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Chapter 10

Gastric Cancer Risk Diagnosis Using Molecular Biological and Serological Markers Based on *Helicobacter pylori*-Related Chronic Gastritis

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1. Introduction

Helicobacter pylori (*H. pylori*) infection causes chronic infection of the gastric mucosae and is a major risk factor for the onset of gastric cancer via a series of steps comprising gastritis, atrophy, metaplasia, dysplasia and cancer [1--20]. However, in Japan and other countries with a high rate of *H. pylori* infection, the presence or absence of *H. pylori* infection alone in diagnosing gastric cancer risk is not appropriate in terms of specificity. Thus, it is necessary to clarify the groups at high risk of gastric cancer based on the natural history of gastric cancer development, and the evaluation of gastric cancer risk through the use of serological and molecular biological evaluation for chronic gastritis accompanying *H. pylori* infection is important [21-23]. This may contribute to both gastric cancer screening and various measures to prevent gastric cancer, such as the formulation of follow-up plans for metachronous gastric cancer [24] after endoscopic resection [25, 26], procedures developed in Japan that have recently been applied in the treatment of early gastric cancer.

This article describes the diagnosis of gastric cancer risk with DNA methylation as an indicator, using gastric mucosa tissue from endoscopic biopsy, which has been studied by the authors as a molecular biological gastric cancer risk marker. Also discussed is the signifi-

cance of serum pepsinogen (PG) as a marker of gastric cancer risk, as recently reported by the authors, and discusses the groups at high risk for gastric cancer based on more detailed evaluation of *H. pylori* -related chronic gastritis.

2. Aberrant DNA methylation in chronic gastritis and application of gastric cancer risk diagnosis

2.1. Concept of DNA methylation

Genetic defects in cancer include both abnormalities in genetic makeup, such as mutations or chromosomal deletions, and epigenetic abnormalities. Deoxyribonucleic acid (DNA) methylation is one type of epigenetic process. DNA methylation occurs physiologically, and is observed in CpG sites where guanine (G) follows cytosine (C) in the gene sequence. CpG sites exist with a low frequency in the genome, but there are exceptional regions where CpG sites are clustered, called CpG islands (CGI). When a CGI is in a gene promoter region, transcription to messenger ribonucleic acid (mRNA) of downstream genes is strongly inhibited (silencing) if that entire CGI is methylated. DNA methylation, along with mutations and chromosomal deletions, is a major factor in the gene inactivation in many cancers [27-29].

Cancer cells show lower methylation of the entire genome and more localized high methylation than normal cells. Low methylation of the entire genome contributes to carcinogenesis through elicitation of genomic instability [30]. Localized high methylation means abnormal methylation in certain CGIs among CGIs that are not normally methylated. Elicitation of high methylation in promoter region CGIs of tumor suppressor genes causes inactivation of that gene. This elicits cell cycle or proliferation signal abnormalities and accumulation of mutations, and contributes to the onset and progression of cancer.

In gastric cancer and other gastrointestinal cancers, silencing of many important tumor suppressor genes has been reported. In gastric cancer in particular, cadherin 1 (*CDH1*), mutL homolog 1 (*MLH1*) and cyclin-dependent kinase inhibitor 2A (*CDKN2A*) are more often inactivated from methylation than from mutations or chromosomal deletion [31].

2.2. Contribution of aberrant DNA methylation in carcinogenesis

Mutations in tumor suppressor genes are a genetic abnormality, and are a cause (driver) of the onset and progression of cancer. In addition, it has been found that many secondary mutations (passengers) are also present in cancer cells with monoclonal proliferation of these cancer cells [32]. Epigenetic abnormalities in DNA methylation play a large role in gastric cancer, and similarly to genetic abnormalities, DNA methylation of tumor suppressor genes can also be a driver (driver methylation) of carcinogenesis. In addition, it has been shown that aberrant methylation in cancer cells also occurs in numerous genes other than tumor suppressor genes (passenger methylation) [33]. Many genes with passenger methylation are genes that originally have low levels of transcription or no transcription and are thought to be an accompanying phenomenon to cancer development. Such aberrant DNA methylation

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in genes is seen in cancer cells, and it has also been shown to be elicited in non-cancerous tissue as well. Numerous studies have thus been conducted on this topic.

2.3. Induction of aberrant DNA methylation in *H. pylori*-related chronic gastritis

In gastrointestinal organs, precancerous conditions are closely related to chronic infection from viral or bacterial infections [34]. Chronic inflammation has also been shown to be an important inducing factor for aberrant DNA methylation [35]. For example, greater accumulation of aberrant DNA methylation is seen in liver tissue of patients with chronic hepatitis C, which is a background factor for hepatocellular carcinoma, than in normal liver tissue [36]. High-level aberrant DNA methylation exists in colon mucosa tissue of patients with ulcerative colitis, which is a background factor for colon cancer [37]. In the pancreas as well, analysis of endoscopically collected pancreatic juice showed accumulation of aberrant DNA methylation associated with the level of abnormality on pancreatic duct images [38].

When evaluating aberrant DNA methylation in non-cancerous regions, it is extremely important to be mindful of the differences between cancerous and non-cancerous tissue [39]. In non-cancerous tissue, which is polyclonal, quantitative evaluation that asks the question "What is the proportion of the existence of aberrant DNA methylation in specific genes?" is important. In other words, measuring the proportion of molecules with aberrant DNA methylation in non-cancerous specimens (DNA methylation level) and estimating the proportion of cells in which aberrant DNA methylation has been induced will lead to accurate evaluation of aberrant DNA methylation in non-cancerous regions. Therefore, quantitative analysis of methylation levels is necessary to evaluate aberrant DNA methylation in the gastric mucosae, a non-cancerous region. Quantitative methylation-specific polymerase chain reaction (MSP) is a very effective means of resolving such problems [40].

The authors conducted a detailed investigation with quantitative MSP to evaluate aberrant DNA methylation in *H. pylori* -infected gastric mucosae, a background factor for gastric cancer [40]. Using gastric mucosae specimens collected endoscopically from healthy individuals positive and negative for *H. pylori* infection, the DNA methylation level was measured in CpG islands in eight regions of seven genes (Lysyl Oxidase (*LOX*), heart and neural crest derivatives expressed 1 (*HAND1*), thrombomodulin (*THBD*), *p41ARC, CDKN2A,* filamin C (*FLNc*), HRAS-like suppressor (*HRASLS*)), in which DNA methylation is seen with high frequency in gastric cancer [41]. The level of DNA methylation was 5.4– to 303- fold higher in the gastric mucosae of individuals positive for *H. pylori* infection than in those who were negative. *THBD* and other passenger methylation showed particularly high DNA methylation levels when compared with driver methylation such as in *CDKN2A*, which is a tumor suppressor gene (Fig. 1). Certain characteristics are seen in genes with aberrant DNA methylation in *H. pylori*-infected gastric mucosae. Low gene expression levels in gastric mucosae are thought to be a possible predisposing factor (methylation susceptibility) in the elicitation of DNA methylation [42]. This strongly suggests that high levels of aberrant DNA methylation are seen in *H. pylori* -infected gastric mucosae and that *H. pylori* infection induces aberrant DNA methylation. It has been shown in animal experiments that *H. pylori* infection

induces aberrant DNA methylation and inflammation of gastric mucosae evoked by infection is important in inducing aberrant DNA methylation [43].

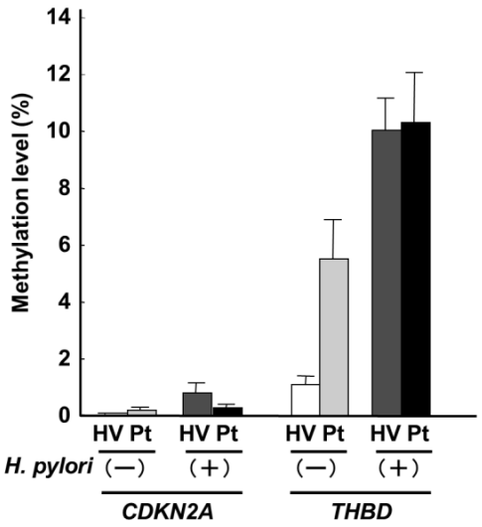

Figure 1. Relationship between *H. pylori* infection, presence or absence of gastric cancer, and gastric mucosae methylation level (citation changed from Reference 40)

Mean methylation level of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and thrombomodulin (*THBD*) genes in gastric mucosae endoscopic specimens measured using the quantitative methylation-specific polymerase chain reaction (MSP) method. In cases when there is no current *H. pylori* infection, non-cancerous gastric mucosae of gastric cancer patients (Pt) shows a higher methylation level than the gastric mucosae of healthy volunteers (HV). In the case of *H. pylori* infection, the methylation level was high, irrespective of whether the person had gastric cancer. High levels of aberrant DNA methylation are seen in *H. pylori* -infected gastric mucosae and that *H. pylori* infection induces aberrant DNA methylation. Ease of methylation differs depending on CpG islands (CGI), and the induction of methylation is much lower in *CDKN2A* than in *THBD*. Error bars indicate standard error.

2.4. Hypomethylation of repetitive elements in *H. pylori*-infected gastric mucosae

Global hypomethylation during gastric carcinogenesis remains unclear, not only when but also where in the genome it takes place. Repetitive elements such as LINE, Alu, and Satα, which occupy some 40% of the entire genome, have abundant CpG and a physiologically high DNA methylation state in normal cells. In cancer cells, however, all of these repetitive elements have a low methylation state. As a result, the overall genome has low methylation (low total amount of 5-methylcytosine), which is thought to lead to genome instability. In *H. pylori* -infected gastric mucosae, it has been shown that low methylation similar to that in cancer is already seen in repetitive elements of Alu and Satα [44]. In enlarged-fold gastritis, for which a relationship with poorly differentiated gastric cancer has been indicated, the DNA methylation level of LINE-1 is reported to be lower than in normal gastric mucosae or

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chronic gastritis without an enlarged fold [45]. There is a strong possibility that hypomethylation of repetitive elements is induced partially in *H. pylori* infected gastric mucosae. Further investigation will be necessary in the future with regard to the role of chronic inflammation associated with *H. pylori* infection in inducing hypomethylation of repetitive elements.

2.5. Accumulation of aberrant DNA methylation in gastric mucosae and gastric cancer risk

The DNA methylation levels of gastric mucosae of healthy individuals and non-cancerous gastric mucosae of gastric cancer patients were investigated with respect to the relationship between aberrant DNA methylation and gastric cancer risk (Fig. 1). The results showed that while the methylation levels were high and there were no significant differences between those who were positive for *H. pylori* infection, methylation levels were low and inhibited in those who were negative for current HP infection, and were 2- to 32- fold higher in the non-cancerous gastric mucosae of gastric cancer patients than in the gastric mucosae of healthy indivudals [40]. In other groups, DNA methylation levels were also investigated in the non-cancerous gastric mucosae of multiple gastric cancer patients, non-cancerous gastric mucosae of single gastric cancer patients, and gastric mucosae of healthy individuals, and it was found that in individuals negative for current *H. pylori* infection the DNA methylation level of *FLNc, HAND1* and *THBD* in particular was correlated with gastric cancer risk [46]. These are known as passenger methylations, but DNA methylation levels were high as a whole, and so quantitative measurement was simple and application as a cancer risk marker is thought to be possible. Mean DNA methylation of tumor suppressor genes *CDKN2A* and *MLH1* was very low, and it would be difficult to use these as cancer risk markers. However, among microRNA genes that function as tumor suppressor genes, some have high DNA methylation levels, as well as correlations with gastric cancer risk [47].

As shown above, passenger genes and microRNA genes are methylated in many cells in gastric mucosae by *H. pylori* infection, and tumor suppressor genes are thought to be methylated in a proportionately smaller number of cells. A high cancer risk state (epigenetic field for cancerization) is thought to be formed by this accumulation [48]. In gastric cancers, an epigenetic field for cancerization is likely to be present and can be detected using appropriate marker genes.

2.6. Progress of gastric mucosae DNA methylation level and gastric cancer risk diagnosis

H. pylori infection potently induces aberrant DNA methylation in gastric mucosae, and its accumulation is associated with gastric cancer risk. To clarify the temporal profiles of aberrant DNA methylation in gastric mucosae, we analyzed time trends of *FLNc* and *THBD* methylation levels in gastric mucosae before, and six weeks and 1 year after *H. pylori* eradication by Quantitative MSP. With respect to the relationship between aberrant DNA methylation in gastric mucosae and *H. pylori* eradication therapy, the authors investigated the trend in DNA methylation before and after *H. pylori* eradication therapy. After bacterial elimination, the DNA methylation levels decreased after six weeks and one year together with the

improvement in chronic inflammation associated with *H. pylori* infection, and a certain level of DNA methylation remained [49].

A model such as in Fig. 2 is assumed if the DNA methylation that disappeared with *H. pylori* eradication is temporarily methylated and the DNA methylation that remained after bacterial elimination is permanently methylated. In other words, in cases of existing *H. pylori* infection, DNA methylation is strongly induced in cells of the entire gland, but if chronic inflammation resolves with *H. pylori* eradication therapy or spontaneous *H. pylori* elimination from the progression of gastric mucosal atrophy, the elicitation of new DNA methylation is decreased. Perhaps the DNA methylation that occurred in differentiated ductal epithelial cells disappears with cell turnover, and the aberrant DNA methylation that occurs in ductal stem cells or progenitor cells remains, even with resolution of chronic inflammation, reflecting the gastric cancer risk. It is possible that individuals with low levels of this permanent methylation also have a low risk of gastric cancer, and conversely, that those with high levels also have a high risk of gastric cancer. A prospective clinical trial is currently underway to test this.

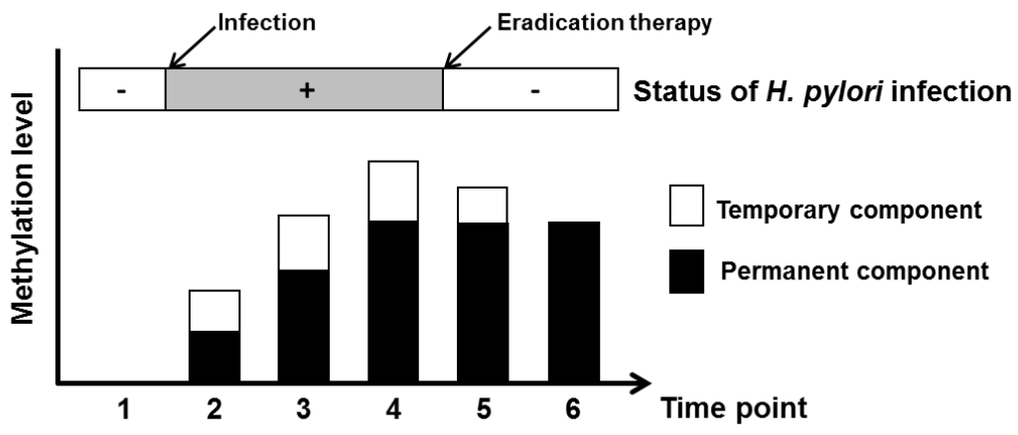

Figure 2. Model of the course of DNA methylation in *H. pylori*-infected gastric mucosae

DNA methylation is assumed to include temporary methylation, which is induced only during the time *H. pylori* infection is present, and permanent methylation that persists even after *H. pylori* infection has been eliminated with eradication therapy. Time point 1: Without *H. pylori* infection, the methylation is initially. Time points: 2–4, *H. pylori* infection induces both permanent and temporary components of methylation, and the total methylation level fluctuates due to fluctuation of the temporary component. Time points 5–6: after *H. pylori* infection discontinues, the temporary component disappears, and the increase in the permanent component stops. Permanent methylation very likely reflects gastric cancer risk.

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3. Identification of gastric cancer risk group by serum PG test

3.1. Evaluation of *H. pylori*-related chronic gastritis by PG test

H. pylori -related chronic gastritis normally spreads from the gastric antrum to the gastric corpus [50, 51], and the progression of chronic atrophic gastritis (CAG) increases the risk for cancer [52-56]. An accurate and reliable evaluation of the extent of CAG is considered to be important for identifying high-risk individuals for cancer; however, it is difficult to accurately diagnose the extent of CAG based on a few biopsy samples, as CAG and intestinal metaplasia represent a multifocal process. Furthermore, histological diagnosis of gastric atrophy depends on subjective judgment without a gold standard [57, 58]. A more convenient and economical test for CAG progression, free of discomfort or risk and based on objective parameters, is necessary.

PG is an inactive precursor of the digestive enzyme pepsin produced exclusively in the stomach. Immunologically, there are two isoenzymes [59]. PG I is produced by chief cells and mucous neck cells of the fundic gland region. PG II, in addition to chief cells and mucous neck cells, is produced in the cardiac glands, pyloric glands and Brunner's glands, with producing cells located over a wide range from the stomach through the duodenum. The majority of PG produced (about 99%) is secreted in the gastric lumen and acts as a digestive enzyme, but a very small amount of PG (about 1%) is also located in the blood and can be measured and evaluated as serum PG. There is general agreement that serum PG levels reflect the morphological and functional status of the stomach mucosa [60, 61].

When the gland margin elevates with the progression of gastric mucosal atrophy diagnosed with chromoendoscopy using Congo red, a high correlation is seen in the incremental decrease in serum PG I levels and PG I/II ratio [62]. Thus, by measuring serum PG I levels and PG I/II ratio, it is possible to objectively evaluate the progress of CAG, which is the bed where gastric cancer develops [63]. In addition, elevated serum PG I and PG II levels and decreased PG I/II ratio are observed at the time of *H. pylori* infection, and have been shown to improve after eradication therapy [64], making them useful as inflammation markers of gastric mucosae.

Various criteria are used in the serum PG test. As criteria for the purpose of gastric cancer screening, a combination of PG I ≤70 ng/ml and PG I/II of ≤3.0, the reference value by Miki et al., has generally been adopted (PG index 1+) [63, 65]. When levels below the reference value are observed, the PG test is judged to be positive. In addition to this reference value, criteria such as PG I ≤50 ng/ml and PG I/II ratio ≤3.0 (PG index 2+), or PG I ≤30 ng/ml and PG I/II ratio ≤2.0 (PG index 3+), are the main criteria used to identify more highly advanced CAG. Since 1992, when PG assay kits became commercially available, a number of screening services provided by workplace or community health services adopted this serum test as a filter

test [21, 66-71]. However, the long-term prognosis of subjects with extensive CAG identified by PG filter test is not fully known.

3.2. Accuracy of detecting gastric cancer using the serum PG test

Using a cohort of healthy middle-aged males, the authors conducted a 10-year follow-up survey of gastric cancer development [72-74]. Based on the results, the accuracy of each criterion for the serum PG test for gastric cancer with onset in the observation period was investigated [73]. With the best reference value (PG index 1+), sensitivity was 58.7%, specificity was 73.4%, and the positive predictive value was 2.6%. The low value for the overall sensitivity was conspicuous. Compared with a report from a meta-analysis of the accuracy of the PG test [75], these results were obviously poorer, with the low value for sensitivity in particular standing out.

One reason for this low sensitivity is thought to be that the patients in past reports of the above-mentioned meta-analysis were groups in which analysis had been performed soon after the start of the PG test and conventional stomach examinations by barium stomach x-ray. In other words, the subjects were groups with gastric cancer prevalence that did not fit well with conventional methods. The low sensitivity in the authors' investigation was thought to be because patients who developed gastric cancer soon after the start of the PG test were excluded, and the observations were of individuals who developed cancer over a longer period of 10 years. From the results of this study, even when performed with the PG test criteria having the best sensitivity, barium stomach x-ray test or some other type of stomach examination was thought to be essential, even for PG test-negative patients, to avoid overlooking gastric cancer, as about 40% of developed gastric cancer was excluded from the subjects of the accuracy test.

3.3. Gastric cancer risk in serum PG test-positive group

As one part of an investigation of the natural history of gastric cancer development, the authors investigated the gastric cancer risk in cohorts identified using each of the serum PG tests [73]. When the subjects were healthy middle-aged males, the annual gastric cancer incidence from the atrophy-negative group was 0.07%, when compared with 0.28% in the PG index 1+ group, 0.32% in the PG index 2+ group, and 0.42% in the PG index 3+ group. The gastric cancer incidence rose incrementally and significantly with the progression of CAG (Fig. 3). The above results thus show that the PG test-positive group is a high risk group for gastric cancer, and even if the development of gastric cancer is not seen at this time, the possibility that they will be affected with gastric cancer in the future is high, and therefore these subjects should continue to undergo detailed examinations and be carefully observed. Thus, the results show again that the PG test is useful as a marker for high risk of gastric cancer.

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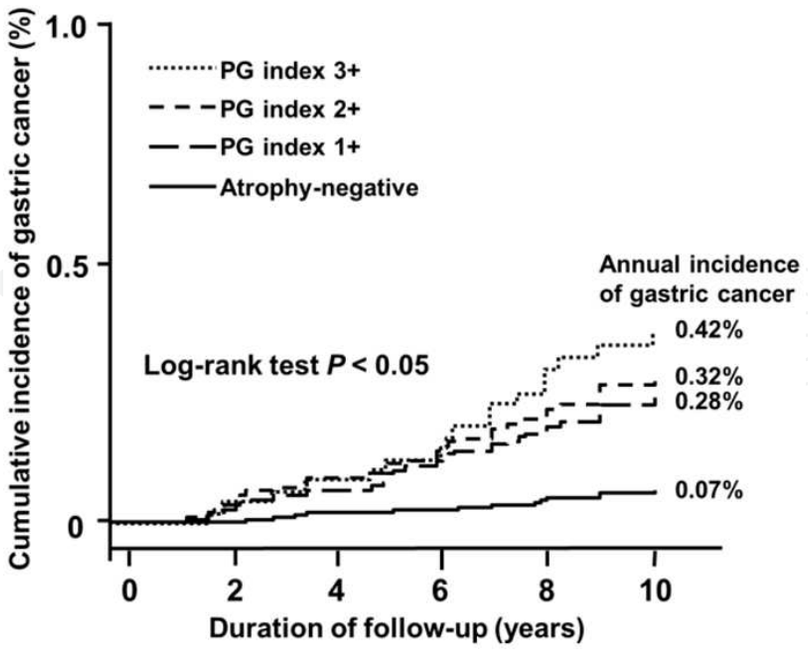

Figure 3. Kaplan-Meier analysis of gastric cancer development in subjects classified using the criteria of the serum PG test

The annual incidence of gastric cancer in cohorts identified using each serum PG test cut-off value (PG I ≤70 ng/ml and PG I/II of ≤3.0 (PG index 1+), PG I ≤50 ng/ml and PG I/II ratio ≤3.0 (PG index 2+), or PG I ≤30 ng/ml and PG I/II ratio ≤2.0 (PG index 3+)) in healthy middle-aged males is shown. Gastric cancer incidence increases in a stepwise fashion and significantly with the progression of chronic atrophic gastritis.

3.4. Identification of high risk for gastric cancer based on staging of *H. pylori*-related chronic gastritis

The relationship between the risk of developing gastric cancer and *H. pylori*-related chronic gastritis staging was also investigated in these same groups [72, 74]. In diagnosing *H. pylori* infection, anti- *H. pylori* antibody titer that can be easily performed with blood tests, similarly to serum PG levels, was used. *H. pylori* -related chronic gastritis stage, including the course from establishment of *H. pylori* infection to establishment of atrophic gastritis, was classified as follows based on a combination of both blood tests. The natural history of *H. pylori* -related chronic gastritis from the establishment of *H. pylori* infection can be expressed as a progression of A → B → C → D in each group. Thus, it is classified into four stages: Group A [*H. pylori* (-) & PG(-)], Group B [*H. pylori* (+) & PG(-)], Group C [*H. pylori* (+) & PG(+)] and Group D [*H. pylori* (-) & PG(+)]. Group A comprised *H. pylori* non-infected healthy men. Group B showed established *H. pylori* infection, but without CAG. Group C had CAG, and Group D had severe intestinal metaplasia due to progression of CAG. However, *H. pylori* in Group D had been spontaneously eliminated, representing so-called metaplastic gastritis.

The results of a 10-year follow-up survey showed that the annual gastric cancer incidence in each group with this staging was 0% in Group A (no occurrence of gastric cancer in 10 years

in this group), 0.11% in Group B (1 in 1,000 individuals developed gastric cancer each year), 0.24% in Group C (1 in 400 individuals developed gastric cancer each year) and 1.31% in Group D (1 in about 80 individuals developed gastric cancer each year). Similar results were observed in a study by Watabe et al.[76]. The above shows that gastric cancer incidence rises incrementally together with the stage progression of *H. pylori*-related chronic gastritis (Fig. 4). In the 10-year follow-up survey, all of those who developed gastric cancer were positive for *H. pylori* infection. This shows that nearly all cases of gastric cancer in Japan derive from *H. pylori*-related chronic gastritis. Based on this fact, it is theoretically possible to identify both high- and low-risk groups (Group A). Using blood test results in this way, the evaluation of the gastric cancer risk in individuals is possible and expected to be useful in grouping individuals for appropriate gastric cancer screening.

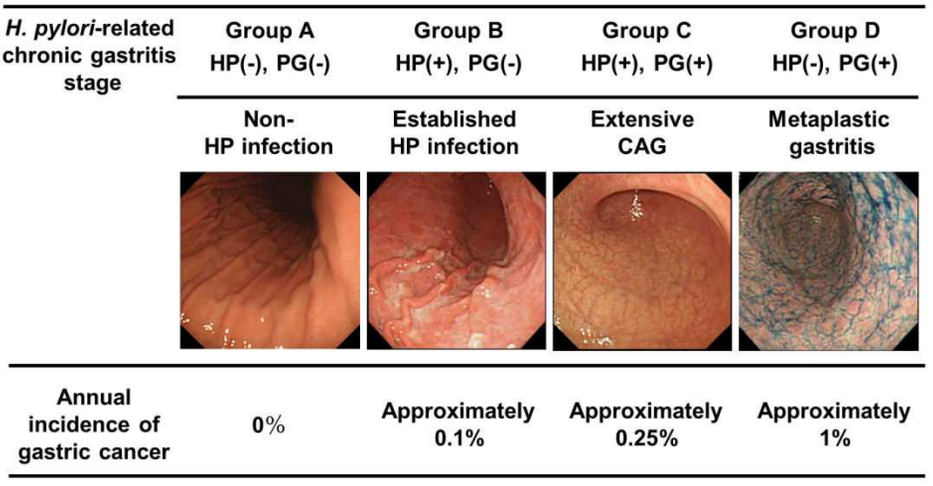

Figure 4. Gastric cancer risk based on *H. pylori*-related chronic gastritis stage classification

H. pylori-related chronic gastritis stage classified based on a combination of both Serum PG test and *H. pylori* antibody is shown. The gastric cancer incidence in healthy middle-aged males increases incrementally and significantly from Group A to Group D in accordance with stage progression.

3.5. Gastric cancer risk in serum PG test-negative group

Even though the serum PG test was shown to be a very useful test as a marker of gastric cancer risk, gastric cancer (particularly diffuse type gastric cancer) was seen in the PG test-negative group. In the authors' investigation, about 40% of gastric cancers were found to be PG test-negative gastric cancers, even using the PG test criteria (PG index 1+) that are thought to have the best balance for test accuracy. This needs to be kept fully in mind when diagnosing gastric cancer risk with the serum PG test. Even in the PG test-negative group, there is thought to be one group in which gastric cancer occurs with a high frequency in Group B of the *H. pylori* -related chronic gastritis staging. Serum PG levels are specific risk markers of gastric cancer development. The risk of developing cancer has been confirmed to increase incrementally with lower PG I levels and I/II ratio in particular [74].

Reviewing the PG-negative group based on this result, it was found to consist of three groups with different risks. Specifically, the PG test-negative group was further divided into three groups: Group α (serum PG I ≤70 ng/ml and PG I/II >3); Group β (serum PG I >70 ng/ml and PG I/II >3); and Group γ (serum PG I >70 ng/ml and PG I/II ≤3). The gastric cancer incidence in Group γ, which showed high serum PG II levels and is thought to have strong inflammation in the gastric mucosae, reached 0.2%, meaning this was a new high-risk group, in which mainly undifferentiated gastric cancer occurs (Fig. 5) [73]. Although the percentage in Group γ was not particularly high in the PG test-negative group, it is a subgroup that should be kept fully in mind. Furthermore, similarly to serum PG II levels, the group with high anti- *H. pylori* antibody titer, which is thought to be an indicator reflecting the level of inflammation, was shown to have a higher incidence of gastric cancer than the low group [74]. In addition, it was shown recently that in Group γ in particular, there are many cases of undifferentiated gastric cancer in individuals with high *H. pylori* antibody titer and endoscopic rugal hyperplastic gastritis[77]. The possibility of gastric cancer inhibition effects from *H. pylori* eradication therapy in such groups has also been demonstrated [78].

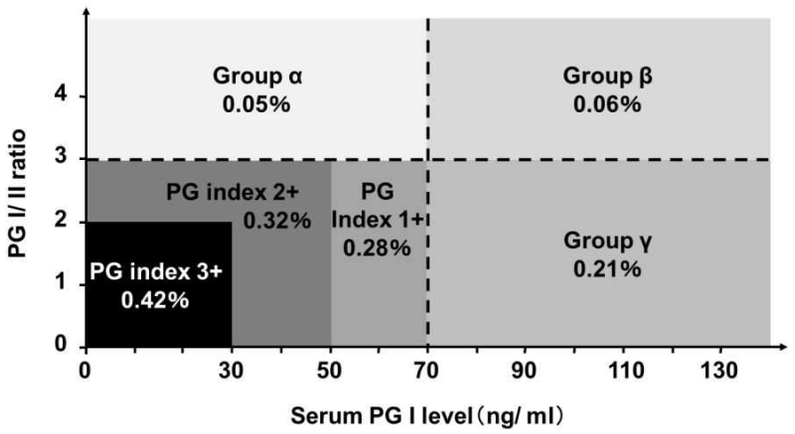

Figure 5. Group determination based on serum PG test and gastric cancer incidence

Schematic presentation of the atrophy-positive criterion and pepsinogen index, which are widely used for the PG filter test in Japan. PG index is used to detect subjects with severe gastric atrophy by subdividing the atrophy-positive criterion identified by PG I of ≤70 ng/mL and PG I/II ratio of ≤3.0 into three groups (PG index 1+ to 3+). Gastric cancer incidence increases in a stepwise fashion and significantly with the progression of chronic atrophic gastritis. In addition, the figure illustrates three subgroups, groups α, β, and γ, in the atrophy-negative criterion. The gastric cancer incidence in Group γ reached 0.2%, meaning this was a new high-risk group, in which mainly undifferentiated gastric cancer occurs.

3.6. Points to bear in mind in diagnosing gastric cancer risk from serum PG test

In the PG test-negative group, the development of cancer in Group A in the *H. pylori*-related chronic gastritis staging (PG test-negative and *H. pylori* judgment-negative) was not seen in a single case in the 10-year follow-up survey by the authors, but there are points to be mind-

ful of in terms of the definitive diagnosis of a patient being in Group A. First is that when *H. pylori* antibody titer measurement kits have low sensitivity, the antibody titer may be negative despite existing HP infection. Second is the need for attention in patients who have undergone *H. pylori* eradication (they are negative for *H. pylori* but the risk of developing gastric cancer is not zero). Third is that, with regard to judging risk from serum PG levels, it cannot be applied in kidney failure patients or patients taking proton pump inhibitors (PPI) following gastrectomy. In addition, the authors have reported that individuals with PG I/II ratio ≤3.0, serum PG I ≤30 ng/ml, or serum PG II >30 ng/ml have a significantly higher risk of gastric cancer [74]. Thus, when the PG I/II ratio is ≤3.0 or serum PG I is ≤30 ng/ml, even in Group A, endoscopic examination should be performed and CAG should be evaluated.

4. Conclusion

Diagnosis of gastric cancer risk using the accumulation of aberrant DNA methylation in the gastric mucosae in endoscopic biopsy tissue and identification of high-risk gastric cancer groups based on serum PG test is discussed (Fig. 6). More effective measures for the prevention of gastric cancer may be possible with specific predictions of gastric cancer risk in individuals based on the natural history of *H. pylori* -related chronic gastritis, and future research results are anticipated.

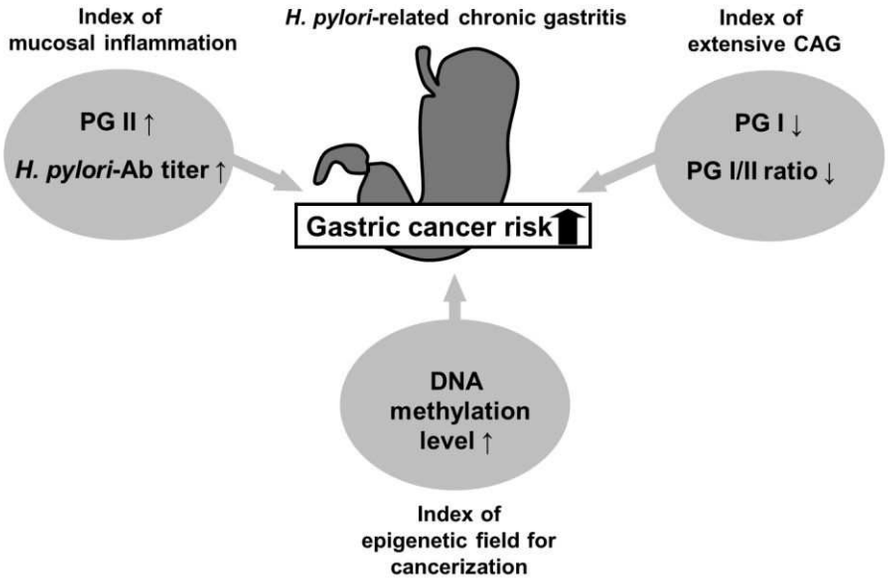

Figure 6. Schematic presentation of gastric cancer risk diagnosis based on *H. pylori* -related chronic gastritis. This article indicated that DNA methylation level of certain genes was associated with *H. pylori* infection and involved in the formation of epigenetic field for cancerization. The serum PG and/or *H. pylori* antibody levels provide an index of gastric cancer development, and that based on these markers the risk for gastric cancer can be objectively determined in each individual with *H. pylori* related chronic gastritis.

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