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Intra-individual variability of total cholesterol is associated with cardiovascular disease mortality: A cohort study

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Abstract *Background and aims:* The relationship between serum total cholesterol (TC) and mortality remains inconsistent. Additionally, intra-individual variability of cholesterol has been of increasing interest as a new indicator for health outcomes. We aimed to examine the association between TC and its variability and risk of mortality.
Methods and results: We performed a retrospective cohort study with 122,645 individuals aged over 40 years in Ningbo, China. The intra-individual variability was calculated using four metrics including standard deviation, coefficient variation, variation independent of mean and average successive variability. Hazard ratios and 95% confidence intervals were estimated for the associations of baseline and variability in TC with risk of mortality by Cox proportional hazards regression models. During 591,585.3 person-years of follow-up, 4563 deaths (including 1365 from cardiovascular disease, 788 from stroke and 1514 from cancer) occurred. A U-shaped association was observed for baseline TC level and risk of total, cardiovascular and cancer mortality, with lowest mortality at 5.46 mmol/L, 5.04 mmol/L and 5.51 mmol/L, respectively. As compared with subjects with TC variability in the lowest quartile, individuals in the highest quartile had 21% higher risk of all-cause mortality (HR = 1.21, 95% CI: 1.05 to 1.40), and 41% higher risk of CVD mortality (HR = 1.41, 95%CI: 1.10 to 1.81).
Conclusion: Both too low and too high baseline TC level were associated with higher risk of total, cardiovascular disease and cancer mortality. Variability of TC could be a risk factor of total and CVD mortality, independent of mean TC level. Future studies are needed to confirm these findings.

Introduction

Blood lipids are the collective term for cholesterol, triglyceride (TG), and lipoids in serum, among which cholesterol and TG have close clinical association primarily. It has been well established that higher cholesterol levels are associated with greater atherosclerotic cardiovascular disease (ASCVD) risk [1–5]. To reduce morbidity

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and mortality, patients were recommended commonly to lower cholesterol level by drug therapy or lifestyle intervention. However, some previous studies have reported an inverse association in the elderly [6–10], and other studies conducted in general population have also reported an inverse or U-shape relationship for total cholesterol (TC) and mortality [11–14].

In addition, recent evidence has suggested that fluctuations of serum cholesterol in individuals may be independently associated with adverse outcomes [15–20]. For example, post hoc analyses of the Treating to New Target(TNT) trial showed that visit-to-visit variability in fasting measurements of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were predictive of coronary and cardiovascular events in patients with known coronary artery disease [15,16]. Similar findings between LDL-C variability and vascular events and all-cause mortality were also reported in post hoc analyses of another clinical trial [17]. However, limited studies have evaluated the role of cholesterol variability as a determinant of mortality among the general population [19] and none in Chinese population.

Herein, we conducted a retrospective analysis in a population-based Chinese cohort of 122,645 adults with 4563 deaths to examine the association of total cholesterol and all-cause and cause-specific mortality. We also examined the potential effects of cholesterol variability during 3 years on sequent risk of mortality.

Methods

Study population

This population-based cohort study was based on Yinzhou Health Information System(YHIS) in Ningbo city. YHIS is a high-quality, efficient health information System within China established in 2004, which has been described elsewhere [21]. Briefly, it is composed of residents' healthcare records, Chronic Diseases Recording System and Hospital Information System, making it possible to retrieve the comprehensive and timely health information for each resident.

According to YHIS, we recruited 143,823 residents aged over 40 years who had at least one medical examination record of total cholesterol during 2010–2014. Of these subjects, 21,178 were excluded due to a history of cardiovascular events (heart disease and stroke), diabetes mellitus, cancer or due to extremely low TC (outliers: those lower than the mean TC $\pm$ 3 SDs). Thus, a total of 122, 645 individuals were included in the primary analysis to examine the association for TC at baseline and mortality. In a secondary analysis, we included subjects with at least three measurements of TC to examine the association between TC variability and risk of mortality ($n = 32{,}237$). All participants gave informed consent for the use of their data.

Data collection and definition

Baseline characteristics data were obtained from the YHIS. Demographic and lifestyle data included age at baseline, gender, marital status, education level, smoking status, drinking status and physical activity frequency and the definitions have been described in the previous study [21]. All physical examinations (including weight, height, and blood pressure) and data collection followed a standard protocol. Serum cholesterol was assayed using fasting serum samples by automatic biochemical analyzer.

We used the mean TC value instead of the first measurement for each subject as their baseline level. We used four metrics for TC variability: (i) standard deviation (SD), (ii) coefficient of variation (CV), (iii) variability independent of the mean (VIM) [22,23] and (iv) average successive variability (ASV) [15], which was defined as the average absolute difference between successive values. VIM was calculated as $100 \times \mathrm{SD}/\mathrm{Mean}^{\lambda}$, where λ is the regression coefficient, on the basis of the natural logarithm of the SD over the natural logarithm of the mean.

Mortality and follow-up

Death status was monitored with official death certificates from Yinzhou Center for Disease Control and Prevention (Yinzhou CDC). Two trained Yinzhou CDC personnel who were blinded to the study participants' status at baseline verified the causes and dates of death independently, which was further validated by an experienced staff. Primary causes of death were identified by the International Classification of Disease, 10th Revision (ICD10). Death status was ascertained by linkage of the selected population to the Yinzhou Death Database, with the most recent update on 31 December 2017.

We considered the cause-specific death as follows:(1) total cardiovascular disease, CVD(ICD10 code: I00–I99); (2)cerebrovascular diseases, commonly known as stroke (ICD10 code: I60–I69); (3) cancer (ICD10 codes: C00–C99); (4) ischemic heart disease, IHD, also called coronary heart disease, CHD(ICD10 code: I20–I25); (5) ischemic stroke (ICD–10 codes: I63 or 69.3).

Statistical analysis

Baseline characteristics are presented as the median (quartile1-quartile3) or n (%). Non-parametric Kruskal-Wallis test was used for continuous variables and Pearson χ^2 test was used for categorized variables. Participants were censored at the last known follow-up date, death or 31 December 2017, whichever came first. The initial date for follow-up was defined from the most recent measurement.

To explore non-linear or irregular shape of association between baseline TC and the risk of total mortality and cause-specific mortality, we used Cox models with penalized splines using non-parametric smoothers [24]. In this model, we used flexible spline terms for total cholesterol and adjusted for the effect of sex and age (as a linear term).The degree of freedom for total cholesterol was selected using the smooth HR package in R based on the corrected Akaike information criterion [25].

We further divided TC levels into 8 groups (<3.63, 3.63∼, 4.14∼, 4.66∼[reference], 5.18∼, 5.70∼, 6.22∼ and ≥6.73 mmol/L, to estimate hazard ratios (HRs) and 95% confidence intervals (CIs)using multivariable Cox proportional hazards model. The category with the lowest mortality was regarded as a reference. Assuming a linear association in TC below 5.18 mmol/l or upper range, we also calculated HRs for an increase of 1 mmol/L taking TC as a continuous variable in each TC range (<5.18 mmol/l or ≥5.18 mmol/l) in the Cox proportional hazards model.

Covariates in the models were as follows: age at baseline (continuous variable), sex (men or women), BMI (underweight or normal or overweight or obesity), systolic blood pressure(continuous variable), marital status (married or others), education level (1–6 years/middle or high school graduate/college or more/others), tobacco smoking (ever or never or unknown), alcohol drinking (ever or never or unknown), physical activity (more than four times/week or one to four times/week or less than one time/week or unknown).We tested the assumption of proportional risk for the Cox models by using cross-product terms (time × TC) and using Kaplan-Meier curves. Fine–Gray competing risks regression models were also used when assessing the associations between total cholesterol and cause-specific mortality [26].

In the secondary analysis, multivariable Cox proportional hazards regressions were used to evaluate the effect of TC variability metrics on mortality with adjustment for the same covariates as in primary analysis plus mean TC value. Each of TC variability (SD, CV, VIM and ASV) was categorized by the quartiles with the lowest quartile as a reference. Linear trends across the quartiles were calculated with the quartile as a continuous variable ranging from 1 to 4.

Subgroup analyses were performed in both analyses to examine whether the association varied by sex (men or women), age at baseline (<60 or ≥60 years), smoking status, alcohol drinking status, BMI (underweight or normal or overweight or obesity), and hypertension (yes or no). Sensitivity analyses were also performed by excluding subjects who had extreme BMI (<18.5 or >40 kg/m^2) or were followed up less than 2 years. To eliminate the possible influence of times of measurements in the variability analysis, subjects with three TC measurements and those with more than three TC measurements were evaluated separately.

Results

The primary analysis

A total of 122,645 subjects were included in the primary analysis. During 591,585.3 person-years of follow-up (median: 4.59 years), 4,563 deaths (including 1365 from cardiovascular disease, 788 from stroke and 1514 from cancer) were identified. The mortality density was 7.71 death per 1000 person years. The mean TC was 4.91 mmol/L and mean age at baseline was 58.04 years. Of the eligible subjects, more than half (56.35%) were women. Subjects with higher TC values were older, were more likely to be women and have a higher prevalence of overweight/obesity, and were less commonly smokers and drinkers. Subjects with higher TC values also had higher median levels of systolic and diastolic blood pressure (Table 1).

Cox models with penalized splines revealed a U-shaped association of TC with all-cause mortality and the minimum mortality reached at 5.46 mmol/L (Fig. 1). Compared with subjects with value of 5.46 mmol/L, those with a lower (<4.75 mmol/L) or higher (>6.35 mmol/L) value had a higher risk of all-cause mortality, subjects with values of 4.75–6.35 mmol/l did not predict significantly higher risk of all-cause mortality. For CVD and cancer mortality, our results also showed U-shaped associations, with nadir at 5.04 and 5.51 mmol/L, respectively. For stroke mortality a similar U-shaped association was also observed with nadir at 4.96 mmol/L. However, no typical U-shaped association was observed for CHD mortality ($P_{for\ nonlinear} = 0.075$). Ischemic stroke mortality had a slightly significant U-shape association ($P_{for\ nonlinear} = 0.048$) (Supplementary Figure I).

Table 2 shows results from the categorical analysis of TC level at baseline and risk of all-cause and cause-specific mortality. Compared with the reference group (TC at 4.66–5.17 mmol/L), subjects in the highest TC group(TC > 6.73 mmol/l) had a 32% higher risk of all-cause mortality (HR = 1.32, 95%CI = [1.13–1.55]), 64% higher risk for CVD mortality (HR = 1.64, 95%CI = [1.26–2.15]), 94% higher risk for stroke mortality (HR = 1.94, 95% CI = [1.39–2.70]) and 48% higher risk for cancer mortality (HR = 1.48, 95%CI = [1.13–1.94]). Subjects with lower TC level (<4.66 mmol/l) also had a significant higher risk of all-cause and cause-specific mortality. When considering competing risks, multivariable HRs for cause-specific death did not materially change (Table 2). We observed linear association of TC and mortality when TC was regarded as a continuous variable in lower(<5.18 mmol/l) and higher(≥5.18 mmol/l) range, separately (Supplementary Table I).

Subgroup analysis showed the relationship between TC and all-cause mortality did not materially alter by sex, BMI and blood pressure, smoking and alcohol drinking status. Stronger association was observed for TC and all-cause mortality was either in smokers or subjects aged <60 years (Supplementary Figure II). When age was further categorized by every ten-year increase, we observed typical U-shaped association in the age between 50–60 and 60–70 years. However, the association tended to be L-shaped in the age of 70–80 years (Supplementary Figure III). Sensitive analysis by excluding individuals with extreme BMIs or those who were followed up less than 2 years did not alter our results (Data not shown).

Table 1 Baseline characteristics according to quartiles of total cholesterol.

	Quartile 1 (<4.32) (n = 30,647)	Quartile 2 (≥4.32 to <4.86) (n = 30,606)	Quartile 3 (≥4.86 to <5.45) (n = 30,705)	Quartile 4 (≥5.45) (n = 30,687)	*P* value
Women (n, %)	13,981(45.62)	16,329(53.35)	18,244(59.42)	20,562(67.01)	<0.001
Age(yr; median, IQR)	55.93 (47.35–64.21)	57.67 (49.46–64.7)	58.51 (51.35–64.89)	59.07 (52.94–65.17)	<0.001
Follow-up(yr; median, IQR)	4.80 (4.34–5.53)	4.59 (4.29–5.46)	4.55 (4.16–5.42)	4.54 (4.13–5.37)	<0.001
Systolic BP (mmHg; median, IQR)	127(114–140)	130(118–142)	130(120–145)	134(120–148)	<0.001
Diastolic BP (mmHg; median, IQR)	80(70–86)	80(71–88)	80(73–89)	81(74–90)	<0.001
BMI (kg/m^2; median, IQR)	22.57 (21.08–24.09)	22.77 (21.26–24.34)	22.95 (21.40–24.65)	23.11 (21.60–24.91)	<0.001
BMI (n, %)					<0.001
Underweight	1322(4.31)	1258(4.11)	1149(3.74)	1028(3.35)	
Normal	21,160(69.04)	20,251(66.17)	19,297(62.85)	18,499(60.28)	
Overweight	7242(23.63)	7965(26.02)	8890(28.95)	9581(31.22)	
Obesity	923(3.01)	1132(3.70)	1369(4.46)	1578(5.14)	
TC (mmol/L; median, IQR)	3.95(3.66–4.15)	4.60(4.46–4.73)	5.13(4.99–5.28)	5.89(5.64,6.29)	<0.001
LDL-C (mmol/L; median, IQR)	2.16(1.84–2.46)	2.68(2.37–2.96)	3.07(2.74–3.39)	3.68(3.26–4.11)	<0.001
HDL-C (mmol/L; median, IQR)	1.19(1.02–1.39)	1.29(1.10–1.50)	1.33(1.15–1.56)	1.40(1.20–1.64)	<0.001
Married[a] (n, %)	26,598(86.78)	26,322(86.00)	26,255(85.50)	26,129(85.14)	0.002
Education[a] (n, %)					<0.001
1–6 years	18,311(59.94)	19,595(64.3)	20,410(66.71)	20,849(68.18)	
Middle or high school graduate	11,594(37.95)	10,202(33.48)	9519(31.11)	8974(29.35)	
College or more	36(0.12)	36(0.12)	21(0.07)	21(0.07)	
Others	608(1.99)	643(2.11)	645(2.11)	737(2.41)	
Smoking[a](n, %)					<0.001
Current	7513(24.61)	6530(21.45)	5794(18.95)	4962(16.24)	
Former	1889(6.19)	1767(5.80)	1727(5.65)	1584(5.19)	
Never	21,124(69.20)	22,148(72.75)	23,059(75.41)	24,002(78.57)	
Drinking[a] (n, %)					<0.001
Often	5245(17.19)	4992(16.36)	4615(15.12)	4034(13.22)	
Occasional	2095(6.86)	1758(5.76)	1591(5.21)	1369(4.49)	
Never	23,179(75.95)	23,680(77.59)	24,357(79.81)	25,131(82.35)	
Physical activity[a] (n, %)					<0.001
More than 4 times/week	11,020(36.39)	10,812(35.78)	10,979(36.2)	11,009(36.34)	
1–4 times/week	10,276(33.94)	10,735(35.53)	10,816(35.66)	10,995(36.29)	
Less than one time/week	8985(29.67)	8668(28.69)	8537(28.15)	8293(27.37)	

IQR, Interquartile Range; BMI, body mass index.
[a] Missing values for marital status (n = 930), education (n = 443), smoking (n = 545), drinking (n = 595) and physical activity (n = 1519).

Secondary analysis

A total of 32,237 subjects were included in the secondary analysis for the association between TC variability and risk of mortality. During a median follow-up of 4.25 years, 1065 deaths were observed. The mortality density was 8.05 deaths per 1000 person years. Survival curve showed there was no significant difference for survival rates between individuals in the primary and secondary analysis(Supplementary Figure IV). The mean TC value was 4.93 mmol/l. The CV values of TC in the quartile groups were (3.28 ± 1.22)%, (6.53 ± 0.84)%, (9.70 ± 1.06)% and(16.84 ± 1.24)%, respectively.

Table 3 presents HRs and 95% CIs for the associations between TC variability and risk of all-cause, CVD and cancer mortality. Compared with the lowest quartile for CV, subjects in the highest quartile had 21% higher risk of all-cause mortality, 41% higher risk of CVD mortality, with evidence of statistically significant monotonic trends (P_{trend} = 0.024; P_{trend} = 0.010, respectively). However, no association was observed for TC variability and cancer mortality. Similar results were seen for other metrics of variability (SD, VIM, and ASV) (Table 3, Supplementary Figure V).

Fig. 2 displays the results from subgroup and sensitive analyses. Positive association for TC variability and all-cause mortality existed either in men or subjects aged ≥60 years or those with hypertension or those with three TC measurements. Similar results were observed for CVD mortality, but no associations were observed for cancer mortality in any subgroup. Exclusion of subjects who were followed up less than 2 years did not alter our findings.

Discussion

This population-based study examined the association between baseline level and intra-individual variability of TC and risk of mortality in the general Chinese population. Overall, individuals with relatively lower or higher TC level had higher risk of all-cause mortality. The magnitude of HRs varied for cause-specific deaths, and the strongest

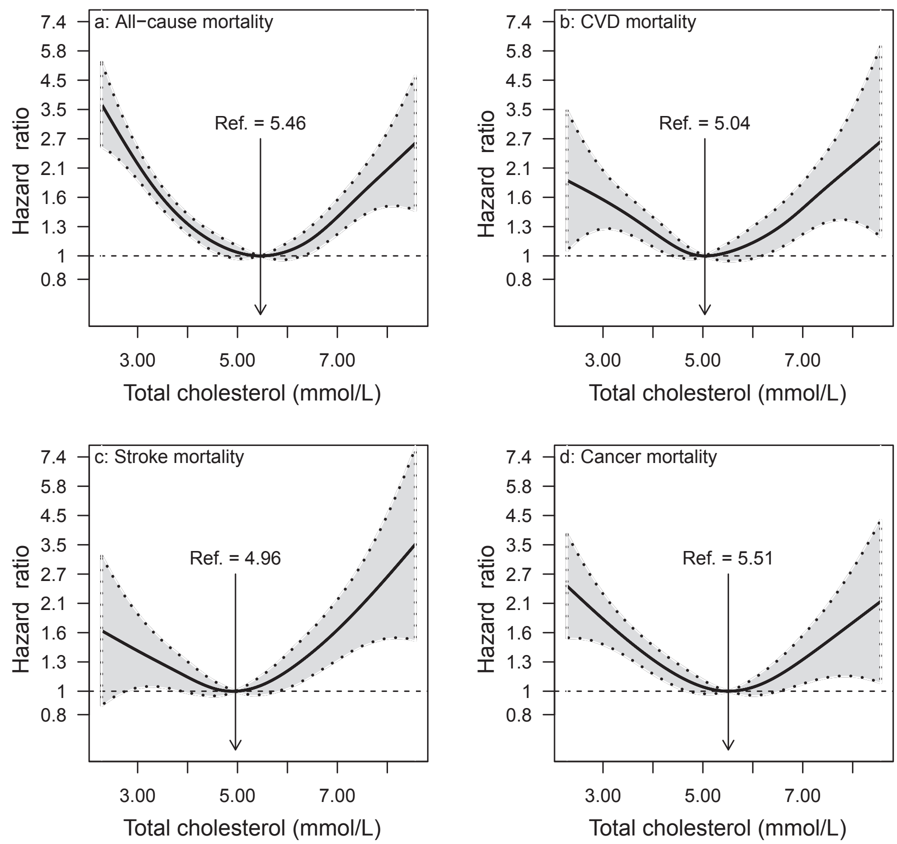

Figure 1 Associations of total cholesterol with all-cause and cause-specific mortality in Cox models with penalized splines. Adjusted for age at baseline (as a linear term), sex, BMI, systolic blood pressure (as a linear term), marital status, education level, tobacco smoking, alcohol drinking and physical activity. CVD, cardiovascular disease. Solid lines = estimated hazard ratio for TC and risk of the mortality; dotted lines = 95% confidence intervals.

association was seen for stroke mortality. Subgroup analysis by age revealed U-shaped relationship between TC and risk of mortality among subjects aged between 50–70 years, and the association became weakened among subjects aged over 70 years. In the second analysis, TC variability was positively associated with risk of total and CVD mortality. Sensitivity analyses by excluding subjects who were followed less than two years did not change these findings.

The relationship between cholesterol and mortality has been of interest for epidemiologists and health professionals for several decades. Although high serum cholesterol, especially high LDL-C level, was regarded as established risk factor for cardiovascular disease, results for the associations between serum cholesterol and risk of mortality remain inconsistent. The Framingham study reported that high cholesterol levels were directly associated with 30-year overall mortality and CVD mortality among subjects under 50 years, and this relationship were not observed for participants aged over 50 years [4]. Some studies conducted in the elderly have showed inverse association for higher total cholesterol levels and decreased all-cause mortality, mainly due to the reduced risk of non-cardiovascular mortality [7,10]. Other studies have also reported inverse association between TC and mortality in general population [12,13]. The inconsistent conclusions from different studies may result from heterogeneous characteristics of population, different categories of total cholesterol and TC fluctuation. Corti and his colleagues suggested that frailty (or disease) in the elderly was more likely to contribute to increased mortality than low cholesterol alone [27]. In addition, limited number of category by quartiles or clinical cut-offs (the guideline for dyslipidemia judgement) may not have enough efficiency to distinguish individuals who have exactly higher risk of mortality. In our study, we divided TC into 8 categories by every 0.51 mmol/L(20 mg/dl) increase, and observed that individuals with high TC level, especially for subjects with TC concentrations more than 6.73 mmol/L, had an elevated risk of mortality. Moreover, results may be influenced by TC variability because lipid concentration actually fluctuated substantially in the body even on day-to-day basis, but most studies only used one measurement of TC as the baseline level. Considering of this point, we used the mean value of TC during the baseline period for each individual as their baseline TC level, which differed from designs of other studies.

Current guidelines for the management of dyslipidemia only provide the reference for high level of cholesterol [28,29], while our study found that low cholesterol can also increase the risk of mortality, which suggested the current TC target could be supplemented, especially

Table 2 Hazard ratios (HRs) and 95% confidence intervals (CIs) across 8 categories of total cholesterol for all-cause and cause-specific mortality.

Cause of death(ICD-10)	Total cholesterol (TC) mmol/L[a]							
	<3.63	≥3.63 to<4.14	≥4.14 to< 4.66	≥4.66 to<5.18	≥5.18 to<5.70	≥5.70 to<6.22	≥6.22 to <6.73	≥6.73
All cause								
N	8516	16,570	26,361	28,064	20,774	12,154	5819	4387
No.of death	457	729	984	917	679	410	201	186
No.of death per 1000 person years	10.93	8.98	7.69	6.78	6.83	7.10	7.33	8.99
Age and sex adjusted HR(95% CI)	1.46 (1.30–1.63)	1.30 (1.18–1.43)	1.15 (1.05–1.26)	1.00(Ref)	1.02 (0.92–1.12)	1.06 (0.94–1.19)	1.08 (0.93–1.26)	1.29 (1.10–1.51)
Multivariable HR (95% CI)[b]	1.41 (1.26–1.57)	1.27 (1.16–1.4)	1.13 (1.03–1.23)	1.00(Ref)	1.01 (0.92–1.12)	1.06 (0.95–1.19)	1.10 (0.94–1.28)	1.32 (1.13–1.55)
CVD								
Age and sex adjusted HR(95% CI)	1.34 (1.07–1.67)	1.41 (1.17–1.69)	1.19 (1.01–1.41)	1.00(Ref)	1.15 (0.96–1.38)	1.23 (1.00–1.52)	1.27 (0.97–1.66)	1.62 (1.24–2.12)
Multivariable HR (95% CI)[b]	1.31 (1.05–1.63)	1.39 (1.16–1.67)	1.19 (1.00–1.41)	1.00(Ref)	1.15 (0.96–1.37)	1.24 (1.01–1.53)	1.28 (0.98–1.68)	1.64 (1.26–2.15)
Multivariable HR (95% CI) competing risk[c]	1.24 (0.99–1.55)	1.38 (1.15–1.66)	1.17 (0.99–1.39)	1.00(Ref)	1.15 (0.96–1.38)	1.23 (1.00–1.52)	1.28 (0.97–1.68)	1.65 (1.26–2.16)
Stroke								
Age and sex adjusted HR(95% CI)	1.17 (0.86–1.57)	1.31 (1.03–1.67)	1.14 (0.91–1.42)	1.00(Ref)	1.18 (0.94–1.50)	1.31 (1.00–1.71)	1.29 (0.91–1.84)	1.91 (1.37–2.66)
Multivariable HR (95% CI)[b]	1.14 (0.84–1.54)	1.3 (1.02–1.66)	1.14 (0.91–1.42)	1.00(Ref)	1.17 (0.92–1.48)	1.31 (1.00–1.72)	1.3 (0.92–1.86)	1.94 (1.39–2.70)
Multivariable HR (95% CI) competing risk[c]	1.06 (0.78–1.44)	1.28 (1.01–1.64)	1.12 (0.89–1.40)	1.00(Ref)	1.18 (0.93–1.49)	1.3 (0.99–1.71)	1.29 (0.91–1.85)	1.94 (1.39–2.70)
Cancer								
Age and sex adjusted HR(95% CI)	1.33 (1.09–1.63)	1.41 (1.19–1.66)	1.15 (0.99–1.35)	1.00(Ref)	1.02 (0.85–1.21)	1.04 (0.85–1.28)	1.12 (0.86–1.47)	1.44 (1.09–1.89)
Multivariable HR (95% CI)[b]	1.29 (1.05–1.58)	1.38 (1.17–1.63)	1.14 (0.97–1.33)	1.00(Ref)	1.02 (0.85–1.21)	1.05 (0.86–1.30)	1.14 (0.87–1.49)	1.48 (1.13–1.94)
Multivariable HR (95% CI) competing risk[c]	1.26 (1.03–1.54)	1.37 (1.16–1.62)	1.13 (0.97–1.32)	1.00(Ref)	1.02 (0.86–1.22)	1.06 (0.86–1.30)	1.14 (0.87–1.50)	1.46 (1.11–1.92)

CVD, cardiovascular disease; ICD-10, International Classification of Disease, 10th Revision.

[a] Increased by every 0.5 mmol/L, equivalent to 20 mg/dl.

[b] Adjusted for age at baseline, sex, BMI, systolic blood pressure, marital status, education level, tobacco smoking, alcohol drinking and physical activity.

[c] Using Fine–Gray competing risks regression models, adjusted for age at baseline, sex, BMI, systolic blood pressure, marital status, education level, tobacco smoking, alcohol drinking and physical activity.

Table 3 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for total, cardiovascular and cancer mortality, by quartiles of total cholesterol variability (SD, CV, VIM and ASV).

Causes of death (ICD-10)		Multivariate HR(95% CI)[a]				P_{trend}
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
All-cause	CV	1.00 (Ref)	0.95(0.82−1.10)	0.89(0.77−1.04)	**1.21(1.05−1.40)**	**0.024**
	SD	1.00 (Ref)	0.87(0.75−1.01)	0.98(0.85−1.13)	**1.22(1.06−1.42)**	**0.005**
	VIM	1.00 (Ref)	0.94(0.81−1.09)	0.96(0.82−1.11)	**1.22(1.06−1.41)**	**0.008**
	ASV	1.00 (Ref)	0.92(0.80−1.06)	0.94(0.82−1.08)	**1.25(1.10−1.43)**	**0.003**
CVD	CV	1.00(Ref)	0.93(0.71−1.23)	0.94(0.71−1.23)	**1.41(1.10−1.81)**	**0.010**
	SD	1.00 (Ref)	0.82(0.62−1.09)	1.04(0.80−1.35)	**1.49(1.16−1.92)**	**0.001**
	VIM	1.00 (Ref)	0.89(0.68−1.17)	0.99(0.76−1.30)	**1.43(1.11−1.84)**	**0.004**
	ASV	1.00 (Ref)	0.99(0.76−1.28)	1.08(0.85−1.39)	**1.50(1.18−1.91)**	**0.004**
Cancer	CV	1.00 (Ref)	0.95(0.74−1.23)	0.92(0.71−1.18)	0.93(0.72−1.21)	0.546
	SD	1.00 (Ref)	0.90(0.70−1.16)	1.02(0.80−1.31)	0.91(0.70−1.19)	0.732
	VIM	1.00 (Ref)	0.99(0.77−1.28)	1.01(0.78−1.29)	0.96(0.74−1.24)	0.796
	ASV	1.00 (Ref)	0.85(0.69−1.06)	0.89(0.72−1.10)	1.03(0.84−1.28)	0.936

The bold entries are considered statistically significant.
[a] Adjusted for age at baseline, sex, BMI, systolic blood pressure, marital status, education level, tobacco smoking, alcohol drinking, physical activity and mean total cholesterol.

in the elderly. Our findings of optimal TC values with the lowest mortality were comparable to a previous study in Korea. A nationwide prospective cohort study of 503,340 Korean adults has reported a U-curve associations for TC and stroke mortality with a nadir at 200−219 mg/dL(equivalent to 5.18−5.70 mmol/L) [30]. Regrettably, we failed to observe significant association between TC and risk of CHD mortality. This result may be caused by insufficient statistical power due to relatively small death cases from CHD.

Our findings of the association between high variability of TC and risk of all-cause and CVD mortality could provide new supportive evidence that fluctuations in serum cholesterol may be new biometrics for health outcomes [15−20]. A recent study assessed intra-individual variability in fasting HDL-C, triglyceride, and LDL-C measurements among 9572 patients in a clinical trial and the results indicated that measures of visit-to-visit variability of triglycerides, HDL-C, and LDL-C were independent predictors of coronary and CV events [16]. In our study, we examined the association among general population with four measures of variability (ASV, SD, CV, and VIM), in which VIM was not correlated with mean TC value, suggesting intra-individual variability of TC was an independent predictor for all-cause and CVD mortality. The hazard ratio for high TC variability and all-cause mortality in our study was close to the result of a nationwide study conducted in Korea, which reported a HR of 1.26 (1.24−1.28) for CV of TC and all-cause mortality (Q4 vs Q1) [19]. Several possible explanations have been proposed to underlie the mechanism of increased risk at higher levels of TC variability. One common explanation is that high cholesterol variability may increase the fluctuations in the composition of atherosclerotic plaques [31,32], influence the plaque stability and finally lead to plaque rupture, which is a main pathological mechanism for myocardial infarction and stroke. In addition, changes in cholesterol can also cause endothelial dysfunction that was regarded as a mediator for atherosclerosis [33−35]. Moreover, cholesterol variability might be simply a risk marker for some pathological progress leading to adverse outcomes. Cholesterol variability can also be linked to use of or non-adherence to lipid-lowering agents and other types of medication. Unfortunately, we were not able to collect information on the use of lipid-lowering medication to distinct whether the fluctuations of TC were caused by non-adherence to agents or other reasons. However, the previous study of a clinical trial where patients tended to be more compliant with treatment indicated that noncompliance was a poor explanation for cholesterol variability [16] and another study showed the variability measures were predictive of events even after controlling for medication adherence [15].

Our study had several strengths and optimizations including large sample size, proper statistical model and TC variability measurements. However, limitations of our study should be noted. Firstly, there may be a concern that individuals with repeated TC measurements might be more sick and were not comparable to the entire population. But we found the mean TC of individuals in secondary analysis (4.93 mmol/L) was very close to that in the primary analysis(4.91 mmol/L), and the mortality rate was slightly higher than the entire population, with nearly overlapping survival curves for these two populations (Supplementary Figure IV). We believe that individuals in the secondary analysis were not highly selected and could be used for analysis. Secondly, most subjects for the analysis of TC variability had only three measurements, which may not be representative as the variability. However, we additionally conducted a sensitive analysis by evaluating participants with three TC measurements and those with more than three TC measurements separately, and our results did not materially alter. Thirdly, our study did not collect information on the use of lipid-lowering medication, which could affect the results both in the primary and secondary analyses. Nevertheless, our study reflected the associations of TC levels (at baseline and TC variability) with mortality in a real-world status, whether

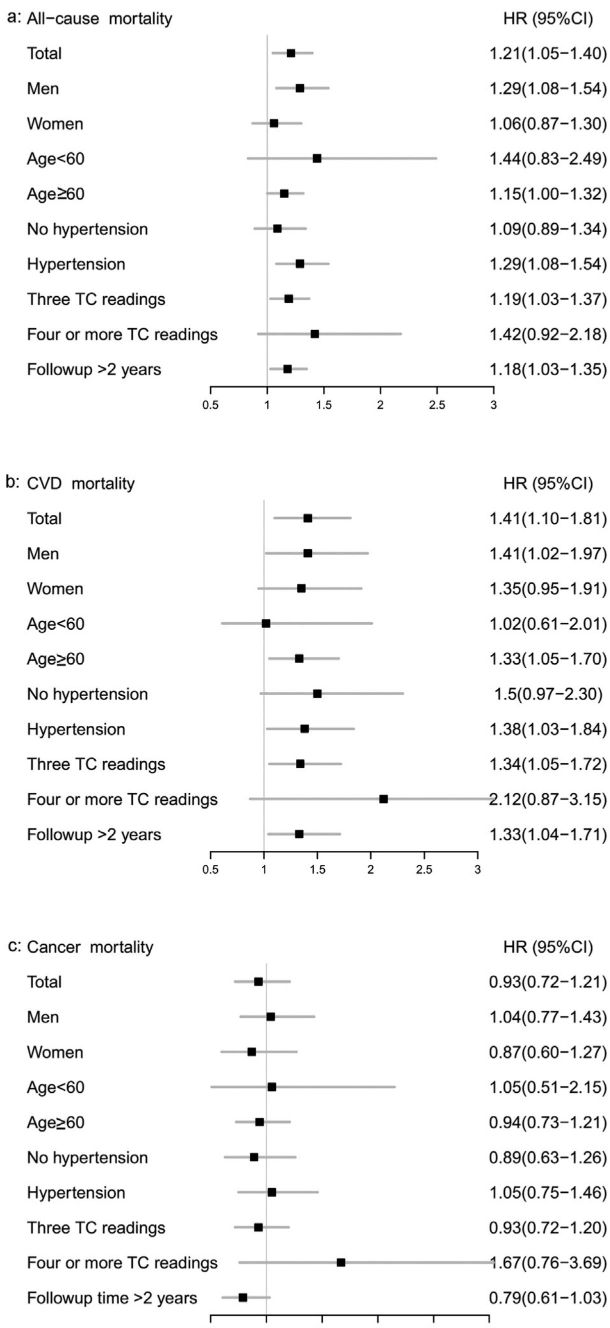

Figure 2 Hazard ratios and 95% confidence intervals for all-cause, CVD and cancer mortality in the highest quartile compared with the lowest quartile of total cholesterol variability (coefficient of variation) in subgroups. Adjusted for age, sex, BMI, systolic blood pressure, marital status, education level, tobacco smoking, alcohol drinking, physical activity and mean total cholesterol.

individuals used the lipid-lowering medications or not. If the optical or stable TC level was maintained by lipid-lowering medications, it indicated the benefit effect of treatments. On the other hand, if high variability of TC was caused by non-adherence to agents or other reasons, it illustrated the adverse effect of these unhealthy behaviors conversely. Fourthly, several factors such as diet and genetics were not measured in our study, which could affect our results. Fifthly, since death was identified as our main outcome, our follow-up duration was relatively short (maximum follow-up: 7.64 years). Finally, subjects in our study were enrolled from one district in Ningbo city, which may not represent our target population. Unfortunately, we cannot conduct a multicenter study due to lack of data from other districts.

Conclusion

In summary, we observed a U-shaped relationship of TC level with risk of all-cause, CVD (especially stroke) and cancer mortality in this Chinese population, indicating that lower or higher serum cholesterol levels could both increase adverse outcomes. We also demonstrated that high level of TC variability was associated with all-cause and CVD mortality. Our findings suggest that TC variability may be an important risk factor in the general population. Future studies are needed to confirm reducing variability of lipid parameters decreases adverse outcomes.

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Ethics Committee of Zhejiang University has approved the research plan and consent has been formally obtained from all patients or their guardians.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.07.007.

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