Five-Year Intra-Individual Variability in C-Reactive Protein Levels in a Japanese Population-Based Study

— The Jichi Medical School Cohort Study at Yamato, 1993–1998 —

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C-reactive protein (CRP) is an acute-phase reactant that is related to future cardiovascular events. However, little is known about the long-term intra-individual stability of CRP in community residents. The 5-year intra-individual correlation of CRP levels was examined in the Jichi Medical School Cohort Study in Japan. CRP measurements were obtained in 1993 and in 1998 from 388 presumptively healthy individuals aged 30–69 years at baseline. The Pearson's correlation coefficient of CRP between baseline and follow-up measurements was 0.43 (95% confidence interval (CI): 0.34–0.51). Additional analyses by sex and smoking status at baseline revealed similar coefficients. The correlation coefficient of CRP was lower than that of other classical risk factors, such as body-mass index (BMI), blood pressure, and total and high-density lipoprotein (HDL) cholesterol. A subgroup of individuals with higher levels of CRP at both baseline and follow-up measurements had higher BMI, hemoglobin A1c, and plasma fibrinogen, and lower levels of HDL-cholesterol than others, even after adjusting for age, sex, and smoking status in a multiple logistic model. In conclusion, the stability of CRP levels was statistically significant in a long-term population-based study. A subgroup with higher levels of CRP who had an aggregation of cardiovascular risk factors was identified by the 2 measurements. (*Jpn Circ J* 2000; 64: 303–308)

Key Words: C-reactive protein; Cardiovascular risk factors; Intraindividual variability; Population-based study

Atherosclerosis is a chronic inflammatory disease that develops in response to cardiovascular risk factors.¹ Circulating blood markers may reflect the activity of the disease.² Meta-analysis has suggested an association between coronary heart disease and blood levels of acute-phase reactants, such as fibrinogen, leukocytes and C-reactive protein (CRP).³ A significant prognostic value of CRP has been inferred from clinical studies in patients with angina pectoris.^{4–6} Nested case-control studies have also identified the baseline plasma concentration of CRP as a risk indicator for cardiovascular disease.^{7–11}

The acute-phase reaction increases plasma CRP levels several hundred-fold within 48 h. The plasma level of CRP is also influenced by various life-styles factors; higher CRP levels have been found in smokers¹² and in individuals under psychological stress.¹³

However, in prospective studies CRP levels at baseline have been regarded as representative of chronic levels, and have been used as long-term risk indicators. Thus, our concern is the long-term intra-individual variability of the CRP concentration rather than short-time fluctuations induced by transient, acute-phase reactions. A few, small studies have evaluated the short-term (5–6 months) intra-individual variability of plasma CRP levels in healthy,

young volunteers.^{14–16} To our knowledge, none of the community-based studies have evaluated the long-term state of CRP. We therefore investigated the 5-year intra-individual variation in CRP levels among healthy subjects in Japan. In addition, we evaluated changes in CRP levels during the follow-up period associated with the baseline status of classical cardiovascular factors.

Methods

Populations

The Jichi Medical School Cohort Study is a prospective population-based study that was designed to explore risk factors for cerebro-cardiovascular disease in 12 Japanese communities. The study was approved by each local government. The study design and some descriptive data were published previously.^{17–20} The cohort study population of the present analysis comprised residents in one community in Niigata prefecture, called Yamato. Residents aged 30–69 years underwent this examination. Mass screening for cerebro-cardiovascular diseases has been conducted in Japan since 1983 in accordance with the health and medical service law for the aged. In June 1993, according to the law, the local government office mailed invitations to eligible individuals. However, those undergoing treatment or care for cardiovascular diseases were excluded. A total of 614 individuals participated in the examination. All were followed up annually by visits, phone calls, mailings and an interview during the annual health examination. Of the participants from 1993, 19 had died, 11 had dropped out because of moving, and so 388 (63.2%) attended the annual examination in June 1998.

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Table 1 Baseline Characteristics of the Study Participants in 1993 who Participated in the Examination in 1998 and Those who did not: The Jichi Medical School Cohort Study at Yamato, 1993–1998*

Characteristics	Examinee in 1998 (n=388)		Non-examinee in 1998 (n=226)		p value
Age (years)	53.5	12.1	49.0	15.3	<0.001
Women (%)	72.2		60.2		<0.003
Smoking status (%)					<0.002
Never smoked	71.1		57.1		
Smoked in the past	9.0		12.4		
Currently a smoker	19.9		30.5		
BMI (kg/m ²) [†]	23.1	3.0	22.9	3.4	
Systolic blood pressure (mmHg)	121.8	18.7	121.7	19.1	
Diastolic blood pressure (mmHg)	74.6	12.2	74.1	12.1	
Total cholesterol (mg/dl)	190.4	35.3	180.3	33.6	<0.001
HDL cholesterol (mg/dl)	51.3	12.8	50.8	12.9	
Hemoglobin A1c (%)	5.42	0.46	5.42	0.65	
Plasma fibrinogen (mg/dl)	231.1	49.0	230.4	54.8	
Factor VIIc (%)	102.1	16.4	98.1	16.2	<0.005
Leukocyte count (/mm ³)	5696	1583	5889	1615	
C-reactive protein (μg/L) [‡]	101.0	120.2	101.0	124.0	

*Each set of values is mean and its standard deviation, unless otherwise specified. [†]Body-mass index is weight in kilograms divided by the square of the height in meters. [‡]Median value and its semi-interquartile range.

Table 2 Association of Cardiovascular Risk Factors at Baseline and at 5-year Follow-up: The Jichi Medical School Cohort Study at Yamato, 1993–1998

Variables	N	Pearson's correlation coefficients (r)	95% confidence interval	p value
Body-mass index	388	0.930	0.915–0.942	<0.001
Systolic blood pressure	388	0.650	0.589–0.704	<0.001
Diastolic blood pressure	388	0.650	0.589–0.704	<0.001
Total cholesterol	367	0.732	0.681–0.776	<0.001
HDL cholesterol	367	0.756	0.708–0.797	<0.001
Leukocyte count	388	0.638	0.575–0.694	<0.001
C-reactive protein*	368	0.430	0.343–0.510	<0.001
Men	104	0.370	0.191–0.525	<0.001
Women	262	0.434	0.331–0.527	<0.001
Smoking status				
Never smoked	76	0.439	0.237–0.605	<0.001
Smoked in the past	33	0.403	0.069–0.656	<0.02
Currently smokers	259	0.431	0.326–0.525	<0.001

*Values transformed into natural logarithms.

Data Collection

Smoking status was assessed by trained interviewers. Body weight was recorded with the subject clothed, and then 0.5 kg was subtracted from the recorded weight. Body-mass index (BMI) was calculated as weight (kg)/height (m)². The systolic and diastolic blood pressure was measured with a fully automated sphygmomanometer (BP203RV-II; Nippon Colin, Komaki, Japan), placed on the arm of a seated individual who had rested in the same position for 5 min prior to the measurement.

Blood samples were taken by trained technicians from the antecubital vein of a seated participant with minimal tourniquet use. Specimens were collected in siliconized vacuum glass tubes containing sodium ethylenediamine tetra-acetic acid (hemoglobin A1c (HbA1c)), 1/10 volume of 3.8% trisodium citrate (fibrinogen and factor VII activity (VIIc)), or no additives (lipids and CRP). Tubes were centrifuged at 3,000 G for 15 min at room temperature. After separation, the serum samples were stored at 4°C in refrigerated containers. Plasma samples were frozen as rapidly as possible to –80°C for storage until laboratory determinations were performed.

Height, weight, blood pressure, total and high-density lipoprotein (HDL) cholesterol, leukocyte count, and CRP

were assayed by the same methods and in the same laboratory as the 1993 examination. Blood samples from 20 individuals could not be obtained because of technical problems.

Laboratory Methods

CRP levels were measured using a nephelometry kit (NA Latex CRP kit, Behringwerke, Marburg, Germany), and a nephelometric analyzer (Behring Nephelometer Analyzer, Hoechst-Behring Diagnostics, Frankfurt, Germany). The assay was standardized against 2 reference materials: the Certified Reference Material (CRM) 470 prepared by the International Federation of Clinical Chemistry (IFCC), and the Japan National Protein Standard, in which value assignments are based on WHO International Reference Preparation 67/86 and CRP 85/506²¹. The interassay coefficient of variation (CV) was 1.18% and the intra-assay CV was 1.36%. The minimal detectable CRP level was 30 μg/L.

Total cholesterol was measured enzymatically (Wako, Osaka, Japan; interassay CV: 1.5%). HDL cholesterol was measured by phosphotungstate precipitation (Wako; interassay CV: 1.8%). Fibrinogen levels were determined using a one-stage clotting assay kit (Data-Fi, Dade, Miami, FL, USA; interassay CV: 2.5%). Factor VIIc was chro-

Table 3 Cross-Classification of Quintile of Baseline C-Reactive Protein by Follow-up C-Reactive Protein: The Jichi Medical School Cohort Study at Yamato, 1993–1998

Quintile of baseline C-reactive protein ($\mu\text{g/L}$)	Range of follow-up C-reactive protein*					Total
	1	2	3	4	5	
Quintile 1 (-38.0)						
N	63	2	1	5	4	75
%	83.8	2.7	1.4	6.8	5.4	100.0
Quintile 2 ($38.1-73.0$)						
N	60	3	4	4	3	74
%	81.1	4.1	5.4	5.4	4.1	100.0
Quintile 3 ($73.1-161.0$)						
N	48	4	4	11	6	73
%	65.8	5.5	5.5	15.1	8.2	100.0
Quintile 4 ($161.1-358.0$)						
N	36	0	7	25	8	76
%	47.4	0.0	9.2	32.9	10.5	100.0
Quintile 5 ($358.1-$)						
N	22	0	6	20	22	70
%	31.4	0.0	8.6	28.6	31.4	100.0
Total						
N	229	9	22	65	43	368
%	62.1	2.5	6.0	17.7	11.7	100.0

*Each interval of ranges defined by quintile at baseline.

Fig 1. Frequency distribution of C-reactive protein level associated with quintile of baseline C-reactive protein level and its 5 ranges at follow-up divided by quintile at baseline: The Jichi Medical School Cohort Study at Yamato, 1993–1998.

mogenically assayed using a human placenta-derived calcified thromboplastin reagent (Chromoquick, Behringwerke), human factor VII-deficient plasma (Behringwerke), and a chromogenic assay autoanalyzer (Behring chromotimer, Behringwerke; interassay CV: 3.8%). HbA1c was measured using a high-performance liquid chromatography method (Kyoto-Dai-Ichi-Kagaku, Kyoto, Japan; CV: 1.1%). Leukocytes were counted using an autoanalyser (Sysmex E-4000, Sysmex, Kobe, Japan).

Blood variables, except for factor VIIc, were measured at

the central laboratory of the Special Reference Laboratory (SRL; Tokyo, Japan), a commercial hematology laboratory. Factor VIIc was measured at Hyogo Prefectural Awaji Hospital, where all values were determined by a single laboratory specialist.

Statistical Analysis

Descriptive parameters are shown as an arithmetic mean and standard deviation, median value and its semi-interquartile range and percentage. Undetectable CRP

Table 4 Comparison of Age, Sex and Smoking Status-Adjusted Mean Value of Cardiovascular Risk Factors Among the 75 Individuals With High CRP Levels at Both Examinations (the RHG) and the Others: The Jichi Medical School Cohort Study at Yamato, 1993–1998

Variables	RHG		Others		p value
	Adjusted mean	SEM	Adjusted mean	SEM	
Body-mass index (kg/m ²)	24.2	0.3	22.9	0.2	<0.001
Systolic blood pressure (mmHg)	125.8	2.1	120.5	1.1	<0.05
Diastolic blood pressure (mmHg)	76.5	1.4	73.8	0.7	
Total cholesterol (mg/dl)	193.0	3.7	189.2	1.9	
HDL cholesterol (mg/dl)	47.2	1.5	52.5	0.7	<0.002
Hemoglobin A1c (%)	5.58	0.05	5.39	0.03	<0.001
Plasma fibrinogen (mg/dl)	247.7	5.1	225.0	2.6	<0.001
Factor VIIc (%)	103.5	1.9	101.9	1.0	
Leukocyte count (/mm ³)	6041	174	5615	89	<0.05

SEM, standard error of adjusted mean; CRP, C-reactive protein; RHG, repeatedly high group.

values were replaced as 20 µg/L. The distribution of the CRP levels was skewed to the left; so log-transformed values were used in the analyses. Student's t test and the chi-square test were used when appropriate. The Pearson's correlation coefficients and the Spearman's rank correlation coefficients between baseline and follow-up CRP were calculated. Differences in CRP levels between the 2 examinations (Δ CRP) were tested by a paired t test. CRP levels at follow-up were divided into 5 ranges based on the quintile of CRP levels at the baseline assay. Linear regression models evaluated Δ CRP associated with baseline measures of the classical cardiovascular risk factors. Baseline CRP levels were included in the models to control for bias due to regression toward the mean²² Differences in adjusted mean values stratified by categorical variables were tested using the analysis of covariance (ANCOVA). To assess the influence of each variable, a logistic regression model was constructed using variables that were significantly correlated with CRP. Odds ratios (OR) were computed as the probability in the subgroup of CRP levels to be in the fourth or fifth range at the 2 examinations associated with an increase of one standard deviation in the concentration of other variables in question. Dummy variables were created for smoking status. Significant difference was defined at $p < 0.05$. Statistical analyses were performed using the SPSS statistical package 8.0 (SPSS, Chicago, ILL, USA) with default settings.

Results

The baseline characteristics of individuals who participated in the 1998 examination and those who did not are shown in Table 1. CRP levels between the 2 groups did not differ significantly. The 1998 participants were more likely to be women or non-smokers. Age, total cholesterol and factor VIIc levels were higher among 1998 participants than among the others. During the 5-year period, 10 of 77 current smokers at baseline had ceased smoking, and none of the non-smokers at baseline had started smoking.

The association of the 2 CRP measurements with other cardiovascular risk factors is shown in Table 2. The Pearson's correlation coefficient of CRP was 0.430 (95% confidence interval (CI): 0.343–0.510). This result was basically unchanged by a non-parametric analysis (Spearman's correlation coefficient; 0.426, 95% CI: 0.339–0.506). Subgroup analyses by sex and smoking status at baseline revealed a similar tendency. The correlation coefficients of CRP were lower than those for other classical risk factors

(Table 2).

The values for the classical risk factors increased during the follow-up period. The mean difference in BMI was 0.32 (95% CI: 0.12–0.44), 5.45 mmHg (3.84–7.05 mmHg) in systolic blood pressure, 13.5 mg/dl (10.9–16.1 mg/dl) in total cholesterol, and 11.3 mg/dl (10.3–12.3 mg/dl) in HDL cholesterol. By contrast, CRP levels at follow-up were significantly lower than those at baseline (Table 3, Fig 1). The median value of Δ CRP was $-43 \mu\text{g/L}$ (95% CI: -53 to $-34 \mu\text{g/L}$). After adjusting for age and baseline CRP levels, no correlation analyses between Δ CRP and other cardiovascular factors at baseline revealed statistical significance (Table 1).

Of the 368 subjects (60.6%) at follow-up, CRP levels were below the minimum detectable level in 223. Eighty-four percent of participants in the lowest quintile of CRP at baseline remained within the lowest range at follow-up. Approximately 75% of the participants in the second or third quintile of CRP at baseline moved to the lowest range. Of the 146 participants in quintiles 4 and 5 of CRP at baseline, 58 (39.7%) moved to the lowest range, and none to the second range, at follow-up. Consequently, we identified a subgroup (repeatedly high group (RHG)) consisting of 75 individuals (51.4%) who remained in the fourth and fifth ranges at follow-up.

Comparison of the baseline characteristics between the RHG and the others is shown in Table 4. After adjusting for age, sex, and smoking status by ANCOVA, the RHG had a significantly higher BMI, systolic blood pressure, HbA1c, plasma fibrinogen, and leukocyte count than the others. HDL-cholesterol levels were significantly lower in the RHG. In the multiple logistic model to assess the influence of each variable, BMI (OR 1.43, 95% CI 1.05–1.95), HDL-cholesterol (OR 0.66, 95% CI 0.48–0.91), HbA1c (OR 1.37, 95% CI 1.02–1.85), and plasma fibrinogen (OR 1.73, 95% CI 1.26–2.38) remained as significant variables for the RHG.

Discussion

Our 5-year prospective study indicated that the Pearson's correlation coefficient of the CRP values between baseline and follow-up measurements is moderate (0.43) and statistically significant, but a little lower than the values of other risk factors measured in this study. Additional analyses by sex and smoking status at baseline revealed similar coefficients. Although the distribution in CRP values seemed to be different between the baseline and follow-up, the results

were confirmed by non-parametric analysis, which tests hypotheses regarding data for which the underlying distribution of the data is not assumed. To our knowledge, this is the first long-term intra-individual correlation of CRP levels in a community-based study.

Compared with results of other community-based studies, the correlation coefficient for CRP in the present study (0.43) is similar to those for diastolic blood pressure (0.46) and blood glucose levels (0.39) in women in the Framingham study²³ and for leisure time physical activity (0.48 for men and 0.41 for women) in a Finnish study²⁴. The coefficient also approximates those for von Willebrand factor (0.48) and plasma fibrinogen (0.45) in a 2.5-year follow-up of male patients suffering from angina pectoris²⁵. The coefficient of determination in our study ($0.46^2 \approx 0.21$) seems to be higher than the contribution of intra-individual variation in CRP to its total variation during a 6-month follow-up of 20 healthy young individuals (0.14).¹⁶ The preceding facts suggest that the long-term intra-individual stability of CRP levels found in the present study is nearly equivalent to the values of the other classical risk factors found in previous studies.

In contrast to changes in other risk factor levels, median CRP values are lower by 43% at follow-up measurement than those of baseline, and the distribution of CRP values seems to be different between baseline and follow-up. The long-term intra-individual variability in CRP measurements consists of true physiological change and change due to error, the latter probably due to regression toward the mean, laboratory assay error, and short-term within-person variation due to the effects of temporary infection, smoking, and other factors.²² Because we analyzed the entire study group, the regression toward the mean would have little effect on the difference in median values between the 2 measurements. We had uniform methods of blood sampling and shipping at both examinations. The CRP assay was carefully standardized against international reference materials.²¹ The minimal detectable level of the assay was almost equal to those of previous studies.^{5,7} Furthermore, the interassay CV in the CRP measurements in our study (1.18%) was lower than that reported in previous studies (4.2–14%).^{7–9,12} Therefore, any error of the methods within an analytic batch would likely be attenuated. A decrease in the proportion of current smokers could also lower CRP levels, but the results were basically unchanged after excluding individuals who ceased smoking during the follow-up period. It is likely that the seasonal variation in epidemic infections also influences CRP levels in a community population, although the 2 examinations were performed in June. A wide fluctuation in CRP levels due to temporal infections may explain in part the unexpected decrease of the median values of CRP during the follow-up period, and probably dilutes a correlation between the CRP level and future cardiovascular events.

Median CRP values in our participants ($101 \mu\text{g/L}$) were approximately 10% of those of healthy populations in Europe and North America ($1,000\text{--}2,000 \mu\text{g/L}$).^{8–12} One reason may be differences in CRP measurement. However, standardization of the CRP assay in the present study against the international reference materials suggests sufficient commutability of the CRP measurement system between this analysis and those of other studies. The difference in CRP values could be due in part to the difference in the severity of underlying atherosclerosis across the popu-

lations. Although we did not evaluate that condition directly, compared with the baseline values in population-based studies in Western countries,^{8–10,12} potent atherosclerotic risk factors such as blood pressure and total cholesterol are low in our population. A cross-cultural comparison study reported that in Japanese populations the levels of plasma fibrinogen, which is regarded as a cardiovascular risk factor, and an acute-phase reactant such as CRP are lower than those in Americans.²⁶ Further studies are needed to extend our findings to populations in other countries.

We found a subgroup with high CRP levels in both baseline and follow-up measurements. The RHG had significantly unfavorable levels of other classical cardiovascular risk factors. A recent case-control study of diabetic patients showed that CRP levels were higher in patients with 4 or 5 cardiovascular risk factors (syndrome X) than in those with 0 or 1 of these factors.²⁷ Our results are also compatible with those of the Cardiovascular Health Study, which showed that a significant association of CRP with future cardiovascular events is limited to individuals in the highest quartile of baseline CRP.⁹ Those studies and our results suggest that high CRP levels identify individuals with aggregate cardiovascular risk factors.

Those who participated in the follow-up examination in 1998 were older and had higher levels of some risk factors (Table 1). Therefore, they may be more likely to have atherosclerotic vascular lesions than the residents who did not participate in the follow-up examination.

In conclusion, we evaluated the long-term intra-individual correlation of CRP using data from a 5-year follow-up study in a Japanese community. The correlation coefficients were statistically significant, but a little lower than those of other classical cardiovascular risk factors. A subgroup with higher levels of CRP at both baseline and follow-up measurements was found, which may identify those persons with aggregate cardiovascular risk factors.

Appendix

The Jichi Medical School Cohort Study Group involved the following investigators: Shizukiyo Ishikawa, Naoki Nago, Eiji Kajii, Manabu Yoshimura, Masahiro Igarashi, Yosikazu Nakamura, Hiroshi Yanagawa, Yoshihisa Ito, Kazuyuki Shimada, Yoshihiro Shibano, Jun Hiraoka, Kazunori Kayaba, Kishio Tsuruta, Tomohiro Saegusa, Seishi Yamada, Takashi Yamada, Kazuomi Kario, Masabumi Mizooka, Takeshi Miyamoto, Joji Ishikawa, Machi Sawada, Tomohiro Deguchi, Kazuo Hara, Kenichiro Sakai, Shuzo Takuma, Akizumi Tsutsumi, Kaname Tsutsumi, and Tsuyako Kumagai.

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