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Serum High-Sensitivity C-Reactive Protein Levels and the Risk of Atrial Fibrillation in Japanese Population: the Circulatory Risk in Communities Study

Mari Tanaka[1], Hironori Imano[1, 2], Yasuhiko Kubota[2], Kazumasa Yamagishi[2, 3, 4], Mitsumasa Umesawa[2, 3, 5], Isao Muraki[1, 2], Renzhe Cui[1], Mina Hayama-Terada[2, 6], Yuji Shimizu[2], Takeo Okada[2], Tetsuya Ohira[7], Tomoko Sankai[8], Takeshi Tanigawa[9], Shinichi Sato[10], Akihiko Kitamura[1, 2, 11], Masahiko Kiyama[2], Hiroyasu Iso[1, 2, 3, 9] and the CIRCS Investigators

[1]Public Health, Department of Social Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
[2]Osaka Center for Cancer and Cardiovascular Disease Prevention, Osaka, Japan
[3]Department of Public Health Medicine, Faculty of Medicine, and Health Services Research and Development Center, University of Tsukuba, Ibaraki, Japan
[4]Ibaraki Western Medical Center, Ibaraki, Japan
[5]Dokkyo Medical University School of Medicine, Tochigi, Japan
[6]Yao Public Health Center, Yao City Office, Osaka, Japan
[7]Department of Epidemiology, School of Medicine, Fukushima Medical University, Fukushima, Japan
[8]Department of Public Health and Nursing, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan
[9]Department of Public Health, Juntendo University Graduate School of Medicine, Tokyo, Japan
[10]Chiba Prefectural Institute of Public Health, Chiba, Japan
[11]Research Team for Social Participation and Community Health, Tokyo Metropolitan Institute of Gerontology. Tokyo, Japan

Aim: This study aimed to investigate the association between the serum high-sensitivity C-reactive protein (hs-CRP) levels and incident atrial fibrillation risk in the general Japanese population, who have lower hs-CRP levels than the Western population, and assess whether the association is modified by sex, overweight, hypertension, and smoking status.

Methods: We conducted a prospective study in 6517 Japanese men and women aged 40–79 years without atrial fibrillation at baseline and enrolled in the Circulatory Risk in Communities Study (2002–2008). The hs-CRP levels were measured using the latex particle-enhanced immunonephelometric assay. Atrial fibrillation was identified using standard 12-lead electrocardiograms and information on physician-diagnosed atrial fibrillation history from the follow-up surveys. We used a Cox proportional hazard regression stratified by community.

Results: During a median follow-up of 11 years, 127 new cases of atrial fibrillation (74 and 53 cases among men and women, respectively) were found. Compared to the lowest quintile of hs-CRP levels, the multivariable hazard ratios (95% confidence intervals) were 2.54 (1.17–5.50), 2.28 (1.06–4.93), 2.92 (1.37–6.23), and 2.77 (1.30–5.91) for the second, third, fourth, and fifth (highest) quintiles, respectively. There was no significant effect modification by sex, overweight, hypertension, and smoking status (*P* for interaction >0.05).

Conclusions: Elevated hs-CRP levels were significantly associated with increased risk of atrial fibrillation in the Japanese population. The association of hs-CRP levels with incident atrial fibrillation did not vary according to sex, overweight, hypertension status, or smoking status.

Key words: C-reactive protein, Atrial fibrillation, Risk factor, Cohort study

Address for correspondence: Hiroyasu Iso, Public Health, Graduate School of Medicine Osaka University, 2-2, Yamada-oka, Suita city, Osaka 565-0871, Japan.
E-mail: iso@pbhel.med.osaka-u.ac.jp

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Introduction

Atrial fibrillation, the most common cardiac arrhythmia in clinical practice, can lead to stroke, heart failure, and other cardiovascular complications[1, 2] and consequently contribute to considerably high mortalities[3, 4]. Over a million individuals are expected to be affected with atrial fibrillation by 2050 because of population aging in Japan[5, 6]. Therefore, it is important to identify populations at a high risk of developing atrial fibrillation.

In our prospective nested case-control study of a Japanese population, we found that the levels of high-sensitivity C-reactive protein (hs-CRP), a major biomarker of systemic inflammation and atherosclerosis[7], was associated with the risk of ischemic stroke[8]. Considering that atrial fibrillation is a common cause of ischemic stroke, the hs-CRP levels could be a predictive marker of atrial fibrillation. In fact, previous cohort studies have reported that the hs-CRP levels predicts atrial fibrillation[9-15]. However, the pathophysiological link between inflammation and the risk of atrial fibrillation remains unclear. Furthermore, only a few long-term population-based cohort studies have examined the association[9-13], and there is limited evidence among Asian populations[14, 15], which have lower levels of hs-CRP than Western populations[16, 17]. It has not been well investigated whether the association is modified by sex, overweight, hypertension, and smoking status, although hs-CRP levels vary with these factors[16-18].

To examine the association between the serum hs-CRP levels and risk of atrial fibrillation and to assess the interactions by sex, overweight, hypertension, and smoking status on the association, we conducted a long-term prospective study among Japanese men and women without atrial fibrillation enrolled in the Circulatory Risk in Communities Study (CIRCS).

Methods

Study Populations

The CIRCS is a community-based dynamic cohort study of cardiovascular risk factors in Japan, the details of which have been previously described[19, 20]. The present study initially included 8257 participants aged 40–79 years in three communities under the CIRCS—Ikawa town, Akita Prefecture (a northwest rural community); Minami-Takayasu district, Yao City, Osaka Prefecture (a mid-western suburb); and Kyowa district, Chikusei City, Ibaraki Prefecture (a mid-eastern rural community); baseline surveys were conducted in 2002–2007 in Ikawa, 2003–2008 in Minami-Takayasu, and 2002 in Kyowa. Initially, individuals were excluded if they had missing data on the hs-CRP levels ($n=2$) or had hs-CRP levels of ≥ 10.0 mg/L ($n=160$), had atrial fibrillation ($n=89$) or cardiovascular disease including stroke and heart disease ($n=783$), or had never undergone the annual health checkup after the beginning of the follow-up period in the present study ($n=706$). Finally, a total of 6517 individuals (2,434 men and 4,083 women) were enrolled in the present study (**Fig. 1**). The protocol was approved by the Ethics Committees of Osaka University, University of Tsukuba, and Osaka Center for Cancer and Cardiovascular Disease Prevention.

Definition of Atrial Fibrillation and Follow Up

The annual cardiovascular disease risk surveys were conducted at each of the healthcare centers in the three communities during follow-up. The subjects were followed-up to determine the first incident of atrial fibrillation in the annual survey by the end of 2017 in Kyowa, 2018 in Ikawa, and 2019 in Minami-Takayasu, respectively. Standard 12-lead electrocardiograms (ECGs) were obtained from all participants in the supine position by well-trained technicians and coded using the Minnesota Code by well-trained physician-epidemiologists. Histories of physician-diagnosed atrial fibrillation were obtained by well-trained public health nurses. Atrial fibrillation was defined using Minnesota Codes 8-3-1 and/or information on history of physician-diagnosed atrial fibrillation from the surveys. We did not discriminate between paroxysmal and persistent atrial fibrillation. History of atrial fibrillation may include paroxysmal atrial fibrillation and a remote event of atrial fibrillation which had converted into sinus rhythm medically or through ablation procedures.

Baseline Examination

Blood was collected from the participants into plastic serum separator gel tubes while in the seated position. The serum was separated by centrifugation within 30 min of blood collection. The serum samples were placed on dry ice at the survey sites in Ikawa and Kyowa and stored at −80℃ until analysis. In Minami-Takayasu, the serum samples were stored under refrigeration until analysis and measured within a few days after the collection. The hs-CRP levels were measured in two laboratories, including the Osaka Medical Center for Health Science and Promotion (OMC), using the latex particle-enhanced immunonephelometric assay (BN ProSpec and BN II; Dade Behring Inc., Tokyo, Japan). The measurement accuracy of these instruments was validated using the international certified reference material for hs-CRP[21] in the standardized CRP program at the OMC[22]. The measurement

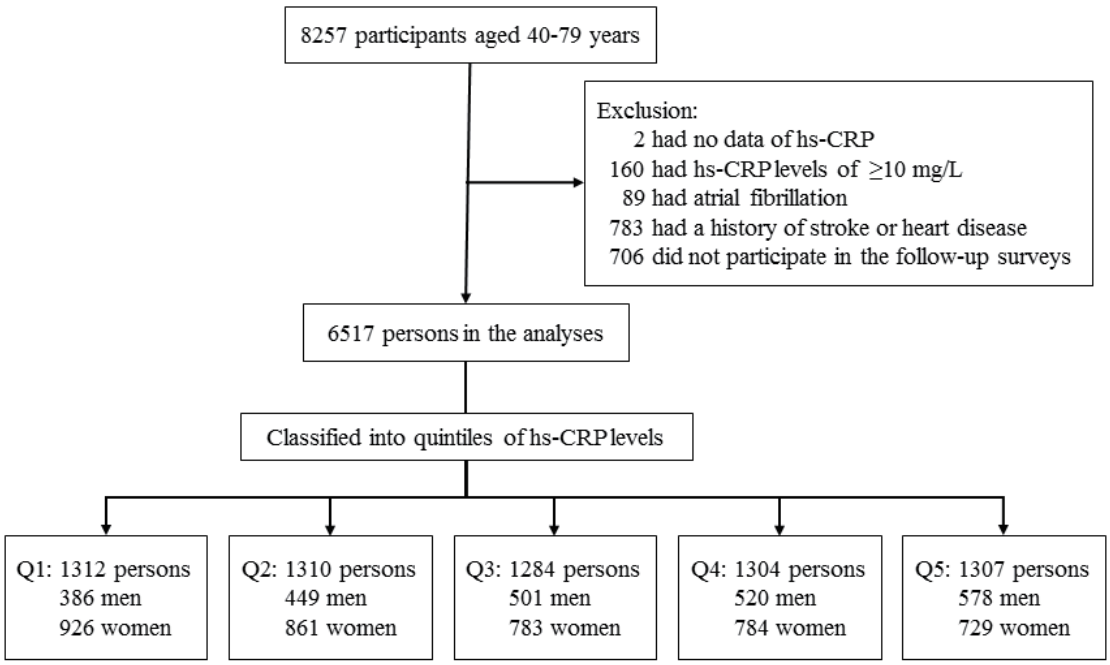


Fig. 1. Flow chart for selection of the study participants

limit range of the hs-CRP levels among the annual risk surveys was 0.149–0.170 mg/L; we treated the value of <0.170 mg/L as 0.170 mg/L in the present study. The body mass index (BMI) was calculated as weight in light clothing (kg) divided by height squared in stocking feet (m^2). Blood pressures in the right arm were measured by trained technicians using standard mercury sphygmomanometers and unified epidemiological methods. Hypertension was defined as systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg. Serum total cholesterol, triglycerides, HDL-cholesterol, and glucose levels were measured using enzymatic methods. The hemoglobin A1c (HbA1c) levels was measured using the latex agglutination immunoassay. When the triglycerides levels was less than 4.5 mmol/L (400 mg/dL), LDL-cholesterol was estimated by the Friedewald's formula[23], as follows: Friedewald-estimated LDL-cholesterol = total cholesterol–(HDL-cholesterol + triglycerides/5). Borderline diabetes mellitus was defined as follows: (1) fasting serum glucose levels of 6.1–6.9 mmol/L (110–125 mg/dL), (2) non-fasting serum glucose levels of 7.7–11.1 mmol/L (140–199 mg/dL), and/or (3) HbA1c levels of 6.0%–6.4% (National Glycohemoglobin Standardization Program [NGSP]). Diabetes mellitus was defined as follows: (1) fasting serum glucose levels of ≥ 7.0 mmol/L (≥ 126 mg/dL), (2) non-fasting serum glucose levels of ≥ 11.1 mmol/L (≥ 200 mg/dL), (3) HbA1c levels of ≥ 6.5% (NGSP), and/or (4) initiation of therapeutic medicine for diabetes mellitus. The fasting state showed at least 8 hours after meals. Dyslipidemia was defined as follows: (1) Friedewald-estimated LDL-cholesterol of ≥ 3.6 mmol/L (≥ 140 mg/dL), (2) HDL-cholesterol < 1.0 mmol/L (<40 mg/dL), (3) triglycerides ≥ 1.7 mmol/L (≥ 150 mg/dL), and/or (4) medication use for dyslipidemia. Each participant was interviewed to determine the number of cigarettes smoked per day, the weekly alcohol consumption in go-units (a Japanese traditional unit of volume equivalent to 23 g of ethanol), postmenopausal status for women, and medication use for hypertension, diabetes mellitus, and dyslipidemia.

Statistical Analyses

The values of the baseline characteristics according to the quintiles of hs-CRP levels were reported as means ± standard deviations for continuous variables and as percentages for categorical variables. And we used the analysis of covariance and the Cochran–Mantel–Haenszel test to compare among the quintiles of hs-CRP levels after adjustment for age, sex, and community. Person-years were calculated from the date of the baseline survey until diagnosis of atrial fibrillation or the final survey during follow-up, whichever came first.

Differences in the estimated cumulative hazard of atrial fibrillation according to the hs-CRP quintiles during follow-up were displayed using Kaplan–Meier curves with the log-rank test. The hazard ratios (HRs)

Table 1. Baseline characteristics of persons according to the quintiles of hs-CRP levels

	Quintiles of hs-CRP levels					*P* value for difference*
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median hs-CRP (range), mg/L	0.17 (0.17-0.19)	0.28 (0.20-0.36)	0.48 (0.37-0.60)	0.80 (0.61-1.16)	2.04 (1.17-9.83)	
No. of persons	1312	1310	1284	1304	1307	
Men, %	29.4	34.3	39.0	39.9	44.2	<0.001
Age, years	56.7 ± 10.1	58.7 ± 9.5	60.4 ± 9.1	60.4 ± 9.3	61.3 ± 9.4	<0.001
Body mass index, kg/m^2	21.8 ± 2.7	22.9 ± 2.8	23.6 ± 2.9	24.3 ± 3.2	24.8 ± 3.5	<0.001
Systolic blood pressure, mmHg	124.9 ± 16.8	127.6 ± 16.8	130.5 ± 16.4	132.0 ± 17.4	133.2 ± 16.9	<0.001
Diastolic blood pressure, mmHg	76.2 ± 10.3	77.5 ± 10.5	78.7 ± 10.6	79.6 ± 10.8	80.0 ± 10.6	<0.001
Use of antihypertensive medication, %	11.4	15.2	20.3	24.2	26.6	<0.001
Hypertension, %	25.7	32.0	40.3	45.9	49.7	<0.001
HbAlc, %	5.3 ± 0.7	5.4 ± 0.7	5.4 ± 0.7	5.5 ± 0.8	5.6 ± 0.9	<0.001
Serum glucose, mg/dL	100.0 ± 19.9	102.0 ± 25.7	103.4 ± 24.2	103.7 ± 22.0	105.4 ± 27.0	0.03
Use of glucose-lowering medication, %	2.5	3.4	3.8	3.3	4.7	0.41
Diabetes mellitus, %	4.7	6.9	7.9	8.9	11.2	<0.001
Serum total cholesterol, mg/dL	210.4 ± 34.6	215.4 ± 34.0	218.0 ± 34.8	219.6 ± 37.0	214.7 ± 36.3	<0.001
Serum triglycerides, mg/dL	88.3 ± 51.7	110.1 ± 76.1	119.0 ± 88.7	132.5 ± 84.3	134.6 ± 94.4	<0.001
Use of lipid-lowering medication, %	5.5	6.7	10.1	8.7	10.3	0.001
Dyslipidemia, %	40.5	52.5	58.5	63.0	62.4	<0.001
Current smokers, %	14.1	18.2	19.7	19.1	25.3	<0.001
Ethanol intake, g/day	7.7 ± 15.7	8.6 ± 17.2	10.5 ± 18.7	10.5 ± 19.6	11.4 ± 20.2	0.03
Postmenopausal among women, %	61.1	75.4	80.8	81.9	84.1	<0.001

Values are reported as mean ± standard deviation or percentage.
*Adjusted for age, sex, and communities.

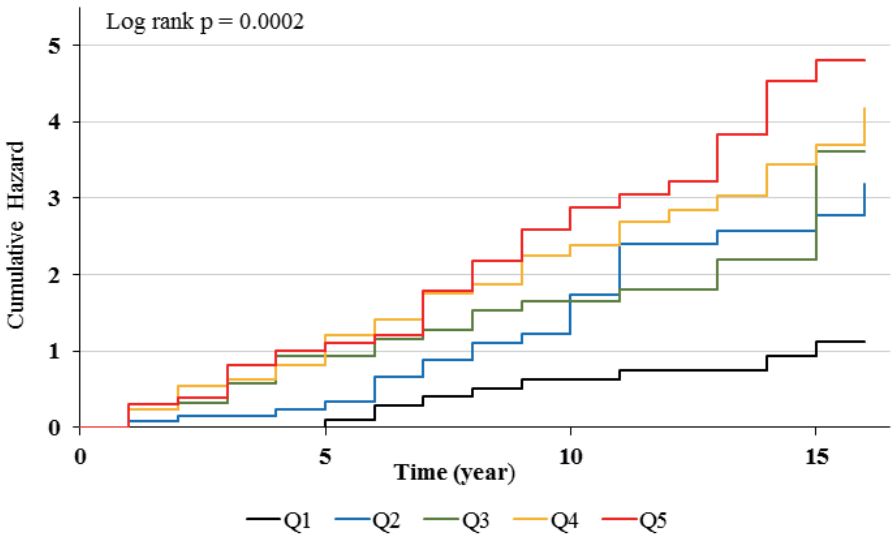
and 95% confidence intervals (CIs) for atrial fibrillation were calculated with reference to the lowest quintile of the hs-CRP levels using a Cox proportional hazard regression model stratified by community, and adjusted for age and sex and for other confounding variables, including BMI (kg/m^2), hypertension (yes or no), serum total cholesterol levels (mg/dL), serum triglycerides levels (mg/dL), glucose levels category (normal, borderline, or diabetes mellitus), smoking status (non-current or current), alcohol intake status (never, former, <23 g/day, 23–46 g/day, 46–69 g/day, or ≥ 69 g/day), use of antihypertensive medication (yes or no), and dyslipidemia treatment use (yes or no). The reasons for the selection of the variables in the multivariable adjustment were as follows: (1) they were generally used in the previous studies which investigated the association between hs-CRP levels and the risk of atrial fibrillation[9-15] and/or (2) they were themselves associated with risk of atrial fibrillation in each of univariate analyses by a Cox proportional hazard regression model in the present study.

The tests for the effect modification by sex, BMI (<25 or ≥ 25 kg/m^2), blood pressure (non-hypertension or hypertension), and smoking status (non-current or current) were conducted with interaction terms generated by multiplying the dummy variable of hs-CRP in each quintile by sex (0 or 1), overweight (0 or 1), hypertension (0 or 1), or smoking status (0 or 1). P-values for the interactions were calculated by the likelihood ratio tests. Moreover, we calculate the HRs and 95% CIs stratified by these factors in the multivariable regression models with each interaction term via the hazardratio statement in proc phreg from the SAS Institute.

All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). *P*-values of < 0.05 were considered to indicate statistical significance on two-tailed analyses.

Results

Table 1 shows the baseline characteristics of 6,517 individuals (2,434 men and 4,083 women) according to the quintiles of the hs-CRP levels. The median hs-CRP levels was 0.47 mg/L (0.54 mg/L in men and 0.43 mg/L in women). Compared with individuals in the lowest quintile of hs-CRP levels, those who were in the second and higher quintiles of hs-CRP levels were more likely to be male, older, smoker, drinker, and high-risk individuals who suffered from hypertension, diabetes mellitus, and dyslipidemia, and to have higher means of BMI and the higher propor-



Quintiles of hs-CRP levels	No. of at risk (No. of event)			
	Baseline	5 year	10 year	15 year
Q1	1312	1085 (1)	814 (6)	507 (9)
Q2	1310	1054 (4)	772 (16)	465 (23)
Q3	1284	1008 (11)	681 (17)	411 (26)
Q4	1304	1029 (14)	716 (24)	396 (31)
Q5	1307	989 (13)	653 (27)	348 (36)

Fig. 2. Kaplan-Meier curves for the cumulative hazard of atrial fibrillation according to the quintiles of hs-CRP levels

tion of postmenopausal women.

The follow-up rate in each subsequent year was 83% in the 1st year to 66% in the 5th year and 45% in the 11th year (median follow-up year). During 65,022 person-years of follow-up (median follow-up of 11 years), a total of 127 incident atrial fibrillation events occurred; 121 (95.3%) events diagnosed by ECG and 6 (4.7%) by self-reported history of physician-diagnosed atrial fibrillation from the follow-up surveys. Of those who were newly diagnosed with atrial fibrillation during follow-up, 58.3% were male.

The cumulative hazard for atrial fibrillation according to the hs-CRP levels quintiles is shown using Kaplan–Meier curves in **Fig. 2**. The highest quintile of the hs-CRP levels was associated with the highest cumulative hazard rate of atrial fibrillation development, whereas the cumulative hazard rate in the lowest quintile remained low throughout the study period. The difference in the cumulative hazard of atrial fibrillation among the hs-CRP levels quintiles increased over time (log rank $P=0.0002$).

Table 2 shows the HRs and 95% CIs for incident atrial fibrillation according to quintiles of hs-CRP levels. Elevated hs-CRP levels were significantly associated with increased risk of atrial fibrillation even after multivariable adjustment; persons with the second or higher quintiles of hs-CRP levels had two- to three-fold increased risk of atrial fibrillation compared with those with the lowest quintile. As shown in **Fig. 3**, these associations were similarly observed in both sexes (P for interaction=0.16), and did not vary by overweight, hypertension, and smoking status (P for interaction: $P=0.71$ for overweight, $P=0.61$ for hypertension, and $P=0.87$ for smoking status, respectively),

Discussion

Our long-term community-based prospective study showed that elevated hs-CRP levels were significantly associated with the risk of atrial fibrillation in the general Japanese population. Furthermore, the significant association was observed in both sexes. The effect modifications by sex, overweight, hypertension, and smoking status on the association were not observed although those without overweight, hypertension or smoking status had a stronger association than those with these factors.

Table 2. Hazard ratios (95% confidence intervals) of the incident risk of aterial fibrillation according to the quintiles of hs-CRP levels

	Quintiles of hs-CRP levels				
	Ql (lowest)	Q2	Q3	Q4	Q5 (highest)
No. of persons	1312	1310	1284	1304	1307
No. of events	9	24	26	32	36
Person-years	13978	13484	12543	12808	12209
Crude incidence rate (/1000 person-years)	0.6	1.8	2.1	2.5	2.9
Age- and sex-adjusted HR (95%CI)	1.00	2.60 (1.21-5.59)	2.64 (1.23-5.64)	3.31 (1.58-6.94)	3.59 (1.73-7.48)
Multivariable HR (95%CI)†	1.00	2.54 (1.17-5.50)	2.28 (1.06-4.93)	2.92 (1.37-6.23)	2.77 (1.30-5.91)

†Adjusted for age, sex, body mass index, hypertension, serum total cholesterol, serum tryglycerides, serum glucose category, smoking status (non-current, current), ethanol intake (never, former, <23 g/day, 23-46 g/day, 46-69 g/day, ≥ 69 g/day), use of antihypertensive medication and dyslipidemia treatment.

To the best of our knowledge, our study is the first to investigate the association between the hs-CRP levels and the development of atrial fibrillation in the general Japanese population. Our findings were consistent with the findings of previous prospective studies in the USA, Denmark, Sweden, and South Korea, which reported that increased CRP levels is an independent risk marker for atrial fibrillation[9-14].

Three previous cohort studies in the USA and Europe reported the following findings: in the Cardiovascular Health Study, individuals with the highest quartile of hs-CRP levels (>3.41 mg/L) had a higher risk of atrial fibrillation than those with the lowest quartile (<0.97 mg/L) among 5491 elderly American men and women (median follow-up duration of 8 years)[9]; in the Copenhagen City Heart Study, individuals with the highest quintile of hs-CRP levels (≥ 3.6 mg/L) had a higher risk than those with the lowest quintile (<1.2 mg/L) among 10,276 middle-aged men and women (median follow-up duration of 14 years)[11]; in the Women's Health Study, women with the highest tertile of hs-CRP levels (>3.4 mg/L) had a higher risk than those with the lowest tertile (≤ 1.1 mg/L) among 24,734 middle-aged health professionals (median follow-up duration of 14 years)[12]. These previous studies have further reported that moderate hs-CRP levels (0.97–3.41, 1.1–3.4, and 1.2–3.6 mg/L, respectively) were not associated with an excess risk of atrial fibrillation[9, 11, 12]. However, in the present study, we found that not only individuals with the highest quintile of hs-CRP levels (≥ 1.17 mg/L) but also those with low-to-moderate hs-CRP levels of ≥ 0.20 mg/L had a higher risk of atrial fibrillation than those with the lowest quintile (≤ 0.19 mg/L). Our results were consistent with the findings from the South Korean study[14]; individuals with the highest hs-CRP levels of ≥ 1.1 mg/L but also those with moderate hs-CRP levels of 0.3–0.4 mg/L had a significantly higher risk of atrial fibrillation than those with the lowest hs-CRP levels of <0.3 mg/L. The median hs-CRP levels in the Japanese and Korean populations was much lower than that in Western countries[14, 16, 17]. Our subjects with hs-CRP levels of >3.0 mg/L accounted for only 6.0% of the total subjects, which was under one-third of the proportion in Western populations[9, 11, 12]. Another South Korean study[15] showed that a high CRP level at a single measurement (>1.0 or >3.0 mg/L: the cut off points propounded by the workshop of CDC/AHA in 2002[24]) was not associated with the risk of atrial fibrillation. From these results, the hs-CRP levels cut-off point to predict risk of atrial fibrillation in the Asian population may be less than 1.0 mg/L and be much lower than that of the Western population.

A potential mechanism for the development of atrial fibrillation by inflammation was suggested based on an animal experimental study; the infiltration of mast cells, key mediators of allergic and immune responses, into the atrium of a pressure-overloaded heart increases the production of platelet-derived growth factor A, which in turn promotes atrial structural remodeling and enhanced atrial fibrillation[25]. However, the mechanism by which a systemic low-grade inflammation (hs-CRP levels of <10 mg/L) causes atrial fibrillation remained uncertain. Marott *et al.* suggested that elevated plasma CRP levels *per se* did not increase the risk of atrial fibrillation in their Mendelian randomization study of 47,000 individuals based on the finding that genetically elevated CRP levels were not associated with the risk of atrial fibrillation[11]. Thus, the CRP levels might be a predictive marker of the risk of atrial fibrillation rather than a causative factor.

Several previous studies have suggested that inflammation is a consequence of atrial fibrillation[26-28]. Induction of atrial fibrillation led to the increment of serum CRP levels at 6 and 24 hours in the patients[26]. CRP may act as an opsonin and partic-

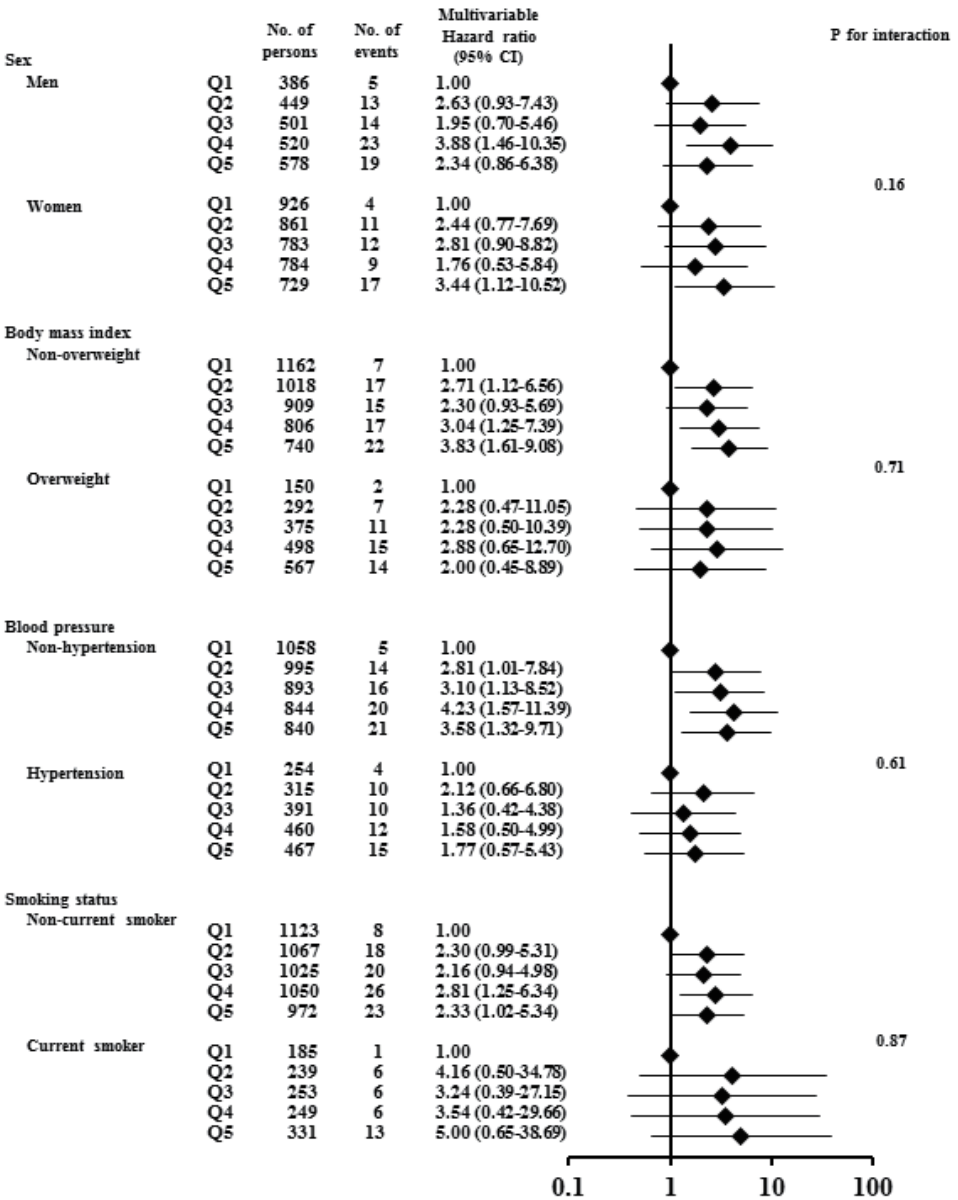


Fig. 3. Forest plot for the multivariable hazard ratios (95% confidence intervals) of the association between hs-CRP level quintiles and risk of atrial fibrillation, stratified by sex, body mass index (< 25 or ≥ 25 kg/m^2), blood pressure (non-hypertension or hypertension) and smoking status (non-current smoker or current smoker), by using a Cox proportional hazard regression model with interaction term

All models were adjusted for age, sex, body mass index, hypertension, serum total cholesterol, serum tryglycerides, serum glucose category, smoking status, ethanol intake, use of antihypertensive medication and dyslipidemia treatment use, and each interaction term was included into the multivariable adjusment. In strata of overweight status, the body mass index variable was replaced with the binary variable (< 25 or ≥ 25 kg/m^2). *P*-values were calculated by the likelihood ratios for the interaction terms between all strata and the quintiles of hs-CRP levels.

ipate in the clearance of apoptotic myocytes[27] in overloaded atria induced by atrial fibrillation[28]. Patients with persistent atrial fibrillation showed higher mean CRP levels at baseline, which continued to be elevated for 1 year, while patients with paroxysmal atrial fibrillation had decreased mean CRP levels at 30 days and 1 year from the baseline[29].

The strengths of our study are that it was a long-term, population-based cohort study with a high follow-up rate. Therefore, our findings could be extrapolated to general Japanese and other Asian populations.

Our study also has several limitations. First, some

misclassification of the hs-CRP levels, i.e., regression dilution bias, needs to be considered because we divided into quintile by the single measurement value of the hs-CRP at baseline. The intraclass correlation coefficient of the log-transformed hs-CRP levels between baseline and the following one year was 0.57 among the individuals who underwent hs-CRP levels measurement for the second consecutive year in our sub sample study (n=3,253), which indicated a moderate positive correlation between them[30]. Therefore, the misclassification of hs-CRP levels would not have a large impact on the present study results. Second, we could not detect paroxysmal atrial fibrillation systematically because we obtained the finding of atrial fibrillation from annual risk surveys. Third, we may have underestimated the number of subjects with atrial fibrillation because some of them may have not reported a history of atrial fibrillation during the surveys. However, the likelihood of the underestimation would be non-differential according to hs-CRP levels and therefore the association was unlikely affected. Fourth, confounding effects by misclassified or unmeasured covariates may remain due to the observational nature of the study. Fifth, we have no good explanation for the increased risk of atrial fibrillation in individuals with apparently normal range of hs-CRP levels, i.e., its second quintile. Further studies will be needed to confirm the hs-CRP levels cut-off to predict increased risk of atrial fibrillation in Asian populations.

Conclusion

The hs-CRP levels were positively associated with the risk of atrial fibrillation in the general Japanese population. This association did not vary by sex, overweight, hypertension, and smoking status.

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Conflict of Interest

The authors report no conflict of interest.

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