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Single and persistent elevation of C-reactive protein levels and the risk of atrial fibrillation in a general population: The Ansan-Ansung Cohort of the Korean Genome and Epidemiology Study☆

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ABSTRACT

Background: Inflammation has been reported to cause atrial fibrillation (AF). However, it remains unclear whether C-reactive protein (CRP) levels predict AF. We investigated whether there was an association between serum CRP levels and the development of AF.

Methods: A total of 10,030 subjects aged between 40 and 69 years were enrolled and followed biennially over a 12-year period in the Ansan-Ansung cohort study. Serum CRP levels were measured at baseline and high-sensitivity CRP (hsCRP) levels were measured at every revisit. AF was identified using 12-lead standard electrocardiography. Inverse probability of treatment weighting was applied to balance the confounders of AF development between groups.

Results: Serum CRP levels were higher in subjects with AF at baseline and those with new-onset AF than in those without AF. Cox-regression analysis showed that high CRP levels (>3 mg/L) and intermediate CRP levels (1–3 mg/L) at baseline were not associated with a higher risk of new-onset AF compared with low CRP levels (<1 mg/L) after adjustments for covariates. The weighted incidences of AF also did not differ according to the CRP levels. In contrast, persistent elevation of CRP or hsCRP levels (≥1 mg/L at all visits) was associated with a higher risk of AF compared with nonpersistent elevation of CRP or hsCRP levels after adjustment for covariates in both unweighted and weighted cohorts.

Conclusion: A high CRP level at a single measurement was not associated with the risk of AF, whereas persistently elevated CRP levels independently predicted the development of AF.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia that has been known to increase overall and cardiovascular mortality [1]. Chronic systemic inflammation and the activated complement system have been recognized as contributors to the development of AF [2,3]. However, the association between increased C-reactive protein (CRP) levels and the risk of AF has not always been consistent in previous studies [4,5]. Many confounding conditions that coincide with AF, including advanced age, obesity, hypertension, diabetes, smoking, heavy drinking, congestive heart failure, coronary artery disease and hyperthyroidism, are also frequently associated with increased serum CRP levels. Furthermore, a single measurement of serum CRP levels may not appropriately reflect the patient's chronic inflammatory state because CRP levels show extreme fluctuations over time [6]. Since most previous studies have investigated the association between CRP levels at baseline and the incidence of AF, serial measurements of CRP levels could be useful for overcoming this issue. Therefore, we investigated whether there was an association between serum CRP levels at baseline and during the follow-up period and the prevalence and incidence of AF in a large longitudinal population-based cohort.

2. Methods

2.1. Study population

This study was conducted with participants from a population-based cohort (the Ansan-Ansung cohort) within the Korean Genome Epidemiology Study (KoGES). The

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Ansan-Ansung cohort is an ongoing longitudinal investigation funded by the Korean government (Korean National Research Institute of Health, Korean Centers for Disease Control and Prevention, and the Ministry of Health and Welfare) to investigate the genetic and environmental etiology of common metabolic and cardiovascular diseases in South Koreans [7,8]. Koreans aged 40–69 years who resided in 2 cities (Ansan and Ansung) were enrolled between June 2001 and January 2003. Detailed information regarding study procedures is available in a previous publication [7].

Comprehensive health examinations and on-site interviews with the participants were obtained in a tertiary hospital located in the region. Participants with acute illnesses or debilitating conditions were excluded before they entered the study. Six serial assessments according to the complete cohort protocol were conducted biennially after the baseline assessment through scheduled visits to the hospital through 2014. At each visit, written informed consent was obtained from all participants. The study protocol adhered to the principles of the Declaration of Helsinki and was approved by the Korean National Research Institute of Health and the Institutional Review Board of Hanyang University Medical Center.

2.2. Assessment of lifestyle, past medical history and physical examination

Information on smoking, alcohol intake, education, income, marital status and the presence of medical conditions including hypertension, diabetes, dyslipidemia, cerebrovascular disease, coronary artery disease, chronic lung disease and asthma were obtained using a questionnaire at every visit by trained questioners according to a specified protocol. Blood pressure was measured using a mercury sphygmomanometer by trained examiners at least 2 times at the level of the heart in a sitting position and averaged. If there was a blood pressure difference ≥5 mmHg between 2 measurements, a third measurement was obtained, and the last 2 measurements were averaged. Waist circumference was measured in the midlevel between the lowest rib and the iliac crest at the end of the expiration in a standing position 3 times and averaged.

Hypertension was defined as a participant diagnosed with hypertension or taking antihypertensive medications. Diabetes mellitus was defined as a participant diagnosed with or taking medications for diabetes mellitus or a hemoglobin A1C level ≥6.5%. Dyslipidemia was defined as a participant diagnosed with dyslipidemia or taking statins without a history of cardiovascular disease or diabetes, a total cholesterol level ≥240 mg/dL, a triglyceride level ≥150 mg/dL or a high-density lipoprotein cholesterol level <45 mg/dL. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration Study (CKD-EPI) equation, and chronic kidney disease was defined as an eGFR ≤60 mL/min/1.73 m^2. Thyroid diseases were defined as a participant diagnosed with a thyroid disease or taking medications for a thyroid disease or a thyroid stimulating hormone level <0.4 or >4.5 mIU/L.

2.3. Measurement of CRP and hsCRP

Measurement of CRP and high-sensitivity CRP (hsCRP) was performed after an overnight fast using an automated analyzers (CRP: Hitachi Automatic Analyzer 7600, Hitachi, Nittobo, Japan; hsCRP: ADVIA 1650, Siemens, Tarrytown, NY, USA). CRP was measured at baseline. AS the measurement of hsCRP levels had become a standard tool for cardiovascular risk stratification since year 2002 [9], hsCRP was measured at each visit that followed to reflect the change in practice. On the first revisit following the baseline assessment, hsCRP was not measured in 65.7% of participants who returned for the first revisit. However, from the second revisit onwards, the rates of missing values in hsCRP measurements were <1% (range 0.0–0.6%). Therefore, persistent CRP elevation was defined as CRP and hsCRP levels ≥1 mg/L at baseline and all revisits except the first one.

2.4. Analysis of electrocardiography and identification of AF

All participants underwent electrocardiography (ECG) at every visit to identify AF. A 12-lead ECG was obtained using a GE Marquette MAC 5000® (GE Marquette Inc., Milwaukee, WI, USA), recorded in a 25 mm/s with 0.1 mV/mm standardization, and interpreted by a cardiologist according to the Minnesota code. AF was defined as a composite of AF or atrial flutter in the standard 12-lead ECG. The rates of missing values in the measurement of ECG ranged from 0.01% to 1.7% at all visits.

2.5. Statistical analysis

The participants were divided into 3 groups according to the CRP levels at baseline. The cut-off points of the baseline CRP levels were set to 1.0 and 3.0 mg/L (<1 mg/L for the low CRP group; 1–3 mg/L for the intermediate CRP group; >3 mg/L for the high CRP group) as proposed previously [10]. Participants whose ECG ($N = 12$) and CRP levels ($N = 3$) were not obtained at baseline were excluded from the analysis. The continuous variables were compared among the groups using ANOVAs, and the categorical variables were compared using Chi-square tests. Kruskal-Wallis tests were employed for variables with skewed distributions including CRP, hsCRP and triglyceride levels. A Kaplan-Meier curve analysis with a log-rank test was employed to compare the incidences of AF among the groups. Multiple logistic regression analysis with a backward variable selection process was performed to evaluate the association between the presence of AF and the serum levels of CRP or hsCRP at each visit. All covariates in the logistic model at each visit were re-assessed at each respective visit before they were included in the model (e.g., diabetes in the model for the 2nd revisit was recategorized using the questionnaire, medication and HgA1c level that were assessed at the 2nd revisit). A Kaplan–Meier curve analysis with a log-rank test was performed to compare the cumulative incidences of AF among the CRP groups. A multivariate Cox regression analysis with a backward variable selection process was performed to evaluate the association between new-onset AF and CRP levels at baseline in the presence of confounding factors. Covariates for the multivariate Cox regression analysis included age, sex, marital status (married vs. unmarried), hypertension, diabetes, dyslipidemia, coronary artery disease, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic kidney disease, chronic lung diseases, thyroid disease, BMI, eGFR, white blood cell (WBC) count, and current smoking and alcohol intake. Inverse probability of treatment weighting (IPTW) using the propensity score was applied to balance the effects of confounding factors when comparing incidences of AF among the CRP groups and calculating hazard ratios (HR) and confidence intervals (CI) of the high and intermediate CRP level groups. The weights were truncated at <1%.

To analyze the effects of persistent CRP or hsCRP elevation on AF development, we created a subgroup of participants whose hsCRP levels were serially measured at every revisit except the first revisit. In the subgroup, participants were divided into 2 groups, either a group with persistent CRP elevation (CRP or hsCRP ≥1 mg/L at all visits except the first revisit) or a group without persistent CRP elevation. The measurement of hsCRP at the first revisit was not included because of the high rate of missing values. A Kaplan-Meier survival curve analysis with a log-rank test was performed to compare the incidences of AF between the 2 groups. A multivariate Cox regression analysis with a backward variable selection process was performed with both unweighted and weighted cohorts using IPTW to evaluate the association between new-onset AF and persistent CRP elevation after adjustment for confounding factors. Two multivariate Cox regression models were evaluated in the subgroup. Model 1 included variables assessed at baseline, and Model 2 included categorical variables assessed at the end of the follow-up period (e.g., diabetes and hypertension) and continuous variables averaged over the follow-up period (e.g., mean BMI and mean eGFR).

All statistical analyses were performed using statistical software R-3.4.0 and its packages, including descr, survival, tableone, survey, ipw and rms. A $p < 0.05$ was considered significant.

3. Results

3.1. Baseline characteristics

Among the 10,030 participants enrolled at baseline, 8603 (85.8%) participants attended the first revisit and 5906 (58.9%) participants attended the 6th revisit. A total of 87,143 person-years were observed over the 12-year period (median follow-up duration 11.5 years). The mean age was 52.3 ± 8.9 years, and 4758 (47.4%) participants were male. The baseline characteristics of the study population are summarized in Table 1. The high (CRP levels at baseline >3 mg/L), intermediate (1–3 mg/L), and low (<1 mg/L) CRP groups included 1835 (18.3%), 4773 (47.6%) and 3419 (34.1%) participants, respectively. The mean age, BMI, waist-hip ratio, systolic and diastolic blood pressures, WBC counts and the levels of hemoglobin A1c, total cholesterol, low-density lipoprotein cholesterol and triglycerides increased with CRP levels. The frequencies of being male or a current smoker or of having hypertension, diabetes, dyslipidemia or chronic kidney disease also increased with CRP levels. The number of participants at each revisit, serum CRP levels at baseline and hsCRP levels at each revisit is summarized in Supplementary Table 1.

3.2. Single measurements of CRP and the presence of AF

AF at baseline and new-onset AF during the follow-up period were found in 40 (0.40%) and 135 (1.35%, 1.55 per 1000 person-years) participants, respectively. The median time interval between the first CRP elevation (≥1 mg/L) and the first AF recognition was 94 (interquartile range, 49–117) months (Supplementary Fig. 1). The frequencies of AF at baseline increased with CRP levels whereas the frequencies of new-onset AF increased only marginally with CRP levels (Table 1). Serum CRP levels were higher in participants with AF at baseline and those with new-onset AF than the levels in those without AF. Serum hsCRP levels were also higher in the participants with AF at baseline throughout the follow-up period, whereas the levels were higher in the participants with new-onset AF only after 4 years, 10 years, and 12 years of follow-up than that in participants without AF (Supplementary Fig. 2 and Supplementary Table 2). Multivariate logistic regression analyses showed that the high hsCRP levels (>3 mg/L) at the third revisit and

Table 1
Baseline characteristics of the participants.

	Low	Intermediate	High	*p*-Value
	CRP <1 mg/L	CRP 1–3 mg/L	CRP >3 mg/L	
	N = 3419	*N* = 4773	*N* = 1835	
Age (years)	48.4 ± 7.1	50.0 ± 7.9	51.1 ± 7.8	<0.001
Male	1566.0 (45.8)	2256.0 (47.3)	934.0 (50.9)	0.002
Married	3063.0 (90.3)	4258.0 (89.7)	1603.0 (88.2)	0.064
Current smoking	834.0 (24.7)	1192.0 (25.3)	522.0 (28.8)	0.004
Smoking frequency (pack/year)	20 [10, 30]	20 [10, 30]	24 [13, 39]	<0.001
Alcohol intake	1767.0 (52.2)	2595.0 (54.8)	978.0 (53.9)	0.080
BMI (kg/m^2)	23.9 ± 2.7	24.9 ± 2.9	25.6 ± 3.2	<0.001
Waist circumference (cm)	78.7 ± 7.7	82.4 ± 8.3	85.1 ± 8.4	<0.001
Waist-hip ratio	0.83 ± 0.07	0.87 ± 0.07	0.89 ± 0.07	<0.001
SBP (mm Hg)	118.2 ± 15.9	123.0 ± 18.0	125.2 ± 17.1	<0.001
DBP (mm Hg)	78.2 ± 11.5	81.6 ± 11.5	84.2 ± 10.9	<0.001
WBC count (10^3/mm^3)	6.2 ± 1.5	6.4 ± 1.6	7.3 ± 2.1	<0.001
Hemoglobin (g/dL)	13.8 ± 1.7	13.7 ± 1.6	13.9 ± 1.5	<0.001
Serum creatinine (mg/dL)	0.92 ± 0.20	0.88 ± 0.19	0.92 ± 0.20	0.002
eGFR (mL/min/1.73 m^2)	89.3 ± 15.0	90.5 ± 14.0	88.1 ± 14.7	<0.001
Total cholesterol (mg/dL)	198 ± 35	199 ± 34	208 ± 39	<0.001
LDL cholesterol (mg/dL)	123 ± 32	123 ± 32	127 ± 39	<0.001
HDL cholesterol (mg/dL)	48 ± 11	45 ± 10	43 ± 8	<0.001
Triglyceride (mg/dL)	114 [86, 161]	135 [101, 186]	151 [110, 217]	<0.001
HgA1c (%)	5.53 ± 0.65	5.68 ± 0.76	5.94 ± 1.27	<0.001
Fasting glucose (mg/dL)	88.0 ± 19.7	89.1 ± 19.9	94.8 ± 36.3	<0.001
Hypertension	236.0 (6.9)	420.0 (8.8)	259.0 (14.1)	<0.001
Diabetes mellitus	408.0 (11.9)	746.0 (15.6)	400.0 (21.8)	<0.001
Thyroid diseases	121.0 (3.5)	158.0 (3.3)	54.0 (2.9)	0.512
Dyslipidemia	1610.0 (47.1)	2790.0 (58.5)	1219.0 (66.4)	<0.001
Chronic lung diseases	85.0 (2.5)	123.0 (2.6)	69.0 (3.8)	0.015
Peripheral artery disease	7.0 (0.2)	22.0 (0.5)	7.0 (0.4)	0.159
Heart failure	13.0 (0.4)	14.0 (0.3)	12.0 (0.7)	0.109
Cerebrovascular event	34.0 (1.0)	53.0 (1.1)	30.0 (1.6)	0.106
Myocardial infarction	23.0 (0.7)	52.0 (1.1)	19.0 (1.0)	0.139
Coronary artery disease	26.0 (0.8)	37.0 (0.8)	16.0 (0.9)	0.902
Chronic kidney diseases	139.0 (4.1)	224.0 (4.7)	121.0 (6.6)	<0.001
AF at baseline	10.0 (0.3)	16.0 (0.3)	14.0 (0.8)	0.023
New-onset AF	36.0 (1.1)	66.0 (1.4)	33.0 (1.8)	0.080
Follow-up duration (month)	140.4 ± 11.0	139.7 ± 15.5	140.2 ± 11.4	<0.001

The data are presented as the mean ± SD or number (%).
Data with a skewed distribution are presented as the median value [the first quartile, the third quartile].
CRP, C-reactive protein; BMI, body mass index; WBC, white blood cell; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AF atrial fibrillation.

the log-transformed hsCRP levels at the sixth revisit were significantly associated with the presence of AF at the respective visits. However, at most visits, higher CRP or hsCRP levels were not associated with the presence of AF after the covariates assessed at the respective visits were adjusted (Table 2; Each multivariate logistic regression model is summarized in Supplementary Table 3).

3.3. Baseline CRP levels and new-onset AF

The Kaplan–Meier curve analysis showed that the unweighted incidence of AF increased with CRP levels among the low, intermediate and high CRP groups (1.9% vs. 2.9% vs. 5.5%, log-rank $p = 0.032$, Fig. 1A). However, unweighted multivariate Cox regression analysis showed that the risk of new-onset AF was not associated with intermediate or high CRP levels after age and other confounding factors were adjusted (Fig. 1B). After we applied IPTW to the original dataset, confounding variables were well-balanced among the 3 CRP groups, and standardized mean differences (SMDs) of all covariates were reduced to <0.1 in the balanced dataset (Supplementary Fig. 3 and Supplementary Table 4). The Kaplan-Meier curve analysis using IPTW showed no differences in the weighted incidence of new-onset AF among the 3 CRP groups (2.2% vs. 2.8% vs. 4.2%, $p = 0.718$; Fig. 1A). A multivariate Cox regression analysis showed that the weighted risk of new-onset AF was also not associated with intermediate (HR 1.13; 95% CI 0.75–1.71) or high CRP levels (HR 1.17; 95% CI 0.75–2.10; Fig. 1B). The baseline CRP levels were transformed into a log-scale, and a multivariate Cox regression analysis was performed using a restrictive cubic spline fit for the log-transformed baseline CRP levels. The risk of new-onset AF increased with CRP levels from 1 to 5 mg/L in a univariate model, but it was not associated with baseline CRP levels in any ranges after all covariates were included (Fig. 1C).

3.4. The association between persistent CRP elevation and new-onset AF

The subgroup included 4499 participants whose hsCRP levels had been serially measured since the second revisit. The baseline characteristics of participants in the subgroup are described in Supplementary Table 5. The incidence rates per 1000 person-years were 3.61 in the group with persistent CRP elevation and 1.36 in the group without persistent CRP elevation. The cumulative incidence of AF was higher in the group with persistent CRP elevation than that in the group without persistent CRP elevation in both the unweighted (8.8% vs. 2.3% respectively, $p = 0.0002$) and weighted cohort (7.5% vs. 2.1%, $p = 0.0008$; Fig. 2A). An unweighted multivariate Cox regression analysis showed that persistent CRP elevation was associated with a higher risk of new-onset AF after the covariates at baseline were included (unweighted model 1; Fig. 2B). The association between persistent CRP elevation and new-onset AF remained significant even after the covariates observed throughout the follow-up period were included (unweighted model 2; Fig. 2B). After the IPTW was applied to the subgroup, the covariates for Model 1 (Supplementary Fig. 4A and Supplementary Table 6) and Model 2 (Supplementary Fig. 4B and Supplementary Table 7) were well-balanced in the weighted cohorts, and most covariate SMDs were reduced to <0.1. A weighted multivariate Cox regression analysis also showed that persistent CRP elevation was significantly associated with a higher risk of new-onset AF after the covariates at baseline (weighted Model 1) and the covariates at the end of follow-up (weighted Model 2) were included (Fig. 2B and Supplementary Table 8).

4. Discussion

In this study, we found that a single measurement of CRP or hsCRP levels was not significantly associated with a higher prevalence or incidence of AF in the general population. Although the crude prevalence or incidence of AF appeared to be different by CRP level, the differences disappeared after adjustment for age and other confounding factors. In contrast, persistent elevation of CRP or hsCRP levels during the follow-up period was independently associated with higher risk of AF development despite thorough adjustment for confounding factors.

4.1. The link between CRP and AF

The link between inflammation and the development of atrial fibrillation has been recognized in many previous studies. The inflammation hypothesis was initially proposed because of the high incidence of AF after cardiac surgery, which is a pro-inflammatory state with complementary system activation [11,12]. Atrial biopsy results in patients with AF revealed inflammatory cell infiltration and oxidative damage within atrial tissue [13,14]. Associations between the incidence of AF and inflammatory mediators including interleukin-6 and tumor necrosis factor-alpha have been reported [2,15,16].

The association between CRP levels and AF has been repeatedly shown in both cross-sectional and longitudinal studies. Aviles et al. reported that high CRP was associated with a higher incidence of AF after a 7.8-year observation in a large cohort study [17]. Several agents known to reduce CRP levels, including statins and angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, have also been reported to prevent the development of AF [18,19]. However,

Table 2
Association between CRP or hsCRP level and the presence of AF at each visit in logistic regression models.

Dependent variable Presence of AF	Model 1 CRP levels as a categorical variable with 3 groups			Model 2 CRP levels as a continuous variable with logarithmic transformation	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
At baseline	Intermediate	0.92 (0.40–2.09)	0.840	1.24 (0.95–1.60)	0.111
	High	1.74 (0.76–3.98)	0.190		
First revisit	Intermediate	2.68 (0.52–13.4)	0.240	1.16 (0.69–1.92)	0.579
	High	0.77 (0.05–13.4)	0.862		
Second revisit	Intermediate	0.86 (0.46–1.61)	0.647	1.16 (0.93–1.46)	0.184
	High	1.04 (0.49–1.22)	0.911		
Third revisit	Intermediate	0.87 (0.48–1.56)	0.632	1.17 (0.94–1.45)	0.158
	High	1.87 (1.00–3.49)	0.049		
Fourth revisit	Intermediate	0.73 (0.43–1.24)	0.244	1.00 (0.82–1.24)	0.955
	High	1.23 (0.66–2.28)	0.520		
Fifth revisit	Intermediate	1.12 (0.69–1.82)	0.644	1.14 (0.94–1.38)	0.178
	High	1.45 (0.80–2.62)	0.216		
Sixth revisit	Intermediate	1.27 (0.81–2.00)	0.291	1.26 (1.04–1.52)	0.016
	High	1.46 (0.81–2.63)	0.206		

The regression analysis was performed with a backward variable selection process (cut-off p-value < 0.05).
From the first revisit, hsCRP was measured instead of CRP.

- Model 1 included CRP (or hsCRP) levels at each visit as a categorical variable with 3 groups (CRP <1 mg/L, CRP 1–3 mg/L, CRP >3 mg/L)
- Model 2 included CRP (or hsCRP) levels at each visit as a continuous variable with logarithmic transformation.

Every model included age, sex, BMI, diabetes, hypertension, dyslipidemia, thyroid disease, asthma, chronic lung disease, chronic kidney disease, peripheral artery disease, coronary artery disease, congestive heart failure, cerebrovascular disease and myocardial infarction investigated at each visit as covariates.

associations between high CRP levels and new-onset AF have not always been consistent. Marott et al. reported in a large genetic cohort study that elevated CRP levels correlated with the risk of AF but genetically elevated CRP levels did not, suggesting that an elevated CRP level itself was not associated with the risk of AF [5]. Sinner et al. reported that CRP levels did not predict the risk of AF in a large cohort study after thorough adjustment for confounding factors of AF [4]. We also observed apparent differences in the prevalence and incidence of AF according to serum CRP levels. However, the baseline characteristics among groups were vastly different. Participants in the high CRP group generally had more serious illnesses and unhealthy lifestyles than did those with low CRP levels. Because those differences could confound the relationship between CRP levels and AF development, we employed multiple statistical analysis techniques including multivariate regression and IPTW to minimize their effects. Then, the apparent differences according to CRP levels disappeared after adjusting for the confounding factors and applying IPTW to the dataset. Occasional associations between higher hsCRP levels and the presence of AF were observed at the 3rd and the 6th revisit, but the associations were only marginal and at most revisits, higher CRP/hsCRP levels did not predict the presence of AF at the respective visits. Although there are still likely unmeasured confounding factors in high CRP levels, our results suggest that a single measurement of serum CRP levels does not predict the development of AF.

4.2. Serial measurements of CRP and the risk of AF

One distinguishing point in our study from previous studies on the relationship between CRP levels and AF, in which the CRP levels were measured once at baseline, is that we measured hsCRP levels repeatedly throughout the follow-up period in >99% of the revisiting participants. Serum CRP levels could easily fluctuate because of occasional infection and the development of various atherosclerosis-related conditions including hypertension, diabetes, obesity and metabolic syndrome, during follow-up [20]. Thus, baseline CRP levels may not sufficiently reflect the level of inflammation throughout the follow-up period, which may explain the lack of association between serum CRP levels at baseline and the incidence of AF. Bogaty et al. suggested that due to intraindividual variability in CRP levels, the use of CRP for stratification of cardiovascular disease risk could be problematic [6,21]. In contrast, our results showed that persistently elevated CRP levels were associated with higher risk of new-onset AF despite thorough adjustment for confounding factors. We even applied IPTW to the subgroup of participants whose hsCRP levels were consecutively measured to balance the confounding factors. Then, we performed weighted multivariate Cox regression analyses with covariates assessed at both baseline and the end of follow-up and still found significant associations between persistent CRP elevation and AF development. Our study also had a follow-up period of 12 years, which is longer than that in most previous studies. Because many confounding conditions could have changed during the follow-up period, we incorporated these changes in our Cox regression analysis. However, the association between persistent CRP elevation and the risk of new-onset AF remained significant. These results suggest that, although an elevation in CRP at a random measurement may not be an indicator of future AF development, chronic systemic inflammation may still have a causal association with the development of AF, independent of many CRP-elevating conditions that could develop during follow-up.

Although a single CRP level did not independently predict the development of AF, our results showed that the baseline CRP levels were higher in participants with AF at baseline or with new-onset AF than the levels in those without AF, and new-onset AF was indeed more frequent in the high CRP group than in the low CRP group. These results implied that CRP elevation at a single measurement may not be a risk factor but may still be a modest risk indicator of AF development.

4.3. The prevalence and incidence of AF

In our results, the prevalence of AF at baseline was 0.4%, and the incidence of new-onset AF was 1.55/1000 person-years during the follow-up, which are much lower values than those of previous reports from Europe and the United States [22,23]. Schnabel et al. reported that the prevalence of AF was approximately 2.0% and that the incidence was 7.5/1000 person-years through 50 years of observation in the Framingham cohort [23]. Because we identified AF using clinical ECG, most cases of paroxysmal AF would not have been identified, and the prevalence of AF was likely underestimated. Schnabel et al. reported that the incidence of AF based on clinic ECGs was 1.6–3.8/1000 person-years,

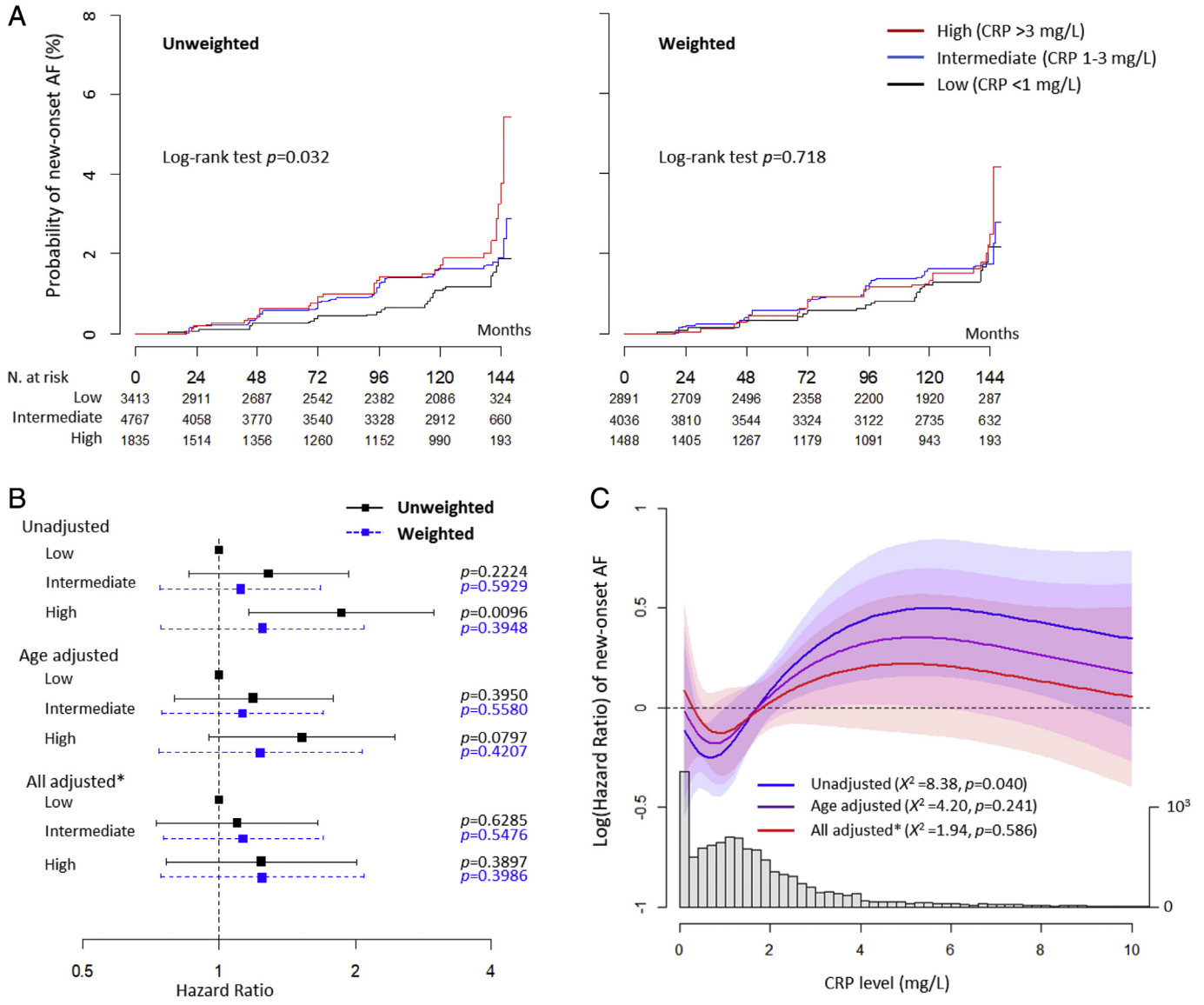

Fig. 1. Risk of new-onset AF according to serum CRP levels at baseline. A) Kaplan-Meier curve analysis showing that new-onset AF occurred more frequently in patients with high CRP levels than in those with low CRP levels ($p = 0.032$, the left panel). However, there were no statistically significant differences in the weighted incidences of new-onset AF among the groups ($p = 0.718$, the right panel). B) After confounding factors were included, the risk of new-onset AF was not significantly different by serum CRP levels at baseline. Weighted Cox regression analysis also showed no significant increase in the risk of new-onset AF in the intermediate and high CRP groups compared with the low CRP group, regardless of the presence of covariates. C) A Cox-regression analysis with restrictive cubic spline fit (knot = 4) for CRP levels at baseline showed that the association between new-onset AF and CRP levels was significant in a univariate model but was not significant when age was or all covariates were included in the model. *Adjusted for age, sex, hypertension, diabetes, dyslipidemia, coronary artery disease, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic kidney disease, chronic lung diseases, thyroid disease, BMI, current smoking and alcohol intake.

which was much lower than that based on all available sources and comparable to that in our study [23]. Ethnic differences may also have influenced the low prevalence of AF in our study. The prevalence of AF has been reported to be 250–325/100,000 in Northeast Asia, which is the lowest in the world [24,25]. Lee et al. recently reported that the incidence of AF was 1.5–1.7/1000 person-years using the Korean National Health Insurance Service database, which is similar to our results [25]. Our study participants were younger than those in other studies [4,5,17,25] by approximately 10–20 years, which may also have contributed to the lower prevalence of AF given that advanced age is a major risk factor for AF.

4.4. Limitations

This study has several limitations. First, because it was an observational study, the association between increased CRP levels and AF could not be directly interpreted as a causal relationship. Although we have adjusted for all relevant covariates in our regression models and applied IPTW to the models to balance the covariates, there may have been unmeasured confounding factors resulting in biased associations. Second, during the 12-year follow-up period, 41% of participants dropped out from follow-up assessments, which may have resulted from serious illnesses involving AF episodes. This drop-out rate may have contributed the low incidence of AF in the cohort. Minor frequencies of missing values were also present in the covariates in the multivariate Cox regression analyses, which may have some impact on the results of the analysis. We reported these rates of missing values in Supplementary Fig. 5. Third, we identified AF biennially using standard 12-lead ECG. This method would arguably have caused under-recognition of paroxysmal AF, which has also been reported to show higher CRP levels than sinus rhythm [11]. However, the identification of paroxysmal AF may not significantly change the results given that

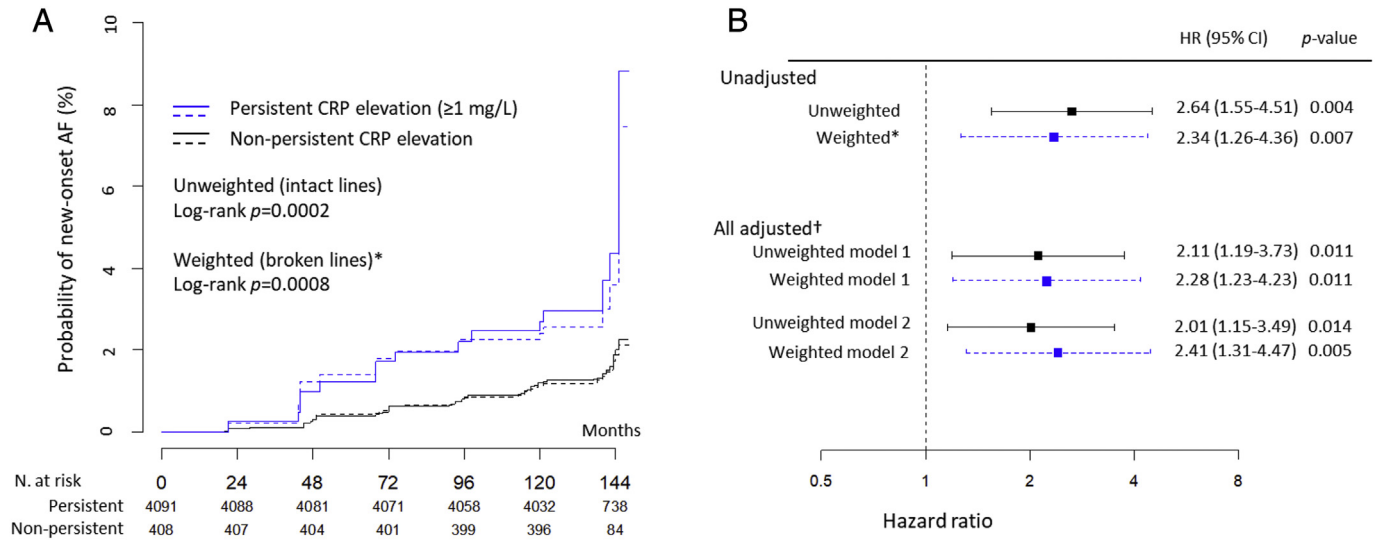

Fig. 2. The association between the risk of new-onset AF and persistent CRP elevation. A) Among subjects whose CRP levels were measured consecutively, the risk of new-onset AF was significantly higher in subjects with persistent CRP elevation than in those without persistent CRP elevation ($N = 4499$). B) Multiple Cox regression analysis also showed that persistent CRP elevation was significantly associated with higher risk of new-onset AF after all covariates were included. - Model 1 included covariates at baseline. - Model 2 included covariates throughout the follow-up period (except sex, mean BMI and age at baseline). *IPTW was applied with the same covariates in the model 1. †Adjusted for age, sex, marital status (married vs. unmarried), hypertension, diabetes, dyslipidemia, coronary artery disease, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic kidney disease, chronic lung diseases, thyroid disease, BMI, current smoking and alcohol intake.

subjects with paroxysmal AF have lower CRP levels than those with persistent AF and that subjects without AF are much more numerous than are those with paroxysmal AF. Persistent AF that occurred, then reverted to sinus rhythm between visits may also have not been recognized as AF in our study. Finally, we measured hsCRP levels instead of CRP levels at the revisits. The difference in the measurement methods of the two CRP levels may have caused some information bias. However, CRP levels are known to highly correlate ($R = 0.98$) with hsCRP levels [26], and could even be directly converted to hsCRP levels, when multiplied by 10 [27]. Moreover, to prevent information bias, we converted the serial hsCRP levels into binary variables using a well-accepted cut-off value (1 mg/L) and did not combine CRP and hsCRP levels into mean or median values.

In conclusion, our results exposed a problem associated with the use of a single measurement of CRP for predicting AF but still support the inflammation hypothesis in the pathophysiology of AF. A single measurement of CRP was not associated with the prevalence or incidence of AF, even though they appeared to be different by CRP levels before adjustment for confounding factors. However, persistently elevated CRP levels were independently associated with the development of AF in a long-term follow-up, which may indicate a causal relationship between chronic systemic inflammation and the development of AF.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.10.070.

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