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High-sensitivity C-reactive protein and cystatin C independently and jointly predict all-cause mortality among the middle-aged and elderly Chinese population

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ABSTRACT

Studies investigating the relationship between high-sensitivity C-reactive protein (hs-CRP), cystatin C, and all-cause mortality yielded inconsistent results. Moreover, the joint effect of hs-CRP and cystatin C on mortality risk is largely unknown for the general population. In this study, we examined the associations between hs-CRP, cystatin C, and all-cause mortality using data from the China Health and Retirement Longitudinal Study (CHARLS). Middle-aged and elderly participants with complete data were enrolled for a 4-year follow-up of total mortality and plasma levels of hs-CRP (n = 11,409) and cystatin C (n = 8680). In study population, the highest quartiles of hs-CRP and cystatin C were significantly associated with increased total mortality risk compared with the lowest quartile, and adjusted hazard ratios (95% confidence intervals) were 2.08 (1.49–2.91) and 1.97 (1.33–2.94) for hs-CRP and cystatin C, respectively. Remarkably, the adjusted hazard ratio (95% confidence interval) of the co-occurrence of elevated hs-CRP and increased cystatin C was 4.17 (2.94–5.92), in contrast to each elevation alone: 1.89 (1.45–2.47) for hs-CRP and 2.08 (1.46–2.97) for cystatin C. Moreover, a subgroup analysis by gender yielded similar associations. Lastly, the addition of hs-CRP and cystatin C to conventional factors significantly improved risk prediction of total mortality (net reclassification index 0.3622, $P < 0.0001$; integrated discrimination improvement 0.0354, $P < 0.0001$). Taken together, findings suggest that plasma hs-CRP and cystatin C serve as independent predictors of all-cause mortality among the middle-aged and elderly Chinese population. Furthermore, the combination of hs-CRP and cystatin C could predict overall mortality better than each component individually.

1. Introduction

With increasing life expectancy and declining fertility, the world's population is expected to experience continuous aging throughout the 21st century [1]. Unfortunately, people aged 45 and over face a considerably increased risk of morbidity and mortality [2]. Therefore, identifying biological mortality markers for the general population, particularly those who are middle-aged and elderly, is of great significance. Although there have been reported many potential markers that were associated with mortality, they are awaiting further assessment and validation.

High-sensitivity C-reactive protein (hs-CRP) is a plasma protein produced in the liver that is commonly considered as a sensitive marker of inflammation [3]. In acute responses to serious infection or major tissue damage, concentrations of hs-CRP can be elevated by up to 10,000-fold [4]. However, many studies have reported the biological roles of hs-CRP at moderate elevated levels in vascular diseases [5–7]. A number of studies have also found that elevated hs-CRP is a predictor of all-cause mortality, but the results of these studies are not consistent [5,8–18].

Cystatin C, a cysteine protease inhibitor produced by all nucleated cells [19], has been suggested to be superior over creatinine as a measure of kidney function [20]. In recent years, many prospective cohorts have found an association between cystatin C and all-cause mortality in the general population [9,21–28]. However, the majority of these studies were conducted in non-Asian populations. Further

Abbreviations: AUC, area under the receiver operating characteristic curve; BMI, body mass index; CHARLS, China Health and Retirement Longitudinal Study; CI, confidence interval; CMU, Capital Medical University; HBS, high blood sugar; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; QC, quality control; SD, standard deviation.

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evidence is needed to be accumulated for their association in Asian populations. In addition, contradictory results have also been reported in the males [24,26,28].

Furthermore, few studies have investigated the combined capacity of cystatin C and hs-CRP in predicting overall mortality in the general population. As a result, this study sought to investigate the relationship between hs-CRP, cystatin C and total mortality based on data from the China Health and Retirement Longitudinal Study (CHARLS). The objectives of this study are two-fold: (1) to separately investigate the potential of cystatin C and hs-CRP as independent predictors of all-cause mortality among the middle-aged and elderly Chinese population; (2) to assess their joint effect on the prediction of all-cause mortality.

2. Material and methods

2.1. Study design

CHARLS is a representative longitudinal study of a middle-aged and elderly Chinese population of adults (aged ≥45 years), conducted by the National School for Development (China Center for Economic Research) at Peking University [29]. This survey is designed to collect a wide range of information on the economic standing, physical and psychological health, demographics and social networks of aged persons. The aim of CHARLS is to better understand the socioeconomic determinants and consequences of aging. The national baseline survey (wave 1), which was conducted between June 2011 and March 2012, included 17,708 respondents and covered 150 counties/districts and 450 villages/urban communities in China. The second wave (wave 2) was conducted in 2013–2014, the third wave (wave 3) in 2014–2015 and the fourth wave (wave 4) in 2015–2016. The respondents in the subsequent three waves included new participants or respondents from previous waves. A detailed description of the design of CHARLS can be found in a previous publication [29]. This study was approved by Biomedical Ethics Review Committee of Peking University. All subjects provided informed consent.

2.2. Study population

The study population included individuals who had plasma levels of cystatin C and hs-CRP measured in wave 1 and were successfully followed up in at least one of the following three waves. In total, 11,664 individuals out of 17,708 respondents in wave 1 were collected blood samples and measured plasma hs-CRP. Of the 11,664 participants who had hs-CRP measurements, 11,409 individuals were followed up in at least one of the subsequent three waves. For cost reasons, a sub-sample (n = 8878) of those who provided blood samples had plasma cystatin C measured. These individuals included all participants aged 70 and over and a random sub-sample of those under 70. The reason for assaying on cystatin C in everyone aged 70 and over is that older adults are generally more commonly accompanied by renal insufficiency [30]. Among the 8,878 participants who had plasma cystatin C measured, 8680 individuals were successfully followed up in at least one of the subsequent three waves.

2.3. Baseline data collection

Venous blood (over 92% fasting) was collected by medically-trained staff and was separated into plasma and buffy coat. Plasma samples were then immediately frozen at −20 °C, stored, and transported to the Chinese Center for Disease Control and Prevention in Beijing within 2 weeks where they were placed in a deep freezer and stored at −80 °C until assayed at Capital Medical University (CMU) laboratory. Hs-CRP was measured by the immunoturbidimetric assay, with an analytical range of 0.1–20 mg/L and between-assay coefficient of variation of < 5.7%. Cystatin C was measured by particle-enhanced turbidimetric assay, with an analytical range of 0.5–8 mg/L and between-assay coefficient of variation of < 5%. Both assays were performed using Roche kits (Roche Diagnostics, Basel, Switzerland) on a Hitachi 7180 chemistry analyzer (Hitachi, Tokyo, Japan). The laboratory used quality control (QC) samples daily during the testing of the CHARLS study samples. All test results from these QC samples were within the target range (within two standard deviations of mean QC control concentrations). The other variables collected included gender, age, smoking, drinking, body mass index (BMI), hypertension (defined by a history of hypertension, or systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg), dyslipidemia (defined by a history of dyslipidemia, or triglyceride ≥2.26 mmol/L, or total cholesterol ≥6.22 mmol/L, or high-density lipoprotein cholesterol < 1.04 mmol/L, or low-density lipoprotein cholesterol ≥4.14 mmol/L), high blood sugar (HBS)/diabetes (defined by a history of HBS/diabetes, or fasting glucose ≥6.1 mmol/L, or non-fasting glucose ≥7.8 mmol/L), a history of stroke, cancer, cardiovascular disease, lung disease, liver disease, kidney disease, digestive disease, asthma, arthritis, psychological problem and memory problem.

2.4. Mortality follow-up

Participants enrolled in wave 1 were followed up in waves 2, 3 and 4. Both interview status (dead or alive) and death time were recorded in wave 2. For waves 3 and 4, only the interview status information was available. For those who had data on all-cause death, the survival time was calculated as the interval between the interview time in wave 1 and the death time. If the exact death time was not available, the survival time was defined as the median of the interval between wave 1 and the specific wave with death information. For those who did not die during the follow-up period, the survival time was the interval between two interview waves.

2.5. Statistical analysis

Plasma levels of hs-CRP and cystatin C were first divided into quartiles. Data were presented as mean ± standard deviation (SD) for continuous variables and frequency (percentage) for categorical variables. Baseline characteristics among quartiles of hs-CRP or cystatin C were compared by the Kruskal-Wallis rank sum test for continuous variables and by the chi-square test for categorical variables. Kaplan-Meier survival curves were used to compare the mortality rates among quartiles of hs-CRP and cystatin C. The Cox proportional hazard ratio model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of hs-CRP and cystatin C. In addition, area under the receiver operating characteristic curve (AUC), net reclassification index (NRI), and integrated discrimination improvement (IDI) were used to evaluate the incremental predictive value of hs-CRP and cystatin C for total mortality at 4 years beyond conventional factors [31]. All statistical analyses were performed by SAS 9.4, with the exception of Kaplan-Meier survival analysis, which was implemented on the R platform. The significance level was set at 0.05.

3. Results

3.1. Baseline characteristics of the study population

Baseline characteristics of the study population by quartiles of hs-CRP are presented in Table 1. Levels of hs-CRP were found to be significantly higher in men than in women. Participants with highest hs-CRP concentrations were older and more likely to smoke compared with those with lower hs-CRP levels. The prevalence of hypertension, dyslipidemia, HBS/diabetes, stroke, cardiovascular disease, lung disease, and asthma all increased with rising quartiles of hs-CRP (all P values < 0.001).

Baseline characteristics of the study population by quartiles of cystatin C are presented in Table 2. Plasma levels of cystatin C were

Table 1
Baseline characteristics of participants by quartiles of hs-CRP.

Characteristics	Quartile 1 (≤0.55 mg/L) (*n* = 2829)	Quartile 2 (0.55–1.04 mg/L) (*n* = 2903)	Quartile 3 (1.04–2.19 mg/L) (*n* = 2818)	Quartile 4 (≥2.19 mg/L) (*n* = 2859)	*P* value
Age-yr	56.83 ± 9.46	58.53 ± 9.62	59.15 ± 9.42	60.62 ± 10.12	< 0.0001
Male sex-no. (%)	1225 (43.30)	1351 (46.54)	1331 (47.23)	1388 (48.55)	0.0007
BMI-kg/m^2	22.37 ± 3.20	23.31 ± 3.71	24.17 ± 3.86	24.24 ± 4.45	< 0.0001
SBP-mmHg	126.89 ± 23.46	130.00 ± 24.34	132.86 ± 25.15	134.24 ± 25.70	< 0.0001
DBP-mmHg	73.77 ± 11.64	75.43 ± 12.30	76.88 ± 12.38	76.90 ± 12.06	< 0.0001
Lifestyle-no. (%)					
Smoking ever	999 (35.48)	1104 (38.12)	1101 (39.18)	1213 (42.62)	< 0.0001
Drinking ever	1071 (38.06)	1138 (39.34)	1064 (37.91)	1120 (39.38)	0.5160
Disease history-no. (%)					
Hypertension	871 (35.15)	1073 (41.41)	1254 (49.43)	1370 (53.70)	< 0.0001
Dyslipidemia	900 (32.35)	1183 (41.32)	1403 (50.63)	1465 (51.90)	< 0.0001
HBS/Diabetes	644 (23.53)	809 (28.80)	906 (33.02)	1042 (37.44)	< 0.0001
Cancer	28 (1.00)	30 (1.04)	27 (0.97)	38 (1.34)	0.5095
Stroke	61 (2.17)	60 (2.08)	99 (3.53)	111 (3.91)	< 0.0001
Cardiovascular disease	298 (10.64)	343 (11.89)	401 (14.35)	437 (15.45)	< 0.0001
Lung disease	244 (8.70)	307 (10.64)	294 (10.50)	371 (13.09)	< 0.0001
Arthritis	967 (34.39)	1060 (36.69)	1023 (36.50)	1086 (38.25)	0.0265
Liver disease	108 (3.86)	113 (3.93)	102 (3.65)	109 (3.86)	0.9549
Kidney disease	163 (5.82)	181 (6.31)	166 (5.95)	198 (6.99)	0.2603
Digestive disease	762 (27.16)	691 (23.92)	602 (21.48)	608 (21.42)	< 0.0001
Asthma	111 (3.95)	135 (4.68)	113 (4.04)	185 (6.53)	< 0.0001
Psychological problem	49 (1.75)	43 (1.49)	45 (1.61)	28 (0.99)	0.0894
Memory problem	57 (2.03)	50 (1.73)	51 (1.82)	58 (2.04)	0.7826
Laboratory measurements					
Cystatin C-mg/L	0.95 ± 0.21	1.00 ± 0.23	1.02 ± 0.27	1.10 ± 0.39	< 0.0001
Total cholesterol-mmol/L	4.88 ± 0.96	4.98 ± 0.97	5.08 ± 1.02	5.00 ± 1.06	< 0.0001
Triglyceride-mmol/L	1.30 ± 1.04	1.48 ± 1.18	1.67 ± 1.36	1.62 ± 1.30	< 0.0001
HDL cholesterol-mmol/L	1.43 ± 0.40	1.34 ± 0.39	1.26 ± 0.37	1.24 ± 0.39	< 0.0001
LDL cholesterol-mmol/L	2.90 ± 0.83	3.01 ± 0.88	3.06 ± 0.93	3.01 ± 0.95	< 0.0001
Glucose-mmol/L	5.79 ± 1.45	6.04 ± 1.82	6.20 ± 2.11	6.47 ± 2.60	< 0.0001

Note: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBS, high blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

positively associated with male sex, age, BMI, smoking, drinking, hypertension, stroke, cardiovascular disease, lung disease, kidney disease, asthma and memory problem (all *P* values < 0.001).

3.2. Association of hs-CRP or cystatin C with all-cause mortality risk

During the 4-year follow-up, there were 554 deaths among 11,409 participants with hs-CRP data. Mortality rates rose with hs-CRP levels in a dose-response manner (Fig. 1A). Compared with the bottom quartile of hs-CRP, the unadjusted HR (95% CI) increased from 1.32 (0.99–1.76) for the second quartile to 1.56 (1.18–2.07) for the third quartile and to 3.09 (2.40–3.98) for the highest quartile (*P* for trend < 0.0001) in the whole population. The HRs were attenuated after the adjustment of multiple covariates, but still remained statistically significant for the highest quartile group. As compared with the first quartile, adjusted HRs (95% CIs) of the fourth quartile were 2.08 (1.49–2.91) in the entire population. In sub-group analysis by gender, similar significant associations were found in both men and women. However, the adjusted HRs in men were much larger than those in women (Table 3).

There were 490 deaths among 8680 participants who had measurements of cystatin C. Similarly, mortality rates were increased with ascending quartiles of cystatin C (Fig. 1B). Compared with the bottom quartile, the unadjusted HR (95% CI) for the second, third, and fourth quartiles were 1.14 (0.80–1.64), 1.85 (1.32–2.59) and 5.20 (3.88–6.98), respectively (*P* for trend < 0.0001). After adjustment for potential confounders, HR (95% CI) of the highest quartile was 1.97 (1.33–2.94) in the entire population compared to the first quartile. In addition, these results did not differ by gender (Table 3).

3.3. Combined effect of hs-CRP and cystatin C on predicting all-cause mortality

To investigate the effect of the coexistence of elevated hs-CRP and increased cystatin C on all-cause mortality, participants were divided into four groups based on the levels of hs-CRP and cystatin C: Group 1 (cystatin C ≤ 1.44 & hs-CRP ≤ 3), Group 2 (cystatin C ≤ 1.44 & hs-CRP > 3), Group 3 (cystatin C > 1.44 & hs-CRP ≤ 3), and Group 4 (cystatin C > 1.44 & hs-CRP > 3). The cutoffs of 3 for hs-CRP and 1.44 for cystatin C were both regarded as high risk levels, which are recommended by the manufacturer of the assays and agreed upon international threshold levels [30]. Compared with individuals in Group 1, there were significant increased death risks for individuals in Groups 2, 3 and 4. Remarkably, a higher risk of death risk was observed for individuals in Group 4 than in Group 2 and Group 3 (Fig. 1C). Compared with Group 1, the adjusted HR (95%CI) for Group 4 was 4.17 (2.94–5.92) in the entire population, in contrast with 1.89 (1.45–2.47) for Group 2 and 2.08 (1.46–2.97) for Group 3 (Table 4). Moreover, this joint effect was not attenuated in the gender-specific analysis and was observed among both men and women (Table 4).

3.4. Incremental predictive value of hs-CRP and/or cystatin C

The addition of hs-CRP and/or cystatin C to conventional factors for the prediction of 4-year total mortality was further evaluated. Compared with the conventional model, the addition of hs-CRP or cystatin C significantly improved the discriminatory power and risk reclassification in the whole population. The values of AUC, NRI and IRI were 0.7907 (*P* = 0.0007), 0.2393 (*P* < 0.0001) and 0.0171 (*P* = 0.0002) for hs-CRP, and 0.7998 (*P* < 0.0001), 0.2946 (*P* < 0.0001) and 0.0219 (*P* < 0.0001) for cystatin C. As expected,

Table 2
Baseline characteristics of participants by quartiles of cystatin C.

Characteristics	Quartile 1 (≤0.86 mg/L) (n = 2138)	Quartile 2 (0.86–0.98 mg/L) (n = 2276)	Quartile 3 (0.98–1.13 mg/L) (n = 2028)	Quartile 4 (≥1.13 mg/L) (n = 2238)	P value
Age-yr	54.73 ± 8.67	57.14 ± 8.65	60.59 ± 9.03	67.28 ± 9.93	< 0.0001
Male sex-no. (%)	670 (31.34)	995 (43.72)	1047 (51.63)	1349 (60.28)	< 0.0001
BMI-kg/m^2	24.05 ± 3.61	23.63 ± 3.79	23.27 ± 3.99	22.75 ± 4.09	< 0.0001
SBP-mmHg	128.15 ± 25.27	130.35 ± 25.92	131.07 ± 21.36	137.34 ± 27.74	< 0.0001
DBP-mmHg	75.39 ± 11.60	75.86 ± 12.19	75.45 ± 11.92	75.62 ± 12.88	0.7682
Lifestyle-no. (%)					
Smoking ever	562 (26.35)	812 (35.74)	898 (44.48)	1124 (50.43)	< 0.0001
Drinking ever	694 (32.55)	876 (38.61)	815 (40.39)	965 (43.31)	< 0.0001
Disease history-no. (%)					
Hypertension	717 (38.24)	864 (43.11)	841 (46.21)	1166 (56.77)	< 0.0001
Dyslipidemia	1047 (49.79)	930 (41.52)	806 (40.36)	934 (42.26)	< 0.0001
HBS/Diabetes	709 (34.59)	653 (29.41)	568 (28.77)	678 (31.00)	0.0002
Cancer	24 (1.13)	23 (1.02)	23 (1.14)	21 (0.95)	0.9072
Stroke	44 (2.07)	49 (2.16)	54 (2.68)	122 (5.50)	< 0.0001
Cardiovascular disease	258 (12.17)	265 (11.75)	285 (14.17)	370 (16.67)	< 0.0001
Lung disease	197 (9.28)	211 (9.33)	253 (12.57)	325 (14.61)	< 0.0001
Arthritis	754 (35.47)	843 (37.25)	741 (36.68)	878 (39.50)	0.0466
Liver disease	59 (2.79)	86 (3.82)	80 (3.99)	97 (4.37)	0.0449
Kidney disease	109 (5.14)	131 (5.81)	131 (6.53)	183 (8.26)	0.0002
Digestive disease	492 (23.14)	568 (25.07)	499 (24.76)	478 (21.47)	0.0182
Asthma	84 (3.96)	96 (4.24)	115 (5.70)	153 (6.89)	< 0.0001
Psychological problem	28 (1.32)	36 (1.59)	24 (1.19)	36 (1.62)	0.5739
Memory problem	30 (1.41)	41 (1.81)	34 (1.69)	79 (3.55)	< 0.0001
Laboratory measurements					
Hs-CRP-mg/L	2.01 ± 4.46	2.12 ± 4.57	2.76 ± 6.89	4.24 ± 10.03	< 0.0001
Total cholesterol-mmol/L	5.11 ± 1.06	5.01 ± 0.94	4.94 ± 0.97	4.91 ± 1.06	< 0.0001
Triglyceride-mmol/L	1.77 ± 1.40	1.44 ± 1.02	1.41 ± 1.11	1.38 ± 1.06	< 0.0001
HDL cholesterol-mmol/L	1.27 ± 0.38	1.34 ± 0.40	1.34 ± 0.40	1.33 ± 0.40	< 0.0001
LDL cholesterol-mmol/L	2.99 ± 0.92	3.05 ± 0.88	3.00 ± 0.88	2.98 ± 0.93	0.0254
Glucose-mmol/L	6.39 ± 2.63	6.07 ± 1.96	5.98 ± 1.60	6.05 ± 1.73	0.0008

Note: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBS, high blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

adding the combination of hs-CRP and cystatin C to the conventional model resulted in a greater improvement of the predictive utility, with the values of AUC, NRI and IRI being 0.8055 (P < 0.0001), 0.3622 (P < 0.0001) and 0.0354 (P < 0.0001), respectively. On the whole, similar results were observed among male and female populations, although hs-CRP did not significantly improve the conventional model in the female population (Table 5).

4. Discussion

Our results suggested that higher levels of hs-CRP and cystatin C were both independently associated with an increased risk of all-cause mortality among the middle-aged and elderly Chinese population. The co-occurrence of elevated hs-CRP and increased cystatin C were found to predispose individuals to a much higher risk of mortality than either factor alone. Moreover, adding hs-CRP and cystatin C to conventional risk factors significantly increased the predictive ability of 4-year total mortality.

In accordance with a number of previous studies [8–13,18,21–25,27–28], higher levels of hs-CRP and cystatin C were found to be associated with higher overall mortality risks in the general population. However, there are several exceptions with inconsistent results. Kistorp et al. (2007) revealed that increased hs-CRP concentrations were associated with overall mortality only in unadjusted models, whereas this association disappeared after adjustment for conventional risk factors [16]. It should be noted that the sample size of this study was much smaller than ours and might not have had enough statistical power to detect any significant association. In sub-group analysis by gender, two studies reported that hs-CRP levels were positively correlated with the risk of all-cause mortality in a male population but not in a female population [14,17]. Nevertheless, other studies found positive associations between hs-CRP and all-cause mortality in women [12,18]. A recent meta-analysis also showed that the effects of elevated hs-CRP levels on all-cause mortality risk were similar in men and women [15]. In our study, plasma levels of hs-CRP were found to be positively correlated with mortality risk in both genders. However, we did observe that the correlation of hs-CRP with mortality was relatively smaller in women than in men, which has also been noted previously [18].

On the other hand, Toft et al. (2012) showed that cystatin C levels were positively associated with in a Norway female population, but there was no such an association observed in males [26]. By contrast, studies by Hart et al. (2017) and Emberson et al. (2010) both reported positive associations between cystatin C and all-cause mortality in men [24,28], which was in line with our findings. We speculate that the inconsistencies in these findings may be explained by diverse cut-off levels, different study populations, various confounders that were adjusted for, or insufficient statistical power. Overall, our study supports a positive link between cystatin C/hs-CRP and all-cause mortality in both genders.

It is worth noting that the combined effect of cystatin C and hs-CRP on the prediction of all-cause mortality in the general population was carefully evaluated and verified in this study. The joint effect of increased hs-CRP and high cystatin C on predicting all-cause mortality was much higher than their respective effects. Moreover, the combination of hs-CRP and cystatin C provided significant incremental predictive value to conventional factors. Actually, the combination of multiple biomarkers has been demonstrated to improve mortality risk prediction [18,22]. Jenny et al. (2007) reported that the combination of cystatin C and fibrinogen could improve prediction of death in men [18]. Zethelius et al. (2008) found that the addition of four biomarkers (troponin I, N-terminal pro-brain natriuretic peptide, cystatin C, and hs-

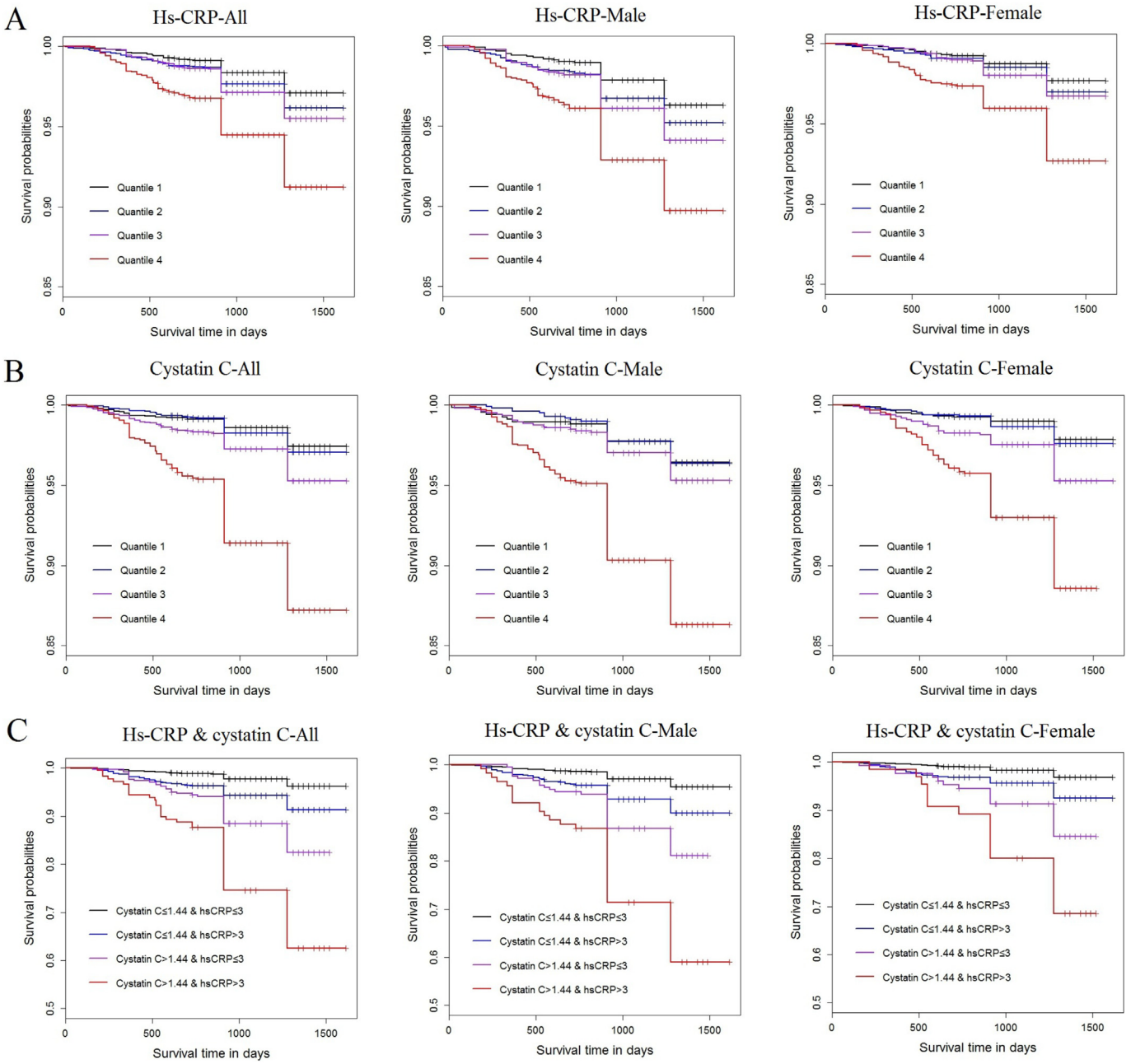

Fig. 1. Kaplan-Meier survival curves for hs-CRP (A), cystatin C (B) and hs-CRP & cystatin C (C).

CRP) to conventional risk factors substantially improved the risk stratification for death from cardiovascular causes in elderly men [22]. To the best of our knowledge, this study is the first one to systematically assess the joint effect of cystatin C and hs-CRP on the prediction of all-cause mortality risk in the general population. Our findings could be of great significance in practice, as monitoring cystatin C and hs-CRP simultaneously may be applicable to better screen high-risk individuals in the future. For those high-risk individuals, conducting more intensive monitoring and providing higher quality health care may decrease the probability of death.

The mechanisms underlying the association between hs-CRP/cystatin C and all-cause mortality are incompletely elucidated. Several explanations may provide some hints. Hs-CRP, as a sensitive marker of inflammation, may reflect underlying inflammatory activities or diseases. Inflammation is known to play an important role in the progression of atherosclerosis [32], the latter being well known as the pathological foundation of cardiovascular diseases. Moreover, many studies have already established the relationship between hs-CRP and a series of diseases such as hypertension [33], coronary artery disease [34], stroke [35] and cancer [36], all of which could contribute to increased mortality. Furthermore, cystatin C has been suggested to be a promising measure of kidney function, when kidney dysfunction has been an acknowledged risk factor for mortality in the elderly [24]. Cystatin C has been shown to be a marker of inflammation as well [37]. Elevated cystatin C is also associated with various important contributors of overall death, including hypertension [38], coronary heart disease [39], stroke [39], and peripheral artery disease [40]. Nevertheless, whether hs-CRP/cystatin C is a direct cause of mortality or a marker of these hidden, potentially fatal diseases awaits further investigation.

There are several limitations in this study. First, complete data about causes of death were not available, preventing a further analysis of the relationship between hs-CRP/cystatin C and cause-specific mortalities. Second, all analyses were based on a 4-year follow-up period.

Table 3
Association of hs-CRP or cystatin C with all-cause mortality risk.

	Total	No. (%) of deaths	Unadjusted		Adjusted[a]	
	number		HR (95%CI)	*P* value	HR (95%CI)	*P* value
Hs-CRP						
All						
Quartile 1	2829	80 (2.83)	1.00	–	1.00	–
Quartile 2	2903	108 (3.72)	1.32 (0.99–1.76)	0.0595	1.13 (0.78–1.64)	0.5315
Quartile 3	2818	123 (4.36)	1.56 (1.18–2.07)	0.0020	1.49 (1.04–2.13)	0.0298
Quartile 4	2859	243 (8.50)	3.09 (2.40–3.98)	< 0.0001	2.08 (1.49–2.91)	< 0.0001
P for trend			< 0.0001		< 0.0001	
Male						
Quartile 1	1225	44 (3.59)	1.00	–	1.00	–
Quartile 2	1351	63 (4.66)	1.31 (0.89–1.93)	0.1699	1.37 (0.82–2.29)	0.2282
Quartile 3	1331	76 (5.71)	1.62 (1.11–2.34)	0.0114	1.77 (1.08–2.92)	0.0243
Quartile 4	1388	139 (10.01)	2.88 (2.05–4.04)	< 0.0001	2.39 (1.50–3.82)	0.0003
P for trend			< 0.0001		< 0.0001	
Female						
Quartile 1	1604	36 (2.24)	1.00	–	1.00	–
Quartile 2	1552	45 (2.90)	1.29 (0.83–2.00)	0.2547	0.84 (0.48–1.46)	0.5303
Quartile 3	1487	47 (3.16)	1.42 (0.92–2.19)	0.1160	1.19 (0.70–2.04)	0.5138
Quartile 4	1471	104 (7.07)	3.22 (2.21–4.71)	< 0.0001	1.70 (1.04–2.80)	0.0361
P for trend			< 0.0001		0.0064	
Cystatin C						
All						
Quartile 1	2138	53 (2.48)	1.00	–	1.00	–
Quartile 2	2276	65 (2.86)	1.14 (0.80–1.64)	0.4684	1.16 (0.74–1.80)	0.5173
Quartile 3	2028	93 (4.59)	1.85 (1.32–2.59)	0.0003	1.26 (0.83–1.93)	0.2795
Quartile 4	2238	279 (12.47)	5.20 (3.88–6.98)	< 0.0001	1.97 (1.33–2.94)	0.0008
P for trend			< 0.0001		< 0.0001	
Male						
Quartile 1	670	23 (3.43)	1.00	–	1.00	–
Quartile 2	995	25 (3.52)	1.01 (0.60–1.71)	0.9645	1.39 (0.72–2.67)	0.3271
Quartile 3	1047	48 (4.58)	1.33 (0.81–2.18)	0.2639	1.21 (0.82–2.29)	0.5646
Quartile 4	1349	180 (13.34)	4.02 (2.60–6.20)	< 0.0001	2.02 (1.11–3.68)	0.0214
P for trend			< 0.0001		0.0061	
Female						
Quartile 1	1468	30 (2.04)	1.00	–	1.00	–
Quartile 2	1281	30 (2.34)	1.14 (0.69–1.89)	0.6118	0.86 (0.46–1.63)	0.6463
Quartile 3	981	45 (4.59)	2.25 (1.42–3.57)	0.0006	1.27 (0.72–2.26)	0.4146
Quartile 4	889	99 (11.14)	5.60 (3.72–8.43)	< 0.0001	1.88 (1.09–3.27)	0.0245
P for trend			< 0.0001		0.0046	

[a] Adjusted for age, smoking, drinking, BMI, hypertension, dyslipidemia, HBS/diabetes, history of stroke, cancer, cardiovascular disease, lung disease, liver disease, kidney disease, digestive disease, asthma, arthritis, psychological problem, memory problem, and cystatin C/hs-CRP.

Table 4
The combined effect of hs-CRP and cystatin C on the prediction of mortality.

	Total	No. (%) of deaths	Unadjusted		Adjusted[a]	
			HR (95%CI)	*P* value	HR (95%CI)	*P* value
All						
Cystatin C ≤ 1.44 & hs-CRP ≤ 3	6744	247 (3.66)	1.00	–	1.00	–
Cystatin C ≤ 1.44 & hs-CRP > 3	1416	120 (8.47)	2.38 (1.91–2.95)	< 0.0001	1.89 (1.45–2.47)	< 0.0001
Cystatin C > 1.44 & hs-CRP ≤ 3	340	58 (17.06)	4.93 (3.71–6.57)	< 0.0001	2.08 (1.46–2.97)	< 0.0001
Cystatin C > 1.44 & hs-CRP > 3	178	65 (36.52)	11.51 (8.76–15.13)	< 0.0001	4.17 (2.94–5.92)	< 0.0001
P for trend			< 0.0001		< 0.0001	
Male						
Cystatin C ≤ 1.44 & hs-CRP ≤ 3	3050	135 (4.43)	1.00	–	1.00	–
Cystatin C ≤ 1.44 & hs-CRP > 3	685	67 (9.78)	2.27 (1.69–3.04)	< 0.0001	2.08 (1.47–2.94)	< 0.0001
Cystatin C > 1.44 & hs-CRP ≤ 3	212	39 (18.40)	4.38 (3.07–6.26)	< 0.0001	2.35 (1.51–3.67)	0.0002
Cystatin C > 1.44 & hs-CRP > 3	113	45 (39.82)	10.52 (7.50–14.74)	< 0.0001	4.41 (2.81–6.92)	< 0.0001
P for trend			< 0.0001		< 0.0001	
Female						
Cystatin C ≤ 1.44 & hs-CRP ≤ 3	3694	112 (3.03)	1.00	–	1.00	–
Cystatin C ≤ 1.44 & hs-CRP > 3	731	53 (7.25)	2.47 (1.78–3.42)	< 0.0001	1.57 (1.03–2.40)	0.0345
Cystatin C > 1.44 & hs-CRP ≤ 3	128	19 (14.84)	5.18 (3.18–8.42)	< 0.0001	1.65 (0.90–3.05)	0.1081
Cystatin C > 1.44 & hs-CRP > 3	65	20 (30.77)	11.35 (7.06–18.28)	< 0.0001	4.86 (2.70–8.74)	< 0.0001
P for trend			< 0.0001		< 0.0001	

[a] Adjusted for age, smoking, drinking, BMI, hypertension, dyslipidemia, HBS/diabetes, history of stroke, cancer, cardiovascular disease, lung disease, liver disease, kidney disease, digestive disease, asthma, arthritis, psychological problem and memory problem.

Table 5
Reclassification and discrimination statistics for 4-year all-cause mortality by hs-CRP and/or cystatin C.

Model	AUC		NRI		IDI	
	Estimate (95%CI)	*P* value	Estimate (95%CI)	*P* value	Estimate (95%CI)	*P* value
All						
Conventional model[a]	0.7810 (0.7566–0.8053)	–	Reference	–	Reference	–
Conventional model + hs-CRP	0.7907 (0.7663–0.8150)	0.0007	0.2393 (0.1415–0.3371)	< 0.0001	0.0171 (0.0081–0.0261)	0.0002
Conventional model + cystatin C	0.7998 (0.7763–0.8233)	< 0.0001	0.2946 (0.1877–0.4014)	< 0.0001	0.0219 (0.0120–0.0319)	< 0.0001
Conventional model + cystatin C + hs-CRP	0.8055 (0.7819–0.8291)	< 0.0001	0.3622 (0.2553–0.4690)	< 0.0001	0.0354 (0.0225–0.0484)	< 0.0001
Male						
Conventional model	0.7499 (0.7159–0.7838)	–	Reference	–	Reference	–
Conventional model + hs-CRP	0.7680 (0.7342–0.8017)	0.0019	0.3028 (0.1734–0.4321)	< 0.0001	0.0326 (0.0153–0.0499)	0.0002
Conventional mode + cystatin C	0.7693 (0.7361–0.8025)	0.0069	0.2577 (0.1169–0.3984)	0.0003	0.0284 (0.0129–0.0439)	0.0003
Conventional model + cystatin C + hs-CRP	0.7808 (0.7474–0.8142)	0.0004	0.4298 (0.2895–0.5701)	< 0.0001	0.0546 (0.0325–0.0768)	< 0.0001
Female						
Conventional model	0.8133 (0.7769–0.8496)	–	Reference	–	Reference	–
Conventional model + hs-CRP	0.8145 (0.7779–0.8511)	0.3070	0.1346 (−0.0206–0.2899)	0.1113	0.0026 (−0.0007–0.0059)	0.1167
Conventional model+ cystatin C	0.8283 (0.7933–0.8634)	0.0178	0.3327 (0.1679–0.4974)	< 0.0001	0.0151 (0.0041–0.0260)	0.0069
Conventional model+ cystatin C + hs-CRP	0.8285 (0.7933–0.8637)	0.0165	0.3773 (0.2130–0.5416)	< 0.0001	0.0170 (0.0058–0.0283)	0.0029

[a] The conventional model included age, smoking, drinking, BMI, hypertension, dyslipidemia, HBS/diabetes, history of stroke, cancer, cardiovascular disease, lung disease, liver disease, kidney disease, digestive disease, asthma, arthritis, psychological problem and memory problem.

Further study is needed to investigate whether baseline serum levels of hs-CRP and cystatin C could predict mortality in a longer period of follow-up, either independently or jointly. Finally, there are some missing values for various covariates, which may prevent the discovery of stronger associations in the multivariate analysis.

5. Conclusions

Elevated plasma hs-CRP or cystatin C could serve as an independent predictor of all-cause mortality among the middle-aged and elderly Chinese population. Furthermore, the combination of hs-CRP and cystatin C could predict all-cause mortality better than each component individually. In the future, monitoring plasma levels of both hs-CRP and cystatin C may be worthwhile to identify high-risk individuals in the middle-aged and elderly population facing a high risk of all-cause mortality.

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Conflicts of interest

None declared.

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