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Short Communication

Seasonal and Inter-day Variation in Serum High-sensitivity C-reactive Protein in Japanese Male Workers: A Longitudinal Study

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Abstract: Although seasonal variation in high-sensitivity C-reactive protein (hsCRP) has been studied cross-sectionally and longitudinally, the results to date have been mixed. Here, to test seasonal variation in this compound with regard to within-subject, inter-day variation, we conducted a longitudinal follow-up study with repeated measurements in Japanese workers with low hsCRP. Blood samples were obtained from four male indoor daytime workers, who were aged 32–57 and commuted to offices in Kawasaki City, on six days within 2-wk windows in February and October, 2008. Serum hsCRP was measured using ultrasensitive latex-enhanced immunonephelometry. Among the subjects with detectable levels of hsCRP, individual median serum hsCRP levels were 38–74% higher in October than in February (p=0.03). This study identified the presence of a seasonal variation in the serum hsCRP level of Japanese workers with low hsCRP levels.

Key words: Inflammation, Intra-individual variation, Measurement error, Misclassification, Shift work

High-sensitivity C-reactive protein (hsCRP), a reliable and cost-effective marker of systemic inflammation, is now used to evaluate the effects of work-related risk factors, including job stress and toxic chemicals[1, 2]. In prospective studies, higher circulating levels of hsCRP have been associated with an increased risk of future cardiovascular diseases, such as coronary heart disease, stroke and myocardial infarction[3, 4]. Shift work is also associated with an increased risk of cardiovascular disease[5]. In Japan excess working hours are a big issue because more than one hundred cases of "*Karoshi*", death from stroke or cardiovascular disease related to overwork, are newly registered each year. Accordingly, the measurement of hsCRP in serum obtained from workers would be useful for protecting against occupationally related stroke or cardiovascular diseases. However, before using the serum level of hsCRP as an index of the risk, we have to evaluate the factors which cause fluctuations in its level. In this study we focused on seasonal variation.

Some researchers have suspected the presence of seasonal variation in serum hsCRP levels[6]. Nevertheless, results of cross-sectional and longitudinal studies examining patterns of seasonal time-specific variation have been mixed[6–9]. The cross-sectional designs were critically limited in that they compared hsCRP levels among different subjects in each season and few longitudinal studies of seasonal variation have been conducted[8, 10–12]. Moreover, few data are available on the magnitude of hsCRP's short-term within-subject inter-day variability[13], while none are available on the significance of seasonal differences vis-à-vis intra-individual inter-day variation. Because time-dependent variations in serum hsCRP, including seasonal fluctuation and daily variation, may incorrectly predict

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worker cardiovascular risk, subsequent misclassification might dilute or bias associations observed in occupational epidemiologic studies. The levels of hsCRP in the Japanese population[14] are much lower than those in many other countries, such as the United States[15]. Accordingly, seasonal variation has to be estimated more carefully, and with controls for inter-day variability. In addition, no data are available on seasonal variation in serum hsCRP in Japanese workers.

Here, to examine seasonal variation in serum hsCRP in consideration of within-subject inter-day variability, we conducted a longitudinal follow-up study with repeated measurements of serum hsCRP concentration in healthy Japanese workers with lower hsCRP levels.

Apparently healthy male workers who commuted to Kawasaki City, Kanagawa Prefecture, which is adjacent to Tokyo, and were without exposure to specific hazardous factors were eligible for participation. Subjects were indoor daytime office workers who were not taking cholesterol-lowering medications; had not been diagnosed with heart disease, cerebrovascular disease, or cancer; were free from other life-threatening illnesses; and were not current smokers. Non-fasting venous blood samples were obtained from each participant, mainly in the morning at 10–11 a.m., on 6 different days within 2-wk windows in February and October 2008. Sampling was conducted such that it was balanced over a week. Four male workers who completed all twelve visits were included in the analyses. Demographic and health information data were collected using self-administered questionnaires. Each participant provided their signed informed consent to participation, and the protocol was approved by the Institutional Review Board of the National Institute of Occupational Safety and Health, Japan.

Blood samples were drawn into Venoject II plastic tubes (Terumo, Tokyo, Japan) which contained fine glass particles and acrylic resin gel. Serum was separated by centrifugation and frozen immediately after blood withdrawal, then stored at –80°C until analysis. Serum hsCRP was measured using ultrasensitive latex-enhanced immunonephelometry on a BN II nephelometer (Dade Behring, Tokyo, Japan) and a reagent, N-Latex CRP II (Siemens Healthcare Diagnostics K.K., Tokyo, Japan) with a lower limit of detection (LOD) of 0.004 mg/dl in a commercial laboratory, Mitsubishi Chemical Medience Corporation (Tokyo, Japan). For quality control, pooled serum samples from volunteers were divided into aliquots and simultaneously analyzed in parallel with each analysis conducted in each season by laboratory analysts who were blinded to the control sample status. Intra-assay CVs for serum hsCRP were 2.9% (mean 0.0152 mg/dl, n=5) and 6.3% (mean 0.016 mg/dl, n=3) for each season. There was no seasonal difference between these two means (p=0.22).

For calculation of basic statistics, serum hsCRP concentrations below the LOD were assigned a value equal to half the LOD, but such subjects without detectable serum hsCRP levels were excluded from the following analyses. Basic characteristics of the subjects were compared by season using Welch's paired t test. Seasonal differences in hsCRP were quantitatively analyzed in consideration of short-term inter-day variation as follows: serum hsCRP levels in each month were compared using the Wilcoxon rank sum test and the difference of subject averages (medians) of serum hsCRP for each subject in the two seasons was tested using the Welch's paired t test or Wilcoxon signed rank test. All statistical analyses and box plots of data were performed using the statistical analysis software R, version 2.9.0 (R Development Core Team, 2009) and the spreadsheet software Excel, version 2003 (Microsoft Corporation, Redmond, WA). All p-values were two-sided and significance was accepted at values of $p<0.05$.

All hsCRP values were within the low range (<0.1 mg/dl). Eleven of 12 serum samples derived from Subject A had no detectable level of hsCRP (<0.004 mg/dl). The basic characteristics of subjects in February and October 2008 are summarized in Table 1. The participants' age range was 32–57 yr. The gender of all participants was male. Anthropometric data showed no significant seasonal changes, but white blood cell count showed a marked seasonal variation (Table 1). Overall mean serum hsCRP was 24% higher in October than in February.

Figure 1 shows the seasonal difference and inter-day variation in serum hsCRP. A significant intra-individual seasonal difference in serum hsCRP level was found in Subject B (p=0.005), whereas the differences were insignificant in Subjects C and D (p=0.31 and 0.24, respectively) according to Wilcoxon's rank sum test. Among the three subjects with detectable levels of serum hsCRP, we found a 38–74% relative increase in median serum hsCRP levels in October compared to February. Using the subject averages (medians) of hsCRP in each season, the seasonal difference was insignificant according to Wilcoxon's signed rank test (p=0.25) but significant according to Welch's paired t test (p=0.03). Within-subject inter-day CVs in serum hsCRP ranged from 10 to 84% (mean 35%), except in Subject A. These values were much larger than the analytical CVs (2.9% and 6.3%).

Their results show that the amplitude of seasonal variation in serum hsCRP was large, even in Japanese workers

Table 1. Basic characteristics of subjects in February and October, 2008

Variable	February mean (SD)	October mean (SD)	*p*-value*
Age [yr]	43.0 (11.6)	43.5 (11.1)	0.18
Body mass index [kg/m^2]	22.8 (4.5)	22.6 (4.6)	0.29
Average sleeping time [h]†,‡	5.55 (0.70)	5.83 (0.93)	0.35
Serum hsCRP (overall mean) [mg/dl]†,§	0.023	0.029	0.03
White blood cell count (overall mean) [μl^{-1}]†	5,958	5,704	0.02
Mean daily outdoor temperature [°C]¶	5.4	13.0	–

* Welch's paired *t* test (two-sided).

†n=6 for each subject in each month. Statistical tests were performed using subject averages.

‡Individual sleeping time was calculated from self-reported bedtime and hour of rising.

§Subject A was excluded from statistical tests of hsCRP because of inadequate detection frequency.

¶Observed in Yokohama City, Kanagawa Prefecture [Japan Meteorological Agency (2008) Climate Statistics. http://www.data.jma.go.jp/obd/stats/etrn/view/monthly_s3_en.php?block_no=47670&view=1. Accessed July 29, 2009].

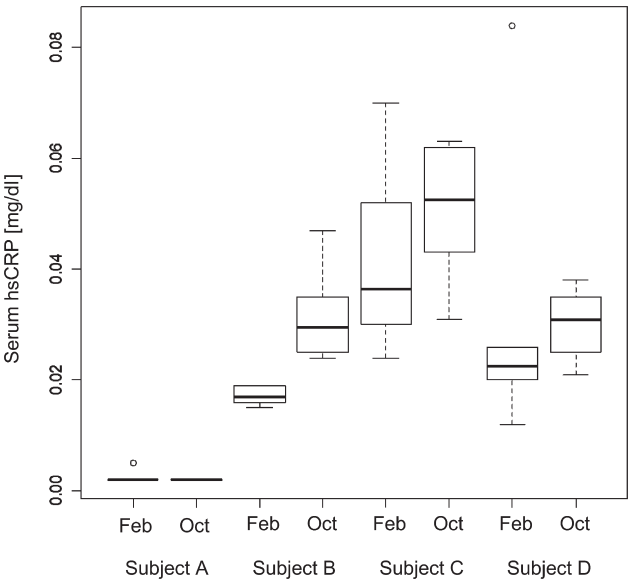

Fig. 1. Within-subject inter-day variation and seasonality of serum hsCRP.

The bold horizontal line represents the median of the distribution; the values above and below this line are divided into quartiles by horizontal lines (the hinges of the box) are the two quartiles nearer the midpoint. When serum hsCRP was below the LOD, a value equal to half of the LOD was assigned.

with low hsCRP, in terms of both within-subject inter-day variability and analytic variation. Levels of hsCRP in October were significantly higher than those in February according to Welch's paired *t* tests.

Our findings add to the growing body of evidence documenting seasonal variation in circulating hsCRP levels[6–9]. Importantly, our study provides the first data on seasonal variation in serum hsCRP in Japanese office workers, whose mean hsCRP level is lower than that of other ethnicities[15]. Our finding that serum hsCRP levels were higher in October than February are consistent with those of the most recent and hitherto largest longitudinal study[8], which showed that serum hsCRP peaked in November in both men and women[8], as well as those of the largest and most recent cross-sectional study, which showed a marginal peak in September[9]. Other longitudinal studies have shown a peak in late winter[10, 11], or no statistically significant variation[12]. This apparent inconsistency in peak month might be due to differences among the countries in which the studies were conducted.

The biological relevance of seasonal variation in hsCRP is uncertain but may reflect a physiological response to changes in daylight hours or indoor or outdoor temperature[9]. Seasonal variations in physical activity may also contribute to the seasonal variations observed[9].

Although circulating hsCRP level has been used in a number of epidemiologic studies[2–4, 15], not all of these took account of seasonal variation in hsCRP in their design. If measurement error was one-sided (e.g. blood samples from cases were collected particularly in the fall), the resulting differential misclassification might fail to identify actual associations. Etiological evaluation might be more appropriate if blood is collected from all subjects in the same specific month. Alternatively, case subjects might need to be individually matched with controls not only by age and gender, but also by the month of blood withdrawal. At the very least, the presence of seasonal fluctuation in hsCRP data should be monitored.

Our study has several strengths. First, it had a longitu-

dinal design with an adequate number of repeated hsCRP measures per subject in each season (n=6, respectively), which enabled within-subject statistical testing between seasons and more reliable estimation of subject averages in each month. Although repeated measurements of hsCRP has been recommended on account of its short term variability[13], even the largest longitudinal study to date used only single measurements in each season[8]. Second, our present longitudinal design with seasonal comparison of serum hsCRP on an intra-individual basis obviated confounding by known and unknown covariates such as anthropometric factors and unmeasured confounders.

Several limitations of the study also warrant mention. First, examinations were made only in October and February, and these two months might not correspond to the respective peak and nadir of circannual fluctuation in serum hsCRP. If so, the resulting smaller difference in serum hsCRP may have caused the null results of statistical tests in two subjects (Subject C and D). An additional limitation is the very small number of subjects. Serial measurements may not always guarantee that unknown factors affecting a subject have an important influence on final results. The generalizability of our results might be restricted to similar worker groups, although the peak season of hsCRP in the present study is at least similar to that reported in the previous largest longitudinal and cross-sectional studies[8, 9]. In future studies, additional measurements of other basic inflammatory markers (erythrocyte sedimentation rate, fibrinogen, …) may help to explain the results. Additionally, taking more than 100 subjects and refining the design and methodology will improve the validity of the results.

In conclusion, our study, conducted with consideration of within-subject inter-day variability, identified the presence of a seasonal variation in serum hsCRP in workers living in Japan. Further research is needed to confirm the pattern of seasonal time-dependent variation of hsCRP in various populations.

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References

1) Clays E, De Bacquer D, Delanghe J, Kittel F, Van Renterghem L, De Backer G (2005) Associations between dimensions of job stress and biomarkers of inflammation and infection. J Occup Environ Med **47**, 878–83.
2) Lin YS, Rathod D, Ho WC, Caffrey JJ (2009) Cadmium exposure is associated with elevated blood C-reactive protein and fibrinogen in the U. S. population: the third national health and nutrition examination survey (NHANES III, 1988–1994). Ann Epidemiol **19**, 592–6.
3) Rifai N, Ridker PM (2001) High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem **47**, 403–11.
4) Pearson TA (2002) New tools for coronary risk assessment: what are their advantages and limitations? Circulation **105**, 886–92.
5) Boggild H, Knutsson A (1999) Shift work, risk factors and cardiovascular disease. Scand J Work Environ Health **25**, 85–99.
6) Kelly GS (2005) Seasonal variations of selected cardiovascular risk factors. Altern Med Rev **10**, 307–20.
7) Sung KC (2006) Seasonal variation of C-reactive protein in apparently healthy Koreans. Int J Cardiol **107**, 338–42.
8) Chiriboga DE, Ma Y, Li W, Stanek EJ, 3rd, Hebert JR, Merriam PA, Rawson ES, Ockene IS (2009) Seasonal and sex variation of high-sensitivity C-reactive protein in healthy adults: a longitudinal study. Clin Chem **55**, 313–21.
9) Rudnicka AR, Rumley A, Lowe GD, Strachan DP (2007) Diurnal, seasonal, and blood-processing patterns in levels of circulating fibrinogen, fibrin D-dimer, C-reactive protein, tissue plasminogen activator, and von Willebrand factor in a 45-year-old population. Circulation **115**, 996–1003.
10) Crawford VL, Sweeney O, Coyle PV, Halliday IM, Stout RW (2000) The relationship between elevated fibrinogen and markers of infection: a comparison of seasonal cycles. QJM **93**, 745–50.
11) Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW (1994) Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. Lancet **343**, 435–9.
12) Frohlich M, Sund M, Thorand B, Hutchinson WL, Pepys MB, Koenig W (2002) Lack of seasonal variation in C-reactive protein. Clin Chem **48**, 575–7.
13) Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E (2001) Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem **47**, 444–50.
14) Arima H, Kubo M, Yonemoto K, Doi Y, Ninomiya T, Tanizaki Y, Hata J, Matsumura K, Iida M, Kiyohara Y (2008) High-sensitivity C-reactive protein and coronary heart disease in a general population of Japanese: the Hisayama Study. Arterioscler Thromb Vasc Biol **28**, 1385–91.
15) Kelley-Hedgepeth A, Lloyd-Jones DM, Colvin A, Matthews KA, Johnston J, Sowers MR, Sternfeld B, Pasternak RC, Chae CU, for the SWAN Investigators (2008) Ethnic differences in C-reactive protein concentrations. Clin Chem **54**, 1027–37.