ± 0.86	2.59 ± 0.81	2.69 ± 0.97	<0.001
Serum sodium (mEq/L)	140.1 ± 3.8	140.1 ± 3.7	140.1 ± 3.8	140.0 ± 4.0	0.769

COPD, chronic obstructive pulmonary disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 2 | The incidence rate of death according to creatine kinase.

	Creatine kinase			
Outcomes	**All levels**	**Low (<111 IU/L)**	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**
Proportion of participants, n	3,446	1,679	800	967
Person-years	13,946.7	6,551.6	3,294.8	4,100.4
All-cause mortality				
Events, n	763	370	134	259
Events, per 1,000 person-years	54.7	56.5	40.7	63.2
Cardiovascular mortality				
Events, n	384	198	67	119
Events, per 1,000 person-years	27.5	30.2	20.3	29.0

The incidence rate was calculated by dividing the proportion of events by the total effective observation time in the risk, which is converted to the number of episodes per 1,000 years.

This study only included patients with CKD with a median eGFR of 40 ml/min × 1.73 m^2, and excluded those with eGFR <15 ml/min × 1.73 m^2. Participants in this study were artificially divided into three groups, affecting the association between creatine kinase and death. Plus, this study included participants at high cardiovascular risk and was vulnerable to biases from reverse causation. Reverse causation may occur when patients with prior cardiovascular disease or increased

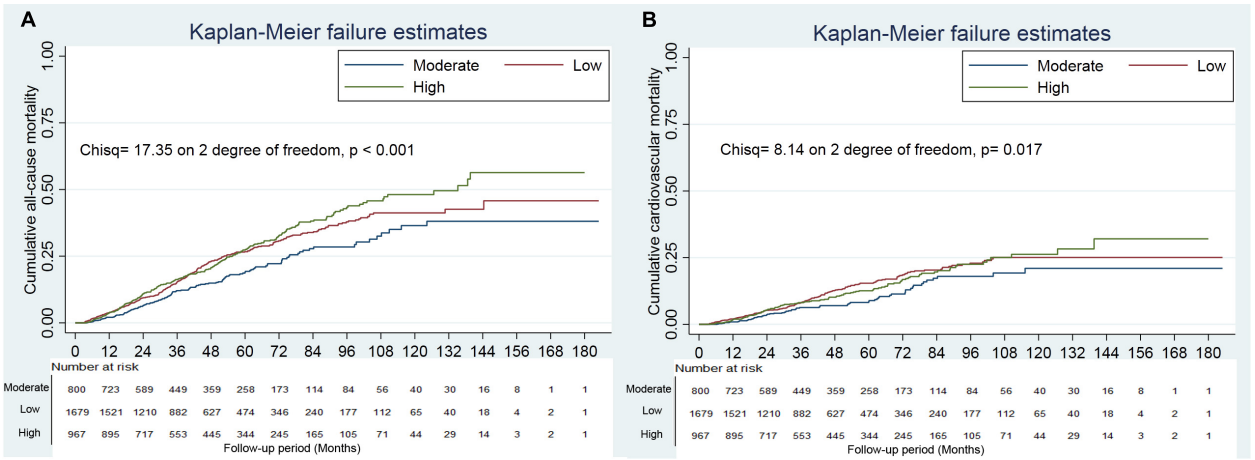

FIGURE 2 | Cumulative mortality incidence by categories of creatine kinase. Panel **(A)** showed cumulative all-cause mortality by categories of creatine kinase. Panel **(B)** showed cumulative cardiovascular mortality by categories of creatine kinase.

TABLE 3 | Association of creatine kinase with all-cause mortality.

	Creatine kinase		
	Low (<111 IU/L)	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**
All-cause mortality, n (%)	370 (22.0%)	134 (16.8%)	259 (26.8%)
Analysis, hazards ratio (95%CI)			
Univariate analysis	1.40 (1.13–1.75)	1.0	1.81 (1.44–2.30)
Multivariate analysis			
Model 1	1.44 (1.15–1.80)	1.0	1.73 (1.36–2.20)
Model 2	1.42 (1.14–1.78)	1.0	1.72 (1.36–2.20)
Model 3	1.40 (1.12–1.76)	1.0	1.73 (1.35–2.00)
Model 4	1.40 (1.12–1.76)	1.0	1.72 (1.35–2.00)
Analysis excluding prior cardiovascular disease*	1.32 (1.04–1.68)	1.0	1.70 (1.31–2.21)
Analysis excluding deaths year 1 and 2*	1.31 (1.04–1.70)	1.0	1.74 (1.32–2.31)

Model 1 included age, sex, body mass index, alcohol intake, current smoking, and systolic blood pressure. Model 2 included variables in model 1 and comorbidities. Model 3 included variables in model 2 and medication use. Model 4 included variables in model 3 and laboratory measurements.
**Adjusted for variables included in model 4.*

TABLE 4 | Association of creatine kinase with cardiovascular mortality.

	Creatine kinase		
	Low (<111 IU/L)	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**
Cardiovascular mortality, n (%)	198 (11.8%)	67 (8.4%)	119 (12.3%)
Analysis, hazards ratio (95%CI)			
Univariate analysis	1.46 (1.09–1.96)	1.0	1.54 (1.12–2.10)
Multivariate analysis			
Model 1	1.47 (1.10–1.98)	1.0	1.46 (1.06–2.00)
Model 2	1.45 (1.08–1.95)	1.0	1.44 (1.05–1.98)
Model 3	1.44 (1.08–1.94)	1.0	1.44 (1.05–1.98)
Model 4	1.45 (1.08–1.94)	1.0	1.44 (1.05–1.98)
Analysis excluding prior cardiovascular disease*	1.40 (1.04–1.88)	1.0	1.46 (1.06–2.00)
Analysis excluding deaths year 1 and 2*	1.42 (1.09–2.05)	1.0	1.55 (1.05–2.30)

Model 1 included age, sex, body mass index, alcohol intake, current smoking, and systolic blood pressure. Model 2 included variables in model 1 and comorbidities. Model 3 included variables in model 2 and medication use. Model 4 included variables in model 3 and laboratory measurements.
**Adjusted for variables included in model 4.*

TABLE 5 | Association of creatine kinase with all-cause mortality in subgroups.

		Creatine kinase			
	Event, n (%)	**Low (<111 IU/L)**	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**	**P-interaction**
All-cause mortality					
<65 years	462 (16.1%)	1.25 (0.95–1.64)	1.0	1.52 (1.14–2.03)	0.145
≥65 years	301 (51.7%)	1.67 (1.06–2.63)	1.0	2.63 (1.58–4.40)	
Male	291 (16.2%)	1.30 (0.91–1.86)	1.0	2.12 (1.46–3.08)	0.875
Female	472 (28.6%)	1.53 (1.13–2.06)	1.0	1.49 (1.08–2.06)	
Hypertension	593 (24.6%)	1.38 (1.06–1.80)	1.0	1.78 (1.34–2.36)	0.547
No hypertension	170 (16.5%)	1.44 (0.93–2.25)	1.0	1.55 (0.96–2.53)	
Diabetes mellitus	286 (43.2%)	1.46 (0.95–2.23)	1.0	2.02 (1.30–3.14)	0.397
No diabetes mellitus	477 (17.1%)	1.37 (1.05–1.79)	1.0	1.61 (1.21–2.16)	
Prior cardiovascular disease	141 (38.3%)	1.64 (0.71–3.77)	1.0	1.89 (0.76–4.69)	0.057
No prior cardiovascular disease	622 (20.2%)	1.42 (1.04–1.95)	1.0	1.38 (0.98–1.94)	
Hyperlipidemia	147 (24.6%)	0.80 (0.46–1.40)	1.0	2.77 (1.58–4.88)	0.034
No hyperlipidemia	616 (21.6%)	1.56 (1.22–2.01)	1.0	1.54 (1.17–2.02)	

All analyses adjusted for age, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, comorbidities, medication use, and lab measurements.

TABLE 6 | Association of creatine kinase with cardiovascular mortality in subgroups.

		Creatine kinase			
	Event rates	**Low (<111 IU/L)**	**Moderate (111–179 IU/L)**	**High (>179 IU/L)**	**P-interaction**
Cardiovascular mortality					
<65 years	246 (8.6%)	1.41 (0.98–2.01)	1.0	1.41 (0.96–2.08)	0.678
≥65 years	138 (23.7%)	1.46 (0.84–2.52)	1.0	1.60 (0.88–2.90)	
Male	157 (8.7%)	2.03 (1.22–3.38)	1.0	2.38 (1.40–4.07)	0.078
Female	227 (13.8%)	1.23 (0.85–1.78)	1.0	1.06 (0.71–1.60)	
Hypertension	308 (12.8%)	1.40 (1.01–1.96)	1.0	1.45 (1.01–2.08)	0.804
No hypertension	76 (7.4%)	1.58 (0.84–2.98)	1.0	1.38 (0.68–2.80)	
Diabetes mellitus	132 (19.9%)	1.50 (0.89–2.78)	1.0	1.58 (0.89–2.78)	0.478
No diabetes mellitus	252 (9.1%)	1.42 (1.00–2.02)	1.0	1.38 (0.94–2.04)	
Prior cardiovascular disease	62 (16.8%)	1.65 (1.03–1.94)	1.0	1.85 (1.01–1.96)	0.180
No prior cardiovascular disease	322 (10.5%)	1.42 (0.72–3.79)	1.0	1.39 (0.76–4.55)	
Hyperlipidemia	50 (8.4%)	0.80 (0.34–1.87)	1.0	2.04 (0.89–4.67)	0.023
No hyperlipidemia	334 (11.7%)	1.56 (1.14–2.14)	1.0	1.33 (0.94–1.88)	

All analyses adjusted for age, sex, body mass index, current smoker, current alcohol use, systolic blood pressure, comorbidities, medication use, and lab measurements.

cardiovascular risk reduce their exercise magnitude due to illness or medical recommendations, reducing serum creatine kinase levels (27). Our study excluded patients with prior cardiovascular disease or those who died in the first 2 years of follow-up did not materially alter our findings. Nonetheless, we acknowledged that reverse causation cannot be completely ruled out. Therefore, a large prospective study is required to verify our results.

Strengths included a large number of patients, high completeness of data, and the availability of detailed covariates to adjust for a broad range of potential confounders. Our study also had several limitations, and findings should be interpreted with these in mind. First, as with all observational studies, a potential limitation of our study was the possibility of residual confounding from unmeasured variables. However, the *E*-value analysis showed that a confounder effect would need to be markedly large to alter the direction of association in the multivariable models. For example, even a strong confounder effect (HR $\geq$ 2.83) would need to be considerably imbalanced between the high and moderate creatine kinase categories to result in an adjusted hazards ratio below 1.0. Weaker confounders could not do so. Second, the single serum creatine kinase measurement at baseline may have underestimated the association between serum creatine kinase levels and mortality because of the regression dilution bias (28). However, regression dilution bias may lead to over-adjustment (29). Third, missing values were replaced by the most recent available deals, not using multiple imputations. Although multiple imputations can randomly fill these missing values, the most recent available values may more appropriately present a patient's clinical status. Fourth, we had not excluded patients taking drugs that might affect serum creatine kinase and did not evaluate

the association between serum creatine kinase and non-fatal cardiovascular events.

In conclusion, a serum creatine kinase before the start of PD between 111 and 179 IU/L was associated with a lower risk of all-cause and cardiovascular mortality than a higher or lower level of creatine kinase, resulting in a U-shaped association curve. Prospective analyses on these associations are needed for causal inferences and to decide whether creatine kinase could serve as a new, clinically helpful biomarker for prognosis in patients with PD.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, the Ethics Committee of the First Affiliated Hospital of Nanchang University, the Ethics Committee of Jiujiang No.1 People's Hospital, the Ethics Committee of Zhujiang Hospital of Southern Medical University, and the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XFW and LZ contributed to the conception, interpretation of data, and drafted the work. XYW, XZ, XF, and FP contributed to the acquisition, analysis, and interpretation of data. NW and YW contributed to the conception and design of the work. XFW and JW contributed to the conception, design of the work, and revised it. All authors read and approved the manuscript and met the criteria for authorship.

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SUPPLEMENTARY MATERIAL

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Biomarkers in Cardiorenal Syndrome and Potential Insights Into Novel Therapeutics

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Heart and kidney failure often co-exist and confer high morbidity and mortality. The complex bi-directional nature of heart and kidney dysfunction is referred to as cardiorenal syndrome, and can be induced by acute or chronic dysfunction of either organ or secondary to systemic diseases. The five clinical subtypes of cardiorenal syndrome are categorized by the perceived primary precipitant of organ injury but lack precision. Traditional biomarkers such as serum creatinine are also limited in their ability to provide an early and accurate diagnosis of cardiorenal syndrome. Novel biomarkers have the potential to assist in the diagnosis of cardiorenal syndrome and guide treatment by evaluating the relative roles of implicated pathophysiological pathways such as hemodynamic dysfunction, neurohormonal activation, endothelial dysfunction, inflammation and oxidative stress, and fibrosis. In this review, we assess the utility of biomarkers that correlate with kidney and cardiac (dys)function, inflammation/oxidative stress, fibrosis, and cell cycle arrest, as well as emerging novel biomarkers (thrombospondin-1/CD47, glycocalyx and interleukin-1β) that may provide prediction and prognostication of cardiorenal syndrome, and guide potential development of targeted therapeutics.

Keywords: cardiorenal syndrome (CRS), biomarker (BM), heart failure, chronic kidney disease, prognosis

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INTRODUCTION

Heart failure (HF) and chronic kidney disease (CKD) are increasing public health issues with a prevalence of 4% and 9% respectively, and a global rise in attributable deaths by 41% for both diseases since 1990 (1, 2). Cardiorenal syndrome (CRS) refers to the concurrent dysfunction of the heart and kidney, which can initiate and perpetuate disease in the other organ through hemodynamic, neurohormonal, and immunological and/or biochemical feedback pathways (3). Combined HF and CKD is associated with high morbidity and mortality (4–9). In people with HF, every 10 ml/min decrease in estimated glomerular filtration rate (eGFR) increases the risk of all-cause death by 7%, while HF hospitalization in people with CKD increases the risk of all-cause death 3–7-fold (8, 9).

CLASSIFICATION OF CARDIORENAL SYNDROME

Currently, CRS is classified into 5 different subtypes based on the perceived primary precipitant of organ injury: (10)

1. CRS type 1: rapid decline in cardiac function (e.g., cardiogenic shock or acute decompensation of chronic HF) resulting in acute kidney injury (AKI).
2. CRS type 2: chronic cardiac dysfunction (e.g., chronic HF) causing progressive decline in kidney function and CKD.
3. CRS type 3: acute decline in kidney function (e.g., AKI or glomerulonephritis) causing acute cardiac dysfunction (e.g., HF, arrhythmia, or myocardial infarction).
4. CRS type 4: CKD causing progressive decline in cardiac function (e.g., left ventricular hypertrophy, HF, or myocardial infarction).
5. CRS type 5: combine cardiac and kidney dysfunction caused by an acute or chronic systemic disorder (e.g., sepsis or diabetes mellitus).

While this classification system is clinical intuitive, it may be difficult to identify the initial insult. Furthermore, this classification system does not incorporate the pathophysiological pathways implicated in CRS such as hemodynamic dysfunction, neurohormonal activation, endothelial dysfunction, inflammation, and fibrosis (11, 12).

Current biomarkers of kidney function such as serum creatinine also have limited sensitivity and specificity. A rise in serum creatinine only occurs after a significant decline in GFR (e.g., 48–72 h after AKI or after 50% of function is lost chronically), is non-specific to the underlying disease process, and is affected by clinical characteristics (e.g., age, weight, gender, ethnicity, volume status, and medication use) that do not reflect true parenchymal injury (13). In this review, we summarize the evidence for different biomarkers in CRS (**Figure 1** and **Table 1**) as well as promising emerging biomarkers that may inform on future management in this debilitating condition.

BIOMARKERS OF FUNCTION (GLOMERULAR FILTRATION AND INTEGRITY)

Albuminuria

Albuminuria is a cheap and widely available biomarker, which may not only reflect glomerular injury but also endothelial dysfunction (14). In three large chronic HF trials, microalbuminuria (30–299 mg/g) and macroalbuminuria ($\geq$ 300 mg/g) were associated with a 1.4–1.8-fold increased risk of all-cause death, cardiovascular death, or HF hospitalization (15–17). Albuminuria predicted these outcomes independent of serum creatinine, HbA1c and left ventricular ejection fraction (LVEF) (16). While treatments exist to reduce albuminuria [e.g., renin-angiotensin-aldosterone system (RAAS) inhibitors], whether targeting albuminuria improves prognosis in CRS requires further study.

Cystatin-C

Cystatin-C, a 13-kDa cysteine protease inhibitor, is produced at a constant rate by all nucleated cells and is freely filtered through the glomerulus, almost completely reabsorbed, and not secreted by the renal tubules. Cystatin-C inhibits collagen- and elastin-degrading cysteine proteases of the cathepsin family and protects against atherosclerosis in apolipoprotein E–deficient mice, though its role in CRS is unclear (18). In both acute and chronic HF, elevated plasma or urinary cystatin-C was associated with a 2–3-fold increased risk of all-cause death, independent of serum creatinine or eGFR (19–22). In acute HF, plasma cystatin-C modestly predicted AKI [area under the receiver operating characteristic curve (AUC-ROC) 0.68] and all-cause death or HF hospitalization (AUC-ROC, 95% confidence interval: 0.73, 0.66–0.80) (23, 24), providing prognostic value in addition to N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and troponin (25). In elderly patients with chronic HF, the highest quartile of cystatin-C doubled the risk of all-cause death, outperforming serum creatinine in multivariate analyses (21). After cardiac surgery, plasma cystatin-C modestly predicted AKI (AUC-ROC 0.68), (26) while urinary cystatin-C has not consistently predicted AKI (27, 28).

Unlike serum creatinine, plasma cystatin-C is not affected by muscle mass but both are affected by volume status. In the Renal Optimization Strategies Evaluation–Acute Heart Failure (ROSE-HF) trial, protocol-driven aggressive diuresis caused worsening of renal function (WRF), based on $\geq$ 20% decrease in eGFR using cystatin C, in 21% of participants but was not associated with an increase in kidney tubular injury markers (29). This suggests tubular markers may have utility in differentiating AKI due to diuretic-induced volume depletion or parenchymal injury in CRS.

Galectin-3

Galectin-3 is a 30-kDa glycoprotein synthesized by cardiac macrophages in response to angiotensin II and aldosterone, which mediates collagen deposition by fibroblasts resulting in cardiac fibrosis (12). Plasma galectin-3 levels are also inversely related to renal function and therefore represents a biomarker for both cardiac fibrosis and GFR. In acute HF, while NT-proBNP was superior to galectin-3 for diagnosis, galectin-3 may be superior to NT-proBNP at predicting 60-day mortality (AUC-ROC 0.74 vs. 0.67, p = 0.05) and was associated with a 14-fold increased risk of all-cause death or HF hospitalization in multivariate analysis (30). In chronic HF, galectin-3 only modestly predicted all-cause death (AUC-ROC 0.612, 0.538–0.685) and all-cause death or HF hospitalization (AUC-ROC 0.58, 0.55–0.61). Pooled analysis of chronic HF trials (mean eGFR 54–58 ml/min/1.73 m^2) showed elevated galectin-3 was associated with a 1.6–2-fold increased risk of all-cause death or HF hospitalization (31–33).

Proenkephalin A

Proenkephalin A is an endogenous opioid secreted by cardiac cells and mediates negative inotropic effects *via* the delta opioid receptor. Plasma proenkephalin is inversely proportional to

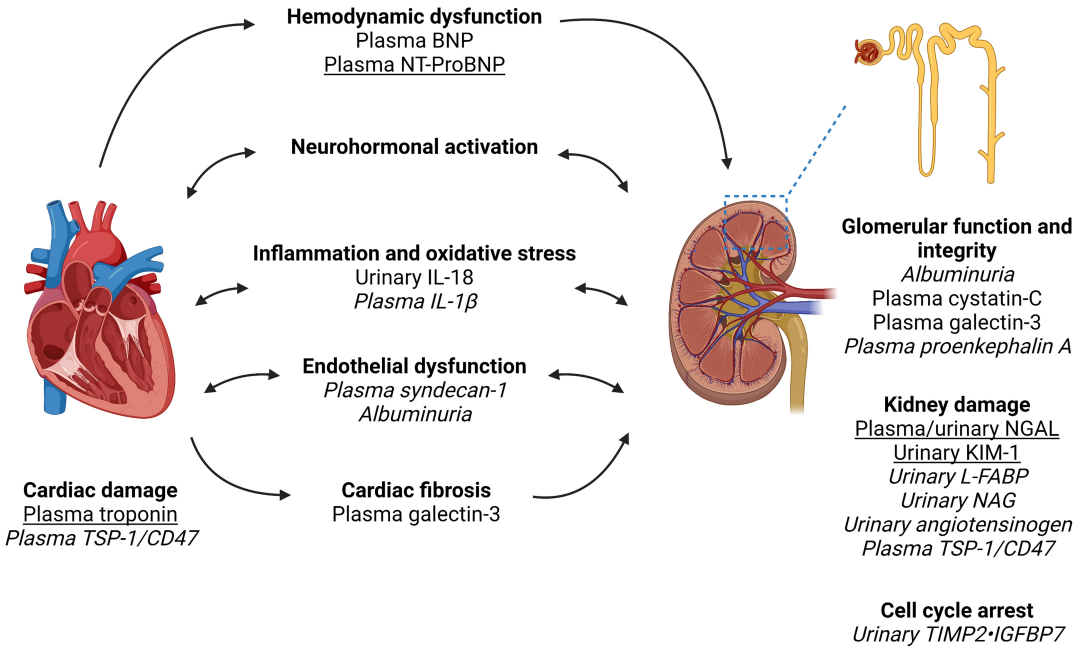

FIGURE 1 | Biomarkers in the pathways of injury in cardiorenal syndrome. Created with Biorender. Cardiorenal syndrome involves the complex bidirectional nature of heart and kidney dysfunction. The pathways involved include hemodynamic dysfunction, neurohormonal activation (primarily the renin-angiotensin-aldosterone system), inflammation and oxidative stress, endothelial dysfunction, and ultimately injury and fibrosis to both heart and kidney. We have outlined the pathways which each biomarker is associated with as well as indicated biomarkers with the most clinical applicability based on the evidence quality of existing literature (underline) vs. biomarkers requiring further research and validation in larger cohort of patients with cardiorenal syndrome (italics). Of note, biomarker performance will depend on kidney function, timing of collection and type of cardiorenal syndrome. Furthermore, a combination of biomarkers may have superior performance to a single biomarker. Therefore, further research is required to identify the type and timing of biomarker(s) analysis in the prediction and prognostication of cardiorenal syndrome. NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; L-FABP, liver fatty acid-binding protein; NAG, *N*-acetyl-beta-D-glucosaminidase; BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; IL, interleukin; TIMP2, tissue inhibitor of metalloproteinases-1 and -2; IGFBP7, insulin-like growth factor-binding protein-7; TSP-1, thrombospondin-1.

GFR and associated with a 1.5-fold increased risk of CKD (highest vs lowest tertile) (34). In acute HF, proenkephalin A modestly predicted AKI (AUC-ROC 0.69) and was independently associated with a 27% increased risk of 1-year mortality or HF hospitalization (35).

BIOMARKERS OF DAMAGE

Kidney Tubule: Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein secreted by immature neutrophils, epithelial cells (including renal tubular epithelium) and cardiomyocytes in response to inflammation. While NGAL has been extensively studied as a marker of renal tubular injury, it also plays a role in mineralocorticoid-mediated cardiovascular fibrosis. NGAL knockout mice demonstrated blunted vascular fibrosis in response to an aldosterone-salt challenge, and reduced cardiac fibrosis, inflammation and left ventricular dysfunction in a myocardial infarction model (36, 37). However, no treatments exist to inhibit NGAL for evaluation in CRS.

In neonatal and pediatric cardiac surgery, plasma and urinary NGAL were detectable 2 h post-surgery (compared to 1–3 days for serum creatinine) and performed exceptionally at predicting AKI (AUC-ROC 0.92–0.998) (38, 39). A small study in adult cardiac surgery demonstrated similar predictive ability for detecting AKI (AUC-ROC 0.98), (40) but this has not been consistent replicated. In a meta-analysis of NGAL in diagnosing AKI, subgroup analysis of 10 studies reporting cardiac surgery-related AKI demonstrated plasma and/or urinary NGAL moderately predicted AKI (AUC-ROC 0.775, 0.669–0.867) (41). In a large cohort study of adults undergoing cardiac surgery, plasma (but not urinary) NGAL significantly improved risk prediction of AKI over the clinical models using demographic factors, surgical factors, eGFR and patient comorbidities (42). These differences highlight the importance of considering time and baseline kidney function when assessing biomarkers. Indeed, for the diagnosis of AKI in 529 critically ill patients in the intensive care unit, cystatin-C, interleukin-18 (IL-18), NGAL, kidney injury molecule-1 (KIM-1), and γ-glutamyltranspeptidase demonstrated optimal performance earlier ($\leq$ 12 h after injury) in patients with preserved kidney function (eGFR $\geq$ 60 ml/min) and later (12–36 h after injury) in patients with reduced kidney function (eGFR $<$ 60 ml/min) (43). In acute HF, plasma and urinary NGAL were associated with a 1.3–2-fold increased risk of long-term mortality, (22, 44) and plasma NGAL outperformed cystatin-C at predicting AKI (AUC-ROC 0.93 vs. 0.68) (23).

TABLE 1 | Biomarkers in cardiorenal syndrome.

Biomarker	Function of biomarker	Predictive value (AUC-ROC per outcome)	Prognostic value ("x" times increased risk of outcome)	Involved in the disease mechanism of CRS	Targeted treatments	References
Biomarkers of function (glomerular filtration and integrity)						
Albuminuria	Marker of glomerular injury	Unclear	Type 2 CRS: -All-cause/CV death or HF hospitalization: 1.4–1.8 times	No	RAAS inhibitors, MRAs, SGLT2 inhibitors	15–17
Plasma cystatin-C	Produced by all nucleated cells. Marker of GFR	Type 1 CRS: -AKI: 0.68 -All-cause death or hospitalization: 0.73	Type 1 and 2 CRS: -All-cause death: 2–3 times	No	No	19–28
Plasma galectin-3	Involved in RAAS-mediated collagen deposition by fibroblasts. Also inversely related to GFR	Acute HF: -All-cause death: 0.74 Chronic HF: -All-cause death: 0.612 -All-cause death or HF hospitalization: 0.58	Type 1 CRS: -All-cause death or HF hospitalization: 14 times Type 2 CRS: -All-cause death or HF hospitalization: 1.6–2 times	Yes	RAAS inhibitors, MRAs	30–33
Plasma proenkephalin A	Involved in opioid receptor-mediated negative inotropic effects. Also inversely related to GFR	Type 1 CRS: -AKI: 0.69	Type 1 CRS: -All-cause death or HF hospitalization: 1.3 times	No	No	35
Biomarkers of kidney damage						
Plasma and/or urinary NGAL	Secreted by neutrophils and epithelial cells in response to inflammation. Mediates cardiac fibrosis by aldosterone	Type 1 CRS: -AKI: 0.775–0.998	Type 1 CRS: -All-cause death: 1.3–2 times -AKI: 5 times	Yes	No	22–23, 38–41, 44–46
Urinary KIM-1	Facilitates phagocytosis of apoptotic renal tubular cells	Type 1 CRS: -AKI: 0.83–0.88	Type 1 CRS: -All-cause death: 2 times Type 2 CRS -All-cause death or HF hospitalization: 1.1–1.5 times	No	No	22, 47–52
Urinary IL-18	Marker of injury from NLRP3-inflammasome on cardiac myocytes and renal tubular cells	Type 1 CRS: -AKI: 0.61–0.75 -AKI-to-CKD: 0.674	Type 1 CRS: -AKI: 3.6 times -All-cause death: 1.2 times	No	No	46–47, 52, 56
Urinary L-FABP	Binds fatty acid oxidation products	Type 1 CRS: -AKI: 0.86 when urinary L-FABP/NAG combined	Unclear	No	No	58
Urinary NAG	Renal proximal tubule brush border marker		Type 2 CRS: -All-cause death: 1.3–1.4 times -HF hospitalization: 1.2 times	No	No	48–49, 58
Urinary angiotensinogen	Marker of intrarenal RAAS activation	Type 1 CRS: -AKI: 0.78 -All-cause death: 0.85	Unclear	Yes	RAAS inhibitors, MRAs	46

(Continued)

TABLE 1 | (Continued)

Biomarker	Function of biomarker	Predictive value (AUC-ROC per outcome)	Prognostic value ("x" times increased risk of outcome)	Involved in the disease mechanism of CRS	Targeted treatments	References
Biomarkers of cardiac damage						
Plasma cTnT	Marker of cardiac myocyte injury	Type 4 CRS: -AMI: sensitivity 92–95%, specificity 88–97% *	Type 4 CRS: -CV events: 2–6 times -All-cause death: not associated	No	No	60–64
Plasma BNP and NT-proBNP	Marker of left ventricular wall stretch	Type 4 CRS: -All-cause death: 0.699–0.818 -All-cause death or CV events: 0.666–0.720?	Type 4 CRS: -CV events: 1.4 times -All-cause death: 1.6 times	Yes	Diuretics	60, 66–67
Cell cycle arrest biomarkers						
Urinary [TIMP2]•[IGFBP7]	Involved in G1 cell-cycle arrest during early phases of cell injury	Type 1 CRS: -AKI: 0.75–0.84	Unclear	No	No	71–72
Novel biomarkers						
Plasma TSP-1	Binds to CD47 to limit nitric oxide-mediated vasodilation	AMI: -HF: 0.82	Unclear	Yes	Anti-CD47 blockade and microRNA-221 targeting TSP-1 in animal models	84, 86–88
Plasma syndecan-1	Marker of glycocalyx injury	Type 1 CRS: -AKI: 0.741 -Severe AKI: 0.812 -All-cause death: 0.788	Type 1 CRS: -All-cause death: 1.3 times Chronic HF: -All-cause death or hospitalization: 2 times (HFpEF) -Not prognostic for HFrEF	Yes	Glycocalyx-protective treatments (albumin, sulodexide, FFP, steroids, etanercept, statins, metformin, heparin)	94–95, 97–111
Plasma IL-1β	Marker of injury from NLRP3-inflammasome on cardiac myocytes and renal tubular cells	Unclear	Unclear	Yes	Anti-IL-1β blockade (canakinumab) in human CV disease	113

CRS, cardiorenal syndrome; AUC-ROC, area under the receiver operating characteristic curve; AKI, acute kidney injury; CV, cardiovascular; HF, heart failure; RAAS, renin-angiotensin-aldosterone system; MRA, mineralocorticoid receptor antagonists; SGLT2, sodium-glucose co-transporter 2; GFR, glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; L-FABP, liver fatty acid-binding protein; NAG, N-acetyl-beta-D-glucosaminidase; cTnT, cardiac troponin T; AMI, acute myocardial infarction; CKD, chronic kidney disease; BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; IL, interleukin; GDF-15, growth differentiation factor 15; ST2, suppressor of tumorigenicity 2; TIMP2, tissue inhibitor of metalloproteinases-1 and -2; IGFBP7, insulin-like growth factor-binding protein-7; TSP-1, thrombospondin-1; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; FFP, fresh frozen plasma.

**cTnT cut-off > 43.2 ng/L for pre-dialysis CKD, > 350 ng/L for kidney failure.*

** Cut-offs differ for all-cause death (stage 1–3 CKD: BNP > 90.8 pg/ml and NT-proBNP > 259.7 pg/ml and stage 4–5 CKD: NT-proBNP > 2,584.1 pg/mL) and all-cause death or HF hospitalization (stage 4–5 CKD: BNP > 157.0 pg/ml and NT-proBNP > 5,111.5 pg/ml).*

Plasma NGAL kinetics may also improve its ability to predict AKI (AUC-ROC 0.91 for delta NGAL change vs. 0.69 for NGAL at baseline) (45). Lastly, urinary NGAL performed 2 days after hospitalization for acute HF differentiated true WRF from pseudo-WRF based on AKI with or without clinical improvement (AUC-ROC 0.83, 0.73–0.93), (22) was associated with a 5-fold risk of AKI, (46) but failed to predict AKI-to-CKD transition (47).

Kidney Tubule: Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM-1) is a transmembrane glycoprotein with immunoglobulin and mucin domains, which is expressed by renal proximal tubule epithelium in response to injury and facilitates phagocytosis of apoptotic tubular cells. In chronic HF, elevated urinary KIM-1 was modestly associated with a 10–15% increased risk of all-cause death or HF hospitalization but did not predict AKI nor AKI-to-CKD transition in acute HF (22, 47–49). In contrast, urinary KIM-1 measured 12 h after cardiac surgery predicted AKI with good performance (AUC-ROC 0.83–0.88), (50, 51) and a high cut-off for urinary KIM-1 (highest vs. lowest tertile) was associated with a doubling of 3-year mortality risk, independent of post-operative AKI (52).

Kidney Tubule: IL-18

Interleukin-18 (IL-18) is an 18-kDa pro-inflammatory cytokine produced by immune cells (e.g., macrophages) and non-immune cells (e.g., vascular endothelial cells and renal proximal tubular cells) in response to tissue injury *via* activation of the NLRP3-inflammasome, which causes programmed cell death in both cardiomyocytes and renal tubular cells (53–55). In acute HF, elevated urinary IL-18 was associated with a 3.6-fold increased risk of AKI in multivariate analysis, (46) and modestly predicted AKI-to-CKD transition at 6 months (AUC-ROC 0.674, 0.543-0.805) (47). In people undergoing cardiac surgery, urinary IL-18 also modestly predicted AKI (AUC-ROC 0.61 at 4 h post-surgery, 0.75 at 12 h, and 0.73 at 24 h), (56) which was superior to clinical models using eGFR, (42) and was associated with a modest 1.2-fold increased risk of long-term mortality (52).

Kidney Tubule: Liver Fatty Acid-Binding Protein (L-FABP), *N*-Acetyl-Beta-D-Glucosaminidase (NAG) and Urinary Angiotensinogen

Fatty-acid binding proteins (FABPs) are a family of 15-kDa cytoplasmic proteins that are involved in the intracellular transport of long-chain fatty acids. L-FABP is located on the renal proximal tubular cells may reduce oxidative stress by binding fatty acid oxidation products (57). NAG is a large lysosomal brush border enzyme (>130 kDa), predominantly expressed on proximal tubular cells, and is not freely filtered. In patients undergoing cardiac surgery, combining urinary L-FABP and NAG at 4 h after surgery with pre-operative clinical factors (including demographics, comorbidities and serum creatinine) improved AKI prediction compared to pre-operative clinical factors alone (AUC-ROC 0.86, 0.74–0.93 vs. 0.79, 0.66–0.88, $p < 0.05$) (58). In chronic HF, elevated urinary NAG was also associated with a 1.2-fold increased risk of HF hospitalization and 1.3–1.4-fold increased risk of all-cause death in multivariate models (48, 49). In acute HF, urinary angiotensinogen also predicted AKI (AUC-ROC 0.78) and all-cause death (AUC-ROC 0.85) (46).

Cardiac Myocyte: Troponin

Cardiac troponin I (cTnI) and T (cTnT) are established diagnostic and prognostic biomarkers in myocardial infarction and HF (59). While troponin levels are elevated in people with CKD due to reduced plasma clearance, elevated cTNT was associated with a 2–6-fold increased risk of cardiovascular events, (60, 61) but not all-cause death after adjusting for kidney function (62). Adjusting cTnT cut-off retained good performance at detecting acute myocardial infarction in people with CKD (cTnT > 350 ng/L (standard cut-off > 14 ng/L) for eGFR < 15 ml/min/1.73 m^2: sensitivity 95%, specificity 97%; cTnT cut-off > 43.2 ng/L for eGFR < 60 ml/min/1.73 m^2: sensitivity 92%, specificity: 88%) (63, 64).

Cardiac Ventricular Stretch: Brain Natriuretic Peptide and N-Terminal proBNP

Pro-BNP is secreted by cardiomyocytes in the ventricle and atria in response to ventricular wall stretch and cleaved into active BNP and inactive NT-proBNP, which are both established biomarkers in HF (65). While NT-proBNP is elevated in people with CKD, elevated NT-proBNP was still associated with a 1.3-fold increased risk of cardiovascular events and 1.6-fold increased risk of all-cause death after adjusting for kidney function (60). In people with CKD, both BNP and NT-proBNP demonstrated moderate predictive ability for all-cause death and/or cardiovascular events at different cut-offs. For stage 1–3 CKD, BNP > 90.8 pg/ml (AUC-ROC 0.699) and NT-proBNP > 259.7 pg/ml (AUC-ROC 0.702) predicted all-cause death. In comparison, for stage 4–5 CKD, BNP > 157.0 pg/ml (AUC-ROC 0.666) and NT-proBNP > 5,111.5 pg/ml (AUC-ROC 0.720) predicted all-cause death or cardiovascular events (66). NT-proBNP kinetics may have improved clinical utility in the CKD population with a doubling of NT-proBNP associated with a 1.4-fold increased risk of cardiovascular events in African-Americans with CKD in multivariate analysis (67).

BIOMARKERS OF CELL CYCLE ARREST

Tissue Inhibitor of Metalloproteinase-2 x Insulin-Like Growth Factor-Binding Protein-7 [TIMP2]●[IGFBP7]

TIMP2 and IGFBP7 are involved in G1 cell-cycle arrest during early phases of cell injury and the product of urinary TIMPIGFBP7 concentrations (NephroCheck) is the first Food and Drug Administration-approved test to assess the risk of AKI based on studies in critically ill patients in the intensive care unit (68–70). Small studies have demonstrated urinary

[TIMP2]●[IGFBP7] predict AKI after cardiac surgery (AUC-ROC 0.84), (71) and in acute HF, where it outperformed KIM-1 (AUC-ROC 0.75, 0.61–0.88 vs. 0.54, 0.37–0.70) (72).

CURRENT CHALLENGES IN THE IMPLEMENTATION OF BIOMARKERS AND EMERGING NOVEL BIOMARKERS

The forementioned biomarkers demonstrate diagnostic utility for AKI and/or cardiac events though implementation remains challenging. Key suggestions for biomarker use from the 23rd Acute Disease Quality Initiative meeting included combining damage and functional biomarkers to identify patients at high-risk of AKI, to improve the diagnostic accuracy of AKI, discriminate AKI etiology, and to assess AKI severity (73). In CRS, biomarkers may assist our understanding of the interaction of heart-kidney injury. Kidney damage biomarkers (e.g., plasma/urinary NGAL, urinary IL-18, urinary KIM-1, urinary L-FABP, urinary NAG and urinary angiotensinogen) and cell cycle arrest biomarkers (e.g., urinary [TIMP2]●[IGFBP7]) may inform in CRS type 1, and cardiac damage biomarkers (e.g., troponin and BNP) in CRS type 3 and 4. In contrast, the utility of biomarkers in CRS type 2 and 5 require further study though potentially more sensitive markers of GFR (e.g., plasma cystatin-C and proenkephalin A) may be inform in CRS type 2, plasma galectin-3 may inform in both CRS type 2 and 4 since it is both a marker of GFR and cardiac fibrosis, and CRS type 5 likely requires a combination of biomarkers since it reflects simultaneous heart and kidney injury. However, interventions in CRS remain limited and few existing biomarkers (e.g., albuminuria, plasma galectin-3, urinary angiotensinogen, plasma BNP and NT-proBNP) offer insights into therapeutic targets that may benefit patients with CRS.

Loop diuretics remain the first-line treatment for fluid removal in HF, however, animal studies raise concerns regarding frusemide-mediated RAAS activation and subsequent myocardial and renal fibrosis (3). While aldosterone-mediated fibrosis in CRS suggest RAAS inhibitors and mineralocorticoid receptor antagonists may be beneficial, the risk of hyperkalemia or AKI may limit their use (74). The thrombospondin-1/CD47 axis, glycocalyx and IL-1β are emerging biomarkers in cardiovascular disease which may also provide prognostic value and therapeutic targets in CRS.

Thrombospondin-1 (TSP-1)/CD47 Axis

TSP-1 is a 480 kDa matricellular protein secreted by tissue in response to hypoxia and binds to the ubiquitously expressed CD47 to limit nitric oxide (NO)-mediated vasodilation, thereby limiting tissue perfusion (75, 76). The TSP-1/CD47 axis has been implicated in renal ischemia reperfusion injury (IRI), (77, 78) atherosclerosis, (79) endothelial dysfunction, (80, 81) pulmonary hypertension, (82) and vaso-occlusive events in sickle cell anemia (83). In microarray analysis of peripheral blood samples, TSP-1 outperformed BNP and cTnT at predicting HF after myocardial infarction (AUC-ROC 0.82 vs. 0.63), (84) and plasma TSP-1 levels are also increased in people with CKD (85).

In human hearts after autopsy and experimental myocardial infarction in mice, CD47 is upregulated on cardiomyocytes and inhibited phagocytosis of apoptotic cardiomyocytes by macrophages (86). CD47 inhibition ameliorated myocardial infarction in mice and rats by enhancing myocardial phagocytosis, resolving monocyte infiltration, increasing endothelial nitric oxide synthase activity and reducing oxidative stress, resulting in reduced infarct size, reduced cardiac fibrosis, and improved left ventricular ejection fraction (86, 87). Anti-CD47 blockade also successfully ameliorated kidney fibrosis in a renal IRI model and reduced expression of fibrosis markers such as transforming growth factor (TGF)-β, SMAD2 and connective tissue growth factor (85). More relevant to CRS, microRNA-221 inhibits TSP-1 upregulation, reduces TGF-β1-mediated cardiac fibrosis, and improves cardiac function and survival in 5/6 nephrectomy rats, a model of CKD (88). Overall, the TSP-1/CD47 axis represents a promising biomarker and therapeutic target in CRS.

Syndecan-1

The glycocalyx is a 0.5–8 μm thick carbohydrate-rich structure composed of glycoproteins (e.g., syndecan-1) bound to glycosaminoglycan side-chains (e.g., heparan sulfate and hyaluronan), which overlies vascular endothelial cells and governs its barrier function as well as antiadhesive and anticoagulant properties (89, 90). Degradation of the glycocalyx has been proposed as an early marker of endothelial dysfunction, which has been increasingly recognized as a critical process in CRS (11, 91–93). In acute HF, elevated serum syndecan-1 at hospital admission predicted AKI (AUC-ROC 0.741), severe AKI (AUC-ROC 0.812) and in-hospital death (AUC-ROC 0.788), and was associated with a 1.3-fold increased risk of all-cause death at 6 months in multivariate analysis (94). In chronic HF, elevated serum syndecan-1 was prognostic in HF with preserved ejection fraction (2-fold increased risk of all-cause death or hospitalization) but not HF with reduced ejection fraction (95).

Restoration of the glycocalyx using a novel selectin-targeting glycocalyx mimetic (DS-IkL) reduced selectin-mediated neutrophil and macrophage infiltration, endothelial cell and fibroblast proliferation, and cardiac fibrosis after myocardial infarction in mice (96). Therapies with proven safety in humans repurposed for glycocalyx regeneration (e.g., albumin, sulodexide, fresh frozen plasma, hydrocortisone, etanercept, rosuvastatin, metformin, and heparin) may also represent potential novel treatments in CRS (97–111).

Interleukin-1β (IL-1β)

Similar to IL-18, IL-1β is involved in activation of the NLRP3-inflammasome in myocardial and kidney injury. Rademaker et al. recently performed RNA sequencing on serial kidney biopsies in an ovine model of acute HF and identified 675 differentially expressed genes with human homologs that were enriched for 9 pathways, of which IL-1β was the top-predicted upstream regulator gene (112). Canakinumab, a humanized monoclonal antibody targeting IL-1β, reduced cardiovascular events by 15% in 10,061 patients with previous myocardial infarction though at the cost of increased fatal infections (113). Similar results were

reported in the CKD subgroup (114). Whether canakinumab or other biomarkers identified in the study by Rademaker et al. have a role in the treatment of CRS require further study.

CONCLUSION

Cardiorenal syndrome is an increasingly common condition in the aging multimorbid population with significant health burden and few effective treatments. Current studies of biomarkers in CRS have largely focused on prognostication and clinical translation has been limited by sparse data comparing them to traditional biomarkers such as serum creatinine. Furthermore, few biomarkers offer insights into the mechanistic basis of disease needed to inform therapeutic strategies. In this review, we propose the TSP-1/CD47 axis, glycocalyx and IL-1β as promising areas for future research in CRS, which have the potential to prognosticate and direct treatments in this complex condition.

AUTHOR CONTRIBUTIONS

EC, NR, and SA conceptualized review topic. EC, KT, JL, SH, ZE, NR, and SA contributed to data interpretation. ZE, NR, and SA contributed to supervision. All authors contributed to important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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SYSTEMATIC REVIEW
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Cardiorenal Syndrome in COVID-19 Patients: A Systematic Review

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Aims: To perform a systematic review assessing the clinical manifestations and outcomes of cardiorenal syndrome or the presence of both cardiac and renal complications in the 2019 coronavirus disease (COVID-19) patients.

Methods: All relevant studies about cardiorenal syndrome or both cardiac and renal complications in COVID-19 patients were retrieved on PUBMED, MEDLINE, and EMBASE from December 1, 2019 to February 20, 2022.

Results: Our search identified 15 studies including 637 patients with a diagnosis of cardiorenal syndrome or evidence of both cardiac and renal complications followingSARS-CoV-2 infection. They were male predominant (66.2%, 422/637), with a mean age of 58 years old. Cardiac complications included myocardial injury (13 studies), heart failure (7 studies), arrhythmias (5 studies), or myocarditis and cardiomyopathy (2 studies). Renal complications manifested as acute kidney injury with or without oliguria. Patients with cardiorenal injury were often associated with significantly elevated levels of inflammatory markers (CRP, PCT, IL-6). Patients with a diagnosis of cardiorenal syndrome or evidence of both cardiac and renal complications had more severe disease and poorer prognosis (9 studies).

Conclusion: The presence of either cardiorenal syndrome or concurrent cardiac and renal complications had a significant impact on the severity of the disease and the mortality rate among patients with COVID-19 infection. Therefore, careful assessment and management of potential cardiac and renal complications in patients with COVID-19 infection are important to improve their outcomes.

Keywords: cardiorenal syndrome (CRS), COVID-19, SARS-CoV-2, cardiac complications, renal complications

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INTRODUCTION

The 2019 coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Current literature indicates that sepsis secondary to COVID-19 infection has typical pathophysiological characteristics, namely early cytokine storms and subsequent immunosuppressive stages (1). Sepsis is frequently associated with cardiovascular complications and acute kidney injury either in isolation or in combination (2).

Angiotensin-converting enzyme 2 (ACE-2) is thought to be the major cell entry receptor for SARS-CoV-2 (3). ACE-2 is also expressed in the heart and kidney, providing a link between coronavirus infection and potential cardiovascular and renal complications (4). A recent epidemiological study (5) demonstrated that acute myocardial injury, cardiac arrhythmias, and shock can occur in 7.2, 18.7, and 8.7% of COVID-19 patients, respectively. Renal involvement is also not uncommon in the course of COVID-19. More than 40% of patients admitted to hospitals with COVID-19 infection had proteinuria (6). Among critically ill patients, acute kidney injury (AKI) is common, affecting ∼20–40% of patients infected with COVID-19 admitted to intensive care units (7).

Although COVID-19 is most commonly associated with COVID pneumonitis, it can also result in several extrapulmonary manifestations, such as thrombotic complications, acute cardiac injury (ACI), acute kidney injury (AKI), gastrointestinal symptoms, and hepatocellular injury (8).

Cardiorenal syndrome can occur in COVID-19 patients, precipitated by arrhythmias, ACI, and AKI (2). Cardiorenal syndrome comprises a spectrum of disorders involving both the heart and kidneys, in which acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other (9).

Limited data is available when evaluating the outcomes of COVID-19 patients with cardiorenal syndrome. Thus, the objective of this systematic review is to analyze and summarize the available literature on COVID-19 patients with both cardiac and renal complications, or cardiorenal syndrome, to gain an improved understanding of these issues in COVID-19 patients.

METHODS

Search Strategy

The literature search was conducted in PUBMED/MEDLINE and EMBASE databases from December 1, 2019 to February 20, 2022 using the following terms: (COVID-19 OR SARS-CoV-2 OR severe acute respiratory syndrome coronavirus 2) AND (acute kidney injury OR acute renal impairment OR acute renal failure OR renal replacement therapy) AND (cardiomyopathy OR CMP OR cardiomyopathies OR myocardiopathy OR cardiac injury OR myocarditis OR heart injury) in the title/abstract. We limited our search to articles written in English. The literature search was conducted independently by three authors (LL, YQC, and DWH). Additionally, all references of selected papers were searched manually. This systematic review followed instructions from the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) statement (10).

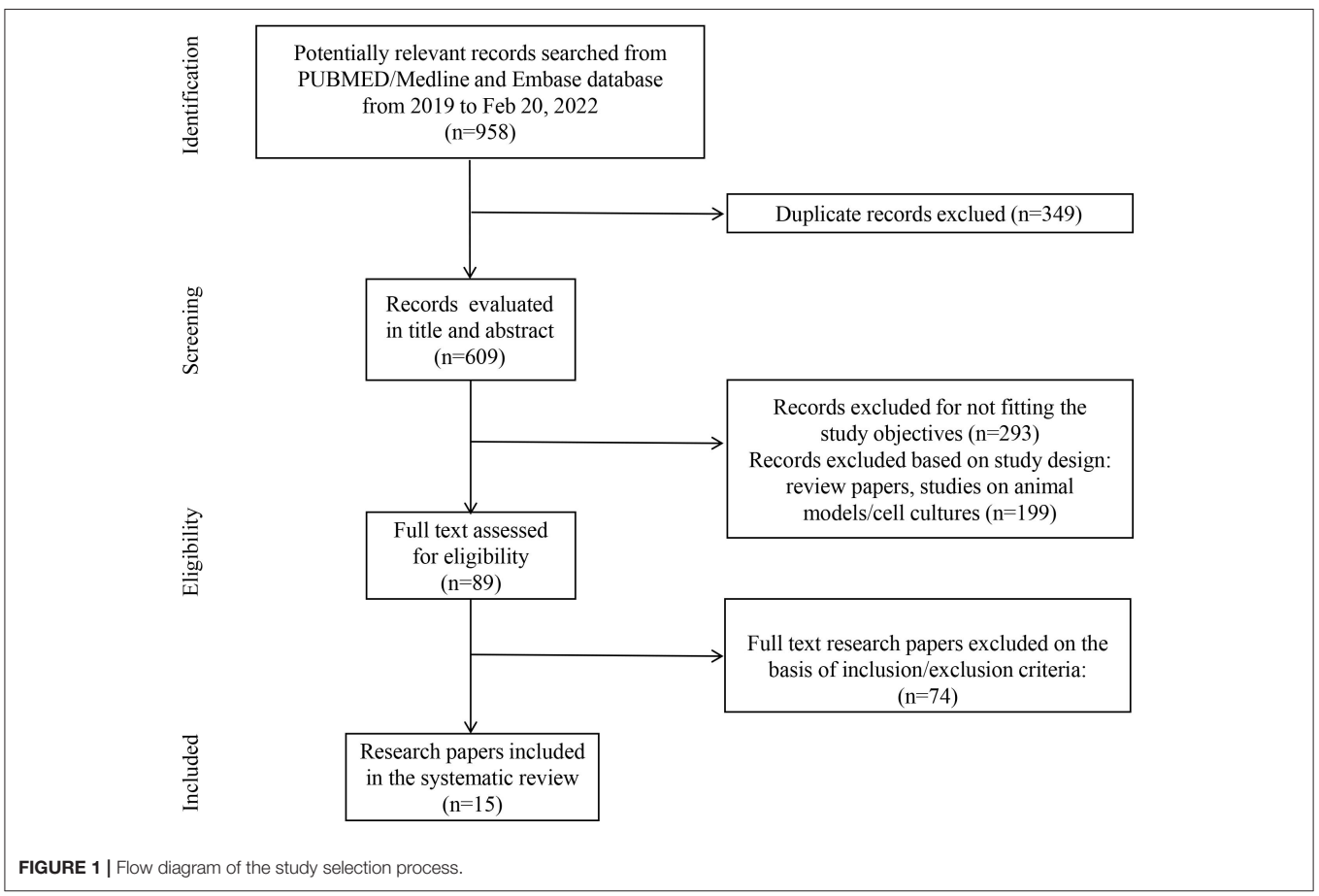

FIGURE 1 | Flow diagram of the study selection process.

TABLE 1 | Characteristics of the included studies.

Author	Country	Type of study	Total participants (n)	Subgroup characteristics	Patients with cardiac and/or renal complications (n)	Gender Male (%)	Age Mean (y)	Underlying diseases	
								Cardiovascular	Renal
Ali et al. (11)	Ireland	Case report	1		1	100%	37	Cardiomyopathy?	N
Li et al. (12)	China	Retrospective study	1,249	6	6	61.9%	36	Hypertension CHD	CKD
Case et al. (13)	USA	Retrospective study	3,389	Tn↑	195	53.8%	68	47.7%Hypertension 37.4% CHD 39.5% CHF 19.0% AF	42.1%CKD
				Tn↑ with AKI	95				
				Tn N	3,194	50.9%	61	53.2% hypertension 13.1% CHD 15.1% CHF 9.6% AF	24.0%CKD
Stefan et al. (14)	Romania	Case report	1		1	0	53	Hypertension Hyperlipidemia	N
Zhu et al. (15)	China	Case report	1		1	100%	55	Hypertension CHD	Renal graft function normal
Naeem et al. (16)	United Arab Emirate	Retrospective study	203	ACI	44	91%	55	55.5% hypertension 9% cardiovascular disease	NA
				ACI and AKI	33				
				No ACI	159	70.5%	46	32.0% hypertension 3.2% cardiovascular disease	
Shi et al. (17)	China	Retrospective study	416	Tn↑	82	49.3%	74	59.8% hypertension 29.3% CHD 14.6% CHF	6.1%CKD
				Tn↑ and AKI	7				
				Tn N	334		60	23.4% hypertension 6.0% CHD 1.5% CHF	2.7%CKD
Rahimzadeh et al. (18)	Iran	Retrospective cohort study	516	AKI	194	85.1%	61	53.6% hypertension 29.4% cardiac disease	8.8%CKD 2.6%KTH
				AKI and ACI	61				
				No AKI	322	49.4%	56	33.9% hypertension 17.7% cardiac disease	0.9%CKD
Rao et al. (19)	USA	Retrospective study	8,574	No AKI	6,011	53.1%	60	52.9% hypertension 8.3% CHF 3.9% MI	8.1%CKD
				No AKI and MACE	279 (4.6%)				
				AKI Stage 1	902	62.5%	69	76.4% hypertension 18.6% CHF 5.7% MI	22.8%CKD

(Continued)

TABLE 1 | Continued

Author	Country	Type of study	Total participants (n)	Subgroup characteristics	Patients with cardiac and/or renal complications (n)	Gender Male (%)	Age Mean (y)	Underlying diseases	
								Cardiovascular	Renal
				AKI Stage 1 and MACE	122 (13.5%)				
				AKI Stage 2	431	63.1%	71	79.6% hypertension 15.3% CHF 6.5% MI	5.3%CKD
				AKI Stage 2 and MACE	81 (18.8%)				
				AKI Stage 3	777	64.9%	65	72.7% hypertension 12.1% CHF 4.4% MI	12.9%CKD
				AKI Stage 3 and MACE	203 (26.1%)				
Pernigo et al. (20)	Italy	Case report	1		1	100%	45	N	N
Ramalho et al. (21)	Portugal	Case report	1		1	100%	50	Dyslipidaemia	N
Saririan et al. (22)	UK	Case report	1		1	100%	61	Hypretension	N
Al-Wahaibi et al. (23)	Oman	Retrospective study	143	Tn↑	31	86.7%	61	61.3% hypretension 16.1% CHF 6.5% CHD	16.1%CKD
				Tn↑ and AKI	21				
				Tn N	112		44	24.1% hypretension 3.6% CHD	6.2%CKD
Parith et al. (24)	USA	Case report	1		1	0	23	N	N
Yasmin et al. (25)	Indonesia	Case report	1		1	0	64	N	N

NA, Not Applicable; ?, Clinically Undetermined; IQR, Interquartile Range; SD, Standard Deviation; ACI, Acute Cardiac Injury; AKI, Acute Kidney Injury; CHD, Coronary Atherosclerotic Heart Disease; CKD, Chronic Kidney Disease; CHF, Congestive heart failure; AF, Atrial fibrillation; MACE, major adverse cardiac event; USA, The united states of America; KTH, Kidney transplant history; MI, myocardial infarction; Tn, troponin; N, Normal; N/A, Not applicable; n, Number; y, Year.

Criteria for Inclusion

We included human studies meeting the following criteria: (1) Patients with COVID-19 were confirmed through positive results for SARS-CoV-2 nucleic acid testing of nasopharyngeal or throat swab specimens; (2) Patients 18 years or older; (3) Patients diagnosed with cardiorenal syndrome or evidence of both cardiac and renal complications. The exclusion criteria applied to the studies were: (1) Pregnant or lactating women; (2) Study type: review, conference abstract, letter to the editor.

Data Extraction

The following variables were extracted from all included studies: first author, the country where the research was conducted, type of study, number of patients, mean age, gender, underlying comorbidities, cardiac and kidney clinical events (such as cardiac arrhythmia, cardiac injury defined as elevated troponin levels, heart failure defined as EF $\leq$ 40%, elevated BNP, or echocardiographic evidence of heart failure, myocarditis, oliguria, anuric, proteinuria, acute kidney injury defined as elevated serum creatinine level, tubular injury), laboratory findings, use of Angiotensin-Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARB), and clinical outcomes. Three authors (LL, YQC, and DWH) independently performed data extraction. Any disagreements were discussed and resolved with the senior authors (AYW and WJQ).

RESULTS

The search identified 15 studies and 637 patients with a diagnosis of cardiorenal syndrome or evidence of both cardiac and renal complications after SARS-CoV-2 infection. They were male predominant (66.2%, 422/637), with a mean age of 58 years old (**Figure 1**; **Table 1**).

The studies were either retrospective (7 studies) or case reports (8 studies). Most patients had multiple comorbidities including hypertension, chronic heart failure, and chronic kidney disease before SARS-CoV-2 infection, but specific data were not provided (**Table 2**).

Cardiac complications manifested as myocardial injury (13 studies), heart failure (7 studies), arrhythmia (5 studies), or myocarditis and cardiomyopathy (2 studies) (**Table 2**). Five studies demonstrated a reduction in left ventricular ejection fraction. Elevated troponin and brain natriuretic peptides were

TABLE 2 | Clinical and laboratory findings of the heart in COVID-19 patients with cardiac and renal complications.

References		Clinical events	Electro cardiogram	Echo cardiogram	Cardiac biomarkers	
					Tn (ng/L)	NT-proBNP (pg/mL)
Ali et al. (11)		Heart failure	Sinus tachycardia Occasional premature ventricular	LVEF 10–15% Dilated left ventricle	N	247 (100–400)
Li et al. (12)		NA	NA	NA	>300	>2,500
Case et al. (13)	Tn↑	NA	NA	NA	2.6–13.82	NA
	Tn N				0.03–0.06	
Stefan et al. (14)		Thoracic pain	N	LVEF 45% Normal dimensions No segmentalkinetics alteration	304–889	301
Zhu et al. (15)		Heart failure Myocardial injury	Atrial fibrillation	NA	1,580	>70,000
Naeem et al. (16)		NA	NA	NA	>60	NA
Shi et al. (17)	Tn↑	13.4% chest pain	T-wave depression and inversion ST-segment depression Q waves	NA	190	1,689
	Tn N	0.9% chest pain	NA		<6	139
Rahimzadeh et al. (18)	AKI	31.4% ACI	NA	NA	10.3	NA
	NoAKI	15.5% ACI			4.3	
Rao et al. (19)	No AKI	3% cardiac arrest 4.6% MACE	NA	NA	10	215
	AKI Stage 1	9.6% cardiac arrest 13.5% MACE			100	1,223
	AKI Stage 2	13.3% cardiacarrest 18.8% MACE			110	848
	AKI Stage 3	19% cardiac arrest 26.1% MACE			100	1,490
Pernigo et al. (20)		Focal myocarditis Hypertensive Cardiomyopathy	Sinus tachycardia Left axis deviation Slight diffuse ST depression	Severe systolic and diastolic left ventricle dysfunction Myocardial thickening LVEF 30%	82	NA
Ramalho et al. (21)		Thrombus in the left ventricle Congestive heart failure	Left axis deviation	LVEF 15% Severe left ventricle dilation	1,345	30.39
Saririan et al. (22)		Myocardial ischaemia	Supraventricular tachycardia ST-elevation after adenosine	Moderate leftventricular systolic dysfunction	6,283–7,459 5,852–2,159	NA
Al-Wahaibi et al. (23)	Tn↑	12.9% atrial tachyarrhythmia 3.2% ventricular arrhythmia 9.7% bradyarrhythmia	NA	NA	NA	NA
	Tn N	0.9%Atrial tachyarrhythmia 1.8%Ventricular arrhythmia 6.5%Brady arrhythmia	NA	NA	NA	NA

(Continued)

TABLE 2 | Continued

References	Clinical events	Electro cardiogram	Echo cardiogram	Cardiac biomarkers	
				Tn (ng/L)	NT-proBNP (pg/mL)
Parith et al. (24)	Cardiomyopathy	A prolonged QT interval of 526 ms	Moderate global left ventricular dysfunction with an LVEF of 34% and moderate right ventricular dilatation with severe right ventricular hypokinesis	80	1,205
Yasmin et al. (25)	Cardiac injury	Fatal pulseless ventricular tachycardia	NA	420	NA

BNP, Brain Natriuretic Peptide; Tn, Troponin; LVEF, Left Ventricular Ejection Fraction; NA, Not Applicable; N, Normal; MACE, major adverse cardiac event.

seen in 9 studies. Renal complications manifested as AKI with or without oliguria. However, severe AKI requiring dialysis therapy was not common (5 studies) (**Table 3**). Patients with cardiorenal injury were often associated with significantly elevated levels of inflammatory markers (CRP, PCT, IL-6) (**Table 4**). Use of ACEI/ARB occurred in 2 studies. Patients with a diagnosis of cardiorenal syndrome or evidence of both cardiac and renal complications had more severe disease and poorer prognosis (9 studies).

DISCUSSION

Patients who developed AKI were more likely to have a cardiac event suggesting a probable role of cardiorenal interaction in the renal dysfunction that occurs in COVID-19. AKI may result in volume overload and cardiac dysfunction, and vice versa since cardiomyopathy may lead to hypotension, renal hypoperfusion, and renal congestion resulting in renal dysfunction (26), and culminating in acute respiratory distress syndrome (ARDS). The cardiorenal syndrome is associated with increased morbidity and mortality in COVID-19 patients, as well as healthcare costs.

COVID-19 may affect the heart and kidney through several mechanisms (**Figure 2**). Firstly, new evidence suggests that SARS-CoV-2 may have direct cytopathic effects on the heart and kidney. ACE-2 is the receptor for SARS-CoV-2 to enter human cells, which is highly expressed in extrapulmonary tissues including the heart and kidney (27). Secondly, excessive release of cytokines due to viral infection, known as cytokine release syndrome or cytokine storm, is the mechanism leading to multiorgan damage in COVID-19. The presence of cytokine storms and pneumonia-related hypoxia can contribute to myocardial and renal ischemia due to changes between oxygen supply and demand. Furthermore, Li et al. (28) has reported that the kinetic changes of cytokines correlate with the prognosis of patients with severe COVID-19. Thirdly, thrombotic microangiopathy seen in COVID-19 may also lead to ACI and AKI. Systemic coagulation dysfunction appears to promote thrombosis with the observation of arterial events in patients with COVID-19, such as renal artery thrombosis or acute coronary syndrome.

Up to a fifth of COVID-19 patients have an acute myocardial injury (12–17% of cases) (29, 30). In patients with SARS-CoV-2 infection, the most common features of myocardial injury were ECG changes and elevated troponin. Echocardiography showed subclinical left ventricular diastolic dysfunction and even decreased ejection fraction (EF) in severe cases (5). As previously seen during coronavirus outbreaks, patients with a low EF are more likely to require mechanical ventilation (31). This is clinically important for hospitalized patients, as expert consensus recommends an early assessment and continuous cardiac monitoring to identify patients with cardiac injury and help predict further COVID-19 complications (32). High-sensitivity troponin is a useful cardiac monitoring tool in COVID-19. Zhou et al. (30) observed a gradual increase in high-sensitivity cardiac troponin I (hs-cTnI) levels in non-survivors (reaching the reference limit on day 11), while hs-cTnI levels in survivors remained low. Piccioni et al. (33) also identified that in patients with COVID-19, high-sensitivity troponin was a negative prognostic indicator. Increased cTnI levels may be associated with endotoxin production, which may be secondary to sepsis, an overall pro-inflammatory state, or direct myocardial infarction through ACE2 receptors in cardiac tissue (34). The increase of IL-6 was parallel to that of hs-cTnI, which increased the possibility of reflecting viral myocarditis. Existing data from China show that one-quarter to one-third of COVID-19 patients have severe heart failure. Zhou et al. (30) reported 23% of heart failure in their series of 191 patients with SARS-CoV-2, while Chen et al. (35) reported 27.5% (33/120) of increased N-terminal pro-B type natriuretic peptide (NT-proBNP).

Although early reports showed a low incidence of AKI (3–9%) among COVID-19 patients in a Chinese population (5), recent data has shown a higher incidence of renal abnormalities. The most prominent findings are proteinuria or hematuria. The most significant findings were albuminuria or hematuria, which was found by test paper evaluation in nearly one-third of patients on the first day of admission, and elevated serum creatinine and blood urea nitrogen in 15.5 and 14.1% of patients (6). Importantly, an elevation

TABLE 3 | Clinical and laboratory findings of the kidney in COVID-19 patients with cardiac and renal complications.

Author		Clinical events	eGFR	Renal biomarkers		Dialysis
			(mL/min/1.73 m^2)	Cr (μmol/L)	BUN (mg/dL)	
Ali et al. (11)		Oliguria Acute tubular injury	<10	657	N	Intermittent hemodialysis
Li et al. (12)		NA	<60	NA	NA	NA
Case et al. (13)	Tn↑	48.7% AKI	58.5% ≤ 30 21.5% ≥ 60	NA	NA	NA
	Tn N	28.5% AKI	28.4% ≤ 30 55.9% ≥ 60			
Stefan et al. (14)		Oliguria Cloudy urine Proteinuria	NA	777.9	239	NA
Zhu et al. (15)		Oliguria	NA	233–308	725.4	NA
Naeem et al. (16)	ACI	75% AKI	66.5	184	NA	NA
	No ACI		94	93		
Shi et al. (17)	Tn↑	8.5% AKI	NA	101.7	NA	2.4% Continuous kidney therapy
	Tn N	0.3% AKI		56.6		0
Rahimzadeh et al. (18)	AKI	61.9% stage 1 18.0% stage 2 20.1% stage 3 63.9% proteinuria	53.48 (35.70–68.25)	118.5	44	NA
	No AKI	29.3% proteinuria		83.1	26	
Rao et al. (19)	No AKI	AKI	NA	97.3	NA	RRT
	AKI stage 1			265.2		0.6% RRT
	AKI stage 2			229.8		2.6% RRT
	AKI stage 3			618.8		36.5% RRT
Pernigo et al. (20)		AKI Acute tubular injury Hypertensive kidney disease	NA	274.1	NA	NA
Ramalho et al. (21)		AKI	NA	145.9	64	NA
Saririan et al. (22)		Anuric	NA	547.2	NA	Continuous veno-venous hemofiltration
Al-Wahaibi et al. (23)	Tn↑	67.7% AKI	NA	NA	NA	48.4% RRT
	Tn N	11.6% AKI	NA	NA	NA	3.6% RRT
Parith et al. (21, 24)		AKI	NA	198.9	NA	NA
Yasmin et al. (25)		AKI	NA	117.6	75.6	NA

Cr, creatinine; BUN, UreaNitrogen; N, Normal; NA, Not Applicable; AKI, Acute Kidney Injury.

of any marker of kidney damage in COVID-19 patients is associated with significantly higher hospital mortality. Several mechanisms may contribute to the kidney injury seen with COVID-19. Other mechanisms that have been reported include sepsis, acute tubular necrosis caused by renal hypoperfusion, cytokine storm, alveolar injury caused by renal medulla hypoxia, cardiorenal syndrome, and rhabdomyolysis (26, 36–38). Magoon et al. has reported less common conditions such as immune-mediated glomerulonephritis and primary glomerular lesions that caused focal segmental glomerulosclerosis collapse (39). Moreover, the hypercoagulable state in COVID-19 may lead to thrombotic microangiopathy and peritubular and glomerular capillary obstruction (38, 40). AKI may also be the result or complication of COVID-19 treatment. Antiviral drugs can lead to tubulointerstitial diseases (41, 42), and biopsy confirmed oxalate nephropathy associated with vitamin C has been reported (43). Certain antibiotics/antibacterial agents have also been implicated in AKI in COVID-19 patients (44).

ACE-2 is the main entry point of most coronaviruses, and its binding domain has a high affinity with SARS-CoV-2. The coronavirus binds to the extracellular domain of ACE-2 on the host cell surface through its spike protein (S protein), and then invades the cells, resulting in the down-regulation of ACE-2 expression on the cell surface (3). After entering cells, viruses

TABLE 4 | Inflammatory index, ACEI/ARB use and the outcomes in COVID-19 patients with cardiac and renal complications.

Author		Inflammatory index	ACEI/ARB use	Outcomes (%)
Ali et al. (11)		CRP < 100 mg/L	ACEI	Cured
Li et al. (12)		PCT 0.1 ng/mL CRP 0.5–37.1 mg/L ESR 24–58 mm/h	NA	Higher mortality rate
Case et al. (13)	Tn↑	NA	NA	56.9% deceased
	Tn N			18.0% deceased
Stefan et al. (14)		CRP 2.2 mg/dL ESR 28 mm/h Ferritin 337 g/dL	NA	Cured
Zhu et al. (15)		CRP 81.6 mg/L IL-6 > 30 pg/ml	NA	Cured
Naeem et al. (16)	ACI	CRP 138.5 mg/L	NA	68.9% deceased
	No ACI	CRP 59 mg/L		5.1% deceased
Shi et al. (17)	Tn↑	CRP 10.2 mg/dLPCT 0.27 ng/mL	NA	51.2% deceased
	Tn N	CRP 3.7 mg/dLPCT 0.06 ng/mL		4.5% deceased
Rahimzadeh et al. (18)	AKI	CRP 69.4 mg/LESR 46 mg/L	28.4%ACEI/ARB	77% severity39.7% mortality
	Non-AKI	CRP 47.4 mg/LESR 41 mg/L	14.3%ACEI/ARB	23% severity7.1% mortality
Rao et al. (19)	No AKI	CRP 6.6 mg/LIL-6 23.0 pg/mL	NA	10.2% deceased
	AKI stage 1	CRP 8.1 mg/LIL-6 38.6 pg/mL		31.1% deceased
	AKI stage 2	CRP 9.1 mg/LIL-6 30.5 pg/mL		38.6% deceased
	AKI stage 3	CRP 10.0 mg/LIL-6 86.0 pg/mL		48.9% deceased
Pernigo et al. (20)		CRP 30 mg/L	NA	Cured
Ramalho et al. (21)		CRP 64.1 mg/dl	NA	NA
Saririan et al. (22)		NA	NA	Deceased
Al-Wahaibi et al. (23)	Tn↑	NA	NA	53.3% deceased
	Tn N			7.1% deceased
Parith et al. (24)		NA	NA	Deceased
Yasmin et al. (25)		PCT 0.1 ng/ml	NA	Deceased

ACEI, Angiotensin-converting Enzyme Inhibitor; ARB, Angiotensin Receptor Blocker; NA, Not Applicable; N, Normal; CRP, C reactive protein; ESR, Erythrocyte sedimentation rate; PCT, Procalcitonin; IL, Interleukin; Tn, Troponin.

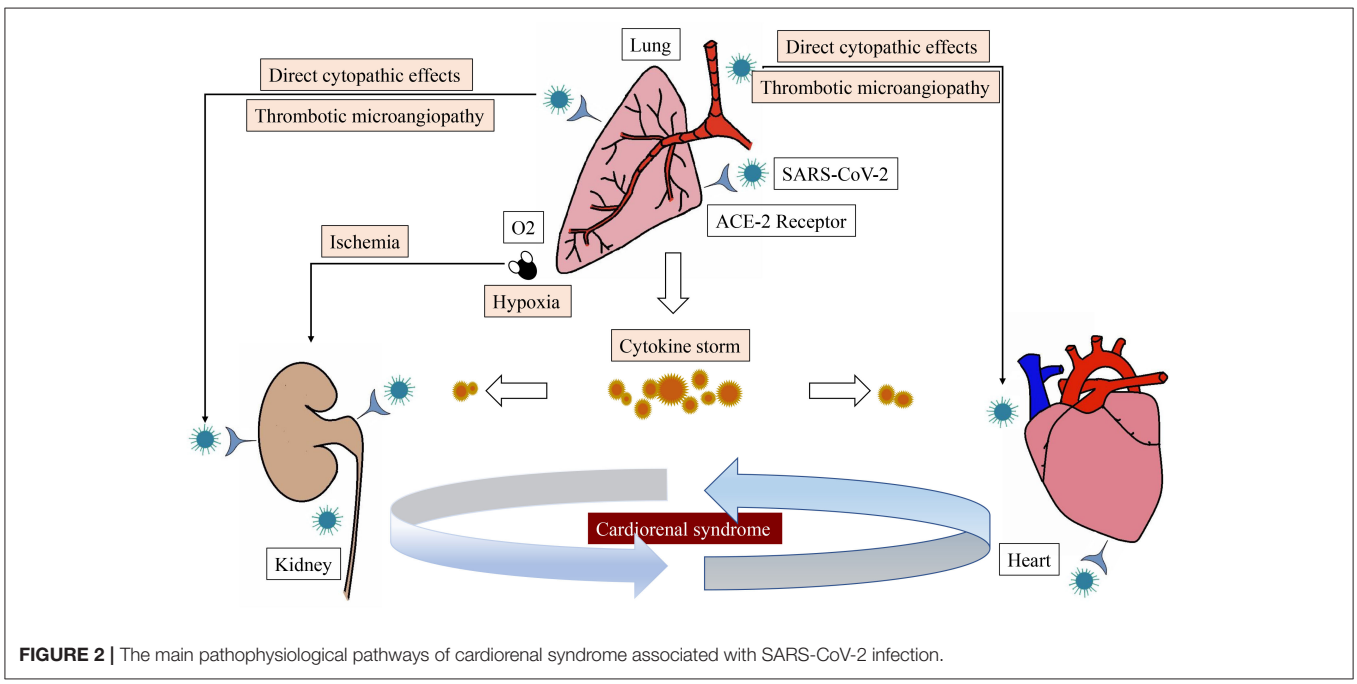

FIGURE 2 | The main pathophysiological pathways of cardiorenal syndrome associated with SARS-CoV-2 infection.

replicate and induce cytotoxicity, which may lead to organ failure. ACE-2 is widely expressed throughout the body, with the highest expression in the gastrointestinal tract and oral epithelium, and is highly expressed in the lung, kidney, and heart (45–47). As mentioned, ACE-2 is highly expressed in the proximal tubule of the kidney (3), which may allow for direct viral cell damage

resulting in tissue injury and renal failure (2). On a cellular level, ACE-2 is widely expressed in cardiac fibroblasts, myocardial cells, and coronary artery endothelial cells (48). The use of an ACEI or ARB for antihypertensive treatment in a rat model has been shown to increase ACE-2 gene expression, protein levels, and activity in hearts (49–51), which may increase the chance of SARS-CoV-2 infection or the severity of COVID-19. Whether these drugs can increase the expression and activity of ACE-2 protein in humans remains controversial. In the absence of convincing clinical data, most professional organizations suggest that ACEI or ARB treatment should be continued for patients with heart failure who have or have the risk of SARS-CoV-2 infection.

CONCLUSIONS

Patients with cardiorenal syndrome or both cardiac and renal complications had a significant impact on the severity of the disease and mortality rate among patients with COVID-19. Therefore, emphasis should be placed on the risk factors for the development of cardiorenal syndrome, its pathophysiologic mechanisms, racial predilection, optimal therapy, and prevention in the COVID-19 patient population. However, there are limited data evaluating outcomes of COVID-19 patients with cardiorenal syndrome. Thus, further research in this area is needed.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

LL, AY, AW, and WQ designed the study. LL, YC, and DH performed the search, study selection, and data synthesis. LL wrote the first draft of the manuscript. AY, AW, and WQ revised the article. All authors contributed to the paper 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.2022.915533/full#supplementary-material

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Helicobacter pylori Infection Maybe a Risk Factor for Cardiac Syndrome X

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Purpose: Cardiac syndrome X (CSX) is a condition with normal coronary angiography but angina pectoris. Chronic inflammation caused by *Helicobacter pylori* (*H. pylori*) infection may play a pathogenic role in CSX. Therefore, we conducted a meta-analysis to explore the relationship between *H. pylori* infection and risk of CSX.

Methods: A systematic search in the Web of Science, Medline, Embase and Chinese databases (CNKI and Wanfang) was conducted up to October 2021. Articles on the association between *H. pylori* infection and the risk of CSX were included and were analyzed by R software (version 4.1.0).

Results: Ten case-control studies involving 703 CSX patients and 731 healthy controls were included. *H. pylori* infection was associated with an increased risk of CSX (OR: 8.29, 95% CI: 4.64–14.82). We also found a significant association in those 25–40 years of age (OR: 1.34, 95% CI: 1.04–1.72), those 40–50 years of age (OR: 11.27, 95% CI: 4.29–29.61), those over 50 years of age (OR: 7.18, 95% CI: 3.59–14.36), those in developing countries [Iran (OR: 12.99, 95% CI: 8.61–19.60) and China (OR: 5.14, 95% CI: 3.09–8.56)]. However, this association was not apparent in a developed country [Italy (OR: 0.93, 95% CI: 0.37–2.33)].

Conclusions: Our study suggested a possible association between *H. pylori* infection and the risk of CSX. Its pathogenicity is stronger in middle-aged individuals and some developing countries. However, more studies are needed to further investigate whether early eradication of *H. pylori* can reduce the incidence rate of CSX, especially in middle-aged individuals and some developing countries.

Keywords: ***Helicobacter pylori*** **infection, Cardiac syndrome X, cytotoxin-associated gene A, meta-analysis, systematic review**

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that causes a variety of gastrointestinal disorders, including chronic gastritis, duodenal ulcer and gastric cancer (1, 2). It may also cause some extra-intestinal diseases, some of which are manifested as respiratory diseases and functional ischemic heart disease (1, 2), recently found to be related to Cardiac Syndrome X (CSX) (3–5).

Virulent strains *H. pylori* can be divided into two subgroups based upon the expression of (6) an immunodominant, 120–145 kDa protein, cytotoxin-associated gene A (CagA). Infection with CagA may be a strong risk factor for the development of CSX by inducing endothelial dysfunction (7).

CSX is characterized by typical exertional angina pectoris, positive electrocardiograph (ECG) or exercise treadmill test, normal coronary angiography with the exclusion of coronary artery spasm. In angina patients undergoing angiography, up to 20% of patients have this symptom (3). Despite extensive studies, the mechanism of this syndrome is still unclear. Nevertheless, it has been proposed that coronary artery endothelial dysfunction is the main pathogenic mechanism underlying CSX (5).

While many studies have attempted to reveal the role of *H. pylori* infection in CSX, their results are contradictory. Thus, we conducted a meta-analysis of relevant articles in an attempt to reveal the association between *H. pylori* infection and the risk of CSX.

MATERIALS AND METHODS

Our meta-analysis strictly adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (8).

Article Search

The meta-analysis was performed with a structured articles search, using the Web of Science, Medline, Embase and Chinese databases (CNKI and Wanfang). The keywords "*H. pylori*" or "*Helicobacter pylori,*" combined with "Cardiac syndrome X," "microvascular angina," "CSX," and "MVA" were used as search terms. There were no language restrictions in the search. The references in the identified articles were checked and if suitable, were also included in the search. The deadline for searching was October 2021.

Article Selection

We repeated all selections. The final inclusion of articles was decided upon by consensus, and when this decision failed, the third author (LXL) made a ruling.

Observational studies (cohort, case–control and cross-sectional) were included if the following criteria were met:

1. Studies were conducted in humans.
2. *H. pylori* infection was determined by serological tests, including antigen-specific enzyme-linked immunosorbent assay (ELISA) and Western blotting, or non-serological tests including rapid urease test, and ^{13}C-urea breath test (UBT) according to the manufacturer's instructions.
3. The diagnosis of CSX included the following criteria: typical history of angina and normal coronary angiography; atypical chest pain with an abnormal myocardial perfusion Image; or exercise electrocardiogram and completely normal results on coronary angiography, with no inducible spasm on ergonovine-provocation test.
4. A control group was included. Studies with matched and sufficient information on the association between *H. pylori* infection and the risk of CSX were selected.
5. For articles with data published more than once, only the article with an adequate study strategies and large number of cases was selected.
6. Patients with evidence of left or right ventricular dysfunction, cardiomyopathy, valvular heart disease, myocardial infarction, concomitant acute and chronic disease were excluded.

Data Extraction

Two well-trained researchers (LSP and LFH) independently extracted the following data based on the pre-specified scheme: first author or second author; average age of case group; year of publication; country; study size; case type; control type; and matching variables. All data were double input.

Data Analysis

Our meta-analysis was conducted according to the recommendations of Cochrane Collaboration and meta-analysis report quality guidelines (9, 10) and was performed using R software (version 4.1.0). The I^2 statistics were used to assess statistical heterogeneity. A value >50% was considered to have significant heterogeneity and random effect models were used to pool data. Otherwise, the fixed effects model was used. The estimated effect measure was the odds ratio (OR) with 95% confidence intervals (95% CIs) for the dichotomous data. If the 95% CI did not contain the value one, the OR was considered to be statistically significant.

The Egger's and Begg's tests were used to assess potential publication bias. Each article in this analysis was sequentially deleted to determine how much it contributed to the overall effect size. The Newcastle-Ottawa Scale (NOS) was adopted to score all articles. Articles that scored ≥7 points were considered "high-quality articles." Several subgroup meta-analyses were performed based on country, mean age, case type, *H. pylori* detection method, socioeconomic status and year of publication.

RESULTS

Search Result

Using our keywords and the references from identified articles, a total of 478 potentially relevant articles were identified. After excluding duplicates, 193 articles remained, of which 152 articles were excluded after checking the title and abstract. After carefully assessing the full text of the remaining 41 articles, 31 articles were determined to not meet inclusion criteria. This was because: (1) They were comments/reviews/case reports without raw data; or (2) They were other special studies, such as *in vitro* studies, animal studies, or epidemic pathological studies focusing on the relationship between *H. pylori* infection and coronary artery disease, rather than CSX. After this exclusion, 10 articles remained in our study (3, 11–19). The selection process for this meta-analysis is shown in **Figure 1**. The detailed information of all identified articles is shown in **Table 1**.

Helicobacter pylori Infection and Cardiac Syndrome X

We found 10 articles that reported an association of *H. pylori* infection with the risk of CSX. The combined random effect odds ratio (OR) was 8.29 (95% CI: 4.64–14.82) (**Figure 2**).

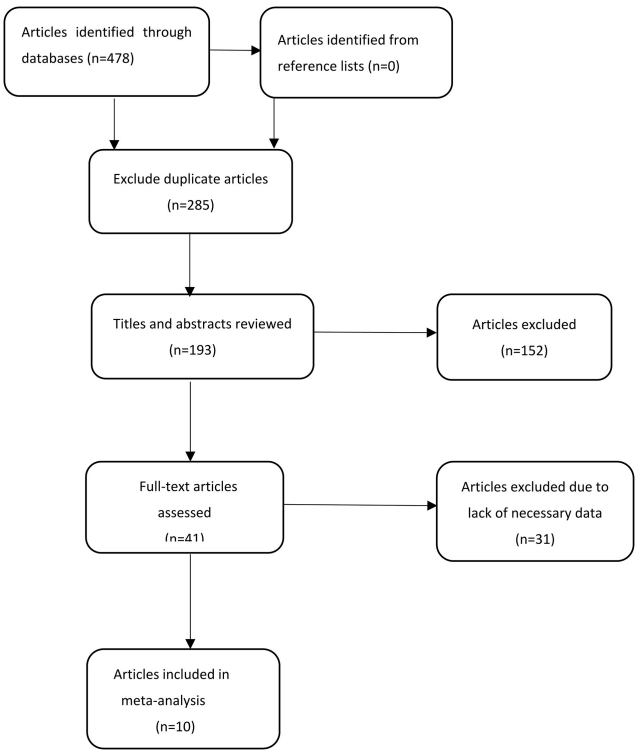

FIGURE 1 | Selection process for articles included in the meta-analysis.

After excluding three articles with low scores (six points), seven articles remained. Another meta-analysis showed that the combined fixed effect OR was 9.22 (95% CI: 6.49–13.10) (**Figure 3**). We also selected seven articles with ≥ four matched variables and performed meta-analysis. The combined random effect OR was 6.06 (95% CI: 2.23–16.43) (**Figure 4**).

The data were separately stratified by country, mean age, case type, *H. pylori* diagnostic methods, socioeconomic status and publication year. Two parallel meta-analyses were performed in each subgroup. Those results are presented in **Table 2**. Results suggested that the association of *H. pylori* infection with the risk of CSX may be much stronger in middle-aged individuals. Similarly, we found that this association is also more common in developing countries (China and Iran), but was not apparent in developed countries (Italy). When we excluded articles with six points, the meta-analysis was performed again. Differences in age and developing countries were still related to bacterial pathogenicity. Whether the articles with six points only or all included articles were analyzed, factors such as age or developing country were consistently observed to influence bacterial pathogenicity.

CagA Strains and Cardiac Syndrome X

We also found three studies focusing on the association of CagA strain infection with the risk of CSX. The combined random effect OR was 3.29 (95%CI: 0.58–18.55 (**Figure 5**). Two studies scored seven points, with an OR 7.70 (95%CI: 0.65–91.37). Because of the lack of data, a subgroup meta-analysis of CagA strains could not be performed.

TABLE 1 | Characteristics of studies in the meta-analysis.

References	Year	Country	Mean age [a]	Study size CSX/CG	Case type	Control type	Study type	Agent	Matched variables[b]	Quality score
Eskandarian (3)	2006	Iran	45 ± 5	40/40	Community	healthy	CS	UBT	1,2,4,5,6	7
Assadi (11)	2009	Iran	53.20 ± 6.16	30/30	Community	healthy	CS	UBT	1,2,3,4	7
Lanza (12)	2004	Italy	57 ± 8	55/60	Community	healthy	CS	Cag-A, UBT anti-HP/IgG	1,2,3,4,5,6	6
Mehraban (13)	2012	Iran	53.8 ± 11.9	88/97	Community	healthy	CS	anti-HP/IgG	1,2	7
Raeisi (14)	2012	Iran	51.8 ± 12.3	60/60	Community	healthy	CS	Cag-A, anti-HP/IgG	1,2,6	7
Seyyed-Mohammadzad (15)	2012	Iran	51.8 ± 12.3	100/100	Community	healthy	CS	Cag-A, anti-HP/IgG	1,2,6	7
Yu (16)	2018	China	51.2 ± 7.1	61/61	Hospital	healthy	CS	anti-HP/IgG	1,2,3,45,6	6
Song (17)	2007	China	45.41 ± 7.99	27/30	Hospital	healthy	CS	UBT	1,2,3,4,5,6	7
Rasmi (18)	2016	Iran	53.8 ± 1.3	88/97	Hospital	healthy	CS	anti-HP/IgG	1,2,4	6
Li (19)	2018	China	60.1 ± 10.49	78/80	Hospital	healthy	CS	UBT	1,2,3,4,5,6	7

CSX, Cardiac syndrome X; CG, control group; CS, case-control study; anti-HP/IgG, anti-Helicobacter pylori /immunoglobulin G; UBT, urea breath test; Cag-A, cytotoxin-associated gene-A. Mehraban et al. study included Group A (Mehraban A: 25-40 years), Group B (Mehraban B: 40-55years) and Group C (Mehraban C: >55years). a Mean age = the mean age of the case group. b 1 = age, 2 = gender, 3 = smoking status, 4 = Blood lipids, 5 = Diabetes mellitus, 6 = Hypertension.

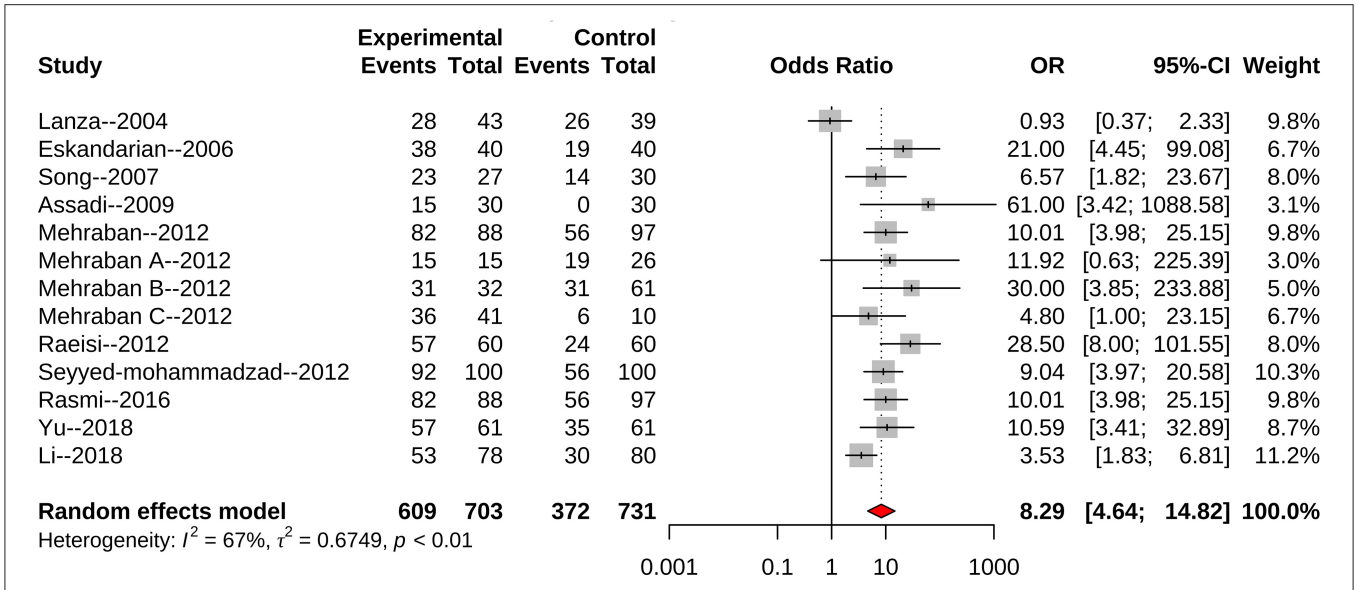

FIGURE 2 | All identified articles evaluating *H. pylori* infection and the risk of CSX.

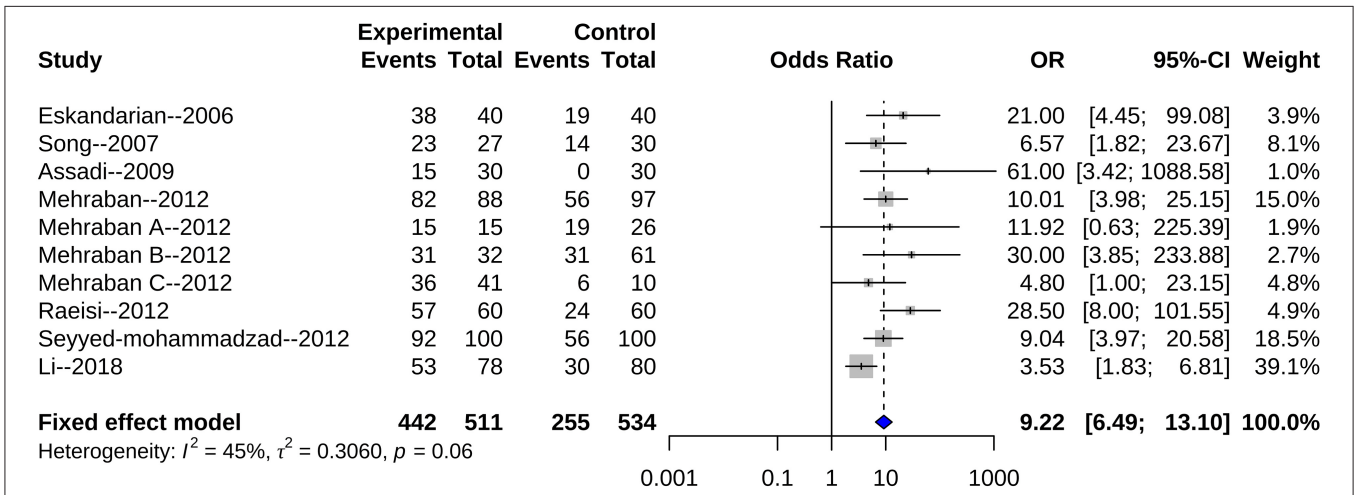

FIGURE 3 | High quality articles evaluating *H. pylori* infection and the risk of CSX.

DISCUSSION

As far as we know, the relationship between *H. pylori* infection and the risk of CSX is still controversial, and our study is the first systematic review and meta-analysis on this topic. Based on the results of our meta-analysis, we estimate that the risk of CSX caused by the bacterial infection increases by ∼87%.

The meta-analysis included 10 articles, nine of which found that *H. pylori* infection increased the risk of CSX. However, one article held the opposite view. Each article in the meta-analysis had a weight range of 3.0–11.2%. Sensitivity analysis was further conducted and concluded that the deletion of any single article would not have a significant impact on the overall effect size (**Figure 6**). There was significant heterogeneity among included articles, which may be attributable to different study designs and different study populations.

In the meta-analysis, all articles achieved satisfactory scores (≥ six points) for quality assessment. Included articles with seven points yielded more reliable results, and there was no difference between studies with low scores (six points) and studies with high scores (seven points). We performed Egger's test ($P > 0.05$) and Begg's test ($P > 0.05$) and the results suggested no potential publication bias in our meta-analysis, assuring confidence in our results.

Several potential confounding factors, such as age, gender, and socioeconomic status, cannot be ignored because they are associated with *H pylori* infection and the risk of CSX. Age and

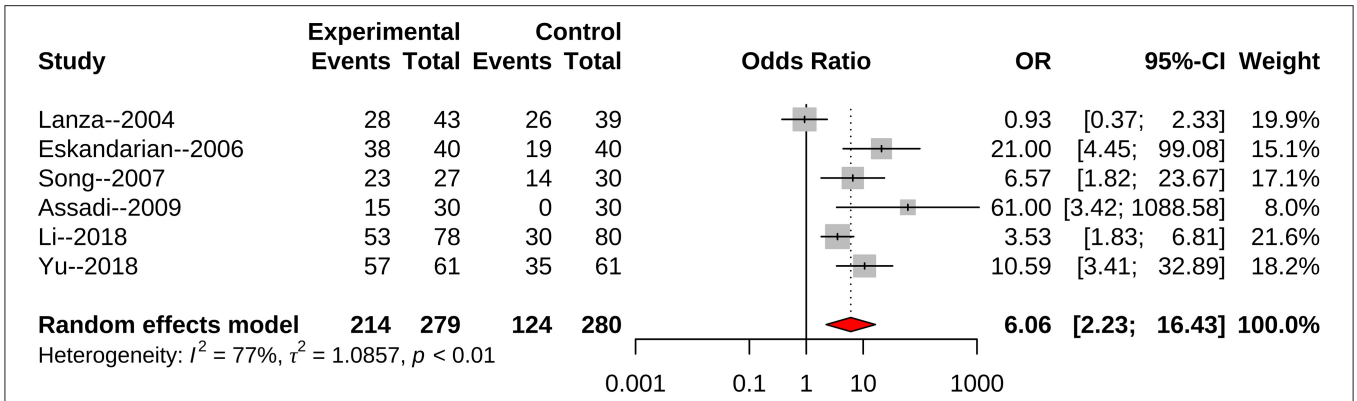

FIGURE 4 | Articles with ≥4 matched variables to evaluate *H. pylori* infection and the risk of CSX.

TABLE 2 | Subgroup analyses about relationship between the risk of CSX and *H. pylori* infection.

Subgroups	Number of studies[a]	OR(95% CI)	*P* value for I^2	Number of studies[b]	OR(95% CI)	*P* value for I^2
Country						
Iran	6	12.99 (8.61–19.60)	0.59	5	13.84 (8.74–21.92)	0.50
China	3	5.14 (3.09–8.56)	0.23	2	4.05 (2.27–7.25)	0.40
Italy	1	0.93 (0.37–2.33)	NG	0	NG	NG
Age						
25–40 years	1	1.34 (1.04–1.72)	NG	1	1.34 (1.04–1.72)	NG
40–50 years	2	11.27 (4.29–29.61)	NG	2	11.27 (4.29–29.64)	0.25
>50years	8	7.18 (3.59–14.36)	0.25	5	8.94 (4.38–20.58)	0.03
Case type						
Community	6	8.34 (5.69–12.23)	<0.01	5	13.84 (8.74–21.92)	0.50
Hospital	4	6.16 (3.96–9.59)	<0.01	2	4.05 (2.27–7.25)	0.40
Diagnostic methods						
UBT	5	5.46 (1.73–17.26)	0.19	4	8.69 (2.90–26.06)	0.05
anti-HP/IgG	6	8.26 (3.93–17.36)	<0.01	3	12.24 (7.49–20.01)	0.48
Socioeconomic status						
Developed countries	1	0.93 (0.37–2.33)	NG	0	NG	NG
Developing countries	9	9.42 (6.87–12.91)	0.12	7	9.22 (6.49–13.10)	0.06
Publish year						
<2010	4	7,41 (1.20–45.76)	<0.01	3	15.20 (6.19–37.32)	0.26
≥2010	6	8.74 (6.24–12.25)	0.12	4	8.96 (4.71–17.03)	0.05

CSX, Cardiac syndrome X; NG, not given; anti-HP/IgG, anti-Helicobacter pylori/immunoglobulin G; UBT, urea breath test; a, all included articles; b, articles with six points.

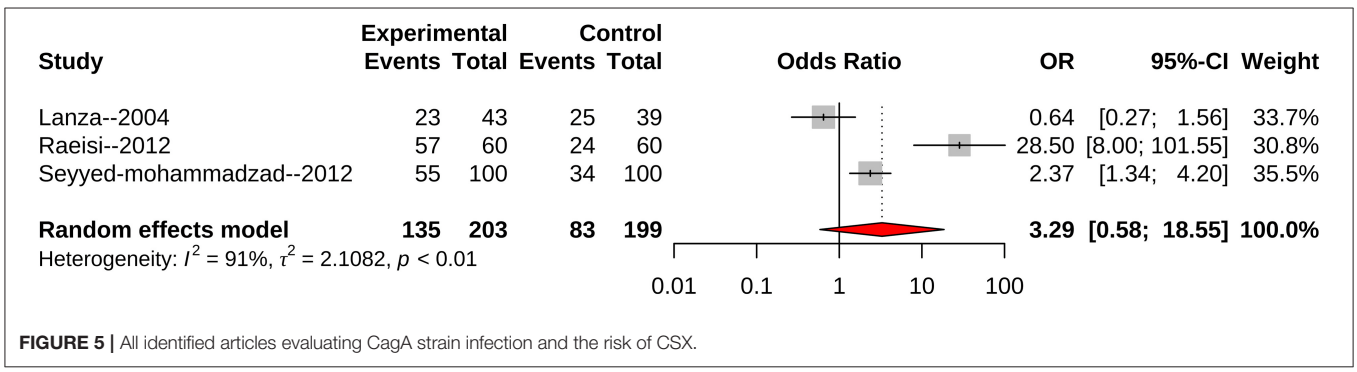

FIGURE 5 | All identified articles evaluating CagA strain infection and the risk of CSX.

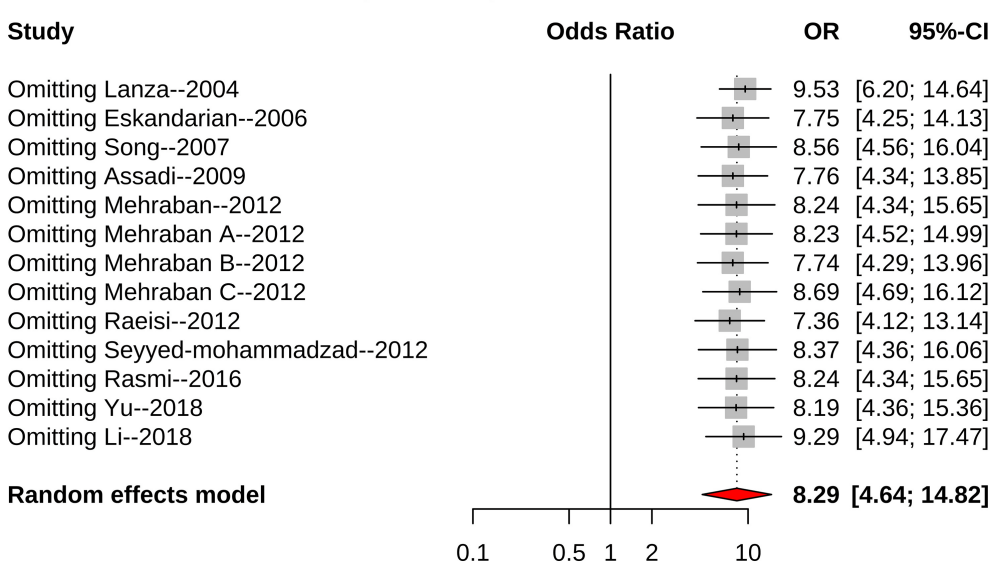

FIGURE 6 | Forest plot for sensitivity analysis.

gender were matched in all articles. A meta-analysis of articles with ≥ four matched variables was performed and the pooled result did not change. Thus, we believe that these confounding factors are evenly distributed in the control group and the case group. Potential confounding factors had no effect on the reliability of the results.

The meta-analysis showed that CagA-positive bacterial infections may not significantly increase the risk of CSX. However, we believe that this conclusion is controversial for two main reasons: first, CagA-positive strains showed high pathogenicity in a number of diseases including atherosclerotic diseases and peptic ulceration (20, 21). Second, the lack of data may be one reason for conflicting results as only three articles were included. As a result, more well-designed studies should be performed.

According to subgroup results, we found that the association between *H. pylori* infection and the risk of CSX was age-dependent, and the incidence rate of CSX was likely greater in middle-aged group than in other groups. This finding is similar to those of previous studies, which have shown that the association between the risk of CSX and *H. pylori* tends to be stronger in middle-aged people (16). We also found some preliminary evidence that the bacteria in developing countries are more pathogenic than in developed countries. Compared with developed countries, the annual recurrence rate of *H. pylori* in developing countries is higher, which may cause CXS to be more pathogenic (22). Additionally, although the *H. pylori*-immunoglobulin G (IgG) antibody test cannot indicate current infection and may overestimate the relationship between the bacteria and the risk of CSX, it is consistent with the results obtained by the urea breath test (UBT) test detects current infection.

After excluding low-scoring articles, several meta-analyses were performed, and differences in these subgroups still existed, supporting the reliability of our results. Previous studies have shown that the incidence rate of *H. pylori* infection was significantly increased with concurrent diseases, such as diabetes and malignant tumors (23, 24). The control group used in our study were all healthy individuals. Therefore, the selection of the control group fully takes into account the differences in *H. pylori* between the groups.

H. pylori infection is able to cause CSX through a variety of mechanisms. Chronic inflammation caused by infection enhances the risk of vascular disease by increasing some acute reactants and inflammatory mediators, leading to endothelial cell damage and blood coagulation (25, 26). Chronic infection caused by *H. pylori* infection, especially CagA-positive strains, can lead to continuously elevated inflammatory metabolites, such as the cytokines Interleukin-1 (IL-1), Interleukin-1 (IL-6), and tumor-necrotic factor (TNF-1), which can affect vascular activity and lead to endothelial dysfunction (5, 27). Also, Chronic *H. pylori* infection can cause a decrease in vitamin B12 and folate absorption, resulting in hyperhomocysteinemia, which promotes the production of intracellular oxygen free radicals and the degradation of nitric oxide, leading to endothelial cell dysfunction (16).

The strength of our study is that it is the first attempt using meta-analysis to identify the association of *H. pylori* infection with the risk of CSX. However, our study also has some limitations. First, due to lack of data, we mainly obtained the age and country information related to CSX individuals. Other factors we could not account for, such as gender and the instrument for measuring *H. pylori*, may also affect the accuracy of our results. Second, our study primarily focuses on three countries, which were divided into developed (Italy) and developing (China, Iran). An expansion of this analysis to other countries is needed to further verify our conclusions. Finally,

the sample size of this meta-analysis is relatively small, which may affect the accuracy of our results. Therefore, additional, larger, well-designed studies should be encouraged to validate our results.

In conclusion, our meta-analysis suggested a possible association between *H. pylori* infection and the risk of CSX. Its pathogenicity is stronger in middle-aged individuals and some developing countries. However, more studies are needed to further investigate whether early eradication of *H. pylori* can reduce the incidence rate of CSX, especially in middle-aged individuals and some developing countries.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

D-HZ, KC, CY, and X-JD designed and analyzed the study. D-HZ, KC, CY, X-JD, and B-BW wrote and revised the manuscript. S-PL, F-HL, Z-XH, and X-LL collected the data. All authors have read and approved the manuscript.

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Microbiota-derived short-chain fatty acids: Implications for cardiovascular and metabolic disease

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Cardiovascular diseases (CVDs) have been on the rise around the globe in the past few decades despite the existing guidelines for prevention and treatment. Short-chain fatty acids (SCFAs) are the main metabolites of certain colonic anaerobic bacterial fermentation in the gastrointestinal tract and have been found to be the key metabolites in the host of CVDs. Accumulating evidence suggest that the end-products of SCFAs (including acetate, propionate, and butyrate) interact with CVDs through maintaining intestinal integrity, anti-inflammation, modulating glucolipid metabolism, blood pressure, and activating gut-brain axis. Recent advances suggest a promising way to prevent and treat CVDs by controlling SCFAs. Hence, this review tends to summarize the functional roles carried out by SCFAs that are reported in CVDs studies. This review also highlights several novel therapeutic interventions for SCFAs to prevent and treat CVDs.

KEYWORDS

short-chain fatty acids, CVD, inflammation, glucolipid metabolism, blood pressure, gut-brain axis

Introduction

In the past 20 years, the incidence of cardiovascular diseases (CVDs) has increased significantly, and brings staggering health and economic burden. Stroke and ischemic heart disease were the main causes of death in China in 2017 (1, 2). Multiple pathological factors influence the initiation and development of CVDs, including atherosclerosis (AS), hypertension, myocardial infarction, heart failure (HF), stroke, and arrhythmia (3–6).

In current years, the gut and its microbiota have been identified as crucial factors in the development of CVDs (7–10). Several bacterial factors, such as the metabolite trimethylamine oxide (TMAO), tryptophan metabolites, and endotoxin, were

demonstrated to affect CVD development (11–13). Changes in gut microbiota (GM) and its constituents, gene abundance, and specific species or flora can affect TMAO. GM disorder can increase TMAO level in the body, thus accelerating the aging of vascular endothelial cells (ECs), affecting the immune system, promoting inflammatory response, and causing the occurrence or aggravation of vascular diseases (14–16). In addition, GM changes also lead to oxidative stress response, sodium metabolism, low density lipoprotein oxidation, and affect the progression of vascular diseases (17, 18).

In short-chain fatty acids (SCFAs), the fermentation products of intestinal microorganisms, especially acetate, propionate, and butyrate were confirmed to reduce intestinal PH value, inhibit pathogenic microorganisms, and maintain intestinal barrier function (19–23). The decease of SCFAs production *in vivo* could lead to the inhibition of the G protein-coupled receptor (GPCR) pathway and the increase of the expression of inflammatory factors, such as INF-γ, leading to lipid metabolism disorder, aggravation of inflammation and vascular remodeling, acceleration of arterial thrombosis, and ultimately the occurrence and aggravation of AS, hypertension, pulmonary arterial hypertension, cerebrovascular diseases, and other diseases (24–27). Due to the development of next-generation sequencing (NGS) technique, metabolomics and bioinformatics analysis, the relationship between SCFAs and CVD has moved from previous associative studies to those that elucidate the cause-effect.

In this review, we gave an overview of gut-derived SCFAs, including acetate, propionate, and butyrate production, transport, and signal transduction and their associations with CVDs. Then, we reviewed the mechanisms of regulating the pathological process, and discussed the role of SCFAs targeted therapy in the progress of CVDs.

The production of short-chain fatty acids and signal transduction

Short-chain fatty acids substrate and biosynthesis

Short-chain fatty acids, major produced by the specific intestinal microbiome in cecum and colon, mainly include acetate, propionate, and butyrate (constitute > 95% of the whole SCFAs) with approximate molar ratio of 3:1:1 in intestinal lumen (28). Indigestible saccharides, such as dietary fibers, none starch polysaccharides (NSP), or resistant starch (RS), that escape digestion in the small bowel are highly anaerobic glycolysis and generate SCFA in the colon (29). Intriguingly, the amino acids from proteolytic are alternative substrate for SCFAs biosynthesis when the routine fibers are in short supply (30). Moreover, to a lesser extent, the minority SCFAs (formate, valerate, and caproate, which make up the remaining < 5%) can be fermented by chain amino acids, such as leucine, valine, and isoleucine (31). The microbiome converting the fermented fibers to major end production is mediated by complex enzymatic pathways. Although more studies are needed to verify the exact commensal microbes producing SCFAs, much information about what kinds of taxa responsible for which metabolites yield is available (**Table 1**).

Short-chain fatty acids absorption

The absorption of SCFAs is efficient and rapid with varying concentration along the whole length of the gut *via* putative mechanism: the monocarboxylate transporter 1 (MCT-1) and the sodium-coupled monocarboxylate transporter 1 (SMCT-1)

TABLE 1 Short-chain fatty acids (SCFA) production, absorption and receptors.

SCFAs	Major metabolic location	Synthetic route	Producers	References
Acetate	Colon, kidneys, sympathetic nervous system, blood vessels, enteroendocrine L cells, the vasculature, immune cells	Acetyl-CoA pathway	Enteric bacteria, e.g., *Akkermansia muciniphila, Bacteroides* spp., *Bifidobacterium* spp., *Prevotella*spp., *Ruminococcus* spp.	Brandsma et al. (148); Battson et al. (149)
		Wood-Ljungdahl pathway	Acetogenic bacteria	
Propionate	Colon, kidneys, sympathetic nervous system, blood vessels, enteroendocrine L cells, the vasculature, immune cells	Succinate pathway	*Bacteroides* spp., *Phascolarctobacterium succinatutens*, *Dialister* spp., *Veillonella* spp., several Firmicutes, and Bacteroidetes	Brandsma et al. (148); Colman and Rubin et al. (150)
		Acrylate pathway	*Megasphaera elsdenii* and *Coprococcus catus*, a few members of the families Veillonellaceae and Lachnospiraceae	
		Propanediol pathway	*Salmonella* spp., *Roseburiainul inivorans, Ruminococcus obeum, Proteobacteria* and members of the Lachnospiraceae family	
Butyrate	Colon, kidneys, sympathetic nervous system and blood vessels, enteroendocrine L cells, the vasculature and immune cells,	Phosphotransbutyrylase/ butyrate kinase route	*Coprococcus comes*, *Coprococcus eutactus*	Donohoe et al. (38); Brandsma et al. (148)
		Butyryl-CoA: acetate CoA-transferase route	*Coprococcus comes*, *Coprococcus catus*, *Coprococcus eutactus*, *Anaerostipes* spp., *Eubacterium hallii*	

receptors (32, 33). The highest SCFAs level is in the cecum and proximal colon at concentrations between 10 and 100 mM as the energy sources for colonic epithelial cells to maintain the intestinal integrity or as a signal molecular (34, 35). Although a majority of SCFAs are metabolized in the colon, a small percentage is absorbed in peripheral blood with the concentration of 19–160 μmoL/L for acetate, 1–13 μmoL/L for propionate, and 1–12 μmoL/L for butyrate (34).

Signal transduction

Besides serving as intestinal fuel (butyrate) or nutrition for colonic mucosa (36, 37), with the development of human diseases, the function of SCFAs also existed in intestinal epithelial cells, immune cells, and adipocytes. Two major mechanisms might be involved. One is as the histone deacetylases inhibitor (HDACi) to connect with the transcriptional machinery. Butyrate is identified as the most potent HDACi activity of the three, followed closely by propionate performing anti-cancer and anti-inflammatory response (38, 39). The other mechanism is coupled with GPCRs, including GPR41 (propionate > butyrate > acetate), GPR43 (propionate = butyrate = acetate), and GPR109A (only respond butyrate). GPCRs are expressed in intestinal epithelial cell, adipocytes, neurons, immune cells, or even vascular endothelium. Although the mechanism has not been fully revealed between SCFAs and associated receptors, increasing studies have highlighted the beneficial effects of SCFAs on CVDs and we will discuss in the next sections.

Short-chain fatty acids in cardiovascular diseases

With the aid of the genomics and other omics tools, science researchers have uncovered the impact of SCFAs on cardiac pathogenesis (**Figure 1**).

Atherosclerosis

Atherosclerosis is a chronic disease of the arterial wall, which is related to myocardial infarction and stroke (40–43).

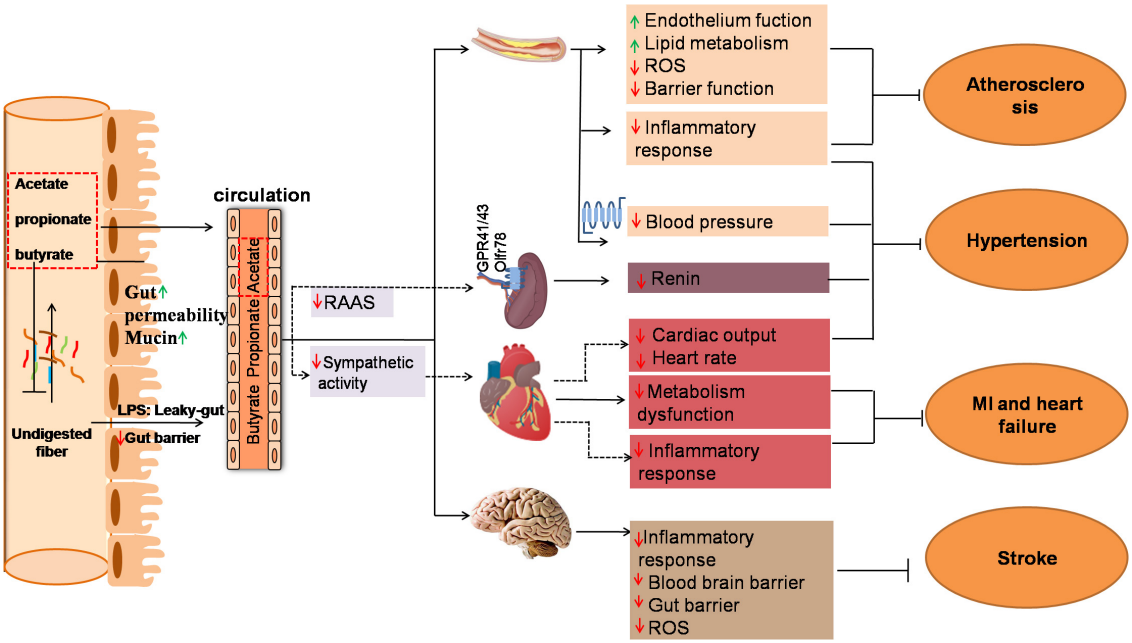

FIGURE 1
Mechanism of gut microbiota produced SCFAs in several key tissues under cardiovascular disease (CVD). Fermentation of undigested fiber in the distal intestine leads to the production of SCFAs. The ration of acetate to propionate to butyrate in the ileum, cecum and colon is ~ 3:1:1. The change of gut microbial diversity, abundance and functions called "dysbiosis" due to overnutrition increase intestinal permeability which allows intestinal luminal endotoxin to translocate to systemic circulation through the "leaky-gut." The elevated endotoxin in vascular, heart and brain lead to "low-grade" inflammation response and ultimately exacerbate CVD such as atherosclerosis, hypertension, myocardial infarction, heart failure and stroke. Propionate and butyrate are generally metabolized in the colon that mainly affect local gut. SCFAs in distal colon could effectively alleviate gut barrier dysfunction by modulating intestinal permeability and secretion of mucus. In addition, small amount of propionate and butyrate and major acetate are absorbed into circulation and modulate the function of coronary artery, kidney, heart, and brain which might effectively improve CVD. Solid lines indicate direct SCFA effects and dashed lines indicate indirect SCFA effects. GPR, G-protein coupled receptor; MI, myocardial infarction; RAAS, Renin-angiotensin-aldosterone System; ROS, Reactive oxygen species.

The underlying pathophysiological mechanisms of AS are lipid deposition, inflammatory response, oxidative stress damage, and endothelial dysfunction (40, 41, 43–45). SCFAs have been proved critical in modulating AS pathological process (**Figure 2**).

Hypolipidemic activity and suppression of foam cell formation

Dyslipidemia level is correlated with the risk of AS and its complications in human populations. In the environment of hyperlipidemia, lipids can be processed into to a mixture of oxidation products and proteins (oxLDL), producing foam cells and stimulating the progression of AS. SCFAs play important roles in lipid metabolism. Studies found that butyrate inhibited the absorption of intestinal cholesterol and promoted the excretion of cholesterol in intestinal cells by regulating the expression of mRNA-associated transporter, which significantly ameliorated AS induced by apoE$^{-/-}$ mice diet (46). It was also found that butyrate pretreatment reduced the atherosclerotic plaque area of mouse aortic arch by 50%, decreased the absorption of oxLDL (oxidized low-density lipoprotein), and reduced the formation and deposition of foam cells in the plaque (47). Other studies have made in-depth research on SCFAs and AS from the perspective of genes. Du uncovered that butyrate lowered the level of several lipogenic genes, such as acyl-CoA thioesterase1(Acot1), Acot2, Perilipin 2 (Plin2) and Plin5 and fatty acid degradation-associated genes including Cyp4a10, Cyp4a14, and Cyp4a (21). Butyrate could induce the transcription of fibroblast growth factor 21 (FGF21) by inhibiting HDAC3 in diet obese mice, thus promoting lipid oxidation, triglyceride clearance, and ketogenesis in the liver (48).

Foam cell formation, which is characterized by accumulation of oxLDL in macrophages, is a sign of early AS, and it binds to scavenger receptors (SR), such as CD 36 and oxLDL (49). ATP binding cassette A1 (ABCA1) can suppress macrophage transformation and contribute to the attenuation of AS (50). Previous studies have demonstrated that butyrate supplementation decreases CD36 expression in peritoneal macrophages from ApoE$^{-/-}$ mice stimulated by oxLDL (51). It has been reported that butyrate accelerates cholesterol efflux by activating the expression of Sp1/ABCA1 pathway, which promotes the reuse of cholesterol in liver macrophages or its final elimination in ApoE$^{-/-}$mice. This is that butyrate regulates cholesterol catabolism by up-regulating biosynthesis of bile acid synthesis rate-limiting enzymes (21).

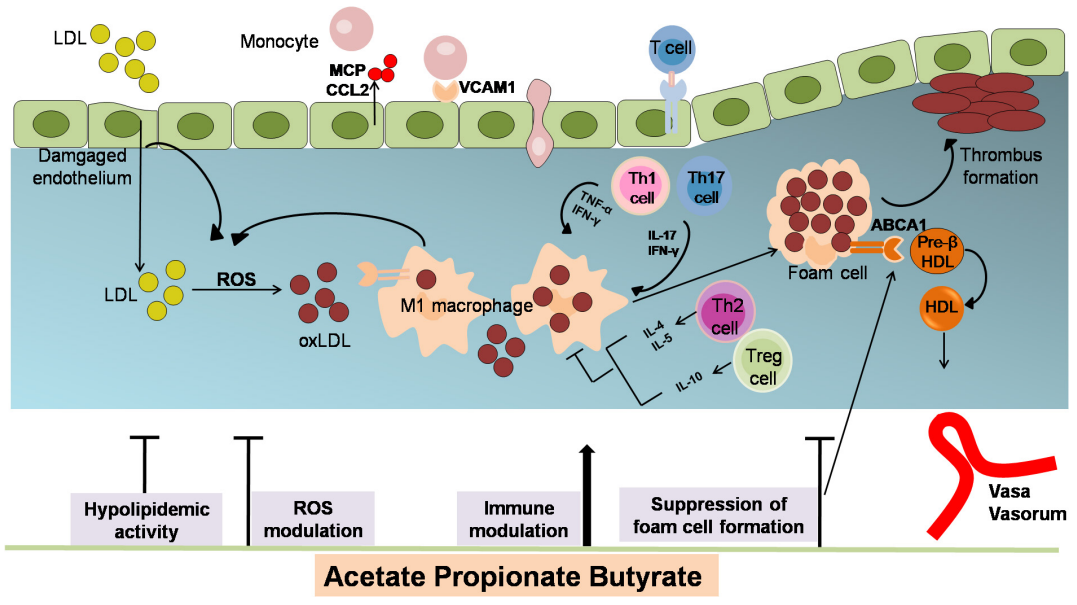

FIGURE 2
The anti-atherosclerotic effects by which SCFAs alleviates the development of AS. Atherogenesis begins with the adhesion of blood leukocytes to the activated endothelial monolayer. Activated endothelial cells express leukocyte adhesion molecules such as VCAMs that capture blood monocytes. The captured monocytes (the most numerous of the leukocytes recruited) matures into macrophages, uptake oxLDL (LDL oxidized by ROS), promote foam cells formation and ultimately yield plaque lesion. Moreover, T cells are observed to differentiate into various subsets of T cells and establish their important modulatory roles. Among them, T helper (TH)−1 and TH17 cells are atherogenic via producing pro-inflammatory cytokines [interferon-γ (IFN-γ)] and activating macrophages, while regulatory T (Treg)cells specific for oxLDL inhibit lesion formation and progression via generating IL-10 and TGF-β.The role of SCFAs in inhibiting atherosclerosis includes attenuating lipid profile and ROS, reducing monocyte adhesion, cholesterol aggregation, macrophage inflammation and foam cells formation. ABCA1, ATP binding cassette A1; CCL2, chemokine (C-C motif) ligand 2; HDL, high density lipoprotein; IFN-γ, interferon-γ; LDL, low density lipoprotein; MCP, chemotaxis protein-1; oxLDL, oxidized low-density lipoprotein; TNFα, tumor necrosis factor; Th, T helper cells; Treg, regulatory T cells; VCAM1, vascular cell adhesion molecule-1.

Short-chain fatty acids modulate immune cell and inflammation

Atherosclerosis is closely associated with chronic vascular inflammation, and the overproduction of inflammatory cytokines and the expression of adhesion molecules are the two important segments in the development of AS. The intensified inflammation of the artery wall can lead to the instability of atherosclerotic plaque and the formation of occlusive thrombus, thus leading to atherosclerotic CVDs events. The beneficial effects of SCFA by modulating the systemic inflammatory response to slow down AS are well established. Inflammatory signaling in vascular endothelium stimulates the biosynthesis of various effector proteins, including endothelial-leukocyte adhesion molecule-1 (*E*-selectin), vascular cell adhesion molecule-1 (VCAM-1), and chemokines, such as IL-8 and chemotaxis protein-1 (MCP-1), which promotes recruitment and retention of circulation monocytes to the injured endothelial monolayer. Therefore, reducing inflammatory transmitters is an important step in preventing the development of AS. Study has reported that SCFA inhibits the production of proinflammatory cytokines through the activation of GPR41 and GPR43, and butyrate promotes preservation of endothelial function *via* attenuating inflammatory factor and thereby exert anti-atherosclerotic action (52). Previous studies have revealed that the intrinsic mechanism of acetic acid on IL-6 and IL-8 is due to signal transduction mediated by GPR 41/43, while the effects of butyrate and propionate on IL-8 production and VACM-1 expression were mediated by HDAC 11. Experiments *in vitro* confirmed that butyrate decreases the production of VCAM1 and chemokine (C-C motif) ligand 2 (CCL2) in ECs upon stimulation of TNFα (47). Similarly, study also uncovered that SCFAs influence LPS- or TNFα-induced endothelial activation by inhibiting the production of IL-6 and IL-8, and reducing the expression of VCAM-1 and subsequent cell adhesion (51). Besides, butyrate has been proved to decrease the release of MCP1/CCL2 in human ECs stimulated by oxLDL and hence decrease the migration monocytes to the lesion area.

After that, monocytes differentiate into macrophages, which aggravated AS by transforming into foam cells and secreting a large number of pro-inflammatory factors. Macrophages express different markers under the induction of different cytokines, and thus differentiate into different subtypes, including M1 and M2 macrophages. Proinflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF-α, are released by M1 macrophages *via* nuclear factor κB (NF-κB) pathway after lipid uptake, and ultimately stimulate foam cells yielding. Conversely, anti-inflammatory (IL-10) cytokines and growth factors (TGF-β) secreted by M2 inhibit the progression of AS (53). Makoto has shown that butyrate and propionate attenuating-α production through blocking NF-κB pathway activation by lipopolysaccharide (LPS) in human peripheral blood mononuclear cells. Butyrate and propionate can rescue ApoA-I transcription in human liver cells under inflammatory conditions by peroxidase-activating receptor (PPAR)-mediated trans-activation inhibition of NF-κB (54). Moreover, butyrate has been shown to increase IL-10 production and decrease TNF-α simulated expression of MCP-1 and VCAM in plaque lesion, and the inner mechanism is also associated with the suppression of NF-κB pathway in ApoE$^{-/-}$ mice (51). Kasahara reported that tributyrin (TB) can inhibit the development of atherosclerotic lesions in germ-free ApoE$^{-/-}$ mice model partly due to the decrement of aortic inflammation indicated by decreasing the relative mRNA levels of TNF-α and VCAM1 in aortic root (55). The study also found that intestinal administration of butyrate reduced endotoxemia and the development of AS and indicated that intervention measures aimed at increasing the representation of butyrate-producing bacteria may provide protection against AS (55). SCFA can also exert its anti-inflammatory effects by interfering with the activation of key signaling proteins, including the MAPK protein ERK (extracellular regulated protein kinase), and can exert its anti-atherosclerotic effects by inhibiting systemic inflammation (56). In terms of the pathological mechanisms of butyrate-modulated inflammation, Kasahara unraveled that butyrate significantly lowered gut permeability, as well as plasma LPS levels. Previous study reported that SCFAs could directly promote T-cell differentiation and produce interleukin-17(IL-17), IFN-γ, and/or IL-10 depending on HDAC inhibitor activity in different cytokine milieu (57). SCFA has different effects on the activation of the endothelial NLRP3 inflammasomes and the associated neointimal formation of the arterial wall. Butyrate may have beneficial effects on vascular inflammation or AS by inhibiting the production of superoxide anions and activation of NLRP3 inflammasomes (52).

Butyrate and propionate have been proved to stimulate the extrathymic generation of Treg cells (58). Unexpectedly, although SCFAs have been found to induce the differentiation of regulatory T cells, butyrate supplementation did not affect levels of CD4^{+}T cells and Treg cells in spleen or para-aortic lymph node samples in AS models (55). Moreover, B2 cells, one of the mature B cells, are TH2-cell-dependent and promote AS by producing specific IgG antibodies against its homologous antigen (such as oxLDL). The anti-oxLDL/oxLDL immune complex is an inflammatory signal that triggers macrophage activation (59). In terms of the effects of SCFAs on the complex, previous evidence has uncovered that butyrate is capable of decreasing contents of IgG anti-oxLDL and attenuating remitting inflammatory response (60). Haghikia revealed a new immune-mediated pathway connecting propionate with intestinal Npc1L1 (Niemann-Pick C1-like 1) expression and cholesterol homeostasis. The findings emphasize the gut immune system as a potential therapeutic target to control dyslipidemia that be a promising strategy for prevention of atherosclerotic CVDs (61).

Inhibition of oxidative stress

Under oxidative stress situation, NO reacts with superoxide to form peroxynitrite anion. The inactivation of NO contributes

to the inflammatory and thereby exerts pro-atherosclerotic action (62, 63). Classical markers of oxidative stress are the superoxide ion (O^{2-}) and nitro tyrosine, whose local and systemic levels are positively correlated with CVDs (64). NADPH oxidase is one of the major enzymes involved in ROS production and has been positively correlated with the progression of AS (65). Aguila demonstrated that the reduction of oxidative stress was related to the lower production by NADPH oxidase (60). *In vitro* studies, butyrate, can reduce the production of reactive oxygen species (ROS). Butyrate pre-treatment of endothelium and peritoneal macrophages yielded a lower oxLDL-stimulated production of superoxide and hydrogen peroxide. Aguilar also demonstrated that oral administration of butyrate positively influences plaque composition by decreasing nitro tyrosine and induces NO synthase (iNOS) formation in lesion site of $ApoE^{-/-}$ mice (60). Previous pieces of evidence have uncovered butyrate treatment that reduces the levels of ROS in vascular smooth muscle cells (VSMCs) by upregulating the level of glutathione-S-transferase (GST) (66). Investigations suggest butyrate is attributed to a blockade of lipid raft redox signaling platforms to produce O2●- upon 7-Ket or CHC stimulations to prevent AS (52). Stamm demonstrated that different oxidants have different vasodilating capacities for the endothelium-dependent vasodilator acetylcholine (ACh) *in vitro* compared to inorganic nitrite. They elucidated oxidants react with NO released by eNOS after acetylcholine stimulation to form the intermediate peroxynitrite, thereby reducing the potency of this endothelium-dependent vasodilator (67).

Taken together, SCFAs are crucial in anti-inflammation, anti-oxidation, and lipid modulation in the progression of AS. SCFAs have been developed as a beneficial microbial product with favorable anti-atherosclerotic effects and are potential therapeutic target for the treatment of AS.

Hypertension

Systemic arterial hypertension is an independent risk factor for CVD (68, 69). Accumulating clinical cohorts have proved SCFAs as an important factor to regulate blood pressure. One cohort included 54 males (38 hypertensive, 7 borderlines, and 9 normotensive) claiming that the stool levels of SCFAs in hypertensive are higher than normotensive individuals based on 24-h ambulatory BP measurements (70). On the contrary, other cohorts have uncovered that the deficient SCFAs production due to long-term low fiber westernized diets increase the prevalence of hypertension. Supporting these results, a cross-sectional study consisted by 29 non-treated hypertensive and 32 normotensive subjects presented a positive correlation between the feces level of SCFAs and systolic and diastolic blood pressure (71). Similarly, in another SPRING study (the Study of Probiotics in Gestational Diabetes) constituted by 205 obese pregnant women at 16 weeks showed that systolic and diastolic blood pressure was associated with altered GM composition and butyrate production. Therefore, they concluded that increasing butyrate producing capacity may be a new perspective for the maintenance of normal blood pressure in obese pregnant women (22). Bartholomeus has suggested that lifestyle modifications leading to augmented SCFAs production could be beneficial for hypertensive CVDs patients (20).

Modulation the classic blood pressure regulatory modes

Chronic activation of the sympathetic nervous system (SNS) and rennin-angiotensin-aldosterone system (RAAS) contributes to hypertension by excessive production of catecholamines, such as noradrenaline and adrenaline, angiotensin II, and aldosterone, which stimulate adrenoceptors, angiotensin II receptor type 1 (AT1), and mineralocorticoid receptors to increase vascular tone, renal sodium and water reabsorption and heart rate. In an angiotensin II (Ang II)-induced rat hypertension model, intramedullary infusion of sodium butyrate lowered mean arterial pressure by suppressing renal receptor (PRR)-mediated intrarenal rennin-angiotensin system indicated by the renal expression of PRR, angiotensinogen, angiotensin I-converting enzyme and rennin (72). In a rodent study, propionate has been demonstrated to modulate rennin release combined with Olfr78 receptor which located in the renal juxtaglomerular apparatus to mediate hypertensive response (73). Similarly, acetate supplementation in hypertension mice downregulated the rennin angiotensin system in the kidney and attenuated the blood pressure. Moreover, high salt consumption aggravates the kidney burden which has consistently been implicated in hypertension. Across over, trial has found that modest sodium reduction increases circulating SCFAs in untreated hypertensives (74). Furthermore, SNS receives signals from brain regions, such as forebrain, hypothalamus, and brainstem, and has projections to all major blood pressure-regulating organs, including the heart, blood vessels, and kidneys. Study has uncovered that propionate may modulate the SNS activity at the level of sympathetic ganglion *via* GPR41 and decrease the heart rate, which is closely related with the cardiac output and blood pressure (75). Researchers found that the hypotensive effect of butyrate may result from decreasing sympathetic activity *via* colon vagus nerve signaling, which depended on the afferent colonic vagus nerve signaling independent of SCFA receptor (76).

Inflammatory response modulation

Elevated levels of circulating cytokines and C-reactive protein (CRP) marked hypertension as a low-grade inflammatory disease involving innate and adaptive immune responses (77, 78). The tissue injury resulted from non-immune mechanisms of hypertension leads to DAMP formation, such as ROS, LPS, and high mobility group box 1 (HMGB1). Within

the innate immune systems, macrophages and DCs detect DAMPs and thus produce pro-inflammatory cytokines and chemokines accumulating in vasculature and kidney. Within the adaptive immune system, effector T cells and B cells in the help of DCs can directly influence renal tubular sodium transport and vascular resistance through pro-inflammatory cytokines releasing. All of these promote blood pressure elevation, while the immunoregulatory pathways involving Treg cells have salubrious effects on BP by producing IL-10. In a study of 441 community-dwelling adults, Cuesta-Zuluaga claimed that individuals with higher butyrate excretion have fewer lipopolysaccharide-binding protein (LBP) level and is associated with hypertension (79). In addition, Ganesh have uncovered that acetate supplementation could prevent OSA-induced gut inflammation and hypertension in obstructive sleep apnea (OSA)-induced rat hypertension model (80). Similarly, in terms of acetate against hypertension, another DOCA-salt hypertensive mice model study has shown that fiber consumption with high acetate concentration lowered IL-1 signaling, an early response pro-inflammatory cytokine originated from macrophages. They also found that acetate downregulated the gene for early growth response protein 1 (Egr 1) mRNA involved in cardiac hypertrophy, cardiorenal fibrosis, and inflammation according to cardiac and renal transcriptome analysis (19). Moreover, propionate has been shown to mitigate systemic inflammation responses quantified as a decrement of splenic effector memory T cell, Th17 cells, and increment of Treg cells in AngII-induced hypertensive model. Bartolomaeus uncovered that the beneficial effects of propionate in AngII-infused hypertensive mice are Treg-dependent as this kind of effect was abrogated in Treg-depleted Ang II-infused mice (20).

G protein coupled receptor regulation

Emerging evidence implicate that SCFAs are associated with reduced blood pressure and less incidence of cardiovascular mortality (66–68). Mortensenet firstly uncovered that SCFAs can dilate isolated human colonic resistance arteries in 1990 (81). SCFA played important parts in rising blood pressure by Olfr (73). Oflr78 and its human ortholog (OR51E2) as a novel SCFA receptor (specifically acetate and propionate) localize in the afferent arteriole part of the juxtaglomerular apparatus (JGA) where they mediate the rennin secretion. The mainly described function of Olfr78 is related to glomerular filtration and blood pressure regulation effect (73, 82). Propionate could combine with Oflr78, elevate cytosolic cAMP, promote renin releasing and exert a chronic hypertensive effect when it entered the circulation *via* colonic absorption (81, 83, 84). Conversely, the effect of propionate on renin release was absent in Olfr78-/- mice65. Pluznick found that propionate administration caused a large, rapid drop in blood pressure in Olfr78-deficient mice, while this hypotensive also manifestation in wide-type mice indicating antagonized effect on Olfr78 exists on BP. This team ultimately has revealed that GPR41co-expression with Olfr78 in the smooth muscle cells of resistance vessels has a hypotensive response on vascular (73). GPR41 appears to induce vasorelaxation with the help of vascular endothelium (85). Consistent with this, oral administration of propionate took a hypertensive effect in Gpr41 null mice (86). GPR41 and Olfr78 likely played opposing roles in the regulation of blood pressure. An antibiotic treatment experiment on wide type and Ofr78-deficient mice highlighted the dual roles of SCFA-mediating hypotensive effects combined with Gpr41 and hypertensive effects antagonized by Oflr78, pressure was significantly increased in Oflr78-deficient mice the mean blood. Altogether, these kinds of opposing responses may produce a "buffering" effect to defense the wide swings in blood pressure due to the normal, physiological variations of SCFAs.

Myocardial infarction

Myocardial infarction (MI) will occur when coronary artery stenosis reaches more than 80%, downstream myocardial cells are ischemic, and oxygen demand is increased under high workload (87, 88). SCFAs were associated with the levels of local and system inflammation, oxidative stress, apoptosis, and metabolism regulation in MI pathogenesis.

Modulation of inflammatory response

Inflammatory processes play an important role in MI (89, 90). SCFAs have been reported to participate in the recruitment, activation, and polarization of leukocyte after MI and this may open a new avenue to improve forms of immunotherapy for MI patients (**Figure 3**). Although SCFAs have been demonstrated to influence neutrophil recruitment, inflammatory mediators, effector functions, and apoptosis related to the immune response (91–94), rarely few studies pointed out the mutual influence of SCFAs and neutrophil variation in the development of myocardial infarction.

Short-chain fatty acids have been demonstrated to drive myelopoiesis in the bone marrow and tissue-resident monocyte infiltration. Dietary supplementation with propionate can modulate immune system and subsequent cardiac repair *via* restoring the level of myeloid cells and CX3CR1 + monocytes infiltration to the peri-infarct zone in antibiotic-treated MI mice (95). They also demonstrate that an SCFA-producing probiotic mixture is associated with both increased levels of myeloid cells in the hearts of antibiotic-treated MI mice and enhancement of propionate levels. *In vitro* studies, SCFAs exert anti-inflammatory response by decreasing the production of cytokines, such as IL-10 and prostaglandin E2 (PGE2) in human monocytes and peripheral blood mononuclear cells (PBMC) (96, 97). In some *in vitro* studies, macrophage incubated with SCFAs decreased the LPS-induced TNF-α, IL-1β, IL-6 secretion, and the inner mechanisms are associated with the NF-κB and

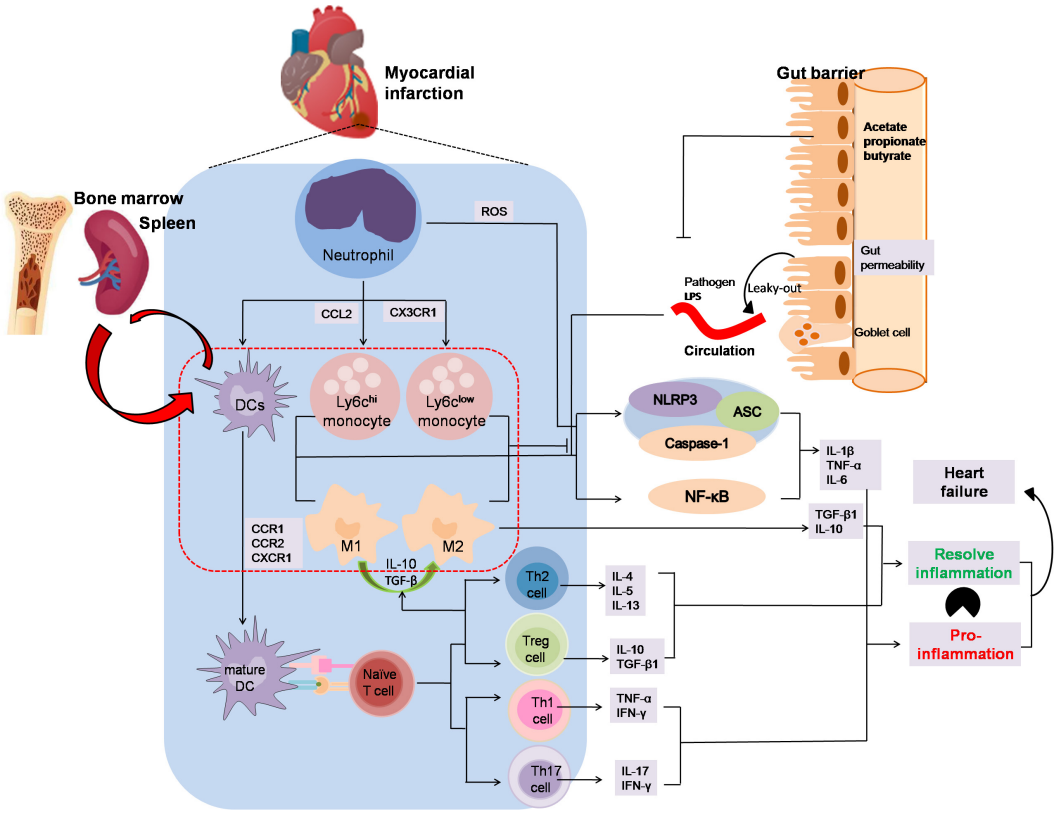

FIGURE 3
The anti-inflammation effects by which SCFAs alleviates the development of myocardial infarction. Neutrophils are the first immune cells to infiltrate infracted myocardium and then activated by adhesion and chemokines released from injured endothelial and ROS from injured endothelium after MI. Ly6c^{hi} monocytes captured by CCL2 are recruited early to engulf necrotic debris and apoptotic myocardial cells mediated by NF-κB or the NLRP3 pathway in the first phase, and anti-inflammatory Ly6c^{low} monocytes dependent on CX3CR1 occur later to participate in myocardial repair by releasing anti-inflammatory factor. DCs activated by CCR1, CCR2 or CXCR1 present antigen to naïve T cell. Th1 and Th17, two of the CD4$^+$ subsets, enhance IFN-γ, TNFα and IL-17 secretion, whereas Th2 and regulatory T cells reacting to activate IL-10 or TGF-βto increase M2 macrophages polarization and dampen inflammation. In addition, the leak-gut effect of dysbiosis may exacerbate the pro-inflammation effect on MI. The imbalance between inflammatory phagocytosis and anti-inflammatory response would aggravate MI. In MI progression, SCFAs might promote M2 macrophages polarization to suppress inflammatory responses and inhibit macrophages cells secreting the pro-inflammatory factor and avoid aggravation of viable areas of the myocardium. Moreover, SCFAs play an important role in T cells polarization and activation, especially Treg cells which could alleviate the inflammation injury in MI progression. ASC, apoptosis-associated speck-like protein containing CARD; CCR, chemokine (C-C motif) receptor; CXCR3, chemokine C-X3-C-Motif Receptor 1; DC, dendritic cell; NLRP3, nucleotide-binding domain leucine-rich repeat proteins 3.

the NLRP3 pathway (98). An intestinal macrophage study demonstrated that the anti-inflammatory effect of butyrate was mediated by HDAC inhibition (99). Jiang demonstrated that butyrate administration down-regulated the expression of inflammatory cytokines (TNF-α, IL-1β), up-regulated the IL-10 levels in the infarct border zone, and ameliorated cardiac function probably through promoting M2 macrophages polarization to suppress inflammatory responses at 3 days post-MI (100). The mechanisms that orchestrate such divergent functions remain unknown. However, a timely resolution of inflammation by manipulating the M2/M1 ratio might be a strategy to prevent infarct expands and left ventricular dilates. Taken together, SCFAs could not only promote monocytes into the injury site to remove the necrosis tissue in the first phase of MI, but also could inhibit macrophages cells secreting the pro-inflammatory factor and avoid aggravation of viable areas of the myocardium.

There may be a link between MI and adaptive immune cells, CD4$^+$T cells deficiency and CD8$^+$T cells are associated with worse outcome in MI patients. SCFAs play an important role in T cells polarization and activation, especially Treg cells. Arpaia found that butyrate facilitated extrathymic generation of Treg cell sin mice (58). In addition to butyrate, *de novo* Treg-cell generation in the periphery was potentiated by propionate (57). Bartholomeus established a cause-effect relationship between anti-inflammatory effects of regulatory T cell and cardioprotective effects of propionate (20). They have found that propionate administration decreased spleen effector T cells, Th17 cells, and increased Treg cells which ultimately protected cardiac damage and remodeling. This cardioprotective effects

of propionate were abrogated in regulatory T cell-depleted mice (15). In conclusion, SCFAs play vital roles in myocardial infarction *via* immune regulation. Nevertheless, most of the literatures linking SCFAs and immune response in CVD are studied in cells or rodent model, additional work is needed to understand which patient population would benefit from SCFAs production.

Metabolism regulation modulation

Deeper insight acquired that SCFAs also impact metabolism, which underlie susceptibility to CVD (101). Only few studies stated its direct effect on myocardial metabolic disorders, most of them are dabbling in maintaining the lipid and glucose homeostasis of obesity and T2DM subjected to metabolic disease. Butyrate, as the most important fuel for the intestine epithelium, could act as an energy source in AMI therapy (102). Cheng found that sodium butyrate injected into ischemic zones in AMI model rat could promote mobilization of cellular energy store and angiogenesis, inhibit ROS generation, and contribute to cardiomyocyte protection by binding to the Sirt3 with the function of NADP + cycle located in the mitochondria (103). Based on these findings, butyrate may be a new therapeutic agent following AMI as one type of nutrient. Zhang has demonstrated that sodium butyrate protected against HFD-induced cardiac ventricular dysfunction and metabolic disorders in T2DM model compared with the HFD-fed mice. They found that butyrate could attenuate metabolic dysfunction by activating p38/PRAK pathway which has been demonstrated to promote the GLP-1 receptor-induced protective effect (63). In another study, propionate-treated modified cardio-protection through improving mitochondrial anomalies which played a central role in energy metabolism control coupled with GPR41 (104). Further study is warranted to elucidate the directly causal effect of metabolism and function between SCFAs and heart both in animal studies and human trial.

Heart failure

Atherosclerosis, hypertension, and other comorbidities such as obesity and T2DM typically impede infarct healing, cause hypertrophy, fibrosis, and subsequently lead to HF. Experiments *in vivo* confirmed that sodium butyrate forcefully attenuates Ang II-induced rat cardiac hypertrophy as indicated by decreased ratio of heart weight/body weight and cardiomyocyte size, attenuated extensive fibrosis, and inflammation, and the inner mechanism is due to repressing the activation of COX2/PGE2 pathway in an HDAC-dependent manner (105). Similarly, Umadevi showed that butyrate reduces natriuretic peptide receptor-A (NPRA) gene (Npr1) copies-elicited cardiac hypertrophic, interstitial fibrosis, and inflammation by inhibiting HDAC1 and HDAC2 (106). Furthermore, elevated cardiac fibrosis and left ventricular hypertrophy are normalized in the presence of acetate supplementation, which is attributed to downregulate the mitogen-activated protein kinases (MAPK) signaling in the heart (19).

Several studies implied that imbalanced composition and function of gut microbiome, known as dysbiosis, increases the risk of incident adverse cardiovascular events, including HF (107–109). The current "gut hypothesis of HF" firstly proposed by Tang implied that decreased cardiac output and adaptive redistribution of systemic circulation led to intestinal hypoperfusion, intestinal villi ischemia, bowel wall edema, and impaired barrier function. This disruption in intestinal barrier function in turn leads to the increment of gut permeability and circulating endotoxins (LPS), augment inflammatory-related respond and escalated HF (110–112). It is worth mentioning that SCFAs play an important role in maintaining intestinal barrier and regulating immune response in HF progression. Clinical trials have shown that patients with HF have a thickened intestinal wall, colon, suggesting intestinal edema, and increased collagen accumulation in mucosal of the small intestine. A441 community-dwelling adult study found that fecal SCFAs was negatively correlated with the serum lipopolysaccharide-binding protein (LBP) concentrations, a biomarker produced to response to LPS microbial translocation and a marker of gut permeability (79). Moreover, butyrate has been reported to increase mucus production and tight-junction protein expression, such as zonulin and occluding, contributing to the decrement of intestinal permeability in a GPR43-dependent manner (113). New mechanistic insight surrounding the impact of SCFAs on gut barrier integrity and cardiomyocyte function is provided *via* inhibiting NLRP3 inflammasome and autophagy (114). Further study is warranted to elucidate the direct effect of SCFAs on gut barrier and cardiac function in HF.

Stroke

Similar to myocardial infarction, cerebral ischemic stroke is caused by focal occlusion or arterial stenosis, as well which leads to the interruption of cerebral blood supply and consequently brain dysfunction (115). Recently, the "brain-gut axis," a bidirectional communication system between the brain and the gut and its microbiota has been demonstrated as a hot area in stroke. Increasing evidence demonstrated that the SCFAs appear to be the most likely missing link along the gut-brain axis and might be able to modulate stroke and post-stroke recovery. Yamashiro found a 13% decrease in total organic acids with SCFAs accompanied by increased abundance of several genera and species correlated with the inflammation independent of age, T2DM, and hypertension in patient with stoke (116). Another study aimed to explore the association between post-stroke cognitive impairment (PSCI) implied that SCFAs could predict 3 months or longer PSCI early and accurately after stroke onset (117). Consistently, SCFAs supplementation is found to be effective treatments for stroke by controlling barrier structure, metabolism, inflammation, and GM dysbiosis.

Interfere with the gut microbiota

Cerebral ischemia causes GM dysbiosis, which may initiate a cascade of events, including increasing intestinal permeability which allows the translocation of GM to systemic circulation through the "leaky-out" that deteriorates the outcome of stroke. The GM itself or metabolites directly or indirectly mediate neural communication and maturation. Remodeling the GM may offer an effective treatment for stroke. In a middle cerebral artery occlusion (MCAO) stroke model, the dysfunction of GM and decreased acetate, propionate, and butyrate occurred in ischemic stroke. Supplementation with butyrate significantly increased the α-diversity of the GM in cerebral ischemic stroke and effectively relieved stroke (118). In addition, butyrate treatment could alter the gut microbial composition of rats with cerebral ischemic stroke enriched the populations of more beneficial bacteria, such as lactobacillus, butyric coccus, and megamonas (118).

Inflammatory response attenuation

Stroke alters the GM composition, and in turn, microbiota dysbiosis has a substantial impact on stroke outcome by modulating the immune response. Microglial cells are the brain's resident immune cells against a variety of external and internal insults of neurodegenerative diseases, stroke, and traumatic brain injuries. SCFAs have been studied to modulate the post-stroke neuronal plasticity mediated by circulating lymphocytes on microglial activation (119). Brain transcriptomic analysis indicated that microglia was the main cellular target of SCFAs on the effect of synaptic, and the inner mechanism is associated with NF-κB pathway (119). Sodium butyrate has a strong anti-inflammatory against LPS-induced responses as indicated by the decreasing levels of TNF-α, NOS2, Stat1, and IL-6 both in rat primary microglia and MCAO model (119). Sodium butyrate decreased the number of monocytes/macrophages *via* inhibiting HDAC activity in permanent middle cerebral artery occlusion (pMCAO) model (120). Taken together, SCFAs may primarily affect lymphocytes already in the peripheral immune compartments which then secondarily mediate microglial changes in the cerebral immune milieu after brain invasion.

Reactive oxygen species and apoptosis

Activated microglial and invading leukocytes exert a cytotoxic function by releasing ROS and apoptotic protein which lead to brain infarction and excitotoxicity. COX-2 catalyzes the production of proteinoid and free radicals. NO produced by iNOS after focal cerebral ischemia regulates the activity of COX-2 (121). Sodium butyrate has been elucidated to inhibit NO and COX-2 expression to attenuate the ischemic injury. Furthermore, HSP70, a critical effect against apoptotic and cell death, and Bcl-2, a typical anti-apoptotic protein, were super induction in ischemic brain by post-insult sodium butyrate due to HDAC inhibition in pMCAO model (120). Butyrate-produced bacteria was demonstrated to improve neurological deficit scores by decreasing the expression of caspase-3, Bax and increasing the ratio of Bcl-2/Bax. In addition, clostridium butyricum pretreatment attenuates cerebral ischemia/reperfusion injury in mice *via* anti-oxidations indicated by the decrement of MDA and the increment of SOD (122).

Blood-brain barrier

After stroke, the integrity of the blood-brain barrier (BBB) is an important consideration for brain protection. Several tight junction proteins between ECs, the major component of BBB, prevent the paracellular diffusion of various molecules in the blood to the brain and its extracellular fluid. Matrix metalloproteinases (MMPs) mediate BBB disruption and vasogenic edema after cerebral ischemia by degrading the extracellular matrix, basal lamina proteins, and tight junctions around the BBB (123). Sodium butyrate can attenuate BBB disruption *via* decreasing BBB permeability and MMP9 activity in the dependent of HDAC activity in transient focal ischemia model (123). In another study, germ-free adult mice non-colonized with clostridium tyrobutyricum (produces mainly butyrate) or bacteroides the taiotaomicron (produces mainly acetate and propionate) enhanced BBB integrity as indicated by the increment of the tight junction proteins occluding and claudin-5. In addition, gavage of sodium butyrate in germ-free adult mice gains the same effect.

Thus, microbiota-produced SCFAs treatments are a promising strategy for stroke, while further studies are needed to illuminate the cause-effect between SCFAs on BBB and stroke.

Arrhythmia

Cardiac arrhythmias are the abnormalities or perturbations in the normal activation or beating of heart myocardium. Few researches have studied the SCFAs on the cardiac arrhythmias. One study showed that butyrate could improve ventricular arrhythmia (VA) following MI *via* shorten P wave duration and QJ intervals. Particularly, butyrate could inhibit sympathetic neural remodeling *via* decreasing the density of nerve fibers for growth-associated protein-43 (GAP-43) and tyrosine hydroxylase (100). Study also demonstrated that propionate reduced susceptibility to VAs in hypertensive rat models. Besides, propionate improved the electric remodeling confirmed by attenuated cardiac gap junction remodeling and lateralization of connexin 43 in cardiomyocytes. The effect of SCFAs on cardiac arrhythmias should be further studied in animal and human studies (20).

Therapeutic intervention

Cardiovascular disease is accompanied by the existence of many risk factors, such as obesity, T2DM, hypertension

interacted with multi-organs, and tissues showing lipid and glucose metabolism disorders, oxidative stress, systemic inflammation, and other performance. Although conventional treatment of CVDs has played a role in clinical practice, many links between the SCFAs and susceptibility for CVD have placed SCFAs as a novel target for therapeutic in the future. Several approaches to manipulate the SCFAs hold promise, including diet regulation, fecal microbiota transplantation, prebiotics, probiotics, and traditional Chinese medicine (TCM).

Dietary interventions

Dietary modulation of nutritional interventions is an effective strategy for CVD prevention and therapy. The World Health Organization stated that the daily consumption of grains, as well as 400 g per day of fresh fruits and vegetables, is recommended in daily intake of fiber. A meta-analysis consisted by 22 cohort studies reveal that dietary rich in fiber is inversely associated with risk of CVD (124), and higher intake fiber may contribute to lower bold pressure in patients with hypertension (125). Mice fed with high fiber diet or supplemented with acetate present a higher level of acetate-producing bacteria, which prevent the development of hypertension and HF (19). In addition, Mediterranean diet characterized by large quantities of fruit, vegetables, cereals, legumes, olive oil, moderate quantities of fish, poultry, and dairy products and low quantities of red meat and wine contribute to high SCFAs levels or SCFAs-producing bacteria, which can consequently modulate CVD pathogenesis. A systematic review examined dietary patterns, such as dietary approaches to prevent hypertension and other diets that delay HF progression. The authors analyzed that the adoption of Mediterranean or the dietary approaches to stop hypertension (DASH-type) diet patterns showed a protective effect on the incidence of HF and/or worsen the cardiac function parameters (126). Similarly, a randomized intervention trial concluded that men and women who consumed a Mediterranean diet for 6 months could improve vascular endothelial function and blood pressure (126). One study specifically demonstrated that habitually following high-level adherence to a Mediterranean diet could significantly increase the levels of fecal SCFAs (127). Another study found that the subject's adherence to the Mediterranean owned a positive correlation with intestinal total SCFAs. Moreover, an increase of 10% of SCFAs accompanied with decreasing inflammatory cytokines, such as VEGF, MCP-1, IL-17, IP-10, and IL-12, after a 3-month low-calorie Mediterranean diet on people had a low-to-moderate cardiovascular risk profile (128). Taken together, consumption of a high fiber diet would be a promising intervention to reduce CVDs risk, and one of the inner mechanisms may be associated with the level of SCFAs.

Traditional Chinese medicine

The TCM has multiple components and targets for the treatment of CVDs, which has a synergistic therapeutic efficacy. In recent years, many studies proved that the active ingredients of Chinese medicine as a therapeutic intervention to treat CVD through interacting with microbial metabolites have been reported.

Studies showed that TCM has gradually become important role in the progression of metabolic syndrome by regulating the intestinal flora SCFAs level. Studies found a variety of TCM polysaccharides that could regulate the production of SCFAs to maintain blood glucose homeostasis in diabetic model. Nie found that the polysaccharide purified and isolated from *Plantago asiatica* L. (PLP) could significantly increase the concentration of SCFAs and decrease the glycemia, lipid profile in high-fat diet-induced T2DM rats, which speculate that the anti-diabetic effect of PLP in T2DM rats may be related with the augmented levels of SCFA (129). Hydroxysafflor yellow A (HYSA) promoted the SCFAs-produced bacteria and increased the production of acetate, propionate, and butyrate, which improve insulin resistance and glucose tolerance (130). Study found that concentration of propionate is negatively correlated with hyperglycemia on high-fat diet-induced T2DM mice with high-dose of total saponins intervention (131). Nie also found that the increasing SCFAs level by PLP was accompanied with decreasing plasma lipid markers, such as total cholesterol and triglycerides, because of propionate inhibiting cholesterol synthesis in rat hepatocyte. Other studies showed that ethanol extract of Ganoderma lucidum and Xiexin Decoction have the potential to reduce serum TG, TC, and LDL-C levels (132–134).

Zhou found that the high-salt diet-induced ejection fraction-preserving rat HF (HFpEF) model treated with Xiao-Qing-Long Tang (XQLT) had a lower Firmicutes/Bacteroidetes ratio, higher acetate, propionate and butyrate concentration compared with the HF group (135). They speculated that the prevention of the HFpEF development by XQLT may be associated with decreased inflammatory cytokines *via* SCFAs (135). In addition, other studies found that simulated gastric juice, simulated intestinal fluid, and human fecal flora incubated with S-3-1 extracted from Sijunzi decoction could regulate the inflammatory-related flora and increase the content of acetate and total SCFAs (136). Other Chinese medicines, such as berberine and Kyolic aged garlic extract, have been proved to inhibit the expression of pro-inflammatory genes, such as TNF-α, IL-1β, IL-6, inducible nitric oxide synthase, and cyclooxygenase-2 (137, 138).

Traditional Chinese medicine has important role in intestinal barrier integrity. Baicalin is commonly used to treat inflammatory bowel diseases (IBD) and hypertension. Wu found the abundance of SCFAs-producing bacteria increased after baicalin interfered with hypertensive rats, the concentration of intestinal metabolites SCFAs increased and

the concentration of butyrate was positively correlated with the expression of tight junction protein (139). It is speculated that baicalin can maintain intestinal integrity and reduce systemic inflammation by up-regulating the production of SCFAs. Garlic extract has probiotic properties which increase the richness and diversity of intestinal microorganisms by increasing the production of intestinal mucus, especially stimulating the growth of lactobacillus and clostridium to reduce blood pressure (139).

Taken together, the TCM could function as metabolic, inflammation, and gut barrier regulator which may hold promise for the prevention or treatment of CVD. We need to ask rigorous mechanism between the TCM, SCFAs, and the CVD.

Probiotics and prebiotics

Probiotics are defined as live beneficial bacteria which are administered to re-establish an appropriate intestinal balance. Another strategy for modulating intestinal microbiota is the use of prebiotics, which are non-microbial entities provided to elicit a favorable impact on microbial community composition and function. Probiotics and prebiotics may potentially act through different mechanisms, including gut permeability, lipid metabolism, and blood pressure with pathogens.

Experiments uncovered probiotics and prebiotic may be potential therapeutic intervention for cardiovascular disorder by modulating the SCFAs level. In an animal study, RS deficiency contributed to a series of pathological alterations in C57BL/6J mice, including reducing acetate-producing microbiome and up-regulating blood pressure (140). In a human clinical trial, stable coronary artery disease patients supplemented with prebiotic was reported to increase plasma propionate level, improve endothelium-dependent vasodilation and systemic inflammation (141). Moreover, daily body restore (DBR), a mixture of nine probiotics organisms of the genera *Lactobacillus* and *Bifidobacterium* and 10 digestive enzymes, was supplemented in mice model of hypercholesterolemia, the level of propionate increased and transverse colon and reversed the lipid profile associated with AS (142). In another study, administration of 14 probiotics in db/db mice generated anti-diabetic effect, which was associated with the increment of propionate, butyrate, and SCFA-producing microbial community levels. Probiotics administration also effectively improves the function of intestinal barrier, insulin resistance. All these results indicate that probiotics might be an effective way to prevent diabetes progression (143). It was found that not all humans respond to dietary changes in a similar manner, and non-responsiveness to either a fiber-rich or weight loss diet was shown to correlate with pre-intervention increased bacterial diversity.

In conclusion, these studies elucidated that individualized treatment programs based on microbiome and SCFAs may provide novel treatment strategies for CVD. Further research to explore the potential mechanism and adverse effects of probiotics and prebiotics in treating CVD is warranted.

Fecal microbiota transplantation

Gut microbial modulation by fecal microbiota transplantation (FMT) is a possible therapeutic intervention designed to displace intestinal pathogens by introducing fecal contents from healthy subjects into the gastrointestinal tract of patients. Some of FMT alter the gut microbiome composition thus increasing the production of certain SCFAs. In an animal study, the Dahl salt-sensitive (S) mice transplanted with cecal contents of Dahl salt-resistant (R) mice had significantly higher systolic blood pressure and mean blood pressure than those of S mice transplanted with autologous (144). In another study, $Ldlr^{-/-}$mice were transplanted with fecal intestinal flora of $Caspase1^{-/-}$ ($Casp1^{-/-}$) mice, and $Ldlr^{-/-}$ mice transplanted with autologous fecal flora of $Ldlr^{-/-}$ mice were served as control group. After 13 weeks of high-fat cholesterol-rich feeding, $Ldlr^{-/-}$ ($Casp1^{-/-}$) mice showed larger atherosclerotic lesion size in the aortic root, higher level of inflammation, and lower cecal concentrations of propionate, acetate, and butyrate compared with $Ldlr^{-/-}$ ($Ldlr^{-/-}$) mice (145). Similarly, in a human clinical study, 18 obese patients with metabolic syndrome infused with a microbiome solution from lean healthy males in small intestine showed a 2.5-fold increase in the number of butyrate production intestinal bacteria in their stool, and an increase of insulin sensitivity compared to the group infused with an autologous gut microbiome solution (146). Microbiota transplantation may play crucial roles in modulating the composition of intestinal flora, regulating blood pressure, increasing insulin sensitivity, reducing inflammation, and arteriosclerosis. A meta-analysis revealed that fecal microbial transplantation is safe as a therapeutic intervention of inflammatory bowel diseases. Whether adverse reactions will occur after MT may be related to the disease being treated and the patient's physical condition (147).

Generally, the application of microbiota transplantation requires caution and more deeper studies are needed on dosing, delivery route, and formulation for intestinal flora transplantation.

Conclusion

Multiple animal and human clinical studies have suggested an important link between intestinal microbial metabolism SCFAs and CVDs. Several pivotal mechanisms might be responsible for the putative positive effects of SCFA on CVDs pathogenesis.

The regulation of intestinal barrier to prevent the pathogens or bacterial endotoxins into the system and modulate the inflammatory response on the immune and periapical tissue by SCFAs takes important roles in the myocardial infarction or HF progression, the plaques formation in AS, blood pressure control, and insulin resistance of T2DM. In addition, the capacity of SCFAs ensuring the energy homeostasis and lipid buffering capacity *via* metabolic regulation and gut-brain axis participating take a crucial part during CVDs progression. It is worthy to note that each SCFA-driven mechanism pathway does not exist independent and better understanding of them would greatly facilitate managing cardiac health especially preventing CVDs. Focusing on the SCFAs manufacturing, diet, TCM, probiotics and prebiotics, and fecal transplantation offer some novel potential therapeutic opportunities for CVDs.

Furthermore, despite these exciting and intriguing findings, few studies have provided casual evidence of a direct participatory role of SCFA and poor intervention therapeutic studies gave an explicit and direct relationship with SCFAs to the development of CVDs. A better understanding of SCFA-host and intervention measures-SCFAs-host are needed in future studies.

Author contributions

XC conceived the idea, critically reviewed, and proofread the manuscript. YL performed the literature search, drafted the manuscript, and drew the figures. YZ assisted in drafting and editing. CS assisted with instructive layout of figures. LL and XC gave constructive comments. All authors read and approved the final manuscript version.

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Conflict of interest

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

Publisher's note

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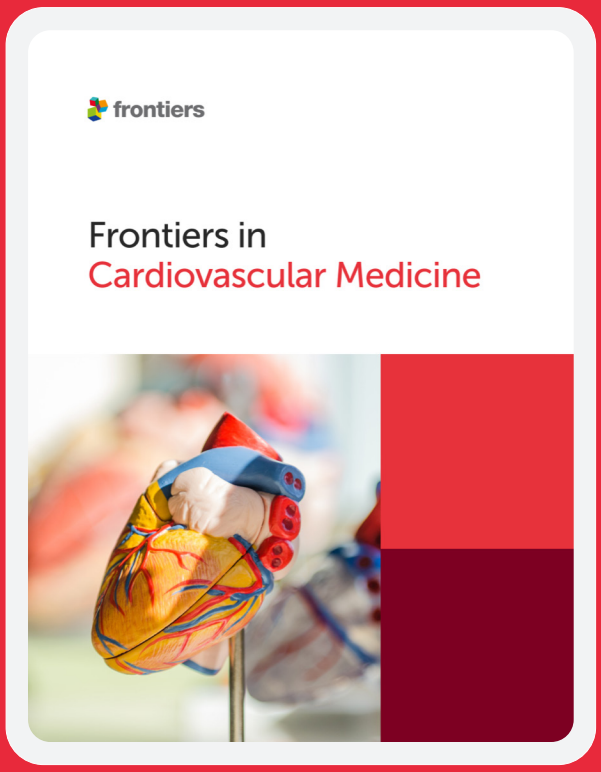

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