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SPECIAL FEATURE

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DNA methylation: a marker for carcinogen exposure and cancer risk

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Abstract Cancers arise as a consequence of multiple genetic and epigenetic alterations. Many genes aberrantly methylated in cancers have been identified in recent years, and their use in cancer diagnosis and therapy is currently under investigation. During our genome-wide screening for a novel tumor-suppressor gene in gastric cancers, we found that only a small amount of aberrant methylation was present, even in non-cancerous gastric mucosae. A subsequent large-scale analysis of the gastric mucosae of healthy individuals and gastric cancer patients using quantitative methylation-specific PCR (qMSP) revealed that *Helicobacter pylori* infection potently induced aberrant DNA methylation in non-cancerous gastric mucosae and that these high methylation levels can decrease following cessation of the *H. pylori* infection. *Helicobacter pylori* infection induced the methylation of specific genes among 48 genes that can be methylated in gastric cancer cell lines. Most importantly, the methylation levels in the gastric mucosae of individuals without *H. pylori* infection correlated with their risk of gastric cancer. These findings show that a field for cancerization is formed by *H. pylori* infection and that this field can be measured using DNA methylation as a marker. The concept of an "epigenetic field for cancerization" has been also demonstrated for colon and breast cancers, and it is possibly present for other cancers and other diseases. Applied knowledge of epigenetic changes in human diseases has now started to make an impact on the prevention, diagnostics, and therapeutics of these diseases.

Keywords Cancer · DNA methylation · Epigenetic · Field cancerization · Field defect · Gastric cancer · *Helicobacter pylori*

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Introduction

Epigenetic modifications are defined as DNA-associated modifications that are faithfully inherited upon somatic cell division, such as DNA methylation at CpG sites, histone modifications, and polycomb complex formation [1]. DNA methylation, in particular, is faithfully replicated upon cell division [2, 3], and is known to serve as a machinery for cellular memory [4]. At the same time, epigenetic modifications show plasticity during development, adaptation, and diseases. Epigenetic modifications are reprogrammed during the formation of germ cells, and dynamic and coordinated changes take place during development and differentiation [5]. Epigenetic changes are also physiologically induced in somatic cells to maintain the memory of exposure to environmental stimuli [6, 7].

Our increasing knowledge of epigenetic changes in human diseases has now started to make an impact on the prevention, diagnostics, and therapeutics of these diseases. From a viewpoint of environmental health and preventive medicine, epigenetic alterations in non-disease tissues are becoming important because it is becoming clear that they can be used as markers for disease risk and past exposure to some disease-inducing factors. In this review, we provide a brief introduction to aberrant DNA methylation in cancers, describe our experimental findings on the presence of aberrant DNA methylation in non-cancerous gastric

mucosae, including a description of its use as a marker for both the risk of gastric cancers and past exposure to *Helicobacter pylori*, an established gastric carcinogen and, finally, discuss the concept of field cancerization and its usefulness an a diagnostic marker in other cancers.

Aberrant DNA methylation in cancers

Aberrant DNA methylation in carcinogenesis

The existence of aberrant DNA methylation in cancer tissues has been known since the early 1980s, but it was not until the early 1990s that it was shown to have a causal involvement in human cancers [1, 8]. Aberrant DNA methylation in cancers is often summarized as (1) genome-overall hypomethylation and (2) regional hypermethylation. Genome-overall hypomethylation is mainly due to hypomethylation of repetitive DNA sequences, such as LINE and Alu (SINE), that constitute a major part of the genome and are normally methylated [9]. Such hypomethylation can cause chromosomal instability and, consequently, tumors [10] as well as aberrant expression of normally methylated genes, such as melanoma antigen genes (MAGEs) [11]. The aberrant activation of oncogenes due to promoter demethylation (hypomethylation) has as yet not been established.

Regional hypermethylation refers to the aberrant methylation of normally unmethylated sequences, most of which are clusters of CpG sites, denoted CpG islands (CGIs). Importantly, when a CGI is located in a gene promoter region, its methylation consistently leads to transcriptional silencing of its downstream gene (Fig. 1). This also applies to many tumor-suppressor genes, such as *CDKN2A* (various cancers), *CDH1* (gastric cancers), *APC* (colorectal cancers), and *BRCA1* (breast cancer). Methylation-silencing of tumor-suppressor genes is now known to be involved in various human cancers [1]. In addition to aberrant DNA methylation being causally involved in carcinogenesis (driver methylation), recent genome-wide studies have revealed the presence of many genes whose methylation is considered to be a consequence of carcinogenesis (passenger methylation) [12–14]. This fact clearly demonstrates the need to carefully analyze the role of any newly detected gene in terms of its role in carcinogenesis.

Clinical use of aberrant DNA methylation

Both diagnostic and therapeutic applications of aberrant DNA methylation in cancers are being developed [15]. One diagnostic application is the use of cancer-specific patterns of aberrant DNA methylation to detect cancer cells and cancer-derived DNA. Different from mutations, aberrant DNA methylation can be detected with high sensitivity – for example, at a sensitivity of one aberrantly methylated DNA molecule among 1000 molecules. The chemical stability of DNA is also an advantage in this application. A second diagnostic application is the association of patterns of aberrant DNA methylation in cancer tissues with tumor characteristics, such as histological type, risk of disease progression, sensitivity to chemotherapy, and molecular alterations [15, 16]. An example of this can be found in neuroblastomas, where the methylation pattern is very closely associated with survival risk [17]. Thirdly, methylation in non-cancerous tissues is now recognized as a marker for cancer risk and exposure to carcinogenic factors, which will be the main topic of this review.

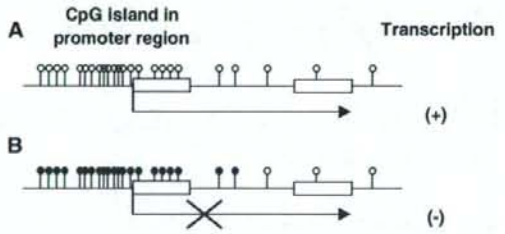

Fig. 1 Methylation of a promoter CpG island (*CGI*) and transcription of its downstream gene. *Open* and *closed circles* Unmethylated and methylated CpG sites, respectively. **a** In a normal cell, most CpG sites within a promoter CGI are unmethylated. **b** Methylation of most CpG sites (dense methylation) of the promoter CGI completely blocks transcription. If such methylation occurs in a tumor-suppressor gene, it leads to inactivation of the tumor-suppressor gene

In terms of therapeutic purposes, epigenetic abnormalities are now used as promising targets. The Federal Drug Agency has approved two demethylating agents, 5-aza-cytidine (5-aza; Vidaza) and 5-aza-2′-deoxycytidine (5-aza-dC; Decitabine), for hematological malignancies [18]. In addition, preclinical trials are ongoing for solid tumors. Demethylating agents currently seem to have therapeutic windows, being active in tumor cells but having few side-effects in normal cells. The concept of "maximum tolerance dose" is not valid for demethylating agents, and an optimal dose for maximum demethylating activity should be achieved [18]. Further investigations are necessary on the most suitable dosing, including the identification of appropriate marker genes and tissue, and on the target specificity in cancer and normal cells.

Aberrant methylation in non-cancerous gastric mucosae

Presence of "aberrant" DNA methylation in non-cancerous gastric mucosae

In human gastric cancers, *CDKN2A* (*p16*), *CDH1* (*E-cadherin*), and *hMLH1* are inactivated more frequently by the

aberrant methylation of promoter CGI than by mutations or chromosomal losses [19]. During previous study carried out in our laboratory in which we identified a novel tumor-suppressor gene, *LOX* [20], we observed that aberrant DNA methylation was present even in non-cancerous gastric mucosae of gastric cancer patients, although at a very low level [21, 9]. It is unlikely that these cancer cells contaminated non-cancerous samples because aberrant methylation in non-cancerous gastric mucosae was observed too often to be contamination.

Methylation in non-cancerous gastric mucosae and *H. pylori* infection

To clarify the meaning of "a small amount" of aberrant methylation in non-cancerous gastric mucosae, we quantified the methylation levels in gastric mucosae of healthy volunteers and in non-cancerous gastric mucosae of gastric cancer patients [22]. Healthy volunteers (individuals without gastric cancer) and gastric cancer patients were classified according to their status of *H. pylori* infection, a major etiological factor for gastric cancers [23, 24], at the time of sampling. The numbers of methylated and unmethylated DNA molecules were counted, using quantitative methylation-specific PCR (qMSP), for eight regions of seven genes, all of which can be methylated in gastric cancers. Methylation levels were calculated as the number of methylated molecules present among the total number of DNA molecules. This value was considered to represent the fraction of cells with methylation in a gastric mucosa.

All of the eight regions showed a similar tendency in terms of methylation levels. Among healthy volunteers, methylation levels were 5.4- to 303-fold higher in *H. pylori*-positive individuals than *H. pylori*-negative individuals [22]. This finding strongly indicates that *H. pylori* infection can potently induce aberrant DNA methylation in non-cancerous gastric mucosae. In addition, *H. pylori*-positive individuals had higher methylation levels than *H. pylori*-negative gastric cancer cases, most of whom were considered to have had prior exposure to *H. pylori* infection (Fig. 2a).

Temporary and permanent components of methylation level induced by *H. pylori*

The above results indicate that high methylation levels will decrease to certain levels after cessation of the *H. pylori* infection. Since endogenous DNA demethylase has not been established and active demethylation in a cell was unlikely to take place, this decrease is considered to be passive demethylation due to cell turnover. A gastric gland consists of one stem cell, multiple progenitor cells, and many differentiated cells [25], and it is expected that methylation in stem cells will persist forever (permanent component) while methylation in progenitor and differentiated cells will disappear (temporary component). It is therefore likely that *H. pylori* infection induced both permanent and temporary components of methylation, and that

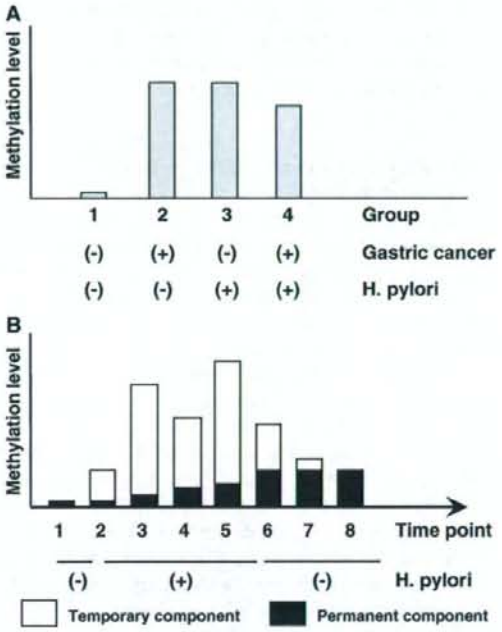

Fig. 2 Methylation induction by *Helicobacter pylori* infection and gastric cancer risk. **a** Schematic representation of methylation levels in the gastric mucosae of individuals with and without gastric cancer, and with and without *H. pylori* infection. Methylation levels were measured in DNA extracted from gastric biopsy specimens. Without *H. pylori* infection, there is a significant difference between healthy volunteers (*group 1*) and gastric cancer cases (*group 2*). With *H. pylori* infection, the methylation level is high in both healthy volunteers (*group 3*) and gastric cancer cases (*group 4*). *H. pylori*-positive individuals had higher methylation levels than *H. pylori*-negative gastric cancer cases, most of whom were considered to have had prior exposure to *H. pylori* infection. Modified from Maekita et al. [22]. **b** A hypothetical temporal profile of gastric methylation levels during the course of *H. pylori* infection in years to decades. Time point 1: Without *H. pylori* infection, the methylation is initially. Time points: 2–5, *H. pylori* infection induces both permanent (*closed box*) and temporary (*open box*) components of methylation, and the total methylation level fluctuates due to fluctuation of the temporary component. Time points 6–8: after *H. pylori* infection discontinues, the temporary component disappears, and the increase in the permanent component stops. It is speculated that the permanent component is due to methylation in stem cells and that the temporary component is due to methylation in progenitor and differentiated cells. The permanent component is correlated with damage in stem cells, and thus with gastric cancer risk

the temporary component disappeared after cessation of the *H. pylori* infection (Fig. 2b).

To support this hypothesis, we eradicated *H. pylori* and measured methylation levels 6 weeks after the eradication. When the eradication was successful, *FLNc* (filamin C) methylation levels decreased to certain levels. When eradication failed, methylation levels fluctuated, depending upon the individual (Nakajima, in preparation). The decreased methylation levels in individuals following successful eradication of *H. pylori* was considered to be due to disappearance of the temporary component, leaving only the permanent component in place.

Methylation levels in gastric mucosae as a marker for gastric cancer risk

In the study described above [22], individuals without *H. pylori* infection, whose methylation levels were considered to reflect the fraction of stem cells with methylation, had methylation levels that were 2.2- to 4.9-fold higher in cases of gastric cancer than in healthy volunteers. We also newly collected non-cancerous gastric mucosae of patients with a single gastric cancer and those with multiple gastric cancers. Patients in the latter group, who were considered to have a higher risk of gastric cancers [26], had a significantly higher *FLNc* methylation level than patients with a single gastric cancer ($P < 0.01$, t test) [27]. These results strongly indicate that methylation levels in non-cancerous gastric mucosae are a good candidate biomarker for gastric cancer risk. In order to confirm their clinical usefulness, a prospective study is currently being planned.

Methylation of specific genes in gastric mucosae by *H. pylori* infection, and its promising potential

In the initial study where eight regions of seven genes were analyzed [22], all eight regions were methylated in the presence of *H. pylori* infection. There are two possibilities explaining this result: (1) these eight regions are regions that can be methylated in gastric cancers, or (2) *H. pylori* infection induces genome-wide, non-specific methylation of CGIs. To distinguish between these two possibilities, we analyzed the methylation of 48 genes in gastric mucosae of individuals with and without *H. pylori* infection. These 48 genes were selected because they can be methylation silenced in gastric cancer cell lines [14]. Some genes were resistant to methylation induction, and some were consistently methylated in individuals with *H. pylori* infection (unpublished data; for review, see [28]. Since low transcription levels are known to trigger promoter methylation [12, 29], it was concluded that *H. pylori* infection can induce decreased transcription of specific genes and that some of these can be methylated. This concept can be expanded to one in which some carcinogenic factors have the potential to induce methylation of specific genes in non-cancerous tissues and that the specific methylation profile of an individual can be used as a marker for past exposure to specific carcinogenic factors.

Epigenetic field for cancerization

The finding that methylation levels in non-cancerous gastric mucosae correlate with gastric cancer risk has the potential to be generalized to cancers of other tissues. Since this finding is closely related with the concept of field defect, or field for cancerization, which has a long history, we first provide a short review of the concept of field for cancerization and then discuss "epigenetic field for cancerization".

The concept of field for cancerization

The concept of "field for cancerization" was first used by Slaughter et al. in 1953 for describing oral cavity cancer [30] and was based on the phenomenon that, even after curative resection of a primary cancer, metachronous (secondary multiple) primary cancers developed further. This occurrence indicated that the background mucosae of a cancer patient were already predisposed to cancer development, providing a field for cancerization. In recent decades, the concept has been applied to cancers of many other organs, especially squamous cell carcinomas of the head and neck (HNSCC) [31, 32], squamous cell carcinomas of the esophagus [33, 34], adenocarcinomas from the Barrett's esophagus [35], stomach cancers [36], breast cancers [37], and skin cancers [38]. The presence of cells with mutations of tumor-related genes, such as *p53*, in the field for cancerization has been shown for head and neck cancers (21–52%) [31, 32] and skin cancers (5/8) [38].

Epigenetic field for cancerization

As the deep involvement of aberrant DNA methylation in human cancers became clear, the occasional presence of aberrant DNA methylation in non-cancerous tissues was recognized in the colon [39, 40], liver [41], Barrett's esophagus [42], and stomach [43]. The presence of aberrant methylation in non-cancerous tissues suggested the involvement of the former in the field for cancerization. However, since DNA methylation can show non-significant fluctuation, analysis of control non-predisposed tissues

from healthy individuals (or patients with cancers at other sites) is essential to demonstrate the association between DNA methylation and the field for cancerization. This was first achieved in the liver [41] and subsequently in the colon [44], stomach [22], and breasts ([45]; Table 1). Our study of the stomach is characterized by a marked difference achieved by quantitative methylation analysis and by the clear presence of an inducing factor, *H. pylori*, in addition to the systemic collection of non-predisposed tissues. These reports from multiple institutions strongly support the existence of an "epigenetic field for cancerization" in addition to a genetic field for cancerization.

Advantages of DNA methylation as a marker for a field for cancerization

There are several advantages to using DNA methylation as a marker of a field of cancerization. First, for some cancers, such as gastric cancers, aberrant DNA methylation of tumor-suppressor genes is more commonly observed than mutations [19]. In such cancers, an epigenetic field for cancerization is likely to be present and can be detected using appropriate marker genes. Second, the fractions of cells with aberrant methylation of marker genes can be much larger than those with mutations. In the case of non-cancerous gastric mucosae of human gastric cancers, the former were in the range of 10^{-3}–10^{-1} [22, 27]. In contrast, the fractions of cells with mutations of a *LacI* marker gene were in the range of 10^{-4}–10^{-3} in the colon and liver of mice heavily exposed to a carcinogen [46]. Third, novel marker genes can be easily isolated because techniques for genome-wide screening for changes in DNA methylation are now available [12]. An ideal marker gene should be methylated in association with methylation of tumor-suppressor genes, but at much higher frequencies (Fig. 3). Finally, methylated DNA molecules can be precisely quantified, even when present at a frequency of 1×10^{-3} [22]. For an assessment of the cancer risk in an individual, plus–minus judgment has limited meaning, and quantitative analysis is essential. Using qMSP of biopsy materials, we were able to predict the gastric cancer risk of individuals.

Inducing factors of aberrant DNA methylation and their detection

Helicobacter pylori was involved in the induction of the field for gastric cancers. Infection by *H. pylori* is known to induce severe chronic inflammation. Chronic inflammation is also present in ulcerative colitis for colon cancers, chronic hepatitis for liver cancers, and Barrett's esophagus for esophagus adenocarcinomas. Therefore, chronic inflammation, possibly specific types, is likely to induce aberrant DNA methylation in normal tissues and thus form a field for cancerization. Interestingly, interleukin 6, whose polymorphisms are involved in the susceptibility of various cancers, is known to induce expression and activity of DNA methyltransferase [18]. Further investigations are necessary to clarify which cytokines are really involved.

Epilogue

The presence of an epigenetic field for cancerization, induced by *H. pylori* infection, is now evident for human gastric cancers. For gastric cancer patients with *H. pylori* infection, we fortunately have a realistic choice: eradication therapy for *H. pylori*. This will prevent further

Table 1 Reports on epigenetic field for cancerization (modified from [53])

Cancer	Inducing factor	Analyzed gene	References
Liver	HBV and/or HCV	*CDKN2A,hMLH1,THBS-1* and five MINT loci	Kondo et al. [41]
Colorectal cancer			
Sporadic	Unknown	*MGMT*	Shen et al. [44]
UC associated	UC	*CDKN2A*	Hsieh et al. [40]
UC associated	UC	*ER, MYOD, CDKN2A*, and *CSPG2*	Issa et al. [52]
Barrett's cancer	Barrett's esophagus	*APC,CDKN2A*, and *ESR1*	Eads et al. [48]
Lung cancer	Smoking?	*CDKN2A, MGMT, DAPK, SOCS1, RASSF1A, COX2*, and *RARβ*	Guo et al. [49]
Gastric cancer	*H. pylori*	*CDKN2A,LOX,THBD, HRASLS,FLNc,HAND1*, and *p41ARC*	Maekita et al. [22]
Breast cancer	Unknown	*CYP26A1*	Yan et al. [45]
Renal cancer	Unknown	*CDKN2A, hMLH1,THBS-1*, and five MINT loci	Arai et al. [47]

UC, Ulcerative colitis; HBV, hepatitis B virus; HCV, hepatitis C virus

exacerbation of an epigenetic field for cancerization and also enable us to measure the risk of metachronous gastric cancers accurately. Follow-up procedures will be modified depending upon the risk. Even if a high risk has already accumulated, there is a possibility that demethylating therapy could reduce the risk. If this scenario is eventually realized, clinical management of gastric cancers will experience a great change.

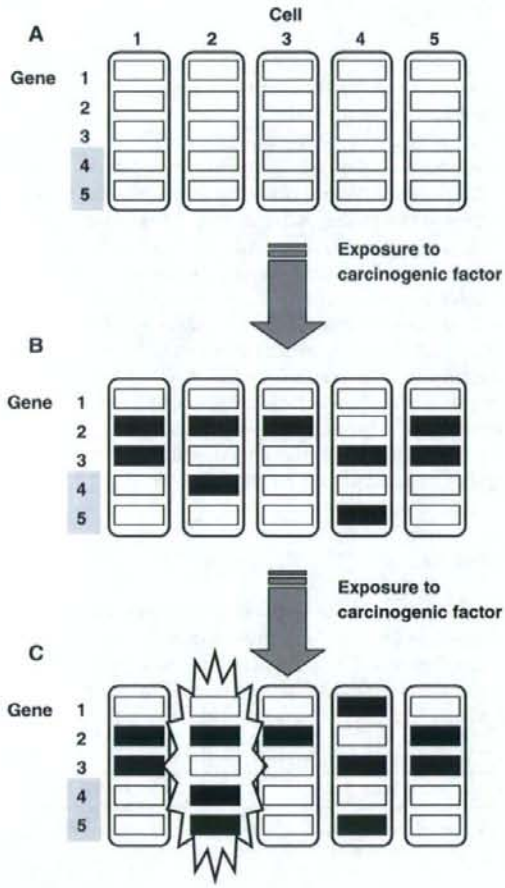

Fig. 3 Gene function and a good marker gene. Genes 4 and 5 are tumor-suppressor genes and, when both are methylated, cell transformation takes place. **a** In a normal tissue, no or little methylation is present. **b** After exposure to a carcinogenic factor, such as chronic inflammation, specific genes become methylated. Good marker genes (genes 2 and 3) are those readily methylated upon exposure to the carcinogenic factor in association with the methylation of tumor-suppressor genes, which are generally resistant to methylation. Specific methylation of genes 2 and 3, not gene 1, is a candidate marker for exposure to the carcinogenic factor. **c** After repeated exposure to the carcinogenic factor, the fractions of cells with methylated marker genes increase, and some cells (cell no. 2) can acquire methylation of multiple tumor-suppressor genes and transform

The concept of epigenetic field cancerization also seems to be valid for colon and breast cancers [44, 45] and, possibly, for liver cancers, esophageal adenocarcinomas, lung cancers, and renal cancers [41, 47–49]. Since the involvement of epigenetic alterations seems not to be limited to cancers [50, 51], there is even a possibility that an epigenetic field defect could be identified for various diseases. It is clear that if disease risk at a time point can be measured by a DNA methylation marker, it will help people to change their lifestyles for more intensive disease prevention.

The clarification of just how much individual carcinogenic factors can contribute to human cancers is an important issue in public health. It is known that some carcinogenic factors leave their fingerprint in the tissues damaged by them – even if they themselves are no longer present – as specific DNA methylation patterns. If more fingerprints could be identified that are as distinct as that left by *H. pylori*, these could be used to identify carcinogenic factors involved in individual cancers. This would enable the appropriate efforts and resources to be focused on the elimination of carcinogenic factors at both individual and public levels.

Research in epigenetics is now very active world-wide. Sweeping changes in the clinical management of cancer patients and elimination procedures of carcinogenic factors are in sight.

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Cancer High-Risk Subjects Identified by Serum Pepsinogen Tests: Outcomes after 10-Year Follow-up in Asymptomatic Middle-Aged Males

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Abstract

Background: **Gastric cancer screening using the pepsinogen filter test is receiving wide recognition in Japan owing to convenience, freedom from discomfort or risk, efficiency, and economy. Because the long-term outcomes of cancer development in extensive atrophic gastritis detected by pepsinogen test are unclear, test-positive and test-negative subjects were investigated in a longitudinal cohort study.**

Methods: **Subjects comprised 5,209 middle-aged men with measured serum pepsinogen levels who were followed for 10 years. Cancer development based on "atrophy-positive" and "atrophy-negative" criteria used for cancer screening was investigated.**

Results: **During the study, 63 cases of cancer developed in the cohort, representing an incidence rate of 125 per 100,000 person-years. Pepsinogen test screening using the most widely used atrophy-positive criterion (pepsinogen I, ≤70 ng/mL; pepsinogen I/II ratio, ≤3.0) displayed 58.7% sensitivity, 73.4% specificity, and 2.6% positive predictive value. Cancer incidence rate was 276 per 100,000 person-years for the atrophy-positive group and 70 per 100,000 person-years for the atrophy-negative group. Incidence rate was higher in groups fulfilling stricter positive criteria detecting more extensive atrophy, reaching 424 per 100,000 person-years. In addition, 9.2% of atrophy-negative subjects with pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0 (reflecting putative inflammation-based high pepsinogen II level) are at high risk for cancer, particularly diffuse-type cancer, with a cancer incidence rate comparable with atrophy-positive subjects (216 per 100,000 person-years).**

Conclusion: **Atrophy-positive subjects by pepsinogen filter test, particularly those fulfilling stricter criteria, and atrophy-negative subjects with low pepsinogen I/II ratio reflecting putative extensive active inflammation constitute populations at high risk for gastric cancer, requiring thorough endoscopic examination.** (Cancer Epidemiol Biomarkers Prev 2008;17(4):838–45)

Introduction

Gastric cancer, despite a recent decline in incidence, is still one of the most common malignancies worldwide and remains a leading cause of cancer death not only in Japan but also in China, Korea, Central and Southern America, and some European countries (1-5). To reduce high mortality and morbidity rates in Japan, mass screening for gastric cancer has been conducted as a public health service since the mid-1960s. The screening program is currently done throughout the country, and ~6 million people annually undergo screening provided by either a community health service or the work place (6-8). Thousands of stomach cancer cases are detected each year, with 5,859,697 people undergoing screening and 5,529 cancers detected in 2004 (detection rate, 0.094%; ref. 8). Cancer screening has thus greatly contributed to reductions in cancer mortality rates in our country (9-13). Although nationwide stomach cancer screening has achieved unparalleled success, the number of people screened has not increased in recent years, with the same people appearing to receive gastric cancer screenings each year; in 2004, the cancer screening covered only 12.9% of the cancer-prone aged population throughout Japan. In addition, cancer screening programs have most commonly adopted gastrophotofluorography with an image intensifier as a filter test. The low resolution of this test is considered problematic because gastrophotofluorography is usually only indicative of abnormalities in the gastric mucosa. More than half of early cancer cases go undiagnosed; whereas sensitivity for advanced cancer is 92%, that for early-stage cancer is as low as 39% (14). Furthermore, gastrophotofluorography is expensive and requires

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technical skills on the part of the radiographic technicians and expert diagnostic abilities on the part of the radiologist. Although the incidence of leukemia has reportedly not increased among participants in the screening program, the risks of X-ray exposure are considered to represent another problem (15, 16). A more efficient screening system is thus sorely needed.

As a trial to improve the screening system, we recently introduced a serum pepsinogen test into gastric cancer screening as an alternative to gastrophotofluorography (17-19). This screening system is based on the hypothesis that chronic atrophic gastritis, including intestinal metaplasia, is a preneoplastic lesion of the stomach (20), together with the results of previous studies indicating that low serum pepsinogen levels reflect the extent of chronic atrophic gastritis (21, 22). In the screening system, individuals positive for extensive atrophic gastritis based on serum pepsinogen levels are further screened by endoscopy. Since 1992, when pepsinogen assay kits became commercially available, a number of screening services provided by work places or by community health services have adopted this serum test as a filter test (23-28). The results of the screening for the past 15 years have shown that addition of the serum test to the screening strategy has markedly increased the number of subjects undergoing screening and has also significantly improved detection rates of gastric cancer and early cancer, in particular, compared with conventional screening using gastrophotofluorography.

The effectiveness of the pepsinogen filter test is thus receiving wide recognition, and the observed high efficiency of the serum test in cancer detection strongly indicates that gastric cancer tends to develop from the atrophic stomach as detected by low serum pepsinogen. However, the long-term prognosis of subjects with extensive chronic atrophic gastritis identified by pepsinogen filter test is not fully known, including cancer incidence rates. In addition, various studies on cancer screening with the pepsinogen filter test have reported a nonnegligible number of test-negative cancers (17-19, 23-28), and the risk for cancer development among pepsinogen test-negative subjects has likewise not been fully elucidated. The present study investigated long-term outcomes of gastric cancer development among pepsinogen test-positive and test-negative subjects based on a 10-year follow-up study.

Subjects and Methods

Study Subjects. Subjects comprised 5,706 male employees ages 40-60 years (mean ± SD, 49.2 ± 4.7 years) who participated in an annual multiphasic health screening program in Wakayama City, Japan, between April 1994 and the end of March 1995. This type of screening program is generally done by various work places throughout Japan to detect incident diseases in the early stages. Subjects with specific symptoms were thus guided to receive medical attention and were excluded from screening. Subjects who had previously undergone gastric resection were also excluded and were examined separately. Symptom-free subjects underwent a series of screening tests and procedures: an interview to ascertain general state of health, physical examination, chest radiography, electrocardiography, blood laboratory tests, urinalysis, and fecal occult blood test. Some of these subjects had been investigated in a previous cohort study (28).

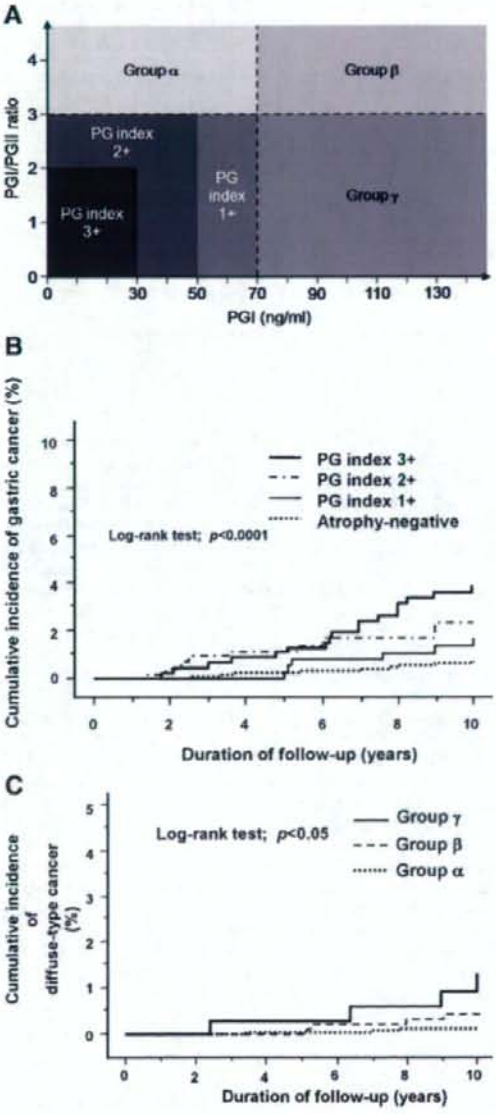
All subjects were followed for the study period of 10 years, from April 1994 to the end of March 2004. Subjects underwent the aforementioned health screening program annually and were also screened to identify incident gastric cancer, as described in the following Gastric Cancer Screening section. The incident day of gastric cancer was defined as the day of the health checkup when the cancer was detected. Duration of the observation period was calculated for each subject from the time of the baseline survey to the diagnosis of gastric cancer.

Analysis of Serum Pepsinogen Levels. Aliquots of separated sera from fasting blood samples collected as routine laboratory tests for general health checkup were stored below −20°C until measurement of serum pepsinogen levels. Serum pepsinogen levels (pepsinogens I and II) were measured using a modification (RIAbeads kit, Dainabott) of our previously reported RIA (29). Subjects with renal failure were excluded from analyses of the results of serum pepsinogen levels. Subjects who had been prescribed medication that might affect gastrointestinal function, such as proton pump inhibitors or nonsteroidal anti-inflammatory drugs, before examination and subjects who had undergone eradication therapy for *Helicobacter pylori* were also excluded.

Gastric Cancer Screening. Cancer screening was done by double-contrast barium X-ray with digital radiography and by serum pepsinogen as filter tests. For upper gastrointestinal barium X-ray, a remote controlled X-ray fluoroscope (TU-230XB, Hitachi Medical Corp.) and real-time digital radiography (DR-2000H; Hitachi Medical) were used. The double-contrast upper gastrointestinal X-ray series used 150 mL of high-concentration barium at 200%, and 11 films were taken for each subject as described previously (28). Subjects were also screened using the serum pepsinogen filter test. Among several test-positive criteria of the pepsinogen filter test used for cancer screening in our country, the criterion of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 is the most widely applied, with atrophy-positive defined when the criterion is fulfilled and atrophy-negative defined when the criterion is not fulfilled (19, 30-33). Additional and stricter criteria of pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0 or pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0 are also used to detect subjects with more extensive atrophy (18, 23, 24). The latter two criteria are used variously, independently or in combination with the aforementioned atrophy-positive criteria, depending on the purpose of screening. When used in combination, each criterion constitutes pepsinogen index 1+ to 3+ within the atrophy-positive group according to the first criterion of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0.

The third criterion, pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0, is defined as pepsinogen index 3+. The second criterion, pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0 but not meeting the criterion for pepsinogen index 3+, is defined

as pepsinogen index 2+. The first criterion, but not meeting the criteria for pepsinogen index 2+ or 3+, is defined as pepsinogen index 1+ (ref. 34; Fig. 1A). In the present study, data on serum pepsinogen levels of subjects were classified basically using the atrophy-positive criterion and pepsinogen index. If a subject was identified as test-positive by serum pepsinogen test, fulfilling the atrophy-positive criterion, that is, pepsinogen index 1+ to 3+, or by barium digital radiography, further examination was conducted by upper gastrointestinal endoscopy (XQ-200, Olympus).



Resected specimens of gastric cancer obtained by endoscopy or surgery were assessed histopathologically and classified according to the classification of Lauren (34) as intestinal or diffuse type. Location of the cancer in the stomach was classified as cardia or noncardia based on clinical or histopathologic records. The ethics committee of Wakayama Medical University approved the study protocols, and informed consent was obtained from all participating subjects.

Statistical Analysis. Data were analyzed using SPSS 11.0 (SPSS) and STATA (STATA Corp.). Differences were tested for significance using the t test for comparisons between two groups, ANOVA for comparisons among multiple groups, and Scheffe's least significant difference test for comparisons of pairs of groups. The χ^2 test was used to compare categorical variables. Long-term effects of pepsinogen test-positive or test-negative criteria on gastric cancer development were evaluated using Cox proportional hazards models.

Results

A total of 5,706 eligible subjects were examined. Of these, 489 subjects who declined to participate in the study program or who met the exclusion criteria described were excluded from the study. In addition, eight cases of gastric cancer that developed within the 1st year of the study were also excluded. The remaining 5,209 subjects

Figure 1. Gastric cancer development based on criteria of pepsinogen filter test used for cancer screening in Japan. **A.** Schematic presentation of the atrophy-positive criterion and pepsinogen index, which are widely used for the pepsinogen filter test in Japan. Pepsinogen index is used to detect subjects with severe gastric atrophy by subdividing the atrophy-positive criterion identified by pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 into three groups (1+ to 3+), as described in the text. In addition, the figure illustrates three subgroups, groups α, β, and γ, in the atrophy-negative criterion, which is used in the present analysis. The atrophy-negative criterion was further classified into the three subgroups as follows: group α, pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of >3.0; group β, pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of >3.0; and group γ, pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0. **B.** Kaplan-Meier analysis of gastric cancer development in subjects classified using the criteria of the pepsinogen filter test. Subjects were classified into four groups according to the atrophy-positive criterion and pepsinogen index and then followed for cancer development for 10 y. Cancer incidence rates for the atrophy-negative group and pepsinogen indices of 1+, 2+, and 3+ were 70 per 100,000, 166 per 100,000, 242 per 100,000, and 424 per 100,000 person-years, respectively. **C.** Kaplan-Meier analysis of diffuse-type gastric cancer development among subjects fulfilling the atrophy-negative criterion. Atrophy-negative subjects were classified into three subgroups, group α, β, and γ, according to the criteria described in the text and in **A**, and were followed for the study period of 10 y. Among these subgroups, incidence rate of diffuse-type cancer was significantly higher in group γ than in groups α and β, at 120 per 100,000 person-years ($P = 0.019$). *PG*, pepsinogen.

Table 1. Gastric cancer incidence rate by various criteria of pepsinogen filter test

Screening criteria	PG I ≤70 and PG I/II ≤3.0		PG I ≤50 and PG I/II ≤3.0		PG I ≤30 and PG I/II ≤2.0		Total
	Negative	Positive	Negative	Positive	Negative	Positive	Subjects
Subjects	3,802	1,407	4,176	1,033	4,782	427	5,209
Person-years	37,010.0	13,416.0	40,626.5	9,799.5	46,419.0	4,007.0	50,426
Age [mean (SD)]	48.8 (4.8)	50.3 (4.4)*	48.9 (4.7)	50.6 (4.3)*	49.0 (4.7)	51.3 (4.2)*	49.2 (4.7)
Follow-up years [mean (SD)]	9.7 (0.8)	9.5 (1.1)	9.7 (0.8)	9.5 (1.2)	9.7 (0.8)	9.4 (1.2)	9.7 (0.9)
Total gastric cancer							
Age [mean (SD)]	50.7 (4.3)	50.9 (3.4)	50.3 (4.2)	51.4 (3.2)	50.5 (4.0)	51.8 (2.9)	50.8 (3.7)
Follow-up years [mean (SD)]	6.3 (2.6)	5.9 (2.6)	6.5 (2.5)	5.6 (2.6)	6.0 (2.7)	6.1 (2.4)	6.0 (2.6)
Cases/incidence rate†	26/70	37/276	32/79	31/316	46/99	17/424	63/125
Intestinal gastric cancer							
Age [mean (SD)]	51.1 (4.0)	51.4 (3.4)	50.6 (3.6)	51.6 (3.4)	50.6 (3.6)	52.4 (3.0)	51.1 (3.5)
Follow-up years [mean (SD)]	6.0 (2.7)	5.7 (2.5)	6.2 (2.6)	5.7 (2.4)	5.8 (2.7)	5.8 (2.2)	5.8 (2.5)
Cases/incidence rate†	16/41	26/194	18/44	24/245	30/65	12/299	42/83
Diffuse gastric cancer							
Age [mean (SD)]	50.2 (4.8)	49.7 (3.2)	50.0 (5.0)	50.6 (2.0)	50.3 (4.8)	50.2 (1.9)	50.3 (4.2)
Follow-up years [mean (SD)]	6.8 (2.5)	6.2 (2.8)	6.7 (2.4)	5.4 (3.3)	6.4 (2.6)	6.7 (2.9)	6.5 (2.6)
Cases/incidence rate†	10/30	11/82	14/35	7/71	16/34	5/125	21/42
Accuracy of screening criteria							
Sensitivity (95% CI)	58.7% (45.6-70.8)		49.2% (36.5-62.0)		27.0% (16.9-39.9)		
Specificity (95% CI)	73.4% (72.1-74.6)		80.5% (79.4-81.6)		92.0% (91.3-92.8)		
Positive predictive value (95% CI)	2.6% (1.9-3.6)		3.0% (2.1-4.3)		4.0% (2.4-6.4)		
Negative predictive value (95% CI)	99.3% (99.0-99.5)		99.2% (98.9-99.5)		99.0% (98.7-99.3)		
Likelihood ratio of a positive test (95% CI)	2.21% (1.78-2.73)		2.53% (1.95-3.27)		3.39% (2.23-5.14)		
Likelihood ratio of a negative test (95% CI)	0.56% (0.42-0.76)		0.63% (0.49-0.80)		0.79% (0.68-0.92)		

Abbreviation: PG, pepsinogen.
*Significantly different from each counterpart ($P < 0.05$).
† Per 100,000 person-years.

(mean age, 49.2 ± 4.7 years) were followed for 10 years and investigated for gastric cancer development. During 50,426 person-years of follow-up, 63 cases of cancer developed, representing an incidence rate of 125 of 100,000 person-years in the cohort (Table 1). Histopathologic features of the detected cancer revealed intestinal type in 42 cases (65%) and diffuse type in 21 cases (35%). Most cases (61 cases, 96.8%) were noncardia cancer, and only 2 cases (3.2%) were located in the gastric cardia. Fifty-four cases (86%) were in the early stage and confined to the mucosa or submucosa. The remaining nine cases were in the advanced stage. Mucosal cancers comprised 74% (40 of 54) of early cancers and were treated by endoscopic resection. The remaining 14 early cancers and 9 advanced cancers underwent surgical resection, and all cases were successfully treated.

Of 5,209 study subjects, 27.0% (n = 1,407) were positive according to the first criterion, pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0, that is atrophy positive. Likewise, 19.8% (n = 1,033) and 8.2% (n = 427) were positive by the second criterion, pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0, and third criterion, pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0, respectively (Table 1). Among the atrophy-positive group, 27.0% (n = 374) were categorized as pepsinogen index 1+, 43.0% (n = 606) as pepsinogen index 2+, and 30.0% (n = 427) as pepsinogen index 3+ (Table 2). Mean age was significantly higher in test-positive subjects than in test-negative subjects in each of the three criteria and increased in a stepwise manner with increasing pepsinogen index, whereas no significant difference was seen in mean follow-up period among subjects, irrespective of test positivity or differences in pepsinogen index. Of 63 cases of gastric cancer that developed during the study period, 58.7% (n = 37) developed in the atrophy-positive group and the remaining 41.3% (n = 26) developed in the atrophy-negative group. With the second and third criteria, 49.2% (n = 31) and 27.0% (n = 17) of cancer cases were test-positive, respectively (Table 1). The accuracy of cancer screening by each of the three criteria was thus as follows: for pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0, 58.7% sensitivity [95% confidence interval (95% CI), 45.6-70.8%], 73.4% specificity (95% CI, 72.1-74.6%), and 2.6% positive predictive value (95% CI, 1.9-3.6%); for pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0, 49.2% sensitivity (95% CI, 36.5-62.0%), 80.5% specificity (95% CI, 79.4-81.6%), and 3.0% positive predictive value (95% CI, 2.1-4.3%); and for pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0, 27.0% sensitivity (95% CI, 16.9-39.9%), 92.0% specificity (95% CI, 91.3-92.8%), and 4.0% positive predictive value (95% CI, 2.4-6.4%). The cancer incidence rate of the atrophy-negative group was 70 per 100,000 person-years compared with 276 per 100,000 person-years for the atrophy-positive group.

Among 37 cancers developed from atrophy-positive subjects, 16.2% (n = 6) were from the pepsinogen index 1+ group, 37.8% (n = 14) from the pepsinogen index 2+ group, and 46.0% (n = 17) from the pepsinogen index 3+ group (Table 2). Kaplan-Meier analysis showed that after 6 years of follow-up, cancer development occurred in the order of pepsinogen index and was highest in the 3+ group, followed by the 2+, 1+, and atrophy-negative

groups (Fig. 1B). A significant stepwise increase in cancer incidence rate was seen with increases in pepsinogen index among the atrophy-positive group from 166 to 424 per 100,000 person-years, and hazard ratio also increased significantly and in a stepwise manner from atrophy-negative to pepsinogen index 3+, reaching 5.16 (95% CI, 2.77-9.51; $P < 0.01$; Table 2). Of the two histologic types of cancer, a significant positive correlation between cancer incidence rate and pepsinogen index was observed only for intestinal-type cancer.

As described previously, 41.3% (26 of 63) of detected cancers developed from the atrophy-negative group. Of the two histopathologic types of cancer, 10 cancers (38.5%) derived from this group were diffuse-type, meaning that nearly half (47.6%) of the 21 diffuse-type cancers detected during the study period developed from nonatrophic stomachs. Whereas the atrophy-positive group comprised subjects with low values for both serum pepsinogen I and pepsinogen I/II ratio, the negative group was composed of not only subjects with high serum pepsinogen I and pepsinogen I/II ratio but also subjects with low serum pepsinogen I or low pepsinogen I/II ratio. The atrophy-negative group can thus be further classified into the following three groups: group α, with pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of >3.0; group β, with pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of >3.0; and group γ with pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0 (Fig. 1A). Because previous studies (including our own) have suggested that a low serum pepsinogen I level or low pepsinogen I/II ratio is related to risk for gastric cancer (18, 35, 36), long-term outcomes of cancer development may differ among these three subgroups. Table 2 shows cancer development in the three groups during the study period. Mean age was significantly higher for group γ than for group α, but no difference in mean follow-up years was seen among the three groups. Mean serum levels of pepsinogens I and II increased significantly and in a stepwise manner from group α to γ, and the elevation was particularly marked for pepsinogen II. Pepsinogen I/II ratio was lowest in group γ. Groups α, β, and γ comprised 58.4% (n = 2,219), 32.4% (n = 1,234), and 9.2% (n = 349) of atrophy-negative subjects, and proportions of cancers that developed in each group were 46.2% (n = 12), 26.9% (n = 7), and 26.9% (n = 7) of proportions in the atrophy-negative group, respectively.

Cancer incidence rates for groups α and β were 55 and 58 per 100,000 person-years, respectively, lower than that in the atrophy-negative group overall (70 per 100,000 person-year), whereas group γ showed a high incidence rate of 211 per 100,000 person-years. A significant increase in hazard ratio was seen from group α to γ (hazard ratio, 3.49; 95% CI, 1.37-8.93), reflecting a significant increase in the hazard ratio of diffuse-type

Table 2. Gastric cancer incidence rate in atrophy-negative and atrophy-positive groups

Group	Atrophy-negative group				P_{trend}	Atrophy-positive group			P_{trend}
	Total	α	β	γ		PG index 1+	PG index 2+	PG index 3+	
Subjects	3,802	2,219	1,234	349		374	606	427	
Person-years	37,010.0	21,702.5	11,976.5	3,324.0		3,616.5	5,792.5	4,007.0	
Age [mean (SD)]	48.8 (4.8)	48.5 (4.8)	49.2 (4.7)	49.8 (4.6)*		49.5 (4.4)	50.2 (4.5)†	51.3 (4.2)†	
Follow-up years [mean (SD)]	9.7 (0.8)	9.8 (0.7)	9.7 (0.8)	9.5 (1.0)		9.7 (0.9)	9.5 (1.2)	9.4 (1.2)	
PG I [mean (SD)]	69.3 (29.6)	51.9 (10.1)	93.1 (30.4)*	96.2 (29.5)*		59.5 (5.8)	38.1 (8.1)†	16.7 (7.7)†	
PG II [mean (SD)]	16.2 (11.4)	10.4 (3.9)	19.9 (8.4)	40.7 (14.2)*		26.6 (6.8)	18.5 (6.7)†	14.0 (5.6)†	
PG I/II [mean (SD)]	5.1 (1.8)	5.4 (1.6)	5.1 (1.7)	2.4 (0.4)*		2.4 (0.5)	2.2 (0.5)	1.2 (0.4)†	
Total gastric cancer									
Age [mean (SD)]	50.7 (4.3)	51.5 (2.9)	51.0 (5.5)	49.1 (5.1)		48.5 (3.7)	50.9 (3.6)	51.8 (2.9)	
Follow-up years [mean (SD)]	6.3 (2.6)	5.9 (2.6)	5.6 (2.2)	7.8 (2.7)		7.0 (2.2)	5.1 (2.8)	6.1 (2.4)	
Cases/incidence rate‡	26/70	12/55	7/58	7/211		6/166	14/242	17/424	
HR (95% CI)§	1	1	0.99 (0.39-2.54)	3.49 (1.37-8.93)	0.019	3.60 (2.17-5.96)	4.55 (2.62-7.43)	5.16 (2.77-9.51)	<0.0001
Intestinal gastric cancer									
Age [mean (SD)]	51.1 (4.0)	51.8 (2.6)	51.7 (6.1)	46.3 (1.5)*		48.5 (4.7)	50.6 (3.7)	52.4 (3.0)	
Follow-up years [mean (SD)]	6.0 (2.7)	5.8 (2.8)	4.0 (1.3)	9.0 (1.0)		7.0 (2.3)	5.6 (2.6)	5.8 (2.2)	
Cases/incidence rate‡	16/41	9/41	3/25	3/91		2/55	12/207	12/299	
HR (95% CI)§	1	1	0.57 (0.15-2.11)	1.99 (0.53-7.39)	0.31	4.47 (2.37-8.42)	5.54 (2.91-10.55)	6.62 (3.18-13.74)	<0.0001
Diffuse gastric cancer									
Age [mean (SD)]	50.2 (4.8)	50.7 (4.0)	50.5 (5.9)	51.3 (6.1)		48.5 (0.7)	53.0 (1.4)	50.2 (1.9)	
Follow-up years [mean (SD)]	6.8 (2.5)	6.1 (2.3)	6.8 (3.0)	7.0 (3.4)		7.1 (2.8)	1.8 (0.7)	6.7 (2.9)	
Cases/incidence rate‡	10/29	3/14	4/33	4/120		4/111	2/35	5/125	
HR (95% CI)§	1	1	2.29 (0.51-10.24)	8.04 (1.78-36.25)	0.021	2.41 (1.02-5.71)	1.96 (0.72-5.36)	3.16 (0.99-10.06)	0.23

Abbreviation: HR, hazard ratio.

* Significantly different from group α subjects ($P < 0.05$).

† Significantly different from pepsinogen index 1+ subjects ($P < 0.05$).

‡ Per 100,000 person-years.

§ In the atrophy-positive group, hazards ratio was calculated in comparison with cancer incidence rate of the atrophy -negative group (total) and, in the atrophy-negative group, was calculated in comparison with that of group α.

cancer but not that of intestinal-type cancer (Table 2). Kaplan-Meier analysis revealed that after 5 years of follow-up, cumulative incidence of diffuse-type cancer was in the order of groups γ, β, and α (Fig. 1C), with an incidence rate of 120 per 100,000 person-years for group γ and significantly increased hazard ratio compared with that in group α (hazard ratio, 8.04; 95% CI, 1.78-36.25).

Discussion

In the present study, 5,209 middle-aged male subjects with measured serum pepsinogen levels were followed for 10 years, and gastric cancer development was investigated based on the criteria of the pepsinogen filter test widely used for gastric cancer screening in Japan. For cancer screening in our country, the following pepsinogen test-positive criteria have been used depending on differences in target population and the purpose of screening: pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0; pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0; or pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0.

Among these, the first criterion (pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0) is considered both the most efficient for identifying extensive atrophic gastritis and the best cancer screening criterion based on the results of previous studies (19, 26, 30-33). In addition, the results of a recent meta-analysis of seven studies analyzing organized population-based cancer screening indicated that pooled sensitivity and specificity of the criterion were 77.3% and 73.2%, offering a better sensitivity/specificity balance than any other criteria and also providing strong support for the use of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 (37). In our observation over 10 years, pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 was considered the best among the criteria currently used for cancer screening, showing 58.7% sensitivity (95% CI, 45.6-70.8%) and 73.4% specificity (95% CI, 72.1-74.6%).

As for the other two criteria, pepsinogen I of ≤50 ng/mL and pepsinogen I/II ratio of ≤3.0 or pepsinogen I of ≤30 ng/mL and pepsinogen I/II ratio of ≤2.0, the sensitivity of screening is <50%, at 49.2% (95% CI, 36.5-62.0%) and 27.0% (95% CI, 16.9-39.9%), respectively. Cancer screening using either of these criteria as an independent criterion for the filter test thus does not seem feasible. As a whole, the sensitivity of these criteria analyzed in the present study was considerably lower than that reported in other studies (37). This is probably due to the fact that previous studies analyzed an initial phase of screening when the pepsinogen filter test was newly applied, in cross-sectional evaluations, whereas the present study analyzed the subsequent phase of screening by prospective evaluation, with eight cases of cancer detected during the 1st year of investigation excluded from the analysis.

The present results strongly indicate that the sensitivity of pepsinogen filter test, which is high on initial prevalent screening, is not equally high for noninitial incident screenings in subsequent years. The reported high sensitivity in these studies was likely caused by overestimating the accuracy of the pepsinogen filter test. Nonetheless, the present results clearly show that the atrophy-positive criterion of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 detects 58.7% of cancers developing over 10 years and also identifies a group of subjects susceptible for gastric cancer. The incidence rate of gastric cancer in the group defined by this criterion was 276 per 100,000 person-years and increased in a stepwise to a maximum level of 424 per 100,000 person-years with an increase in pepsinogen index from 1+ to 3+. The observed correlation between incidence rate of gastric cancer and grade of atrophy as indicated by pepsinogen index verifies the hypothesis that risk for cancer increases with progression of chronic atrophic gastritis (20) and that serum pepsinogen level offers a reliable marker for cancer development and coexisting atrophic gastritis.

Meanwhile, a nonnegligible proportion (41.3%) of gastric cancers developed in the atrophy-negative group during the study period. In this group, the percentage of diffuse-type cancers (38.5%) was significantly higher than that in the atrophy-positive group (29.7%), and nearly half of diffuse-type cancers (47.6%) developed in this group during the study period, in good accordance with the hypothesis that this type of cancer develops in the stomach following chronic inflammation without passing through an intermediate step of atrophic gastritis together with intestinal metaplasia (38-40).

Unlike the atrophy-positive group, this group displays heterogeneous serum pepsinogen levels, containing subjects with both high and low levels of serum pepsinogen I and pepsinogen I/II ratio, and can be classified into three subgroups: group α, with pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of >3.0; group β, with pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of >3.0; and group γ, with pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0. Among these subgroups, cancer incidence rate was highest in group γ at 216 per 100,000 person-years, comparable with that in the atrophy-positive group and even higher than that for pepsinogen index 1+ (166 per 100,000 person-years). Establishment of *H. pylori* infection results in increased serum pepsinogen levels, and this elevated pepsinogen level (particularly pepsinogen II in the nonatrophic stomach) is considered to reflect the severity of gastritis (41, 42). Gastric inflammation is thus likely to be increasingly severe in groups α, β, and γ, in that order, as revealed by respective pepsinogen levels. In addition, the mean age in each of the three subgroups increased in the order of groups α, β, and γ, and the number of subjects in these subgroups decreased in the same order.

Furthermore, the proportion of *H. pylori*–negative subjects was larger in the order of group α (33%; 733 of 2,219), group β (19%; 229 of 1,234), and group γ (1%; 4 of 349). The process of gastritis thus seems to advance from group α thorough group β and finally to group γ after the establishment of *H. pylori* infection. These results strongly indicate that a group of subjects with putative extensive active gastritis in the nonatrophic stomach are at high risk for cancer comparable with that in subjects with extensive atrophy. These subjects comprised 9.2% (349 of 3,802) of the atrophy-negative group and can be identified using a criterion of pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0.

However, this criterion detects only about one quarter of cancers (26.7%) developing in the nonatrophic stomach. This is probably because serum pepsinogen level, especially pepsinogen II, does not show marked elevation in most *H. pylori*–related multifocal gastritis unless *H. pylori*–induced active inflammation becomes widespread, and we cannot detect subjects in whom the active inflammatory process is focally severe enough to commit epithelial cells to neoplastic transformation.

Taken together, the present results clearly indicate that the atrophy-metaplasia-dysplasia-cancer sequence described by Correa (20) represents the main route of stomach carcinogenesis in Japan, and the pepsinogen filter test using a criterion of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 offers a reliable method for identifying individuals at high risk for the sequence-derived cancer, with an incidence rate of 276 per 100,000 person-years. In addition, we have revealed another group at high risk for cancer without gastric atrophy, identified by a criterion of pepsinogen I of >70 ng/mL and pepsinogen I/II ratio of ≤3.0. The characteristics of this minor group are that it comprises only 6.7% (349 of 5,209) of total cancer-prone aged subjects, shows a high cancer incidence rate comparable with that in subjects with extensive atrophy (216 per 100,000 person-years), and tends to develop diffuse-type cancers with higher malignant potential. Based on the present results, screening targeting both of these high-risk groups would provide 69.8% sensitivity (95% CI, 56.8-80.4%), 66.7% specificity (95% CI, 65.4-68.0%), and 2.5% positive predictive value (95% CI, 1.8-3.3%).

The major aim of cancer screening is to detect treatable early-stage cancer in asymptomatic individuals. For early detection of premalignant or malignant lesions, endoscopic visualization is considered the best method. Endoscopy is invasive, uncomfortable, and expensive and is thus only offered to subjects with positive results on a filter test in cancer screening. In Japan, mass screening for gastric cancer has primarily adopted gastrophotofluorography as a filter test. Compared with this traditional filter test, serum pepsinogen test is easier to perform, provides quicker results, and produces no patient discomfort (17, 19).

Furthermore, the test is inexpensive; the cost for detection of a single cancer was much less than that for conventional screening, comparable with that for surgical resection of a single cancer case (19, 28). Gastrophotofluorography reportedly offers 57% to 90% sensitivity, 77% to 91% specificity, and 0.9% to 2.0% positive predictive value (43). The present results indicate that specificity of the pepsinogen filter test using the criterion of pepsinogen I of ≤70 ng/mL and pepsinogen I/II ratio of ≤3.0 as a cutoff is slightly lower (73.4%), representing a higher false-positive rate than reported for gastrophotofluorography.

However, as clearly indicated in the present study, pepsinogen test-positive subjects are at high risk for cancer, and this low specificity (high false-positive rate) may be partly attributable to the relatively long latency between initiation of the carcinogenic step and clinically established cancer development. The incidence rate of cancer in pepsinogen test-positive subjects is 276 per 100,000 person-years, meaning that one cancer develops in about every 36 subjects during the 10-year period. Regular and strict long-term endoscopic surveillance of this group thus seems warranted. Whether eradication of *H. pylori* is effective for preventing cancer development in these pepsinogen test-positive subjects warrants further investigation.

In conclusion, atrophy-positive subjects and atrophy-negative subjects with putative extensive active inflammation as defined by pepsinogen test criteria constitute populations at high risk for gastric cancer and need thorough examination by endoscopy. Because the results of previous studies have clearly indicated that use of the same cutoff for pepsinogen test results in comparable outcomes in different sets of individuals and in different countries for the detection of preneoplastic or neoplastic lesions (37), the present data are probably useful not only for subjects in high-risk areas of gastric cancer outside Japan but also for subjects in low-risk areas. Mass cancer-screening programs may not be feasible, but strict follow-up of high-risk subjects will probably be effective even in Western countries (44). Further studies are necessary, particularly analyzing follow-up programs of subjects identified by pepsinogen tests and including examination of cost-effectiveness.

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Risk of gastric cancer in asymptomatic, middle-aged Japanese subjects based on serum pepsinogen and *Helicobacter pylori* antibody levels

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A total of 5,209 asymptomatic, middle-aged subjects, whose serum pepsinogen (PG) and *Helicobacter pylori* antibody levels had been assessed, were followed for 10 years. Subjects with positive serum *H. pylori* antibodies (>50 U/mL) had an increased cancer risk (HR = 3.48, 95% CI = 1.26–9.64). Risk of gastric cancer increased as the antibody level increased; the *H. pylori*-positive group with antibody levels >500 U/mL had the highest incidence rate (325/100,000 person-years). Cancer development also increased with a reduced serum PG I level or a reduced PG I/II ratio; the risk was significantly elevated with serum PG I level ≤30 ng/mL (HR = 3.54, 95% CI = 1.95–6.40) or PG I/II ratio ≤3.0 (HR = 4.25, 95% CI = 2.47–7.32). Furthermore, the risk of diffuse-type cancer increased as PG II level increased; it was significantly elevated with PG II level ≥30 ng/mL (HR = 3.81, 95% CI = 1.10–13.21). Using *H. pylori* antibody and PG levels, subgroups with an especially high or low cancer incidence rate could be identified. *H. pylori*-negative or indeterminate subjects with low PG level (PG I ≤30 ng/mL or PG I/II ratio ≤2.0) or *H. pylori*-positive subjects with antibody levels >500 U/mL and a low PG level were among the subgroups with a high cancer incidence rate (over 400/100,000 person-years). In contrast, *H. pylori*-negative subjects with a PG I level >70 ng/mL or a PG I/II ratio >3.0 had the lowest risk; none of these subjects developed cancer. Thus, serum PG levels and/or *H. pylori* antibody levels can be used to predict the risk of cancer in individuals with *H. pylori*-related gastritis from the general population.

Key words: gastric cancer; pepsinogen; *Helicobacter pylori*; chronic atrophic gastritis; intestinal metaplasia

Stomach carcinogenesis in high-risk populations, including the Japanese population, is believed to begin with chronic active inflammation of the stomach mucosa, proceeding to extensive atrophy together with intestinal metaplasia, then to dysplasia, and finally to cancer.[1] Currently, *Helicobacter pylori* (*H. pylori*) is considered a major factor in the establishment of the carcinogenic sequence in the stomach.[2–12] *H. pylori*-related gastritis usually starts in the antrum and expands proximally towards the body of the stomach.[13–15] During this process, both multifocal atrophy and intestinal metaplasia develop, eventually leading to chronic atrophic gastritis (CAG) with extensive intestinal metaplasia. Several studies dealing with endoscopic biopsies or chromoendoscopic testing found that the progression of CAG increases the risk for cancer.[12,16–20] Thus, an accurate and reliable evaluation of the extent of CAG is considered to be important for identifying individuals at high risk of cancer. The diagnosis of CAG is based on histopathology of the stomach mucosa. However, gastric endoscopy with biopsy is an invasive test and inappropriate for mass population screening. In addition, since CAG together with intestinal metaplasia is a multifocal process, it is difficult to accurately diagnose the extent of CAG based on a few biopsy samples. Furthermore, histological diagnosis of gastric atrophy depends on subjective judgment without a gold standard.[21,22] Thus, a test for CAG progression that is more convenient, free of discomfort or risk, economical and based on objective parameters is needed.

There is general agreement that serum pepsinogen (PG) levels reflect the morphological and functional status of the stomach mucosa.[23,24] We have previously shown that a reduction in the area of fundic gland mucosa with CAG progression was well correlated with a stepwise reduction in the serum PG I level or PG I/II ratio, and that the PG levels are considered reliable markers for CAG progression.[25] Therefore, it is highly probable that these serum tests can effectively identify a cancer-susceptible population. Indeed, previous seroepidemiological studies revealed that the risk of gastric cancer was significantly increased in subjects with extensive CAG diagnosed by PG levels.[26–30] These results strongly indicate that the serum PG levels reflect an individual's risk for gastric cancer. However, there have been few reports describing the relationship between PG levels and the cancer incidence rate. Meanwhile, the extent and severity of mucosal inflammation is believed to be involved in a series of molecular events that lead to cancer and that depend on multiple bacterial and host factors, including the immune response.[31,32] To date, several animal model and human studies have indicated that serum *H. pylori* antibody levels reflect the severity of *H. pylori*-related gastritis.[33–36] Thus, cancer development may be related to the serum antibody level. In the present study, the correlation between the gastric cancer incidence rate and serum PG levels and/or *H. pylori* antibody levels was assessed in a middle-aged, general male population based on the results of a 10-year longitudinal cohort study.

Material and methods

Study population

The subjects were 5,706 male employees (mean age [SD], 50.4 [5.4] years; range, 40–60 years) who had an annual multiphasic health checkup at their workplace in Wakayama City, located in the western part of Japan. In 2005, the gastric cancer mortality

Abbreviations: CAG, chronic atrophic gastritis; CI, confidence interval; DR, digital radiography; ELISA, enzyme-linked immunosorbent assay; *H. pylori*, *Helicobacter pylori*; HR, hazard ratio; IgG, immunoglobulin G; PG, pepsinogen; SD, standard deviation.

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TABLE I – BASELINE CHARACTERISTICS OF THE SUBJECTS STRATIFIED BY SERUM *H. pylori* ANTIBODY LEVEL

	Total	*H. pylori* antibody level (U/ml)					p (trend)[1]
		Negative <30	Indeterminate ≥30 and ≤50	Positive			
				Total	>50 and ≤500	>500	
Total Subjects	5209	999	554	3656	2817	839	
Person-years	50426	9784	5540.5	35101.5	27092	8009.5	
Age [mean (SD)]	49.2 (4.7)	48.3 (4.5)	46.9 (5.1)	49.8 (4.6)[2]	49.8 (4.6)[2]	49.7 (4.6)[2]	
Follow-up years [mean (SD)]	9.7 (0.9)	9.8 (0.7)	10.0 (0.2)	9.6 (1.0)	9.6 (0.9)	9.6 (1.1)	
PG I [mean (SD)]	60.6 (30.4)	58.2 (20.7)	55.9 (30.0)[2]	62.0 (32.5)[2]	61.2 (32.3)[2]	64.9 (33.2)[2]	
PG II [mean (SD)]	17.0 (10.6)	9.6 (4.4)	11.0 (6.6)[2]	20.0 (11.0)[2]	19.3 (10.6)[2]	22.5 (11.8)[2]	
PG I/II [mean (SD)]	4.2 (2.1)	6.4 (1.7)	5.6 (1.9)	3.4 (1.6)[2]	3.5 (1.7)[2]	3.1 (1.4)[2]	
Total gastric cancer							
Age [mean (SD)]	50.8 (3.7)	51.0 (3.5)	51.8 (2.1)	50.8 (3.9)	51.6 (3.7)	49.7 (3.9)	
Follow-up years [mean (SD)]	6.0 (2.6)	6.4 (3.6)	7.8 (1.0)	5.9 (2.5)	5.4 (2.6)	6.4 (2.5)	
Cases/incidence rate[3]	63/125	4/41	4/72	55/157	29/107	26/325	
HR (95%CI)		1	3.16 (0.75–13.22)	3.48 (1.26–9.64)	3.10 (0.94–10.21)	9.56 (2.89–31.65)	<0.0001
PG I [mean (SD)]	53.1 (43.3)[4]	35.5 (12.5)	35.7 (13.2)	55.6 (45.7)[2,4]	48.4 (28.3)[2,4]	64.3 (59.9)[2]	
PG II [mean (SD)]	21.6 (18.1)[4]	19.0 (3.9)	18.3 (9.7)	22.0 (19.2)	17.6 (9.2)	26.9 (25.5)	
PG I/II [mean (SD)]	2.7 (1.5)[4]	1.9 (0.7)	3.0 (3.0)[2]	2.7 (1.4)[2,4]	2.8 (1.4)[2,4]	2.6 (1.5)[2]	
Intestinal-type gastric cancer							
Cases/incidence rate[3]	42/83	3/31	3/54	36/103	19/70	17/212	
HR (95%CI)		1	3.84 (0.70–20.97)	2.98 (0.91–9.72)	2.98 (0.69–12.82)	9.16 (2.11–39.78)	0.0009
PG I [mean (SD)]	47.5 (47.2)[4]	34.6 (15.1)	37.7 (5.4)	49.4 (50.6)[2,4]	39.6 (21.3)[4]	60.2 (69.6)[2]	
PG II [mean (SD)]	20.1 (20.9)[4]	20.6 (2.7)	14.5 (7.4)[2]	20.6 (22.5)	14.1 (4.7)[2,4]	27.8 (31.3)[2]	
PG I/II [mean (SD)]	2.6 (1.7)[4]	1.7 (0.8)	3.7 (3.2)[2]	2.7 (1.6)[2,4]	2.9 (1.5)[2]	2.4 (1.7)	
Diffuse-type gastric cancer							
Cases/incidence rate[3]	21/42	1/10	1/18	19/54	10/37	9/112	
HR (95%CI)		1	1.85 (0.12–29.54)	4.99 (0.66–37.45)	3.36 (0.43–26.39)	10.37 (1.31–82.14)	0.019
PG I [mean (SD)]	64.3 (32.4)[4]	38.2	29.7	67.5 (32.4)[2,4]	62.8 (34.7)[2]	72.8 (30.8)[2,4]	
PG II [mean (SD)]	24.4 (10.0)[4]	14.1	29.7[2]	24.7 (10.2)[2]	24.2 (12.0)[2]	25.2 (8.6)[2]	
PG I/II [mean (SD)]	2.7 (1.1)[4]	2.7	1.1	2.8 (1.1)	2.6 (1.5)[4]	2.9 (0.7)	
Lung cancer							
Age [mean (SD)]	49.0 (4.7)	47.3 (5.4)	45.0 (7.1)	50.0 (4.2)	50.0 (4.2)	0	
Follow-up years [mean (SD)]	7.6 (1.1)	7.8 (0.5)	7.0 (1.4)	7.5 (1.3)	7.5 (1.3)	0	
Cases/incidence rate[3]	20/40	4/41	2/36	14/40	14/52	0	
HR (95%CI)		1	0.81 (0.26–2.62)	0.94 (0.33–3.25)	1.57 (0.62–3.93)	0	
PG I [mean (SD)]	74.7 (45.9)[4]	55.0 (16.9)	78.3 (18.7)	79.9 (53.3)[2]	79.9 (53.3)	0	
PG II [mean (SD)]	17.5 (10.8)	8.8 (2.8)	12.5 (1.1)	20.6 (11.5)[2]	20.6 (11.5)	0	
PG I/II [mean (SD)]	4.8 (2.0)	6.5 (1.6)	6.3 (0.9)	4.1 (1.8)[2]	4.1 (1.8)	0	

[1]Trend for the increase in HR with increase in serum *H. pylori* antibody level.–[2]Significantly different from the negative subjects ($p < 0.05$).–[3]Per 10.000 person-years.–[4]Significantly different from the total subjects in each subgroup stratified by serum anti-*H. pylori* antibody level ($p < 0.05$).

rate for the area was 53.0/100,000 person-years, compared with 39.9/100,000 person-years for the whole of Japan. In fact, in the same year, Wakayama ranked fourth in terms of gastric cancer mortality among the 47 administrative divisions of Japan. Between April 1994 and March 1995, fasting blood samples were collected as part of the routine laboratory tests for a general health checkup (baseline). Aliquots of the separated sera were stored below −20°C until they were used. In Japan, health checkup programs are done to identify selected diseases in their early stage of development. Therefore, subjects who had specific symptoms, required prompt medical care or had a previous gastric resection were excluded from the study. Symptom-free subjects took part in the following tests and procedures: an interview to determine their general health status, a physical examination, chest X-rays, an electrocardiogram, blood laboratory tests, urinalysis and a fecal occult blood test.

Serologic analysis

Serum PG levels were measured using PG I/PG II RIA-Bead Kits (Dainabbot Co., Tokyo, Japan), which involve a modified radioimmunoassay method that we previously established.[37] Subjects with extensive CAG were diagnosed on the basis of the previously described PG test-positive criteria (PG I ≤70 μg/L and PG I/II ratio ≤3.0).[38,39] These criteria have a sensitivity of 70.5% and a specificity of 97%.[38] Subjects who had been prescribed medications that might affect gastrointestinal function, such as proton pump inhibitors or nonsteroidal antiinflammatory drugs, prior to the examination, and subjects who had undergone *H. pylori* eradication therapy were excluded from the analysis of the PG test results. Anti-*H. pylori* IgG titers were measured using ELISA (MBL, Nagoya, Japan). Subjects with antibody titers >50 U/mL were classified as *H. pylori*-infected; those with antibody titers <30 U/mL were regarded as infection negative; and subjects with a titer level that was ≥30 and ≤50 U/mL were considered indeterminate. The sensitivity and specificity of the ELISA used in the present study was 93.5% and 92.5%, respectively.[40] The *H. pylori*-infected group was further divided into 2 subgroups depending on the antibody titer: a low-titer group and a high-titer group, which included subjects with titers >500 U/mL.

Surveillance method

The subjects were screened annually to identify incident gastric cancer cases during the 10-year period between April 1994 and March 2004. Gastric cancer surveys were conducted using a combination of screening methods; all subjects were screened using the PG test and double contrast barium digital radiography (DR). The DR system includes remote-controlled X-ray fluoroscopy (TU-230XB, Hitachi Medico, Tokyo, Japan) and real-time digital radiography (DR-2000H, Hitachi Medico). A total of 150 mL of high-concentration barium (200 w/v %) was used for the double contrast upper-gastrointestinal X-ray series; 11 films were taken for each subject. This system is superior to conventional barium X-ray in image quality and diagnostic accuracy; the cancer detection rate by DR was 1.9 times higher than by conventional screening.[41] Those with positive X-ray findings and/or a positive PG test based on the aforementioned criteria were further examined by panendoscopy (Types XQ200, Olympus, Tokyo, Japan). On the basis of their location, the detected cancers were classified into cardia or non-cardia. The resected specimens obtained by endoscopy or surgery were assessed histopathologically and classified using Lauren's classification into intestinal-type or diffuse-type.[42] The incident day of gastric cancer was defined as the day of the health checkup when the cancer was detected. The length of the observation period was calculated for each subject from the time of the baseline survey to the diagnosis of gastric cancer. The incidence of various cancers (including gastric cancer) and resultant death in the cohort was determined and analyzed yearly and was confirmed by workplace clinic and health insurance records. The ethics committee of Wakayama Medical University approved the protocol, and informed consent was obtained from all participating subjects.

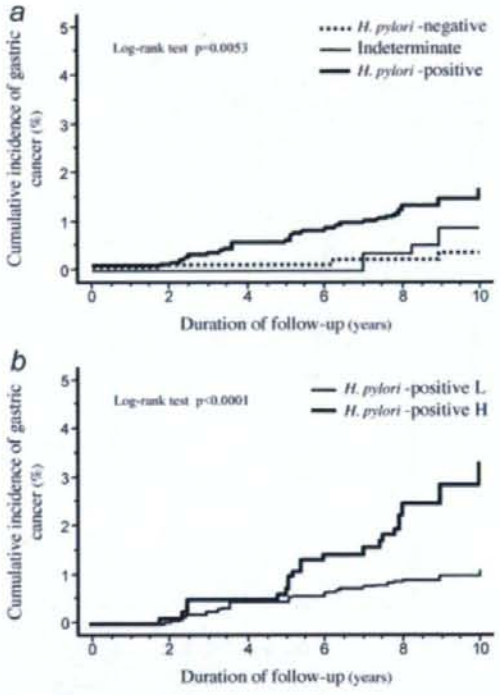

FIGURE 1 – Risk of gastric cancer and *H. pylori* infection. (*a*) The development of gastric cancer and serum *H. pylori* antibody levels. Subjects were classified into 3 groups (*H. pylori*-negative, indeterminate, and *H. pylori*-positive) based on the serum *H. pylori* antibody level as described in the text. The cumulative incidence of gastric cancer in the 3 groups was plotted using the Kaplan-Meier analysis; the differences between the groups were assessed using the log-rank test. The cancer incidence rates for the *H. pylori*-negative, indeterminate, and *H. pylori*-positive groups were 41/100,000 person-years, 72/100,000 person-years, and 157/100,000 person-years, respectively. The differences between the *H. pylori*-positive group and the other 2 groups were significant ($p = 0.0053$). (*b*) The development of gastric cancer in the *H. pylori*-positive group based on antibody levels. The *H. pylori*-positive group was divided into 2 subgroups: the *H. pylori*-positive L (low-titer) subgroup and the *H. pylori*-positive H (high-titer) subgroup based on the titer levels as described in the text. The cumulative gastric cancer incidence was analyzed using the Kaplan-Meier analysis. The cancer incidence rates for the *H. pylori*-positive L and H subgroups were 107/100,000 person-years and 325/100,000 person-years, respectively. The difference between the 2 subgroups was significant ($p < 0.0001$).

Statistical analysis

The data were analyzed using SPSS 11.0 (SPSS, Chicago, IL) and STATA (STATA Corp., College Station, TX). The differences were tested for significance using the t test for comparisons between 2 groups, analysis of variance (ANOVA) for comparisons among multiple groups, and Scheffe's LSD test for comparisons of pairs of groups. The χ^2 test was used to compare categorical variables. The long-term effects of the serum PG levels and the *H. pylori* antibody titer levels on gastric cancer development were evaluated using Cox proportional hazards models.

Results

Among the 5,706 eligible subjects, those who met the exclusion criteria were not included in the study. The 8 cases of gastric cancer that developed within the first year of the study were also excluded. Table I shows the baseline characteristics of the remaining 5,209 study subjects. The mean (SD) age of the subjects at the start of the study was 49.2 (4.7) years, and the mean (SD) follow-up period was 9.7 (0.9) years. During the study period, 63 cases of gastric cancer developed (incidence rate = 125/100,000 person-years) and 7 patients died from the cancer. To date, nearly 4 years after the study, there has not been a single cancer case that is believed to have escaped detection by the annual screening during the study period. Macroscopically, only 2 cancers were located in the cardia; most (96.8%) were non-cardia cancers. Histopathologically, 66.7% (42/63) of the detected cancers were intestinal type, and the remaining 33.3% (21/63) were diffuse type (Table I).

H. pylori *antibody level and cancer risk*

Irrespective of their histopathology, the majority (87.3% [55/63]) of cancers developed in the *H. pylori*-positive group (Table I). *H. pylori* infection was associated with a significantly increased risk of gastric cancer (HR = 3.48, 95% CI: 1.26–9.64). Figure 1 shows the Kaplan-Meier analysis of the subjects stratified by serum *H. pylori* antibody level. After 7 years of follow-up, there was a stepwise increase in the cumulative incidence of gastric cancer as the antibody level increased from negative to positive. The incidence rates of gastric cancer in the *H. pylori*-negative, the indeterminate, and the positive groups were 41/100,000, 72/100,000 and 157/100,000 person-years, respectively (Fig. 1*a*, Table I). In the *H. pylori*-positive group, the cancer development was significantly higher in the high-titer subgroup than in the low-titer subgroup (Fig. 1*b*, Table I). In addition, there was also a significant stepwise increase in the HR (95% CI) with an increase in the antibody level; in the high-titer subgroup, the ratio was 9.56 (2.89–31.65), and the p value for the linear trend was 0.0001. This dose-dependent positive association between antibody level and cancer risk was observed in both histological cancer types.

PG I levels and cancer risk

Next, the relationship between the serum PG I level and cancer development during the study period was investigated. The study subjects were divided into 3 groups based on their PG I levels, as follows: group I-50 with PG I >50 ng/mL, group I-30 with PG I >30 ng/mL and ≤50 ng/mL, and group I-0 with PG I ≤30 ng/mL. The Kaplan-Meier analysis of the subjects in the 3 groups showed that after 5 years of follow-up, cancer development increased in a stepwise manner as the PG I level decreased (Fig. 2*a*); the incidence rate of gastric cancer ranged from 85/100,000 person-years in group I-50 to 341/100,000 person-years in group I-0 (Table II). Likewise, there was a stepwise increase in the HR (95%CI) for cancer with reduction in PG I level, showing a significant elevation in group I-0 (3.54 [1.95–6.40], $p = 0.0001$). With respect to the histopathological cancer type, only in the intestinal-type cancer was a significant stepwise increase in the incidence rate and HR noted with reduction in the PG I level. Next, the subjects were

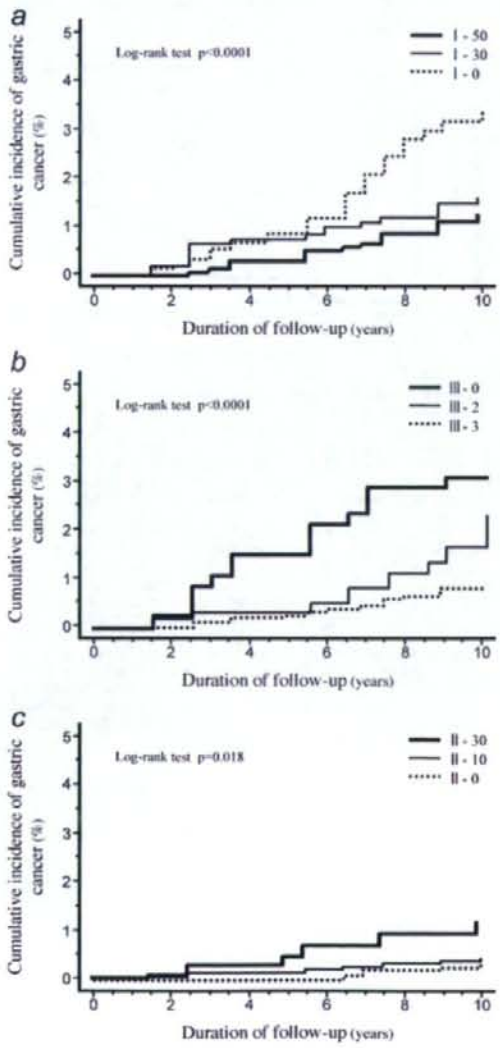

FIGURE 2 – Risk of gastric cancer and serum pepsinogen (PG) level. (*a*) Gastric cancer development and the serum PG I level. Subjects were classified into 3 groups (I-50, I-30, and I-0) based on the serum PG I level as described in the text. The cumulative incidence of gastric cancer in the 3 groups was plotted using the Kaplan-Meier analysis; the differences among the groups were assessed using the log-rank test. The cancer incidence rates for groups I-50, I-30, and I-0 were 85/100,000 person-years, 130/100,000 person-years, and 341/100,000 person-years, respectively. The difference between group I-50 and the other 2 groups was significant ($p < 0.0001$). (*b*) Gastric cancer development and the serum PG I/II ratio. Subjects were classified into 3 groups (III-3, III-2 and III-0) based on the serum PG I/II ratio as described in the text. The cumulative incidence of gastric cancer in the 3 groups was plotted using the Kaplan-Meier analysis; the differences among the groups were assessed using the log-rank test. The cancer incidence rates for groups III-3, III-2 and III-0 were 60/100,000 person-years, 209/100,000 person-years, and 302/100,000 person-years, respectively. The difference between group III-3 and the other 2 groups was significant ($p < 0.0001$). (*c*) Diffuse-type gastric cancer development and the serum PG II level. Subjects were classified into 3 groups (II-30, II-10 and II-0) based on the serum PG II level as described in the text. The cumulative incidence of diffuse-type gastric cancer in the 3 groups was plotted using the Kaplan-Meier analysis; the differences among the groups were assessed using the log-rank test. The cancer incidence rates for groups II-30, II-10 and II-0 were 119/100,000 person-years, 35/100,000 person-years, and 7/100,000 person-years, respectively. The difference between group II-30 and the other 2 groups was significant ($p < 0.018$).