Original Contribution

White Blood Cell Count and Risk of Gastric Cancer Incidence in a General Japanese Population

The Hisayama Study

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The authors examined the association between white blood cell (WBC) count and the development of gastric cancer in a 19-year follow-up study of 2,558 Japanese subjects aged ≥40 years (1988–2007). The subjects were stratified into 4 groups according to baseline WBC quartile (≤4.4, 4.5–5.2, 5.3–6.3, or ≥6.4 × 10^3 cells/μL). During follow-up, 128 subjects developed gastric cancer. The age- and sex-adjusted incidence of gastric cancer increased linearly with higher WBC level: 1.7, 2.6, 3.9, and 5.4 per 1,000 person-years, respectively, for the 4 quartile groups (P for trend < 0.01). The risk of gastric cancer was 2.22-fold (95% confidence interval: 1.19, 4.14) higher in the highest WBC quartile group than in the lowest group after adjustment for confounding factors. With respect to Helicobacter pylori infection status, H. pylori-seropositive subjects in the highest WBC quartile group showed a significantly greater risk of gastric cancer than those in the lower 3 quartile groups, whereas such an association was not observed in H. pylori-seronegative subjects. There was no evidence of heterogeneity in the association (P for heterogeneity = 0.65). The study findings suggest that higher WBC levels are a risk factor for gastric cancer, especially in subjects with H. pylori infection.

Helicobacter pylori; inflammation; leukocytes; proportional hazards models; prospective studies; risk factors; stomach neoplasms

Abbreviations: CI, confidence interval; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.

Although the incidence of gastric cancer has been on the decline for the past century, this neoplasm is still the second most common cause of death from cancer worldwide (1). While early diagnosis and treatment of gastric cancer have significantly improved the prognosis, the 5-year survival rate in patients with advanced gastric cancer is still poor (2). There is thus a pressing need for improved methods to identify persons at high risk of gastric cancer, so that affected persons can be diagnosed and treated as early as possible.

Gastric cancer is considered a multifactorial disease that involves various risk factors in its development (3–7). Among these risk factors, Helicobacter pylori infection has been shown to be the most significant. However, each year, approximately 0.5% of persons with H. pylori infection actually develop gastric cancer (8), possibly suggesting that H. pylori is not the only etiologic factor for gastric cancer and that other cofactors modify the relation between H. pylori infection and this disease.

Circulating white blood cell (WBC) count is a stable, well standardized, widely available, and inexpensive biomarker of systemic inflammation (9). Several prospective studies have shown that an increase in WBC count within the clinically normal range is associated with increased risk of several chronic diseases, including cardiovascular disease, hypertension, and diabetes (9–11). In recent years, chronic inflammation has also been hypothesized to play a role in the pathogenesis of cancer. In fact, several prospective studies...
have revealed that an increment in WBC count was associated with increased risks of cancer in the lung, large bowel, and breast, as well as cancer overall (12–14). To the best of our knowledge, however, none of the previous studies evaluated the association of WBC level with the development of gastric cancer. Accordingly, our aim in this study was to examine prospectively whether WBC count is an independent risk factor for the development of gastric cancer or whether it modifies the relation between H. pylori infection and gastric cancer in a general Japanese population, taking other comprehensive risk factors into consideration.

MATERIALS AND METHODS

Study population

The town of Hisayama is located in a suburban area adjacent to Fukuoka City, a large urban center on Kyushu Island in the southern part of Japan. The population of the town is approximately 7,500 and has been stable for the past 40 years. The age and occupational distributions of the Hisayama population have been almost identical to those of Japan as a whole from the 1960s to the present, based on data from the national census (15). The dietary patterns of the residents are also similar to those of participants in the National Nutrition Survey, for which subjects were selected from 300 areas throughout Japan (16). In 1988, a screening survey for the present study was performed in Hisayama. A detailed description of this survey has been published previously (3, 15). In this survey, 2,742 Hisayama residents aged 40 years or older (80.9% of the total population in that age group) underwent a health check-up. After the exclusion of 130 persons with a history of gastrectomy or gastric cancer, for 49 of whom WBC data were not available and 5 of whom died during the examination period, 2,558 subjects (1,048 men and 1,510 women) were enrolled in the study.

The study protocol was approved by the Kyushu University Institutional Review Board for Clinical Research, and written informed consent for medical research was obtained from the study subjects.

Follow-up survey

This population was followed for 19 years, between December 1988 and November 2007, through annual health examinations. Particularly comprehensive health check-ups were conducted every 5 years. For all subjects who did not undergo regular check-ups or who moved out of town, health status was checked every year by mail or telephone. In addition, a daily monitoring system was established by the study team and local physicians or members of the town’s Division of Health and Welfare. All of the participants were followed up completely over a 19-year period. Local clinics and hospitals in and around Hisayama were surveyed for cases of gastric cancer by referring to medical records of barium radiographic examinations, upper endoscopic examinations, and biopsy diagnoses. We also checked all records from annual mass screenings for gastric cancer that included upper gastrointestinal series. Furthermore, to find any concealed gastric cancer, autopsies were performed on 601 (72.1%) of the 833 subjects who died during the follow-up period. The diagnoses of all cases of gastric cancer were confirmed by histologic examination of tissue obtained during surgery, including gastrectomy and endoscopic mucosal resection, or autopsy. Pathologic diagnosis and classification of identified gastric cancers were conducted according to the guidelines proposed by the Japanese Gastric Cancer Association (17) and the histologic classification of Laurén (18). Thus, the subtypes of gastric cancer were categorized as either intestinal or diffuse.

During the 19-year follow-up period, gastric cancer developed in 128 subjects (84 men and 44 women), including 4 subjects with concealed cancers (3.1%) first diagnosed at autopsy. Among these cases, there were 12 subjects (9.4%) who had 2 synchronous gastric cancers (double cancers), for a total of 140 lesions. Of these lesions, 112 cases were classified as involving intestinal-type tumors and the remaining 28 as diffuse-type tumors.

Laboratory testing and risk factor measurement

At the baseline examination, WBC count was measured using the impedance method (19). Based on the distribution of WBC levels, subjects were classified into quartile groups: \( \leq 4.4, 4.5–5.2, 5.3–6.3, \) and \( \geq 6.4 \times 10^9 \) cells/\( \mu L \). To assess the independent effect of WBC count on the occurrence of gastric cancer, the following baseline factors in addition to age and sex were used for analysis as confounding factors. Information about the history of peptic ulcer disease, alcohol intake, and smoking habits was obtained by means of a questionnaire administered to each subject, and the latter 2 items were categorized as current use or not. Height and weight were measured with the subject in light clothes without shoes, and obesity was defined as body mass index (weight (kg)/height (m\(^2\)) \( \geq 25.0 \). Diabetes was determined according to medical history, glucose level (fasting glucose level \( \geq 7.0 \text{ mmol}/\text{L} \) or postprandial glucose level \( \geq 11.1 \text{ mmol}/\text{L} \), or a 75-g oral glucose tolerance test (the 1988 World Health Organization criteria), which was administered to most of the subjects aged 40–79 years (15), with plasma glucose measured by the glucose-oxidase method. Serum cholesterol levels were determined using an enzymatic autoanalyzer. WBC count, blood glucose level, and total cholesterol level were analyzed within 24 hours after obtaining the blood samples. A portion of the serum was stored at \(-20^\circ C\) until it was used in the measurement of immunoglobulin G antibodies to \( H. pylori \) in 1997 and high-sensitivity C-reactive protein (hs-CRP) in 2002. Serum immunoglobulin G antibodies to \( H. pylori \) were assayed by means of a quantitative enzyme immunoassay, and the assay values were interpreted as either positive or negative based on the manufacturer’s instructions. Serum hs-CRP levels were analyzed using a modification of the Behring latex-enhanced C-reactive protein assay on a Behring nephelometer (BN-100; Dade Behring, Tokyo, Japan). Data on dietary factors were obtained by the semiquantitative food frequency method, which was validated in a prior study (20). Daily nutrient intakes, including intakes of total energy, salt, vitamin A, vitamin B1, vitamin B2, vitamin C, and dietary fiber, were calculated using the 4th revision of the Standard Tables of Food Composition in Japan (21). The nutritional
Statistical analysis

The mean values of possible risk factors were adjusted for age and sex using the analysis of covariance method and were tested for trends across WBC levels using multiple regression analysis. The frequencies of risk factors were adjusted for age and sex using the direct method and were tested for trends using the Cochran-Mantel-Haenszel $\chi^2$ test. Subjects were censored on the date of their death, the date of their undergoing gastrectomy for reasons other than gastric cancer, or the end of follow-up for those still alive. The incidence rate of first-ever gastric cancer was estimated with the person-year method after adjustment for age and sex. All of the study subjects were used as a standard population for age- and sex-adjustment. The differences and trends in gastric cancer incidence among WBC levels were tested by means of a Cox proportional hazards model. Hazard ratios and their 95% confidence intervals were also estimated with the Cox proportional hazards model, where calendar time was used as the time scale (23). In the multivariate analysis, we selected the clinically or biologically plausible risk factors for gastric cancer occurrence listed in Table 1 (3–7, 24). Then we performed backward selection, which started from the full model including all relevant variables and deleted the variables showing the smallest statistical contribution to the model at each step until all remaining variables had individual $P$ values less than 0.20 (25), and obtained the final list of confounding factors used for multivariate adjustment. We tested for heterogeneity in the association between subgroups by adding an interaction term to the relevant Cox model. We also evaluated the risk estimates by fitting the Cox proportional hazards model with the time-dependent variable of WBC count every 5 years in order to take into consideration the influence of variation in WBC count during the follow-up period, where the baseline data were used for covariates. Missing values for WBC count over time were imputed by carrying the last observation forward. Statistical analyses were conducted using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina). A 2-tailed $P$ value less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the age- and sex-adjusted mean values or frequencies of potential risk factors for gastric cancer by quartile of WBC count at baseline. Younger subjects had...
The differences and trends in incidence among WBC levels were tested by means of a Cox proportional hazards model. For the upper quartiles of WBC count the frequencies of male sex, H. pylori infection, total cholesterol, smoking habits, and dietary factors, including intakes of total energy, vitamin A, and dietary fiber. Thus, we investigated the effects of elevated WBC level on gastric cancer occurrence according to H. pylori infection status (Table 3). Among H. pylori-seropositive subjects, the multivariate-adjusted hazard ratio for gastric cancer among subjects with WBC levels of \( \geq 6.4 \times 10^3 \) cells/\( \mu L \) was significantly higher than that for subjects with WBC levels of \( \leq 6.3 \times 10^3 \) cells/\( \mu L \) (HR = 1.80, 95% CI: 1.19, 2.73; P = 0.006), whereas no significant relation was observed in H. pylori-seronegative subjects (HR = 1.10, 95% CI: 0.32, 3.77; P = 0.88). There was no evidence of heterogeneity in the relation between H. pylori infection status and WBC level (P for heterogeneity = 0.65).

Furthermore, we addressed the association between WBC level and risk of gastric cancer occurrence by H. pylori infection status and smoking and alcohol drinking. Elevated WBC level appeared to be a significant risk factor for gastric cancer only among current smokers or drinkers with H. pylori infection. However, these findings were considered unreliable because of small numbers of events in these subgroups, particularly those without H. pylori infection.

Finally, we addressed the relation between WBC level and gastric cancer occurrence according to histologic type in subjects with H. pylori infection. Subjects with WBC levels of \( \geq 6.4 \times 10^3 \) cells/\( \mu L \) had a greater risk of gastric cancer than those with WBC levels of \( \leq 6.3 \times 10^3 \) cells/\( \mu L \), regardless of histologic type (intestinal type: age- and sex-adjusted HR = 2.22, 95% confidence interval (CI): 1.19, 4.14; P = 0.01) (Table 2). Every increment in WBC level of \( 1.0 \times 10^3 \) cells/\( \mu L \) was associated with a 1.13-fold (95% CI: 1.02, 1.25) greater risk of gastric cancer after adjustment for the above-mentioned confounding factors. A comparable relation was observed in the sensitivity analysis after we excluded subjects who developed gastric cancer within the first 3 years of follow-up in order to avoid the influence of concealed gastric cancer at baseline (multivariate-adjusted HR = 2.16, 95% CI: 1.11, 4.20; P = 0.02). In the analysis using the time-dependent Cox proportional hazards model with WBC counts varying over time, subjects in the highest quartile of time-varying WBC level had a significantly greater risk of gastric cancer than those in the lowest quartile (HR = 2.02, 95% CI: 1.10, 3.72; P = 0.02).

Subjects with H. pylori infection had a 2.33-fold (95% CI: 1.35, 4.01; P = 0.002) greater risk of incident gastric cancer than those without H. pylori infection after adjustment for age, sex, total cholesterol, smoking habits, and dietary factors, including intakes of total energy, vitamin A, and dietary fiber. The present analysis demonstrated that an elevated WBC level was a significant risk factor for the development of gastric cancer in a general Japanese population. This association remained significant even after adjustment for other risk factors: age, sex, H. pylori infection, total cholesterol level, smoking habits, and dietary factors. The same was true when we took into account variation in WBC count during the follow-up period. Moreover, the coexistence of elevated WBC levels and H. pylori infection increased the future risk of gastric cancer. To the best of our knowledge, this is the first cohort study to examine the association between WBC level and gastric cancer incidence. Most importantly, the relation between WBC level and gastric cancer was observed only among subjects with H. pylori infection, although there was no evidence of heterogeneity in the association between H. pylori infection status and WBC levels, probably because of limited statistical power.

To date, several epidemiologic studies of elevated WBC levels and cancer risk have been reported (14, 26–28). Most studies, except a large prospective cohort study conducted in South Korea (28), revealed positive associations between WBC count and cancer mortality (14, 26, 27). With regard to site-specific cancers, one prospective study showed that colon cancer morbidity and mortality increased with higher WBC levels (13), and another study showed that higher...
WBC levels were associated with the risks of lung, colorectal, and breast cancer (12). The present study demonstrated that higher WBC levels were significantly associated with a greater risk of gastric cancer even after adjustment for confounding factors. Thus, it may be reasonable to suppose that an elevation in WBC count, which reflects nonspecific chronic inflammation, is associated with the development of most cancers.

In the present study, the significant relation between WBC level and gastric cancer was observed only in subjects with *H. pylori* infection. *H. pylori* infection is a strong risk factor for the development of gastric cancer through chronic gastritis (3, 29). The number of peripheral blood leukocytes reflects the extent of mucosal inflammation induced by *H. pylori* infection (30). These findings raise the possibility that peripheral WBC count is a useful indicator for estimating the severity of gastric mucosal inflammation caused by *H. pylori* infection, which is likely to act as an intermediate factor between *H. pylori* infection and gastric cancer development.

The biologic mechanisms through which elevated WBC levels increase the risk of cancer in subjects with *H. pylori* infection remain to be established. *H. pylori* infection has been shown to increase the production of inflammatory cytokines such as interleukin-8, interleukin-6, and tumor necrosis factor α from epithelial cells in the gastric mucosa (31). A case-control study demonstrated that an interleukin-8 polymorphism was associated with higher expression of interleukin-8 protein in the gastric mucosa, more severe neutrophil infiltration, and increased risk of atrophic gastritis and gastric cancer (32). Additionally, experimental studies showed that neutrophils stimulated by *H. pylori* extracts produced interleukin-8 in vitro (33) and that interleukin-8 enhanced the production of reactive oxygen metabolites from neutrophils (34). Thus, the neutrophil itself seems to contribute to neutrophil migration into the gastric mucosa and up-regulation of oxidative mucosal injury. Excess reactive oxygen and nitrogen species often cause extensive tissue damage and DNA damage (35, 36), which may induce mutational changes in oncogenes and tumor suppressor genes.

### Table 2. Hazard Ratios for Gastric Cancer According to Quartile of Circulating White Blood Cell Count, Hisayama Study, Japan, 1988–2007

<table>
<thead>
<tr>
<th>Quartile of White Blood Cell Count, ×10³ cells/µL</th>
<th>No. of Subjects</th>
<th>No. of Events</th>
<th>Person-Years at Risk</th>
<th>Age- and Sex-Adjusted HR (95% CI)</th>
<th>Multivariate-Adjusted* HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1 (≤4.4)</td>
<td>637</td>
<td>15</td>
<td>10,284</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Quartile 2 (4.5–5.2)</td>
<td>627</td>
<td>22</td>
<td>10,173</td>
<td>1.30 (0.67, 2.51)</td>
<td>1.10 (0.56, 2.14)</td>
</tr>
<tr>
<td>Quartile 3 (5.3–6.3)</td>
<td>665</td>
<td>39</td>
<td>10,439</td>
<td>2.01* (1.10, 3.69)</td>
<td>1.64 (0.88, 3.04)</td>
</tr>
<tr>
<td>Quartile 4 (≥6.4)</td>
<td>629</td>
<td>52</td>
<td>9,620</td>
<td>2.78** (1.53, 5.03)</td>
<td>2.22* (1.19, 4.14)</td>
</tr>
</tbody>
</table>

*P for trend< 0.001

**Abbreviations:** CI, confidence interval; HR, hazard ratio.

* In multivariate analysis, results were adjusted for age, sex, *H. pylori* infection, total cholesterol, smoking habits, and dietary factors (intakes of total energy, vitamin A, and dietary fiber) by means of the Cox proportional hazards model.

### Table 3. Hazard Ratios for Gastric Cancer According to Circulating White Blood Cell Count and *Helicobacter pylori* Infection Status, Hisayama Study, 1988–2007

<table>
<thead>
<tr>
<th>White Blood Cell Count</th>
<th>Age-, Sex-, and <em>H. pylori</em> Infection-Adjusted*</th>
<th>Multivariate-Adjusted&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6.3 × 10³ cells/µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Events</td>
<td>No. of Subjects</td>
<td>HR 95% CI  P Value</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>1.77* (1.23, 2.54) 0.002</td>
</tr>
<tr>
<td><em>H. pylori</em> infection status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>1.27 (0.41, 3.98) 0.68</td>
</tr>
<tr>
<td>Positive</td>
<td>63</td>
<td>1.84* (1.25, 2.71) 0.002</td>
</tr>
</tbody>
</table>

<sup>a</sup> *P < 0.01* (for white blood cell count ≥6.4 × 10³ cells/µL vs. ≤6.3 × 10³ cells/µL).

<sup>b</sup> The *H. pylori* infection variable was excluded from the relevant model in the subgroup analysis.

<sup>a</sup> In multivariate analysis, results were adjusted for age, sex, *H. pylori* infection, total cholesterol, smoking habits, and dietary factors (intakes of total energy, vitamin A, and dietary fiber) by means of the Cox proportional hazards model.

genes and subsequently lead to the development of cancer in the gastric mucosa with *H. pylori* infection (37–39). Further investigations will be needed to address these issues.

The strengths of our study include its longitudinal population-based study design, long duration of follow-up, completeness of follow-up, accuracy of gastric cancer diagnosis, consideration of WBC count variation during the follow-up period, and inclusion of comprehensive risk factors, including *H. pylori* infection. The limitations of the present study should also be noted. First, differential WBC counts were not performed in this study. Thus, we could not determine which types of WBCs were more prone to reflect the occurrence of gastric cancer. Second, we were unable to deny the possibility of subclinical gastric cancer at baseline, since we did not perform a screening survey of the stomach in each subject at the time of recruitment. However, in a nationwide mass screening in Japan (40), the prevalence of gastric cancer in healthy subjects was reported to be low (0.12%). Moreover, the sensitivity analysis carried out after exclusion of subjects who developed gastric cancer during the initial 3-year follow-up period did not show any material differences in the findings. Thus, the influence of such occult cases of gastric cancer would have been small. Third, serum preserved at −20°C was used in the measurement of immunoglobulin G antibodies to *H. pylori* and hs-CRP. Certainly, the preservation of serum for a long period may lead to inaccuracy in such measurements, but an epidemiologic study has shown the stability of protein in serum preserved at −20°C for 10 years or more (41). Thus, we do not believe that this limitation altered our findings substantially.

In conclusion, the present study is the first population-based cohort study to demonstrate a linear relation between WBC level and the risk of gastric cancer occurrence. Our findings suggest that persons with *H. pylori* infection and higher WBC levels should be considered a high-risk population for gastric cancer occurrence. It is recommended that members of this high-risk group undergo regular endoscopic screening, which may achieve the anticipated goals of early detection and treatment for gastric cancer.

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Conflict of interest: none declared.

REFERENCES


