Gastric Cancer

With Special Focus on Studies from Japan

Akiko Shiotani
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Preface

Helicobacter pylori was declared a human carcinogen in 1994. Evidence has now accumulated to show that at least 95% of gastric cancers are etiologically related to *H. pylori*. In Japan, there has been a progressive and rapid decline in the prevalence of *H. pylori* infection, and the number of gastric cancer deaths has begun to decline in recent years. Japanese insurance policy approved eradication therapy for *H. pylori*-positive gastritis after endoscopic examination in February 2013. The high incidence of gastric cancer in Japan initially resulted in the establishment of a countrywide gastric cancer screening program to detect early and treatable cancers. Population-based endoscopic gastric cancer screening started in September 2014. On *H. pylori* eradication in Japan, potassium-competitive acid blocker (P-CAB) has been approved in February 2015. P-CAB is able to achieve longer and stronger acid suppression, and the superiority of P-CAB-based triple therapy over proton pump inhibitors (PPIs) has been confirmed.

The book comprises of five parts: epidemiology, pathogenesis, risk clarification, therapy, and prevention focusing on gastric cancer in Japan. In the first part, the two chapters indicate gastric cancer epidemiology in Japan and outside Japan. There are three chapters describing *H. pylori* virulence factors and epigenetic and proteomic modulations related to gastric carcinogenesis and clinicopathological features of gastric cancer. *H. pylori* eradication reduces or eliminates mucosal inflammation and reverses or reduces *H. pylori*-associated molecular events such as aberrant activation-induced cytidine deaminase expression, double-strand DNA breaks, impaired DNA mismatch repair, and aberrant DNA methylation. However, increased risk of gastric cancer remains even after *H. pylori* eradication. The theme of the third part is risk clarification and cancer screening before and after eradication. For high-risk groups, especially those with severe atrophy, long-term follow-up endoscopic surveillance for gastric cancer becomes more important than eradication and should be offered. There are three chapters on treatment of gastric cancer regarding current status of endoscopic treatment, operation, and chemotherapy in Japan. Endoscopic submucosal dissection (ESD), which was developed to make possible the en bloc removal of large, flat, superficial cancer lesions, has become a standard technique in Japan and other East Asian countries. The final part consists of two

chapters about gastric cancer prevention. Earlier eradication of *H. pylori* is considered to be more effective in preventing gastric cancer by inhibiting the progression of mucosal atrophy. Japanese gastric cancer elimination projects including test and treat in young generation are thought to promote a decrease of gastric cancer-related deaths.

Kurashiki, Okayama, Japan Akiko Shiotani

Contents

Part I
Epidemiology

Chapter 1
Japan

Kato Mototsugu

Abstract Gastric cancer remains one of the most common cancers in the world, being the third most common cause of cancer death and the fifth most common malignancy. Incidence rate of gastric cancer is highest in East Asia including Japan, Korea, and China. More than half of new gastric cancer cases in the world have been diagnosed in East Asia. Gastric cancer is not a disease of the past in Japan like other developed Western countries. Although mortality and morbidity rates of gastric cancer have been dramatically declining in Japan, the absolute number of gastric cancer deaths has remained constant for a few decades. The number of deaths has begun to decline in recent years. Interestingly, gastric cancers detected in Japan have characteristic features. Mortality rates in Japan are considerably lower than incidence rates due to the impact of diagnosis and treatment of early-stage gastric cancers.

Keywords Gastric cancer · Early gastric cancer · Mortality · Morbidity · *H. pylori* · Salt · Diet · Atrophy · Intestinal metaplasia

1.1 Mortality

Over time in Japanese trends of age-standardized cancer mortality rates, mortality rates of gastric cancer in both male and female have been dramatically declining throughout the observation period (Fig. 1.1) [1]. This phenomenon is in contrast with the rising mortality rates of other cancers such as lung, colon, prostate, and breast. Gastric cancer has been the leading cause of cancer death for a long time, but it has now dropped to second place in male and fourth place in female. In 1950 gastric cancer deaths accounted for about 48% of cancer deaths, while in 2011 they accounted for about 14% [2]. Mortality rates of gastric cancer worldwide have been declining for decades. This decrease is due to changes in food preservation methods (from salted to refrigerated or frozen) and to everyday availability of fresh vegetables and fruits.

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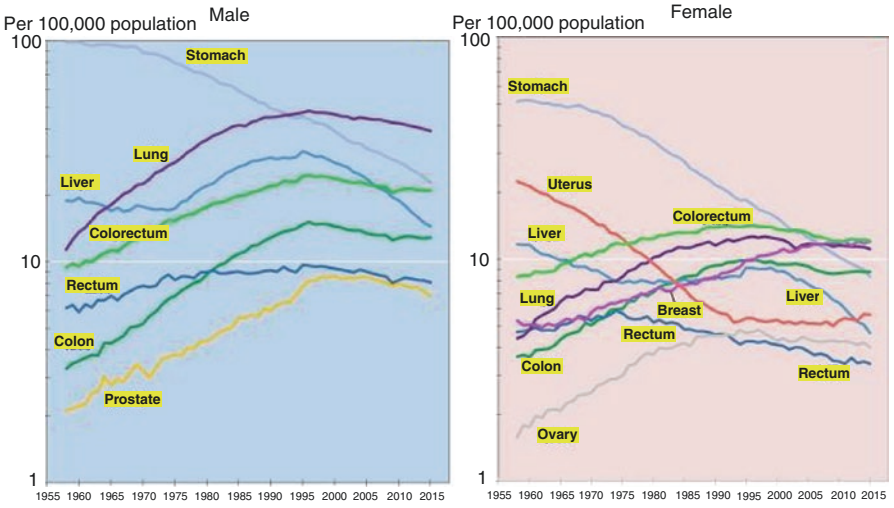

Fig. 1.1 Age-standardized mortality rate of cancer by site in Japan. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

An age-standardized mortality rate of gastric cancer performed in Japan every 10 years, from the 1960s to the 2010s, shows decrease of mortality rate in all ages over time (Fig. 1.2a) [1]. In the 1960s, the peak of mortality rate was between 75 and 79 years old. However, every 10 years, the peak age has increased, currently reaching over 85 years old. When the mortality rate of each age class is represented by the birth year, the mortality rate is lower for late births in the same age group (Fig. 1.2b) [1]. Infection rates of *H. pylori* in Japanese individuals born before 1950 were uniformly high; however, even the mortality rate of those generations was affected by their birth years [3]. Therefore, a change in lifestyle contributed to the decline in mortality rates of gastric cancer. A decline in the prevalence rate of *H. pylori* appears to be a factor that influenced mortality rates only later.

Although age-standardized mortality rate of gastric cancer has declined, the absolute number of gastric cancer deaths has remained constant between 50,000 and 55,000 over the past 50 years (Fig. 1.3) [1]. A trend of the number of gastric cancer deaths by age in Japan reveals that they peaked from 65 to 75 years old until the 1990s (Fig. 1.4) [1]. Subsequently, in the 2000s the number of gastric cancer deaths in individuals over 85 years old increased dramatically. The shift of gastric cancer patients and death to an extremely elderly population are attributable to the rapid aging of Japanese population (Fig. 1.5) [4]. Originally the effect of decreasing the number of deaths caused by a decline in the age-adjusted mortality rate was offset by an increase in the number of gastric cancer deaths among a super elderly population.

The absolute number of deaths from gastric cancer was 48,632 in 2013, 47,903 in 2014, 46,659 in 2015, and 45,509 in 2016. These numbers show a decreasing trend following the introduction of insurance coverage for *H. pylori* eradication as a consequence of *H. pylori*-induced gastritis [1]. A fall of 9.2% over the last 4 years

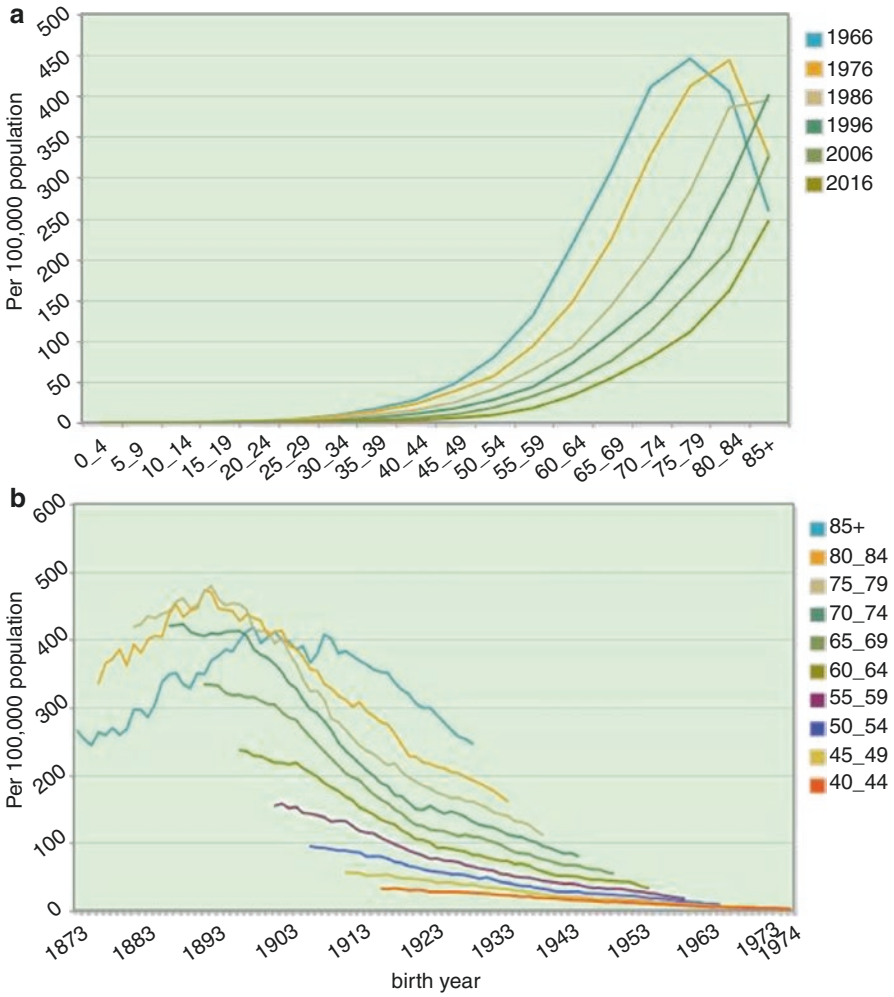

Fig. 1.2 Trend of age-standardized mortality rate by age and by birth year in Japan. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

showed a significant decrease, compared to the expected number of gastric cancer deaths using previously observed data shown by the National Cancer Center (Fig. 1.6) [4, 5]. From 300,000 to 600,000, patients have *H. pylori* eradicated every year after insurance approval in year 2000. Six million patients with gastritis have had *H. pylori* eradicated in the 4 years following the availability of insurance coverage. It is estimated that 12 million Japanese have had *H. pylori* eradicated so far [5]. It seems that reduction of gastric cancer incidence following *H. pylori* eradication over a 10-year period has contributed to the recent decrease in the number of gastric cancer deaths.

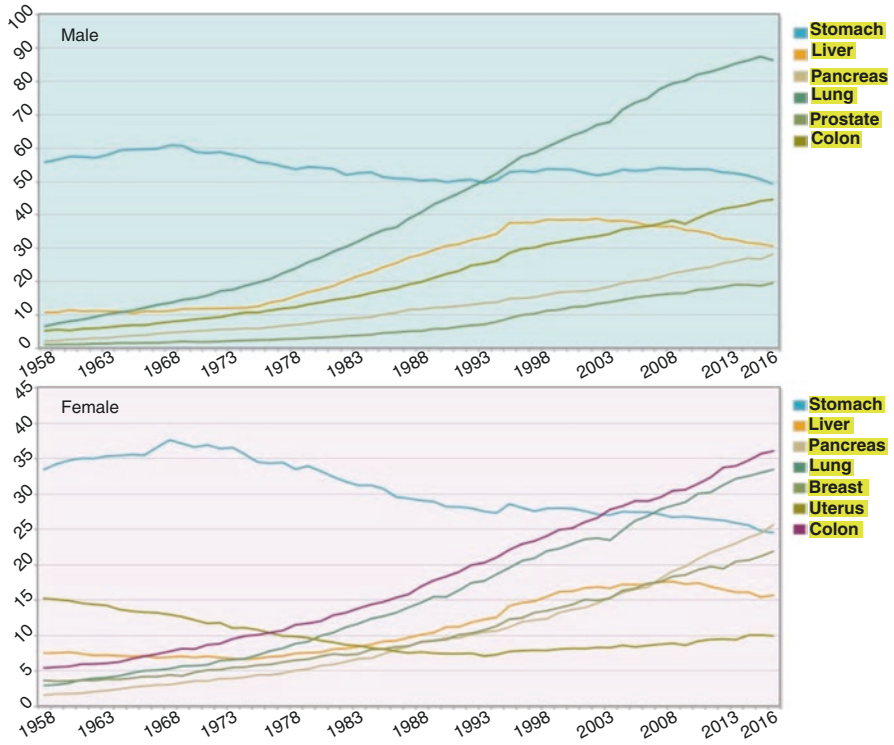

Fig. 1.3 Absolute number of cancer deaths by site in Japan. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

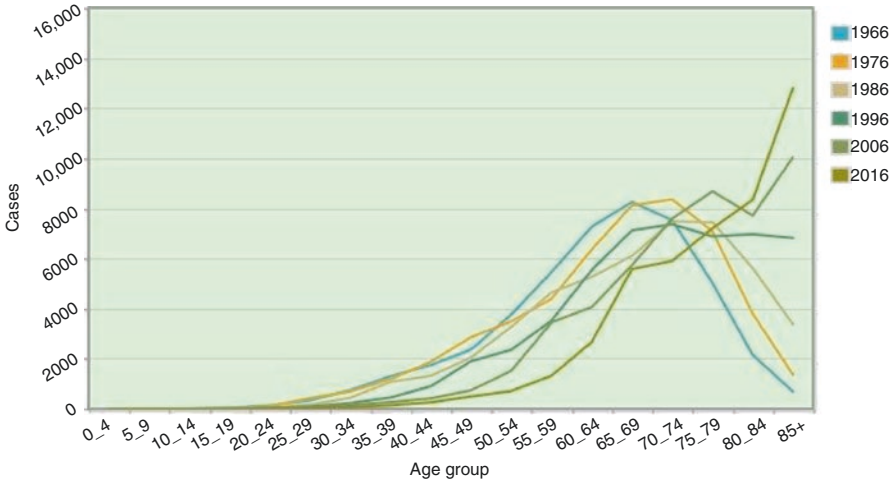

Fig. 1.4 Trend of the absolute number of gastric cancer deaths by age in Japan. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

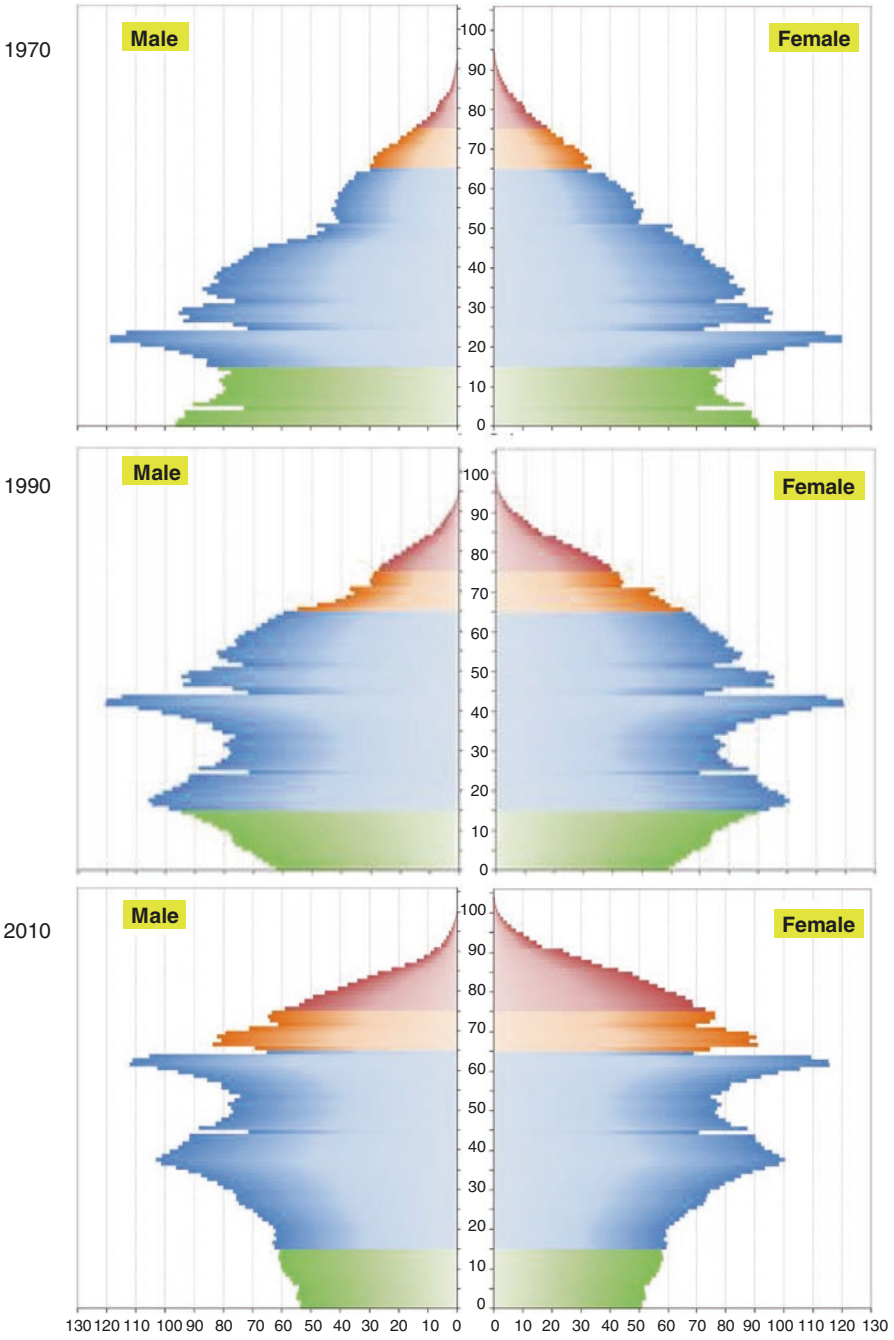

Fig. 1.5 Population pyramid of Japan from 1940 to 2010

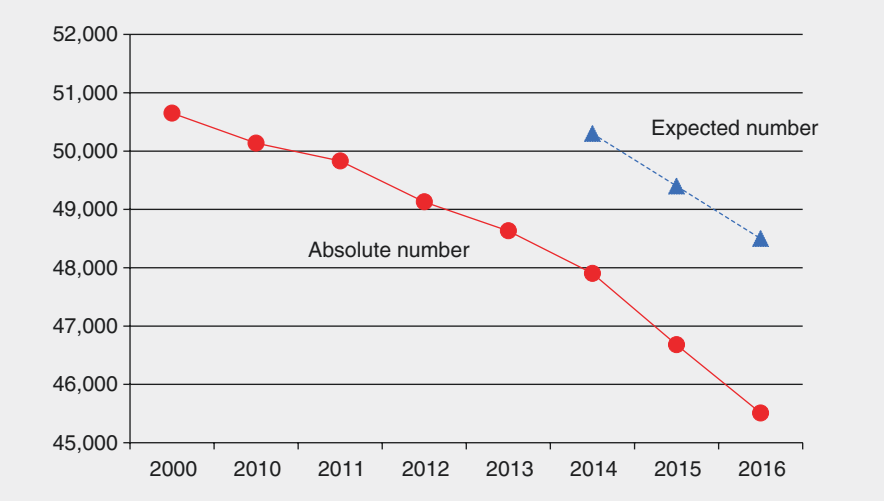

Fig. 1.6 The difference between absolute and expected number of deaths from gastric cancer in Japan

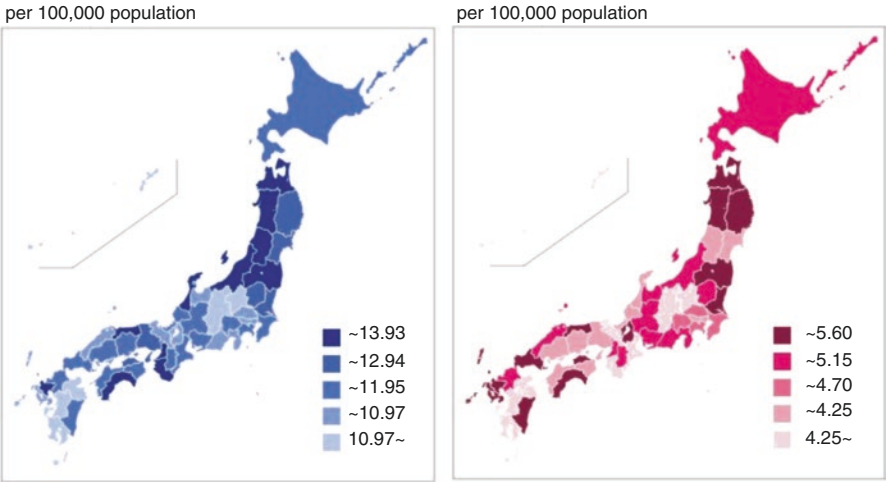

Fig. 1.7 Age-standardized mortality of gastric cancer by prefecture in Japan. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

The distribution of gastric cancer varies across geographical regions. The mortality rate of gastric cancer in Japan is high in both males and females, highest in the northeast region by Akita Prefecture and Yamagata Prefecture, and lowest in the southwest area by Okinawa Prefecture (Fig. 1.7) [1]. This regional difference is also associated with food culture. For example, high salt consumption is prevalent in high-risk areas of gastric cancer [6]. Although the prevalence rate of *H. pylori* in

Okinawa Prefecture is in the national average, there are reports that an *H. pylori* strain with lower pathogenicity is the main cause of infection rather than the East Asian strain [7].

1.2 Morbidity

Similarly to other countries, age-standardized estimated morbidity rates also have been declining for decades in Japan (Fig. 1.8) [1]. However, to date the absolute number of gastric cancer incidences in both male and female shows an increasing

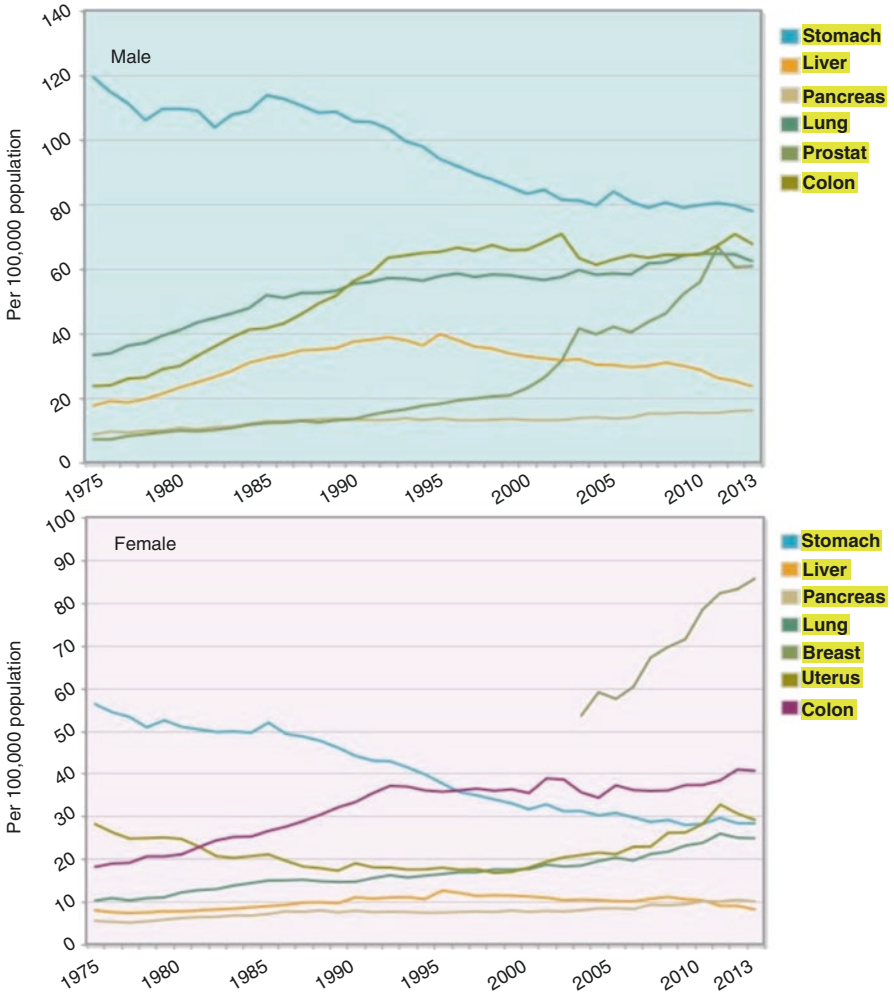

Fig. 1.8 Age-standardized estimated morbidity rate of cancer by site in Japan

trend (Fig. 1.9a) [1]. Since the incidence of gastric cancer is influenced by both population size and age distribution, a paradoxical increase of gastric cancer incidence is due to the high proportion of elderly citizens in the Japanese population. Based on a trend of age distribution of gastric cancer incidence, more recently there has been an increase of the incidence of gastric cancer between the ages of 65 and 85 (Fig. 1.9b) [1].

It is generally known that gastric cancer risk is high in men. In a comparison of age-standardized mortality rates of gastric cancer, the ratio of men and women is 2.8 in Japan [2]. While women experience a higher morbidity rate than men up to the age of 30, gender difference becomes remarkable in middle age and older [1]. Environmental factors including *H. pylori* infection, smoking habit, and alcohol consumption are more strongly associated with middle-age and older men than host factors. The IARC working group reported that the marked geographical variation

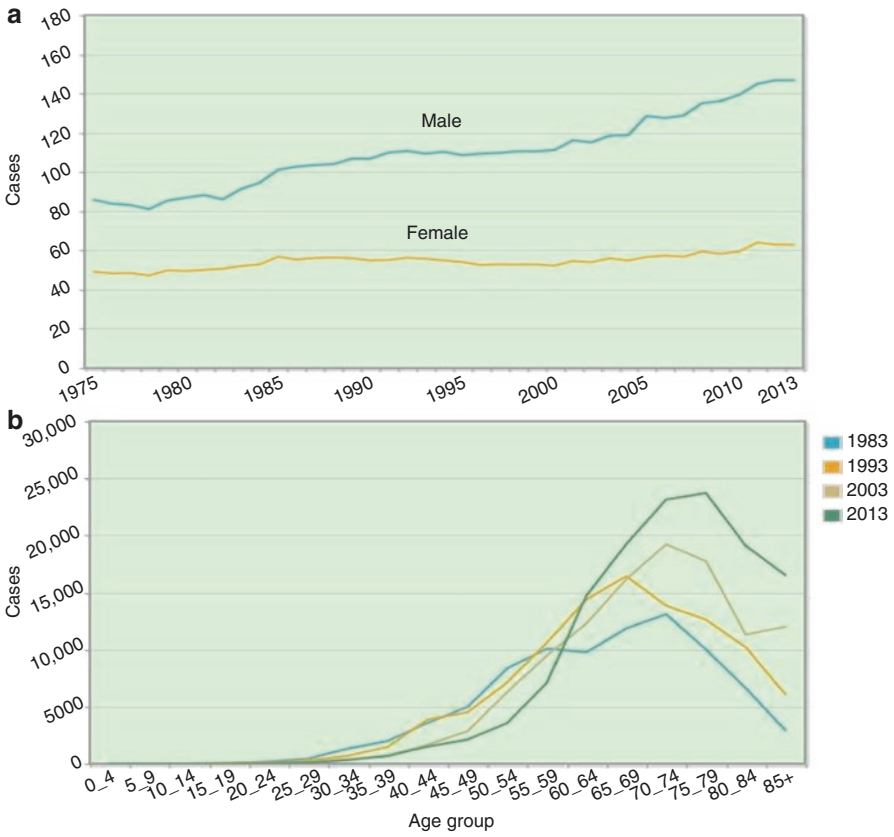

Fig. 1.9 Trend of estimated number of gastric cancer incidence in Japan. (**a**) By gender. (**b**) By age. Source: Center for Cancer Control and Information Services, National Cancer Center, Japan

of gastric cancer and the remarkable decline in incidence may be related to the reduction of ubiquitous exposures worldwide [8]. Improvements in sanitation and preservation and storage of foods, changes in the prevalence of *H. pylori* infection, and use of antibiotics are thought to be responsible for these phenomena.

1.3 Clinico-Epidemiological Features

Gastric cancers detected in Japan have characteristic features when compared to other countries, with the exception of the Republic of Korea. Although prognosis of gastric cancer is generally poor, mortality rates in Japan are considerably lower than incidence rates. This data reflects the impact of early diagnosis and treatment of gastric cancer [9].

The clinical concept of early gastric cancer was established in 1962 by the Japanese society of Gastroenterological Endosopy [10]. Early gastric cancer was defined as tumor invasion limited to the mucosal and submucosal layers regardless of regional lymph node metastasis. Some prospective and retrospective follow-up studies using endoscopic examination showed that within 1–5 years, mucosal cancer progressed to submucosal cancer or advanced cancer [11, 12]. Follow-up studies estimated that 50% of mucosal cancer developed into submucosal cancer during the follow-up period of 91 months. Twenty-five percent of mucosal cancer and submucosal cancer developed into advanced cancer between 19 and 91 months [11]. Follow-up studies of Western patients diagnosed with high-grade dysplasia showed that 60–80% of high-grade dysplasia progressed to carcinoma within a very short mean follow-up time of 6 months [13]. In other words, high-grade dysplasia was already carcinoma.

The WHO and Japan have different TNM classification of gastric cancer. Tis in WHO has carcinoma in situ, intraepithelial tumor without invasion of the lamia propria, and high-grade dysplasia [14]. T1a in the WHO is defined as a tumor that invades the lamina propria or muscularis mucosa. T1a in Japanese classification includes both Tis and T1a under the WHO classification [15]. The Vienna classification of gastrointestinal epithelial neoplasia is a compromise between the Western and Japanese points of pathologic diagnosis. High-grade dysplasia/adenoma in Western pathology and noninvasive carcinoma and intramucosal carcinoma in Japanese pathology fall in the same category 4 (mucosal high-grade neoplasia) according to the Vienna classification [16, 17].

Trends of clinico-epidemiological features of Japanese gastric cancer were analyzed based on the Gastric Cancer Database that collected 19,306 gastric cancers from 1946 to 2014 [18]. As to the degree of progression of gastric cancer, stage I increased since the 1970s following the introduction of endoscopic diagnosis for early gastric cancer (Fig. 1.10a) [18]. Nowadays, stage IA (intramucosal carcinoma) represents about 50% of gastric cancers in Japan. Almost all gastric cancers stage IA are resected using endoscopic treatment. A 5-year relative survival rate of stage I is higher than 95% in Japan. Detection of early gastric cancer contributes to the

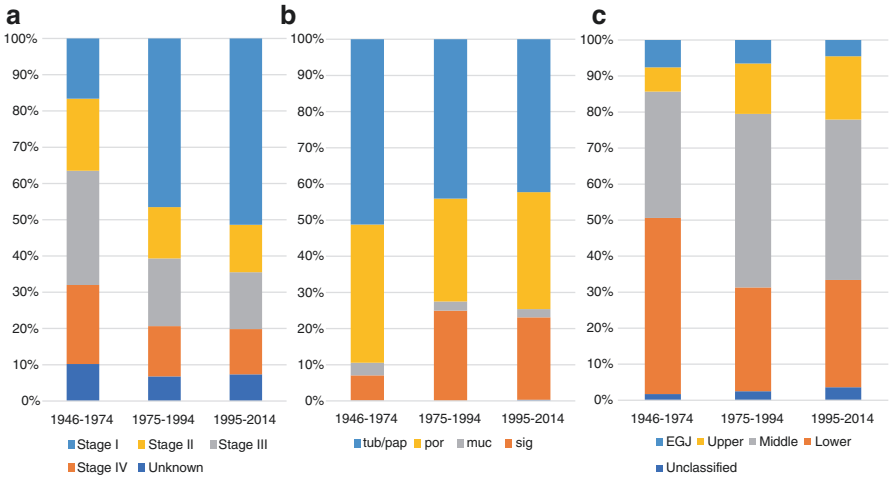

Fig. 1.10 The trend of proportional frequencies about features of Japanese gastric cancer. (**a**) Clinical stage. (**b**) Histological finding. (**c**) Primary site

improvement of prognosis. There is a significant difference between Japan and other countries with the exception of the Republic of Korea [19].

In terms of histological finding frequency, the order of tubular and papillary adenocarcinoma (tub/pap), poorly differentiated adenocarcinoma (por), signet ring cell carcinoma (sig), and mucinous adenocarcinoma (muc) have not changed; however, the proportion of tub/pap has been decreasing over time (Fig. 1.10b) [18]. Corpus predominant gastritis is associated with the occurrence of tub/pap. Since the majority of Japanese patients with *H. pylori* infection have corpus predominant gastritis, the proportion of tub/pap is higher than in Western countries [20].

In terms of the primary site of gastric cancer occurrence in Japan, the occurrence in the lower region has been decreasing over time, while the frequency of occurrence on the distal region represents 75% or more, and occurrence in the esophagogastric junction is less than 5% and remains stable over time (Fig. 1.10c) [18]. Cardia cancer is not related to *H. pylori* in general, but in Japan cardia cancers often have the background of *H. pylori*-induced severe atrophy [21].

1.4 Risk Factors

H. pylori infection is necessary but not sufficient for the carcinogenesis of gastric cancer. Many factors are associated with gastric cancer development. Carcinogenesis factors include the following: environment, host genetics, and aging. Environmental factors include low acid output, high-salt diet, and tobacco use. *H. pylori* infection plays an important role in gastric carcinogenesis; specifically almost all gastric cancers develop from a background of *H. pylori*-infected mucosa. De novo-type gastric cancer without chronic gastritis is rare, representing about less than 1% of total gastric cancer cases [22].

H. pylori infection occurs under the age of 5, and in 80% of cases, it is a domestic infection, exchanged between mothers and children. *H. pylori* prevalence rate of each generation is determined by the age of 10, and infection rate does not rise with age. *H. pylori* prevalence rate in Japan has characteristics of a biphasic relationship between the high rate age and the age when the rate is rapidly declining from the time of advanced economic development (Fig. 1.11) [3]. Recent nationwide report showed that *H. pylori* infection rate has steadily declined in Japan [23]. When examining the infectious rate of *H. pylori* for each 10-year-old class based on reported results, the percentage of *H. pylori* infection decreased by 10 to 15% in 10 years in each age group [3, 23–25]. Figure 1.12 is the estimated prevalence rate of *H. pylori* for each age at 20-year intervals from 1950 to 2070 in Japan.

H. pylori infection causes atrophic changes and intestinal metaplasia on the gastric mucosa. Histological and endoscopic degree and extent of these findings are strongly associated with the risk of gastric cancer development. Nodular gastritis is a specific type of gastritis distinguished by lymphoid follicle formation associated with *H. pylori* infection. It is known that nodular gastritis poses a risk of gastric cancer in young females, predominantly a diffuse type cancer [26].

Many epidemiological studies suggested that gastric cancer risk may be increased with high intake of various traditional salt-preserved foods and decreased with high intake of fruit and vegetables [27]. There is a positive correlation between salt intake and gastric carcinogenesis in Japanese epidemiological studies [28]. Since salt promotes chemical carcinogenesis in stomach with *H. pylori* as a co-promoter, high-salt diet increases the risk of gastric cancer development in *H. pylori*-infected

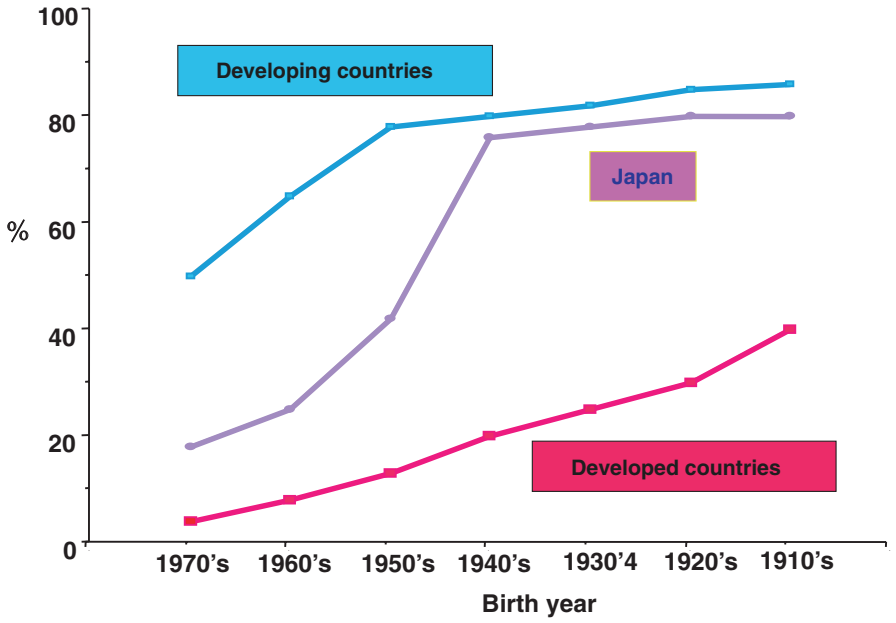

Fig. 1.11 Prevalence of *H. pylori* infection in Japan. The pattern of *H. pylori* prevalence lies between the developing and developed countries

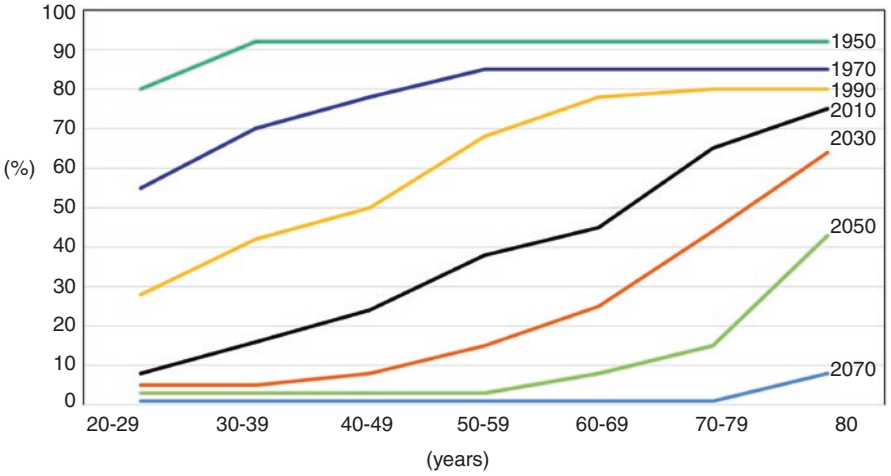

Fig. 1.12 The estimated prevalence rate of *H. pylori* for each age at 20-year intervals from 1950 to 2070

population, not in *H. pylori*-negative population [29]. A systematic review of Japanese epidemiological studies showed that the relative risk for current smokers was estimated to be 1.56 for the total population [30].

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Chapter 2
Gastric Cancer Worldwide Except Japan

Mimi C. Tan, Maya Balakrishnan, and David Y. Graham

Abstract Until recently, gastric cancer was the most common cause of cancer deaths. Despite the rapid fall in incidence, gastric cancer is still the fifth most common cancer and third leading cause of cancer-related mortality worldwide. The lifetime risk of gastric cancer to age 74 remains between 1 and 3% in most European countries and as high as 5–20% in some parts of Asia. The discovery of *Helicobacter pylori* as the cause of atrophic gastritis, the lesion that predisposes to gastric cancer, has resulted in increasing attempts to eliminate gastric cancer by eradicating *H. pylori*. Here we review the worldwide changes in gastric cancer incidence and the current lifetime risk.

Keywords Gastric cancer · Epidemiology · *Helicobacter pylori* · Epidemiology · Risk factors

2.1 Introduction

Until the mid- to late twentieth century, gastric cancer was the most common cause of cancer-related mortality in most countries [1]. In the United States (USA), gastric cancer retained that title until 1952 when the age-adjusted cancer mortality rate of lung cancer surpassed gastric cancer in men [2]. Gastric cancer's prominence in the nineteenth century is evidenced by the large body of literature from that era dedicated to it. For example, the 1903 English translation of Riegel's book *Diseases of the Stomach* cites 158 references from 1878 to 1896 and refers to Leube in Ziemssen's *Handbook of Special Pathology and Therapy* for the older literature up to the late 1870s [3]. Examples include Marc d'Espine who reported that between 1838 and 1855, 45% of fatal cancers in Geneva were gastric [4]. Virchow, using

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autopsy material, calculated that between 1852 and 1855 in Wurzburg, Germany, 34.6% of all fatal cancers were gastric [3]. In 1889, Haberelin in Switzerland found 11,422 gastric cancers (41.5%) among 27,500 cancers that ended fatally [5]. The UK Registrar General's annual report from 1897 to 1900 found that gastric cancer was the leading cause of death (20.6%) among 12 different malignant diseases [4].

Worldwide statistics remained rare until 1915 when Frederick L. Hoffman, statistician for the Prudential Insurance Company of America and chairman of the Committee of Statistics, American Society for the Control of Cancer, published a book entitled *The Mortality from Cancer Throughout the World* [1]. Hoffman's book was one of the first that attempted to pull together all available data and to detail plans for subsequent collection of cancer statistics. He provided data from 23 countries and innumerable subpopulations pointing out the difficulties in obtaining reliable data. His book confirmed that in most countries, including Western countries and Japan, gastric cancer was the most common cause of cancer-related death [1].

2.2 Gastric Cancer and Gastritis

The late nineteenth century was also a time of intense interest in gastric physiology and diseases, including gastric cancer and peptic ulcer disease. One focus was on histologic damage to the stomach, gastritis, and its relation to disease [6]. The association of gastric cancer with chronic gastritis and gastric atrophy was well established in the late 1800s and early 1900s [7, 8]. Both Charles Mayo and Arthur Hurst are credited with the statement that gastric carcinoma never involves a healthy stomach [9, 10]. In 1879, von den Vender made the association of gastric cancer with achlorhydria [8, 11], which was confirmed and greatly expanded (e.g., by Comfort in 1934) [12]. von den Vender's observation stimulated extensive research examining acid secretion and gastritis [8, 11]. In the Schorstein Lecture of 1929, Sir Arthur Hurst reported that gastrectomy specimens of patients with chronic ulcer and carcinoma had consistently shown gastritis to be present throughout the stomach [9]. He further noted that "chronic gastritis, which precedes the onset of carcinoma, is in fact the most common predisposing condition" [9] and that "the ideal form of prophylaxis [for gastric cancer] would be not merely to recognize and treat the common precancerous gastric conditions, chronic gastritis and chronic gastric ulcer, but to prevent their development" [9].

By 1950, the gastritis-carcinoma sequence had been firmly established, with gastritis recognized as the soil from which cancer arose [8, 13]. Research on gastritis continued with many studies worldwide seeking to discover the cause of gastritis, which would likely also be the cause of gastritis-associated diseases, including peptic ulcer disease and gastric cancer. Unfortunately, the discovery of *Helicobacter pylori* and proof that it was the major cause of gastritis was not immediately integrated into this plethora of data regarding gastritis. Instead, this new finding attracted many new investigators who seemed ignorant of prior research. Additionally, most

early epidemiology studies regarding gastric cancer and *H. pylori* were based on *H. pylori* serology, which, in retrospect, provided misleading data as serology often becomes negative following development of gastric atrophy [14–16]. Thus, early studies significantly underestimated the attributable risk of *H. pylori* on gastric cancer. Proof that gastric cancer was strongly linked to gastric atrophy, which was previously established by early researchers, had to be rediscovered before it caused investigators to relate *H. pylori* to gastric cancer. Rather than confirm that the long sought-after cause of gastritis, gastric cancer, and peptic ulcer had been found, observations (e.g., such as the association of *H. pylori* with gastric cancer) were treated as unique, deserving to be published in the most prestigious journals. This delay in recognizing that the discovery of *H. pylori* closed the loop in the long search for the cause of gastric cancer and peptic ulcer disease resulted in the delayed realization that elimination of *H. pylori* could eliminate both diseases.

It is now well accepted that the most common cause of gastric cancer is infection with *H. pylori* and that the major risk factor is the development of atrophic gastritis [8, 17–19]. Factors that affect *H. pylori* infection and/or atrophic gastritis can influence gastric cancer incidence. The impact of environmental factors, such as diet or *H. pylori* infection, on the incidence and pattern of gastric disease is best appreciated when analyzing birth cohorts [20]. Increased access to clean water and improved sanitation over time have resulted in reduced *H. pylori* infection rates among younger generations compared to older generations [18]. Additionally, the anatomic pattern of *H. pylori* gastritis (pangastritis vs. antral predominant) leads to different disease presentations. Pangastritis with atrophy presents as gastric ulcer and cancer, whereas antral predominant gastritis presents as duodenal ulcer disease. Thus, a change in the pattern of disease from predominantly gastric ulcer and gastric cancer to duodenal ulcer among Western countries in the late nineteenth and early twentieth century signified a change in the pattern of gastritis [18]. In contrast, the overall decline in gastric cancer and both gastric and duodenal ulcers in the latter part of the twentieth century reflected the progressive fall in *H. pylori* prevalence [18].

2.3 The Gastric Cancer Cascade

In 1975, Correa described a cascade of superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and finally gastric cancer [21, 22]. He termed the basic pattern of gastritis “multifocal atrophic gastritis.” It is now recognized that his description of a multifocal process was related to his method of determining the pattern of intestinal metaplasia (e.g., staining for sucrase activity) (Fig. 2.1). While multifocal staining identified intestinal metaplasia, it could not recognize the lawn of underlying atrophy then called pyloric or pseudopyloric metaplasia that represented the primary atrophic event [23, 24]. Pyloric metaplasia is now easily recognized histologically by immunohistochemical staining of corpus mucosa for spasmolytic polypeptide (SPEM) [25]. Islands of intestinal metaplasia develop

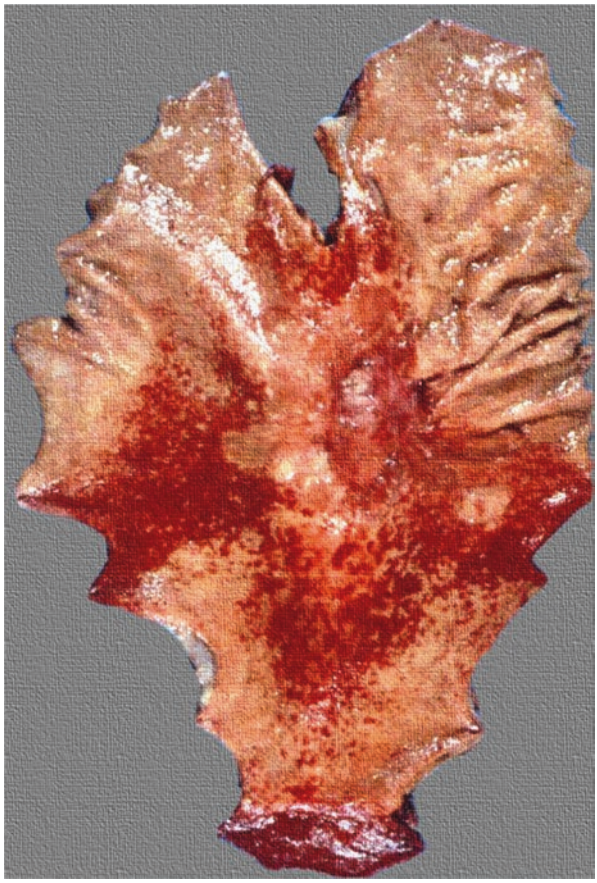

Fig. 2.1 Illustration of a gastric resection specimen stained for sucrase to identify the multifocal pattern of intestinal metaplasia

within this lawn of SPEM within which gastric cancer arises (Fig. 2.2) [23, 24]. The cascade is now best described as *H. pylori* infection, superficial gastritis, atrophic gastritis, metaplastic epithelia, intramucosal neoplasia, and finally invasive cancer. Most no longer believe that intestinal metaplasia evolves into gastric cancer. However, the stem cell for gastric cancer remains unknown [26]. Nonetheless, mucosal atrophy and loss of parietal cells in the corpus results in hypochlorhydria or achlorhydria which forms the milieu in which gastric cancer develops [13, 27]. Extensive atrophy of the gastric corpus also leads to decreased numbers of chief cells, which primarily produce pepsinogen (PG) I. Serum PGI levels < 70 μg/L or PGI/PGII ratio < 3 is widely used as a biomarker for severe atrophic gastritis involving the corpus [28, 29].

The severity and extent of atrophy is now often classified using a five-point scale (0–4), the Operative Link for Gastritis Assessment (OLGA) or Gastric Intestinal Metaplasia Assessment (OLGIM) staging systems, used for cancer risk stratification [30, 31]. The OLGIM system, which stages the severity of intestinal metaplasia in the corpus and antrum, has been reported to show better interobserver agreement

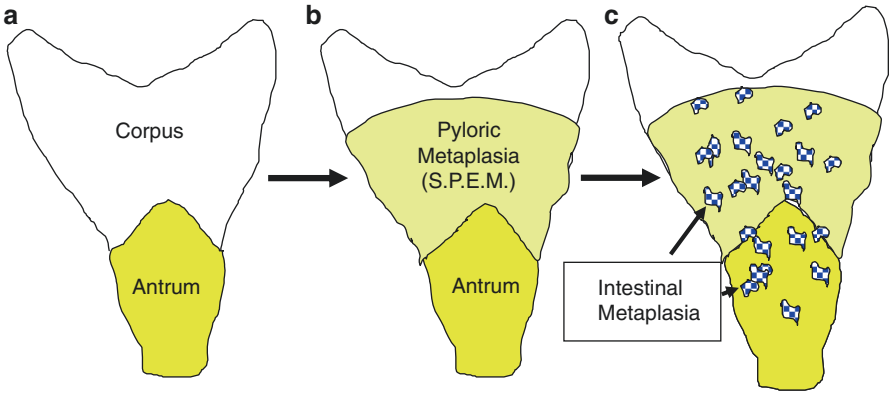

Fig. 2.2 Antral mucosa (yellow) (**a**) or pseudopyloric metaplasia (light yellow) (**b**). Atrophy advances as proximally into the corpus as a lawn of pseudopyloric metaplasia that can also be recognized by immunohistochemical staining for spasmolytic polypeptide (SPEM). Islands of intestinal metaplasia (blue hatch) subsequently develop within the lawn and over time may expand (**c**)

compared to the OLGA [30]. Scores of 3 or 4 are associated with a markedly increased risk of gastric cancer [31]. Higher OLGA staging has also been shown to correlate with the results of PGI/PGII ratio testing. Intestinal metaplasia is an easily recognizable manifestation of atrophy and, in the right circumstance, identifies the presence of severe atrophy that may warrant surveillance in high-risk populations [28]. Conversely, focal gastric intestinal metaplasia without background atrophy can also develop after mucosal injury and is not associated with heightened gastric cancer risk [29].

2.4 Atrophic Gastritis, Intestinal Metaplasia, and Gastric Cancer Risk

Worldwide, gastric cancer risk reflects the prevalence and age of onset of atrophic gastritis [17]. For example, in a Swedish cohort, the annual incidence of gastric cancer was 20/100,000 person-years in those with non-atrophic mucosa, 100/100,000 person-years in atrophic gastritis, and highest in those with intestinal metaplasia (129/100,000 person-years) and intramucosal neoplasia (263/100,000 person-years) [32]. In a US cohort of 4146 patients in northern California with atrophic gastritis, as evidenced by the presence of intestinal metaplasia, the incidence of gastric carcinoma was 72/100,000 person-years, which was 2.6 times the risk seen in the general population [33]. The incidence of gastric carcinoma rose to 767/100,000 person-years in those with low-grade intramucosal neoplasia. In a similar study in southern California, the age-adjusted incidence of gastric carcinoma arising in a cohort with atrophic gastritis, evidence by the presence of intestinal metaplasia, was 172/100,000

person-years compared to 9.67/100,000 person-years in the reference population [34]. The risk of carcinoma with gastric atrophy is especially heightened in non-Whites. In the Northern California study, Hispanics independently predicted the development of gastric carcinoma in the context of atrophic gastritis after adjusting for age and *H. pylori* status [33].

2.5 Worldwide Epidemiology of Gastric Cancer

In 1975, gastric cancer was the most common cause of cancer worldwide [35]. Since then, gastric cancer incidence has declined. Now, gastric cancer is the fifth most common cancer and third leading cause of cancer-related mortality worldwide [as reported by the World Health Organization (WHO) International Agency for Research on Cancer (IARC) 2012 data] [35]. The incidence appears to be decreasing due to improved diet and food storage (less use of salt and more fresh fruits, vegetables, and vitamin C) [36, 37], decreased smoking [37], improved sanitation, and reduced transmission of *H. pylori* infection [38].

As early as 1915, it was clear that the risk of gastric cancer was not uniform and was reduced among populations with predominantly vegetarian diets [1]. For example, among 1000 autopsies performed in India, there was a single case of gastric cancer, and among 396 cases of carcinoma seen at the Mayo Hospital in Lahore, Pakistan between 1882 and 1903, there was no case of gastric cancer [1].

Figures 2.3, 2.4, 2.5, 2.6, and 2.7 illustrate the cumulative lifetime risk of gastric cancer, the risk to age 74, in males from 2012 by country. The cancer incidence shown was reported from the WHO IARC GLOBOCAN project, which is the highest quality data on cancer incidence for 184 countries [35]. The 2012 GLOBOCAN estimates of cancer incidence were reported based on *Cancer Incidence in Five Continents* volumes IX (1998–2002) and X (2003–2007) [39, 40]. Additionally, Fig. 2.7 shows the reported gastric cancer incidence for subpopulations at highest risk based on *Cancer Incidence in Five Continents* volumes VIII (1993–1997) and X (2003–2007) [40, 41].

For all countries with data available in 2012 and 1997, gastric cancer risk has decreased over time with the exception of Uganda, Algeria, Cuba, China, Vietnam, India, and Denmark. The highest cumulative lifetime risk of gastric cancer is in La Reunion, Mauritius, and Kenya in Africa (Fig. 2.3); Chile, Guatemala, and Costa Rica in Central/South America (Fig. 2.4); South Korea, Mongolia, and Japan in Asia (Fig. 2.5); and Belarus, Russia, and Albania in Europe (Fig. 2.6). The lifetime risk to age 74 remains less than 1% in most African countries and between 0.5 and 3% in most European and American countries. Some subpopulations with highest cumulative risk of gastric cancer are shown in Fig. 2.7. The highest rates of gastric cancer are in specific counties of China, Japan, and South Korea, with the highest cumulative risk (22%) in Yangcheng County, China.

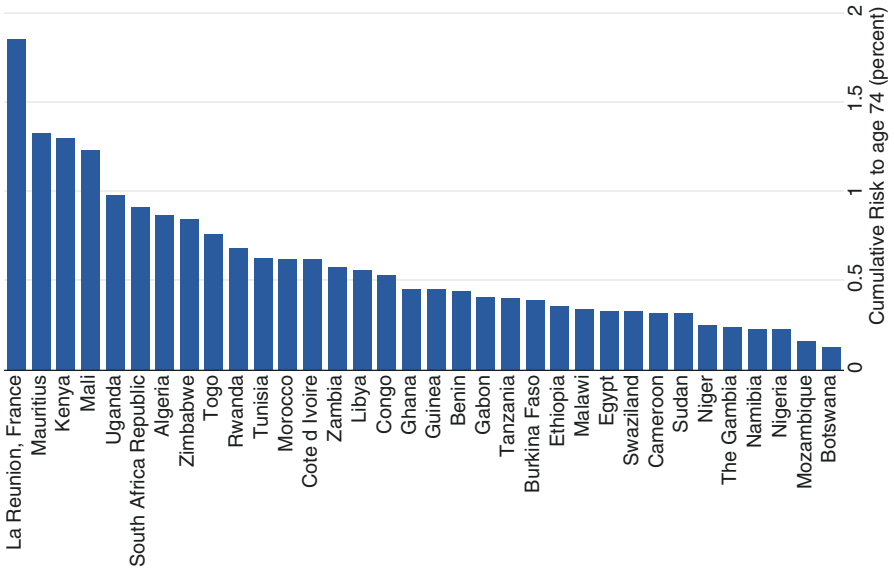

Fig. 2.3 Cumulative lifetime risk of gastric cancer up to age 74 in males by country in Africa for 2012 calculated from [35]

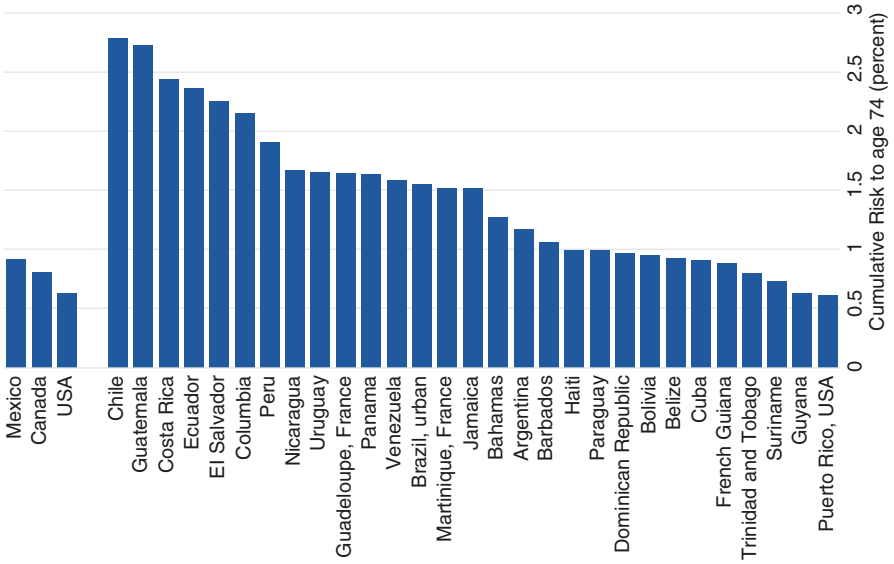

Fig. 2.4 Cumulative lifetime risk of gastric cancer up to age 74 in males by country in North and Central/South Americas for 2012 calculated from [35]

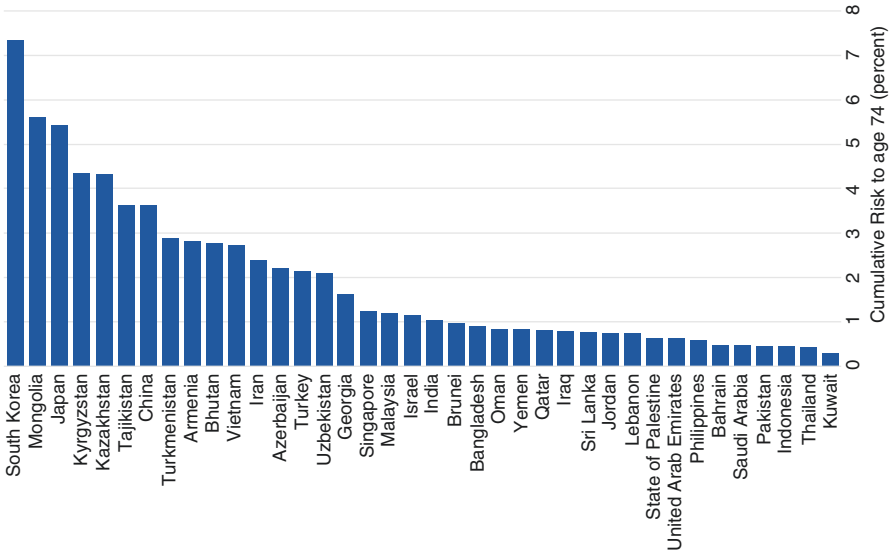

Fig. 2.5 Cumulative lifetime risk of gastric cancer up to age 74 in males by country in Asia for 2012 calculated from [35]

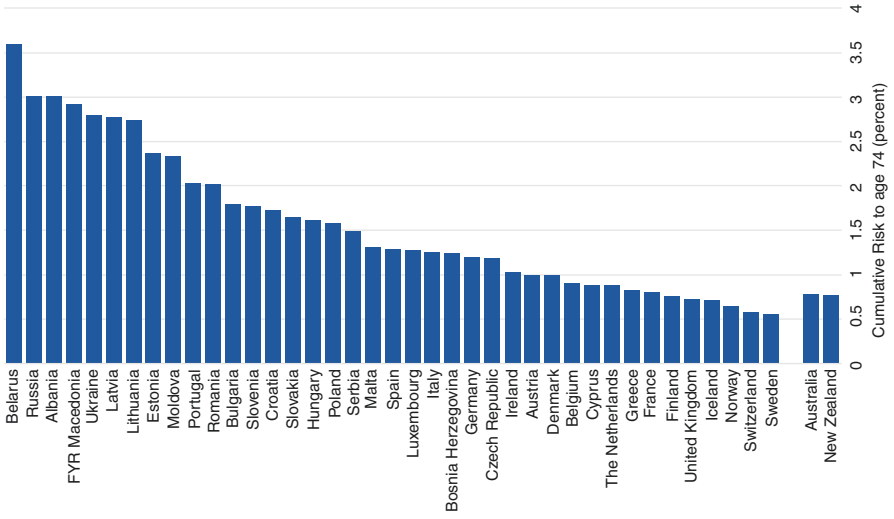

Fig. 2.6 Cumulative lifetime risk of gastric cancer up to age 74 in males by country in Europe and Oceania for 2012 calculated from [35]

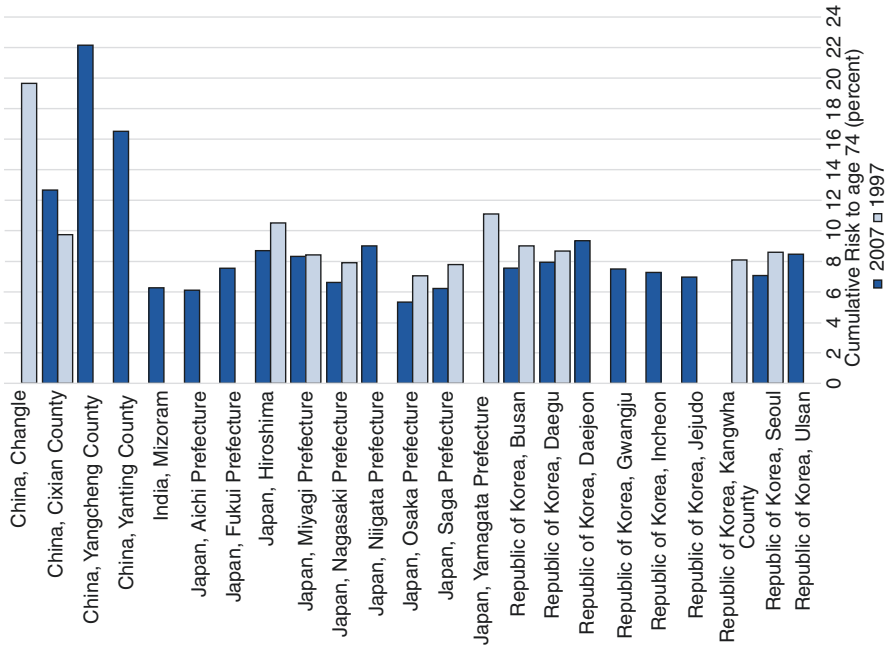

Fig. 2.7 Cumulative lifetime risk of gastric cancer up to age 74 in Asian subpopulations at highest risk for gastric cancer for 2007 (dark bar) and 1997 (light bar) calculated from [40, 41]

2.6 Quality in Reporting of Cancer Incidence and Mortality

Gastric cancer epidemiologic data quality has varied over time and still varies across countries. Early on, in the nineteenth and much of the twentieth century, gastric cancer reporting and data collection were not standardized. During that era, gastric cancer statistics relied on autopsy-derived collectible statistics. The reporting was likely biased based on who underwent autopsies (which varied by socioeconomic or other factors), which autopsies were recorded (e.g., population deaths vs. hospital-specific deaths), and which autopsies may not have reflected disease patterns on a population level. Additionally, cited cause of death in the early literature may have inaccurately classified cancer-related death (e.g., cause of death recorded as cachexia rather than gastric cancer).

When interpreting gastric cancer incidence, the age structure of the background population must be accounted for. Since the majority of gastric cancers occur after age 60, populations with shorter life expectancies are expected to have a lower

gastric cancer incidence. This phenomenon may partly explain the low incidence observed in African countries. Also, failure to age-adjust in a population with a shorter life expectancy can markedly underestimate the gross incidence of disease, predominantly in the elderly.

The World Health Organization (WHO)-reported cancer incidence is age-standardized to allow for comparisons between groups and time intervals. However, the quality of cancer incidence data varies greatly between countries. Some countries provide high-quality data from >50% of the population (e.g., USA), whereas other countries have high-quality data from <10% of the population (e.g., China). Some developing countries are classified as having only lower-quality regional data available (e.g., Kenya). Therefore, since standardization in reporting of cancer varies widely across countries, variability in estimation of cancer incidence may exist.

2.7 Conclusions

Variability in gastric cancer statistical reporting has posed some limitations in interpreting temporal trends in gastric cancer patterns. Over the past three decades, the WHO IARC has provided high-quality gastric cancer data, but there continues to be variability in the quality of data reported by each country. Gastric cancer was the leading cause of cancer-related death until 1975. Since then, gastric cancer incidence has been declining worldwide, likely due to improved diet and food storage, decreased smoking, and decreased *H. pylori* transmission. Currently, South Korea, Mongolia, and Japan have the highest lifetime risk of gastric cancer. Eradication of *H. pylori* will eventually make gastric cancer a rare disease [42].

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Part II
Pathogenesis

Chapter 3
Helicobacter pylori and Related Virulence Factors for Gastrointestinal Diseases

Evariste Tshibangu Kabamba and Yoshio Yamaoka

Abstract *Helicobacter pylori*—a worldwide spread bacterium—is still infecting more than half of humans. This bacterium is closely associated with serious human diseases such as gastric cancer. As only few infected humans develop the most severe clinical outcomes, there have been important efforts for identifying and understanding factors predicting bacterial virulence. Here, we discuss main features of virulence factors that have emerged from decades of intensive researches in the world. From tens of candidate virulent factors found out by epidemiological studies and explored by laboratory experiments, the *cag* pathogenicity island, the VacA, and several outer membrane proteins such as BabA are so far the most studied. Many other candidate virulent factors such as the serine protease HtrA and *H. pylori*-related prophages have been identified recently and would be attracting an increasing interest. Topics regarding the virulence of *H. pylori* species have accounted for the most dynamic among related researches, especially as access to genome sequences is increasing. Therefore, we will attempt to highlight the most recent findings in direct line with each discussed *H. pylori* virulence factor.

Keywords *Helicobacter pylori* · Virulence factors · *cag* pathogenicity island · VacA · Outer membrane proteins · HtrA · Prophages

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3.1 Prologue

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium infecting the mucosa of the human stomach. Actually, this bacterium was initially identified as associated with chronic gastritis at the time of its first description in the 1980s [1]. Later it was causally linked to more serious gastric pathologies including gastric cancer, the most common digestive cancer and the second cause of death and expenditure for cancer worldwide [2]. The role of *H. pylori* in gastric carcinogenesis was established when large epidemiological studies such that of Uemura N et al. reported a higher incidence of gastric cancer in *H. pylori*-infected individuals, which confirmed previously published reports [3–5]. Since 1994 the bacterium has been classified as a class I carcinogen by WHO, and the cure of *H. pylori* has emerged as an effective strategy for preventing gastric cancer [6]. However it has been widely shown that the prevalence of *H. pylori* did not match the distribution of gastric cancer in the world and that only less than 1% of overall infected patients were likely to develop gastric cancer [2]. To clarify these observations, a huge number of studies have been conducted to identify bacterial components determining disease outcomes among infected patients. Such bacterial components have been referred to as virulence factors. Since the 1990s, virulence factors have constituted an intensive topic of research in *H. pylori*-related field with the aim to clarify the pathogenesis of the infection, to explain, or to predict the risk for the development of severe gastroduodenal diseases including gastric cancer and peptic ulcer. Thus currently the pathogenesis of the *H. pylori* infection is thought to be driven by several virulence factors facilitating the colonization and inducing the inflammation as well as the host cell damage. Therefore, studies on *H. pylori* virulence factors promise to better understand the distribution of the gastric cancer risk and to impact the allocation of effective health-care efforts against the disease burden. Several candidate virulence factors have been found in *H. pylori.* This chapter is a brief sketch of the most important *H. pylori* virulence factors. We often attempt to summarize so far the biological activity and the epidemiological role of each virulence factors.

3.2 The CagA and *cag* Pathogenicity Island (PAI)

The *cag* pathogenicity island (PAI) is a ~40-kb genomic insertion that likely was acquired horizontally and integrated into the chromosomal glutamate racemase gene of a subset of *H. pylori* strains. It encodes approximatively 30 genes that are split in some strains into a right segment (*cag*I) and a left segment (*cag*II) by a novel insertion sequence (IS*605*) or, in a minority of strains, by an intervening chromosomal sequence [7]. Genes located within the *cag* PAI are found to encode collectively for a rigid syringe-like apparatus, termed the *cag* type IV secretion system (*cag*-T4SS), including a cytotoxin-associated gene A protein effector (CagA) (Fig. 3.1a). The gene encoding for the CagA (*cagA*) is localized on the terminal end

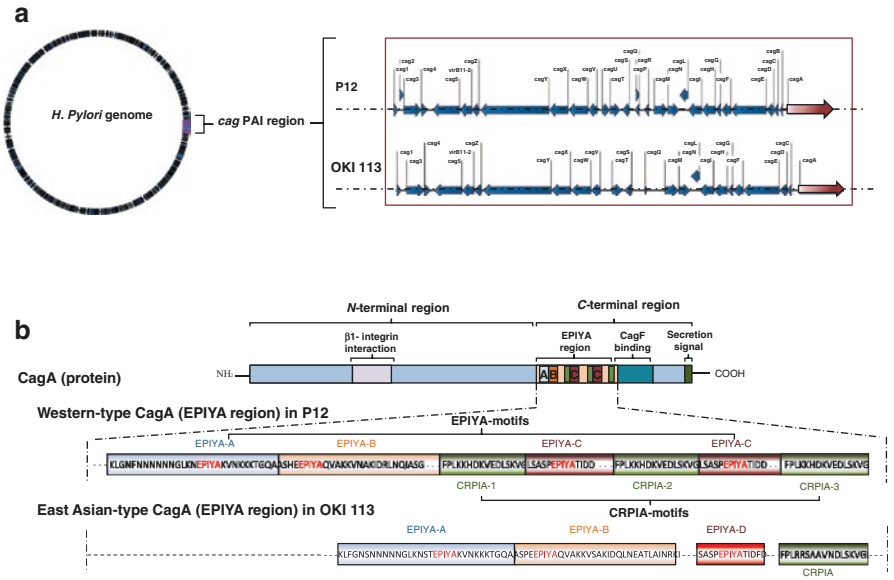

Fig. 3.1 Overall structures of the *cag* PAI (**a**) and the CagA protein (**b**) in *H. pylori* strains P12 and OKI 113 (**a**) The structure of the *cag* PAI region (~37 kb). The region comprises 28 genes that encodes for the *cag*-T4SS including the CagA protein effector. (**b**) The structure of the CagA protein (~1214 amino acid residues). The N-terminal part of CagA harbors a putative β-integrin binding region. The C-terminal region comprises the EPIYA region, the region binding to the secretion chaperone CagF, and the C-terminal secretion signal. In *H. pylori* strains from Western countries, the EPIYA region may contain EPIYA ABCC motifs and three MKI/CM/CRPIA motifs as for *H. pylori* strain P12 (NC_011498.1). However, typical strains from East Asian countries have EPIYA ABD motifs and one MKI/CM/CRPIA motif as for the *H. pylori* strain OKI 113 (NC_020508.1)

of the *cag* PAI and is considered as a molecular marker for the presence of the *cag* PAI region in *H. pylori* strains. The CagA is an immunogenic 120–140 kDa cellular effector that is demonstrated to be translocated into host cells through the *cag*-T4SS. Upon delivery into host cells, the CagA interacts with a large repertoire of cellular signaling pathways including those leading to carcinogenesis [8]. Elucidating the biological function and the epidemiologic role of the CagA-*cag* PAI tandem has raised concerns among researchers until to become, since its first description in the 1990s, the most studied virulent factor of *H. pylori*.

Actually, an important step was reached when a major role of the CagA protein (and the *cag* PAI) in the development of gastric cancer emerged from epidemiological studies in 1990s especially in Western countries [9–11]. Thus *H. pylori* strains producing the CagA protein (assumed as *cagA*-positive strains) were demonstrated to be more pathogenic than those not producing the protein (assumed as *cagA*-negative) [12]. Later, this allowed mapping the gastric cancer risk in the world [2]. However, in East Asia where the incidence had always been among the highest in the world, most of *H. pylori* strains had a *cagA* gene irrespective of the disease [13]. Attempts to explain more accurate trends in geographical distribution of the

incidence of gastric cancer brought out the first description of the sequence variations within the 3′ region of the CagA [14, 15]. Actually from its first description, the CagA size was known to vary in different strains by a mechanism involving duplication of regions within the protein's gene [16]. We found that these variations were due to repeats of Glu-Pro-Ile-Tyr-Ala motifs (termed EPIYA motifs) and their flanking sequences differentiating Western and East Asian strains and established a molecular tool for illustrating the gastric cancer risk distribution worldwide [2, 15]. Therefore four distinct EPIYA segments—EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D—each of which contains a single EPIYA motif, have been identified in the EPIYA-repeat region of CagA (Fig. 3.1b) [2]. The EPIYA-repeat region of CagA from Western *H. pylori* isolates is in an arrangement of EPIYA-A, EPIYA-B, and EPIYA-C segments (ABC-type CagA, traditionally called Western CagA). CagA from East Asian *H. pylori* isolates comprises also EPIYA-A and EPIYA-B segments but an EPIYA-D segment instead of the repeatable EPIYA-C. Thus the EPIYA-repeat region of East Asian CagA is in an arrangement of EPIYA-A, EPIYA-B, and EPIYA-D segments (ABD-type CagA, traditionally called East Asian CagA) [15, 17]. Then the roles of biological function of EPIYA segments were elucidated by Hagashi et al. [18]. Actually by using a series of EPIYA mutants of CagA, they revealed that SHP-2 specifically binds to the tyrosine-phosphorylated EPIYA-C or EPIYA-D segment. This starting point has led to the current model of the biological activity of CagA upon delivery into host cells. This model shows highly complex signaling pathways altered by translocated CagA throughout multiple receptor kinases (c-Met and EGFR) and non-receptor kinases (Src, Abl, Csk, aPKC, Par1, PI3K, Akt, FAK, GSK-3, JAK, PAK1, PAK2, and MAP kinases) in the human gastric epithelium, manipulating processes ranging from cell adhesion and polarity to apoptosis, inflammation, or cell cycle progression [8]. Fundamentally the sequence fanking the tyrosine phosphorylation site of EPIYA-D segment perfectly matches the consensus high-affinity binding sequence for the SH2 domains of SHP-2, whereas that fanking the tyrosine phosphorylation site of the EPIYA-C segment differs from the consensus sequence by a single amino acid at the pY + 5 position. As a result, East Asian CagA, which contains the EPIYA-D segment, exhibits stronger SHP-2 binding than does Western CagA, which contains the EPIYA-C segment [18, 19]. Within some Western strains, the EPIYA-C segment is variably multiplied in tandem of mostly two or three repeats associated with different levels of the disease risk [17]. Those having a greater number of EPIYA-C segments could exhibit stronger activity to interact with SHP-2 and are more closely associated with precancerous lesions and GC [17, 19, 20]. Thus, based on the structure of the EPIYA-C tandem, Nagase et al. have devised Western CagA into type I Western CagA constituted by a single EPIYA-C segment (~70% of Western strains) and type II Western CagA containing multiple EPIYA-C segments [21]. Further analyses of the CagA repeat regions came to identify a distinct J-Western-type CagA subtype specifically among strains from East Asia harboring a Western-type CagA [22]. Overall epidemiological studies have suggested a decreasing gastric cancer risk from ABD, ABCCC, ABCC, and ABC-type CagA [2].

On the other hand, the full structure and the molecular mechanisms regulating the *cagA* function within the *cag* PAI region have been intensively studied. Actually the *cagA* molecule which harbors a unique structure with no sequence homology to any known proteins in databases has been further characterized recently with the disclosure of the crystal structure of its N-terminal segments [23]. Thus the N-terminal structured part of CagA consists of several domains and harbors the putative integrin-binding region [23, 24]. The unstructured C-terminal region displays the well-known repeated sections containing the so-called EPIYA and CM (CagA multimerization) or CRPIA (conserved repeat responsible for phosphorylation-independent activity) motifs, as well as a region binding to the secretion chaperone CagF and the C-terminal secretion signal (Fig. 3.1b) [25]. Moreover, the *cagA* promoter region which had been described [26] was further characterized by Ferreira et al. recently [27]. Thus functional sequence motifs located in the promoter region (the +59 AATAAGATA and the −10 TATAATGA sequence motifs) have been described and linked to CagA expression levels, and interleukin-8 (IL-8) secretion by infected gastric cell line, as well as to severe clinical outcomes [27]. Since these sequence variations have discriminated different levels of gastric cancer risk between Colombian strains from European and African origins, the discussion should be extended in future studies to strains from other geographical origins. Another important *cagA*-related feature brought out by recent data is the number of copies found within strains. Jang et al. have showed that *H. pylori* isolates can carry multiple tandem copies of *cagA* that enounce CagA expression and activity and may impact on the development of gastric disease [28]. Consistently with Jang et al., Draper et al. have just showed, while using close strains named PMSS1 and SS1, that the number of *cagA* changes dynamically and modulates CagA activity [29]. Thus future epidemiological studies should address not only the sequence variation within CagA (EPIYA and CM/CRPIA motifs) but also the functionality of the whole *cag* PAI/T4SS as a requirement for the CagA biological effects, the *cagA* promoter variations, and the number of *cagA* copies as a useful marker for predicting disease risk. In the same perspective, a β-lactamase-dependent reporter system allowing precise and quantitative determination of translocation of CagA into host cells has fortunately been just developed [25]. This phosphorylation-independent assay has opened the door to further insight in the future understanding of the in vivo function or the epidemiological role played by the *H. pylori cag*-T4SS and the amount of translocated CagA.

3.3 The Vacuolating Cytotoxin (VacA)

The other most extensively studied virulence factor is the vacuolating cytotoxin A (VacA), an exotoxin that was originally named due to its capacity to induce host cell vacuolation. At the time of its discovery, a bacterial toxin with similar activity had not yet ever been described; thus, since then many studies have been conducted to clarify its function and structure [30]. Actually it is established that the

VacA structure includes a 33 kDa N-terminal domain linked to cytotoxicity and a 55 kDa C-terminal domain involved in the binding to cell surface receptors [31]. Though almost all *H. pylori* strains harbored a *vacA* gene, the allelic polymorphism found within the protein molecule shows clinical significance and toxic activity when displaying differently the combination of its three regions: the signal peptide (s1 and s2 variants), the intermediate (i1, i2, and i3 variants), and the middle regions (m1 and m2 variants). Two novel polymorphic sites, the deletion (d1 and d2 variants) and the c-regions (c1 and c2 variants), located in the 3′-end region of VacA have been reported recently (Fig. 3.2) [32]. Similarly to sites described previously, some variants of these two novel regions have been associated to high risk of gastric cancer [33, 34]. However, the biological function of the regions in different VacA functions has still not been identified yet. Globally, the VacA has been described as a multi-receptor protein that has pleiotropic effects, including membrane depolarization, mitochondrial dysfunction, autophagy, activation of mitogen-activated protein kinases, inhibition of T cell function, and the induction of apoptosis [35]. These functions contribute to a persistent colonization of *H. pylori* and to the pathogenesis of several upper digestive tract diseases. Recently, further descriptions of the VacA-related pathways and functions have been reported. Amilon et al. have described a putative stem-loop structure in the 5′ untranslated region that influences the transcription of *vacA* and leads to higher expression and toxic activity of the VacA [36]. An extra-digestive location of functional VacA in lung has led to suggest a role of VacA in the pathogenesis of respiratory diseases through IL-8 and IL-6 induction [37]. Then, new host factors interacting or regulating the VacA-induced apoptosis have been reported. Yahiro et al. have described a new signal pathway of VacA-induced apoptosis through cytoplasmic accumulation of connexin 43 (Cx43), a ubiquitous connexin family member taking of gap junction and cell-cell channel formation [31]. In addition, Chang et al. have just showed a role of the cortactin, an actin-binding protein, in the regulation of the apoptosis induced by VacA [38].

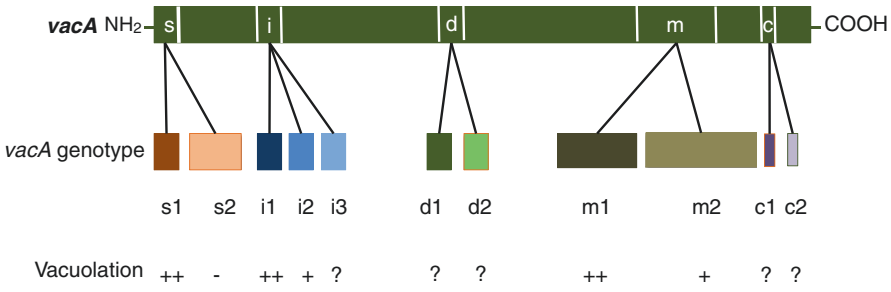

Fig. 3.2 Current allelic diversity described in the VacA sequence. The VacA structure comprises five regions of sequence diversity referred as signal (s), intermediate (i), middle (m), deletion (d), and c-regions (c). The vacuolating activity of the VacA varies based on different alleles. In vitro *vacA* s1/m1/i1 alleles give higher vacuole formation than s2/m2/i2, respectively. The function of novel polymorphic regions, c and d regions, as well as i3 subtype has not been studied yet

3.4 Outer Membrane Proteins (OMPs)

H. pylori harbors a large catalogue of ~64 predicted OMPs with a potential role in the bacterial adherence to the gastric mucosa and which is divided into five paralogous gene families (Fig. 3.3) [39].

The most studied OMPs are proteins belonging to the family of *Helicobacter outer membrane proteins (*Hop) such as Hop S (currently BabA), Hop P (or SabA), Hop H (or OipA), Hop C/B (or AlpA/B), Hop Z, and Hop Q. On a global view, the OMP profile of *H. pylori* strains differs significantly from that of other Gram-negative species as it is extraordinarily abundant (encoded by ~4% of the bacterial genome) and no major OMPs predominate; rather multiple lower-abundance OMPs are observed [39]. Taken individually, OMP genes (e.g., such that of SabA) are among the most structurally divergent genes existing in the *H. pylori* genome [39]. Gradually evidences of the important molecular role of OMPs in the bacterial evolution and pathogenicity have been accumulated over the years. Some of the OMPs are now well-established as adhesins with known host receptors (e.g., BabA/B, SabA, AlpA/B, and HopQ) (Table 3.1).

Evolutionary studies show that *H. pylori* takes considerable advantage of the extreme allelic diversity, the genetic variability, and the functional plasticity of these OMPs to evolve and persist in host and finally to expand within human species [40–42]. Beside the adherence function, new roles of OMPs in the bacterial virulence

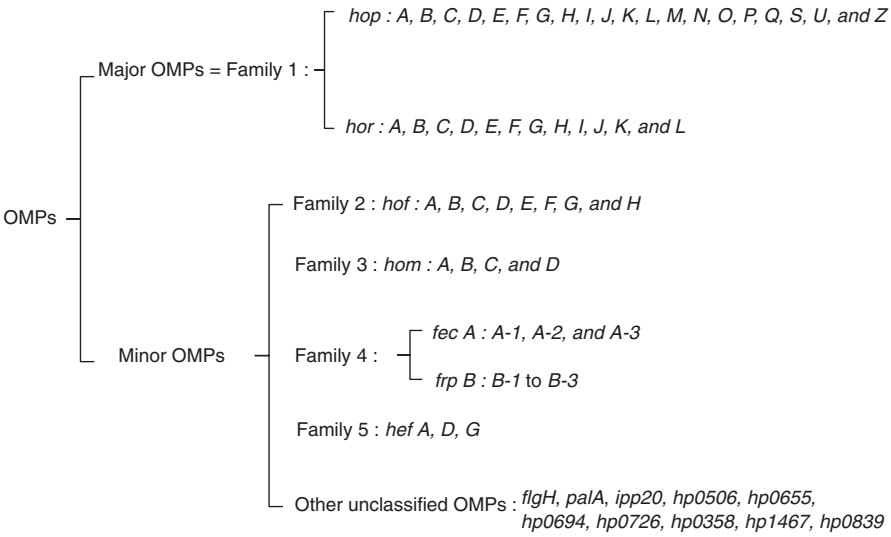

Fig. 3.3 Classification of *Helicobacter pylori* OMPs molecules according to Alm et al. [39]. A huge set of at least 64 OMPs have been predicted in *Helicobacter pylori* species which are divided into five paralogous gene families. This figure summarizes the main OMPs already reported but do not present several putative OMPs. These proteins are likely adhesins or porins and might interact with the host or the extracellular environment

Table 3.1 *Helicobacter pylori* outer membrane proteins with correspondent host receptors as far as it is known

OMPs	Receptors
BabA (HopS)	Mucin MUC5B Agglutinin glycoprotein-340 (gp-340) Proline-rich glycoprotein containing Fucα1-2Galβ motif Secretory immunoglobulin A containing fucose-oligosaccharide motifs Salivary agglutinin DMBT1 Lewis b blood group antigen (Leb) and terminal fucose, H1-antigen, A-antigen, and B-antigen Mucin MUC5AC with N-acetylgalactosamine-β-1,4-*N*-acetylglucosamine Mucin MUC1 Mucin MUC2
SabA (HopP)	Sialyl Lewis X, sialyl Lewis A, Lewis X
HopQ	Carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 1, 3, 5, 6
AlpA/B (HopC/B)	Collagen IV, laminin

have been emphasized by studies analyzing their interaction with biological function of the *cag*-T4SS or their direct effect over host cells [43–48]. Actually from epidemiological data, highly virulent *H. pylori* strains are found to express these OMPs along with proteins from the *cag* PAI. This is consistent with observations showing that high pathogenic strains, especially that encoding the *cag* PAI, are also high-adherent strains harboring numerous OMPs with even higher abilities to even enhance the expression of OMPs' ligands on gastric epithelial cells [49]. Thus adhesins likely ascertain intimate bacterial contact to gastric epithelial cells, while the *cag*-T4SS which forms an extracellular pilus-like structure allows the translocation of the effector protein CagA to induce pathogenic pathways leading to severe gastroduodenal diseases such as gastric ulcers and gastric cancer [48].

The *blood group antigen-binding adhesion* (BabA) is the most studied *H. pylori* OMP. From its initial identification, BabA was established as an OMP that mediates the bacterial adherence to ABO/Lewis b (Le^b) blood group antigens in the gastric pit region of the human stomach mucosa [50, 51]. Later several molecules have been identified as BabA receptors found in the oral cavity and in the stomach (Table 3.1) [50]. The *babA* gene has two paralogous genes, *babB* and *babC*, with which it can be located variably onto three different chromosomal loci defined as A, B, and C [52]. There might even be a yet not identified chromosomal loci for the *babA* gene [50, 53]. The clinical relevance of the *babA* gene is well established as the gene had been associated with an increased risk of gastric cancer and peptic ulcer. However, very few is still known about the function of *bab*C and *babB*, as well as the effect of different loci [54]. Evidences for a virulent role of BabA rely also on epidemiological data associating the *babA* gene to other virulence genes as well as on the fact that BabA-mediated adherence of *H. pylori* would be a potentiator of *cag*-T4SS activity and induction of proinflammatory cytokines (like *CCL5* and *IL-8)* and precancer-related factors (like *CDX2* and *MUC2*) [50, 55]. Recently, additive evidences were provided by an epidemiological study that showed a significant association between

the combination of OipA, BabA, and SabA and the diagnosis of *H. pylori*-associated gastric cancer [56]. However, overall accumulated efforts from many years have been emphasized by recent investigations demonstrating that the *H. pylori* BabA sequence, expression, and corresponding binding phenotypes are highly diverse and dynamic [57–62]. Moreover, it had been demonstrated that the *bab* genes have some dinucleotide (CT) repeats in 5′-region leading to phase variation phenomenon with resulted proteins frameshifted by premature stop codons [63]. Along with its paralogous genes, the *babA* gene is also able to undergo the formation of chimera proteins with altered protein expression [50]. Thus, cautions should be made while interpreting results from epidemiology because all the above phenomena likely impact the association between the gene and clinical outcomes. Then, Sweeney and Guillemin have rightly suggested extending the discussion in such studies, to *babA* sequence and expression variation, host glycans, and disease incidence in populations of different hosts and *H. pylori* ancestry [59].

The *Helicobacter outer membrane protein Q* (HopQ) is an OMP that was first predicted as *HP1177* or *omp27* after prior complete sequencing of *H. pylori*, before being demonstrated as actually existing on the surface of the bacteria and influencing the adherence to human epithelial cells [64]. Since two alleles of the *hopQ* gene were described with epidemiological association between the *hopQ I* allele and the *cagA* gene [65, 66], this OMP is increasingly attracting the interest of researches. Thus, by screening a large scale of *H. pylori* mutants, Belogolova E. et al. identified HopQ as a non-*cag* PAI-encoded cofactor of T4SS function, essential for CagA translocation and for CagA-mediated host cell responses such as formation of the hummingbird phenotype and cell scattering. Their work also showed that deletion of *hopQ* reduces the T4SS-dependent activation of NF-κB, the induction of MAPK signaling, as well as the secretion of IL-8 in the host cells [67]. Moreover, Jiménez-Soto LF et al. identified the HopQ along with other *H. pylori* OMPs, such as HopI or AlpAB, as factors restricting and controlling subsequent CagA translocation into host cell independently to the β1 integrin receptor availability [68]. Efforts for clarifying molecular mechanisms of OMP interactions with CagA translocation have led concomitantly to demonstrate that HopQ was implicated into a *cag* PAI-related virulence-enounced interaction with human receptors from the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family [47, 48].

The *H. pylori outer inflammatory protein A* (OipA, also called HopH) is an OMP encoded by the HP0638 gene into the genome of the strain named 26695. We originally described the gene and its function in relation with the IL induction and clinical relevance [69–71]. Like the *babA* gene, the *oipA* has been recognized as a hyper-mutable gene with some dinucleotide CT sequence repeats (DSRs) located in its 5′ coding region. Thus the gene may undergo phase variation which may modify its reading frame. These events lead to phenotypic variations corresponding to the presence or absence of the encoded protein assimilated to switch “ON” or “OFF” status [71, 72]. We suggested that the resulting phenotypic variations could account in the association between the gene genotype and clinical outcomes [71]. However, the *oipA* had been shown with controversial features over its adhesion function and its ability to induce a pro-inflammatory response when using a gastric epithelial cell

line or animal models [73–76]. Nevertheless, based on epidemiological studies, a functional OipA was established to correlate with highly virulent strains expressing the *cag PAI* and the *vacA*-s1/m-1 type [73, 77]. Even the OipA-related receptor is still yet definitively identified; OipA-related host cell signaling has been reported. Thus this OMP was suggested to trigger pathways related to inflammation induction, actin remodeling, and cell apoptosis throughout the epidermal growth factor receptor (EGFR)/focal adhesion kinase (FAK), the phosphoinositide-3 kinase (PI3K)-dependent Akt activation, as well as the forkhead transcription factors of class O (FoxO) [78, 79]. Recently, new arguments for the virulence role of OipA have been gained by treating gastric cell lines with various concentrations of OipA molecules, as well as *oipA* "off," "on," and knockout strains [80, 81]. While concluding on the binding property of OipA, this group shows also toxic effect as well as apoptosis-triggered cascade via signaling pathways enouncing the Bax/Bcl-2 protein ratio and the cleaved caspase-3 level followed by a mitochondrial apoptotic cascade [80]. Above all, OipA has been also proposed as a suitable oral vaccine candidate against *H. pylori* infection [82]. We do believe that research on the OipA protein has great potential for understanding the pathogenesis and for promoting effective strategies against *H. pylori.*

3.5 Duodenal Ulcer-Promoting Gene (*dupA*)

The *H. pylori* duodenal ulcer-promoting gene (*dupA*) encompasses two continuous sequences, *jhp0917* and *jhp0918*, located in the plasticity region, a pathogenicity island of the bacterial genome [83–85] as described initially in the strain named J99 [86]. The *jhp0917* gene encodes a protein of 475 amino acids but lacks a region homologous to the C-terminus of *virB4*, whereas *jhp0918* gene encodes a product of 140 amino acids that is homologous to the missing *virB4* region [83–85]. The epidemiological role of the *dupA* gene has been debated among researchers. Actually the *dupA* gene (*jhp0917–jhp0918*) was originally reported as a marker for the development of duodenal ulcer disease; but its possible association with the development of gastric cancer was not unanimously reported by epidemiological studies [83, 85, 87–91]. Similarly in a systematic review, Shiota et al. analyzed more than 2466 patients and confirmed the importance of *dupA* gene for DU, especially in Asian countries, whereas no link was found with gastric ulcer and GC [92]. However it has been suggested that some important host factors likely affecting the function of the gene would explain these controversies [90, 93]. In addition, the sequence polymorphism of the *dupA* gene observed later appears currently as important to be considered for describing any link to disease outcomes [94]. In fact *dupA* genes had been clustered into short and long types regarding the presence or not of an extra 600 bp in the sequence likely predicting better the virulence of strains [95]. Moreover, several frameshift mutations have been found in the *dupA* gene sequence and can likely alter its produced protein function and structure, as well as its association with clinical outcomes [96–98]. The biological function of the *dupA* gene is still not fully

clarified and includes an eventual interaction with other *vir* homologues in the plasticity region to form a type IV secretion system similar to that of the *cag* PAI [84]. The gene has been also controversially associated to IL-8 production by gastric epithelial cells, in DNA or protein uptake/transfer, and bacterial survival to low pH [83, 98, 99]. Overall, currently sufficient evidence can associate the *dupA* gene to an increased risk for duodenal ulceration rather than gastric cancer [94].

3.6 Induced by Contact with Epithelium (*iceA*) Gene

The induced by contact with epithelium (*iceA*) gene was identified with two allelic families, *iceA1* and *iceA2* [100]. Its allele *iceA1* demonstrated an upregulation induced by the contact of *H. pylori* with gastric epithelial cells and exhibited sequence homology with *nlaIIIR*, a gene encoding a CTAG-specific restriction endonuclease in *Neisseria lactamica* [100, 101]. In prior reports, the *iceA1* genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation [100, 101]. However, contemporary reports were controversial with the difficulty to reproduce the same observations in different populations [13, 102–105]. However, while conducting a meta-analysis including 50 studies with a total of 5357 patients, we had observed that the *iceA1* unlike the *iceA2* genotype was positively associated with peptic ulcer especially in Western countries [106]. Until recently controversial data about the *iceA1* clinical relevance were still released [107–111]. Further studies on the *iceA* gene are still needed to help in understanding the discrepancies between existing data.

3.7 *H. pylori* Prophage

Bacteriophages (phages) are viruses that infect often bacteria [112]. The phage-bacteria interplay includes an insertion step into the host genome that may lead to either bacterial lysis or prophage domestication. It gives rise to an evolutionary arms race between these viruses and their hosts as it is possible that they shape the host genome in terms of the diversity or even virulence evolution [113]. Shortly after the discovery of *H. pylori*, Marshall et al. described intracellular phage-like particles observed in human gastric mucosa [114]. Subsequent observations of phages in *Helicobacter* spp. had been relatively rare [115–119]. The putative pathogenic role of *H. pylori* prophage has been revived since the first isolation of an integrated prophage, similar to phages of the *Siphoviridae* family, from a gastric MALT lymphoma patient [120]. Then, it is gradually admitted that *H. pylori* strains harbor frequently prophage sequences [121–123]. Recently, Kyrillos A et al., by screening phage orthologous sequences among 335 *H. pylori* strains, found a correlation between the presence of a phage-related sequence likely acquired by horizontal gene transfer and that of the two major virulence factors, CagA and VacA

[124]. Another *H. pylori* prophage has been reported in the genome of a *cag* PAI-negative strain isolated from a patient suffering from gastric cancer [125]. Since non-pyloric *Helicobacter* prophages are revealed to encode for antibiotic resistance genes and virulence factors [126], we can predict that the genetic content and the putative pathogenic role of *H. pylori* prophage will attract particular attention from researchers in the near future.

3.8 The *H. pylori* HtrA

The *high-temperature requirement A* (*htrA*) gene encodes for a serine protease released in the extracellular environment by *H. pylori* during infection. Since the proteolytic activity of the extracellular *H. pylori* HtrA had been shown to cleave the cell adhesion protein, its possible cross talk with the CagA activities and its direct effect over the infection process have been intensively studied [127, 128]. Identification of the tumor suppressor E-cadherin as an HtrA substrate came to underline the significant role of HtrA activity in *H. pylori*-induced carcinogenesis and in disruption of adherens junctions allowing bacterial transmigration across the epithelium [127]. Recently Schimidt et al. have further elucidated the activity of the HtrA over the E-cadherin [129, 130]. In the meantime, Tegtmeyer et al. were showing the *htrA* gene locus as being conserved among 992 *H. pylori* clinical isolates and that the proteolytic activity of HtrA was essential for bacterial survival [131]. Finally Harrer et al., by overexpressing the HtrA when introducing successfully a second functional *htrA* gene in *H. pylori* P12 and 26695 strains, have just demonstrated that risen HtrA enounces cleavage of E-cadherin, bacterial transmigration, and delivery of the *cag*-T4SS effector protein CagA into polarized epithelial cells [132]. Taking into account all these findings, the HtrA protease has brought out a novel model explaining how *H. pylori* access to the basolateral compartment for deployment of the *cag*-T4SS and injection of the oncoprotein CagA in host cells [8, 132, 133]. Therefore, the *H. pylori* HtrA-triggered E-cadherin is without a doubt a bacterial virulent factor. Further studies would come to highlight the eventual epidemiological role of such an important virulent factor.

3.9 Epilogue

We have described the progress made in the *H. pylori* virulence-related field with fascinating promises under development in this area. The complexity of their biological activities and their possible interconnections calls for huge efforts in related research and cautions while interpreting their association to clinical outcomes. New virulent factors as well as important insights into well-known factors also are still to be brought to light. We do believe that the use of molecular, immunological, and whole-genome analysis approaches will improve our understanding and

identification of bacterial genetic factors that may influence *H. pylori* virulence and disease outcome and, ultimately, provide the basis for future therapeutic strategies in the near future.

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Chapter 4
Gastric Carcinogenesis

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Abstract Gastric cancer is a leading cause of cancer-related death, particularly in Asia. A number of risk factors associated with gastric carcinogenesis have been identified by epidemiological, clinical, and molecular studies. Several epigenetic and proteomic modulations are known to be primary drivers promoting carcinogenesis and the progression of gastric cancer. In recent years, the role of these modulations in gastric carcinogenesis has been widely studied. Early gastric cancer can be treated and even completely cured surgically using endoscopic submucosal dissection (ESD). However, the prognosis of advanced or distantly metastasized gastric cancer is poor. Highly advanced gastric cancer is difficult to completely cure using chemotherapy. Therefore, prevention or early detection of gastric cancer is crucial. Understanding how epigenetic or proteomic modulations affect gastric carcinogenesis has importance in detecting, treating, and preventing gastric cancer. Further study is expected to provide us the basis for targeted molecular therapy or novel biomarkers that evaluate the prognosis or risk of gastric carcinogenesis. In this chapter, recent results of studies on epigenetic and proteomic modulations related to gastric carcinogenesis and clinical outcomes are described, with a special focus on Japanese research.

Keywords *Helicobacter pylori* · Inflammation · Epstein-Barr virus (EBV) · Epigenetic modulation · miRNA

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4.1 Risk of Gastric Carcinogenesis

In Japan, early-stage gastric cancer tends to be detected through a nationwide screening program and new endoscopic technology [1]. However, gastric cancer is the fourth most common cancer and second leading cause of cancer death worldwide, and it remains the leading cancer in Japan [2]. *Helicobacter pylori* infection, smoking, and high salt intake are well-known risk factors for the development of gastric cancer [3–5]. Additionally, recent research involving animal studies has shown that alcohol has a carcinogenic effect in the stomach [6, 7]. Some prospective cohort studies have demonstrated a positive association between alcohol consumption and the risk of gastric cancer in Western populations [8, 9]. In Japanese populations, although many studies have evaluated the association between alcohol consumption and the risk of stomach cancer, many of these studies were retrospective or only investigated the frequency of drinking [10–13]. Recently, Tamura et al. showed that alcohol consumption is associated with an increased risk of stomach cancer among Japanese men by examining the association between quantitative alcohol intake and the risk of gastric cancer using data collected prospectively from a large Japanese population [14].

Matsuo et al. reported that the association between alcohol intake and the risk of gastric cancer is remarkable for patients with a variant allele of aldehyde dehydrogenase 2 (ALDH2) by a case-control study [15]. Interestingly, it is known that this variant allele is dominant in the Japanese population [16, 17]. However, cohort studies considering genetic background in relation to ALDH2 polymorphisms have not been examined. Further research is needed to elucidate the effect of alcohol consumption on the risk of gastric cancer.

4.2 Epigenetic Modulation in Gastric Carcinogenesis

Epigenetic changes have been shown to affect gastric carcinogenesis. Epigenetic modulation in gastric carcinogenesis is associated with epidemiological and clinicopathological factors [18]. Recently, the clinical significance and prognosis of epigenetic modulation in gastric carcinogenesis were evaluated. Recent research has examined the association between epigenetic modulation and gastric carcinogenesis, particularly with respect to histone modulation, DNA methylation, and microRNA (miRNA). Understanding how epigenetic modulations affect gastric carcinogenesis is important for detection, treatment, and prevention of gastric cancer.

Histone modulation, including methylation, acetylation, phosphorylation, and ubiquitination, affects oncoprotein expression. Recently, it was reported that expression of histone-lysine N-methyltransferase Suv39H1 and trimethylated histone H3 methylated lysine 9 (H3K9) is increased in gastric cancer, and trimethylated H3K9 is positively associated with tumor stage and metastatic status [19].

Transcriptional repression by methylation of CpG islands is an important mechanism in many types of cancers. Aberrant methylation of CpG islands has been detected in nonneoplastic tissues with chronic inflammation [20, 21]. In *H. pylori*-infected gastric mucosa, chronic inflammation and atrophy are strongly induced [22]. This would cause epigenetic changes characterized by promoter methylation of multiple genes [23, 24]. Eradication of *H. pylori* contributes to improvement of chronic inflammation or atrophy and prevents the development of gastric cancer [25–27]. Therefore, it is thought that *H. pylori* eradication could reverse methylation of the CpG islands of certain genes [28, 29]. On the other hand, even after successful eradication of *H. pylori*, there have been several cases where gastric cancer was identified [30, 31]. This evidence suggests that the residual methylation status in gastric mucosa might be associated with the development of gastric cancer after *H. pylori* eradication [29, 32]. To investigate this theory, Tahara et al. examined the promoter methylation status of nonneoplastic gastric mucosa after *H. pylori* eradication using a total of 140 gastric specimens from 99 participants, 6 months after eradication [33]. In this study, the methylation status of five candidate genes (*MYOD1*, *SLC16A12*, *IGF2*, *RORA*, and *PRDM5*) was examined [33]. Interestingly, the atrophic type specimens, with informative endoscopic features of intestinal metaplasia, showed high methylation status for all five genes compared with that of the restored type specimens [33]. This evidence indicates that the formation of intestinal metaplasia causes residual DNA methylation, and it is considered an irreversible epigenetic event in *H. pylori*-infected gastric mucosa. On the other hand, the clinical significance of histone modulation in gastric cancer patients is still unknown, and further research is necessary.

Recent studies have revealed a relationship between miRNA expression and the invasion and metastasis of gastric cancer. Scirrhous gastric cancer has a rapid invasive infiltration and high incidence of peritoneal dissemination, resulting in a very poor prognosis. The 5-year survival rate of patients with peritoneal dissemination is only 2% [34]. Therefore, understanding the mechanisms involved in the spread of gastric cancer to the peritoneal cavity and the development of a novel therapy are required. Recently, several studies have identified miRNAs involved in the regulation of epithelial–mesenchymal transition (EMT)-related transcription factors [35, 36]. Specifically, the miR-200 family has been demonstrated to be involved in the EMT process during cancer progression and metastasis [37]. The miR-200 family consists of five members and is divided into two clusters: miR-200a/b/429 and miR-200c/141. The targets of the miR-200 family have been identified as ZEB1 and ZEB2, which are direct repressors of E-cadherin, which is an EMT marker [38]. In gastric cancer, miR-200b is considered an essential regulator of the EMT by inhibiting migration and invasion through the downregulation of ZEB1 and ZEB2 in gastric cancer cells [39]. In several types of cancers, the miR-200 family has been reported to inhibit expression of ZEB1 and ZEB2 mRNA [40–42]. Interestingly, the promoter region of miR200 family is caused by aberrant DNA methylation in various cancer cells, leading to reactivation of ZEB1 and ZEB2 involved in the EMT process [43–45]. On the other hand, stromal fibroblasts, referred to cancer-associated fibroblasts (CAFs), are the major cellular constituents of tumor stroma. CAFs

play a pivotal role in malignant progression, including in the initiation, proliferation, invasion, and metastasis of various cancer cells [46–49]. Previous studies have shown that gastric CAFs are associated with the progression, growth, and spread of scirrhous gastric cancers [49, 50]. Kurashige et al. evaluated whether CAFs of gastric cancer are associated with the progression and invasion of the corresponding cancer cells through epigenetic changes of miR-200b [51]. The results of this study revealed that CAFs of gastric cancer reduced miR-200b expression and promoted tumor invasion and migration [51]. Epigenetic changes of miR-200b can be detected in inoculated high-frequency peritoneal dissemination cells using a mouse model [51]. Additionally, Kurashige et al. showed that the clinical gastric cancer samples that had low miR-200b expression had significantly higher peritoneal metastasis and poorer prognosis compared with those with high miR-200b expression [51]. These findings demonstrate that CAFs reduce miR-200b expression and promote tumor invasion by changing miR-200b expression in gastric cancer [51].

Stimulation of the EMT in cancer cells is mainly caused by disappearance of adhesion molecules such as E-cadherin. Upon stimulation of the EMT, cell polarity and cell-cell adhesion are disrupted, leading to the acquisition of a mesenchymal phenotype which activates migratory and invasion capabilities. Thus, many researchers are focusing on clarifying the EMT regulatory network. Recently, several miRNAs have been identified as EMT-suppressive miRNA. In contrast, few EMT-inducing miRNAs have been identified. Yanaka et al. identified a novel EMT-inducing miRNA through function-based screening of 328 synthetic miRNAs [52]. This screening approach is known to be suitable for exploring the oncogenic and tumor suppressive effects of miRNAs on cancer cells [53–56]. Using this approach, Yanaka et al. identified miR-544a as an EMT-inducing miRNA [52]. MicroRNA-544a has been demonstrated to be associated with the regulation of E-cadherin (CDH1). Yanaka et al. showed that overexpression of EMT-inducing miR-544a induces VIM (vimentin), SNAI1, and ZEB1 expression and reduces CDH1 expression [52]. The reduction of CDH1 by inducing miR-544a expression induced the nuclear import of β-catenin and the stabilization of β-catenin in nucleus, resulting in an EMT phenotype [52]. The signaling pathway associated with miR-544a might become a prognostic marker and therapeutic target for metastatic gastric cancer.

4.3 Protein Modulation in Gastric Carcinogenesis

Thrombospondin-1 (TSP1) is well known to contribute to tumor migration, invasion, and transforming growth factor (TGF-β) activation [57]. TSP1 is a multifunctional, 450 kDa, extracellular matrix glycoprotein [58]. Recently, Kashihara et al. examined the role of TSP1 in gastric carcinogenesis by analyzing a total of 39 patients with gastric cancer who had undergone gastrectomy [59]. They showed that the expression of TSP1 is high in patients with mucosal atrophy and gastric cancer. TSP1 activates the NF-κB pathway through binding to CD36, and the induced NF-κB signaling plays an important role in inflammation [60]. Severe inflammation occurs in *H. pylori*-infected mucosa. Kashihara et al. demonstrated that the

expression of TSP1 is high in *H. pylori*-infected gastric mucosa. Additionally, Alvarez et al. reported that methylation of TSP1 can be detected in *H. pylori*-infected chronic gastritis and gastric cancer [61]. These findings indicate that expression of TSP1 induced by inflammation is associated with the gastric carcinogenesis. However, it remains unclear how TSP1 signaling is associated with the development of gastric cancer, and further investigation on this matter is needed.

Pyruvate kinase (PK) is a rate-limiting enzyme in glycolysis and generates ATP and pyruvate by transferring the phosphate from phosphoenolpyruvate to ADP. In mammalians, PK consists of four isoforms (L, R, M1, and M2), and these isoforms are present in each different cell. The PKM isoform is converted to PKM1 or PKM2 through alternatively splicing of pre-mRNA. PKM1 is expressed in most differentiated tissues, whereas PKM2 is found primarily in embryonic tissues and tumor cells [62]. PKM1 is present as highly enzymatically active tetrameric forms. In contrast, PKM2 exists as either an active tetramer or inactive dimer and is present as a dimer with low activity in cancer cells [63–67]. In cancer cells, as a result of low activity of PKM2, glycolysis slows, and the pools of glycolytic intermediates are provided to generate the nucleotides and amino acids that are essential for cellular growth [68–70]. Cancer cells produce an increased amount of lactate by glycolysis even in the presence of oxygen, a phenomenon termed “aerobic glycolysis”, referred to as the “Warburg effect” [71]. In the Warburg effect, PKM2 is an important glycolytic enzyme [63, 64]. Therefore, PKM2 knockdown in cancer cells reduces tumor formation ability by reversing the Warburg effect [64]. Additionally, high levels of PKM2 expression have been detected in various tumors, indicating that a switch of the PKM isoform from PKM1 to PKM2 is an essential event for cancer development. Shiroki et al. reported that PKM2 expression is induced in gastric cancer tissues without a change in isoform expression [72]. It has been reported that PKM2 expression contributes to a reduced prognosis in gastric cancer [73, 74]. PKM2 promotes the growth of gastric cancer cells by regulating Bcl-xL expression or epidermal growth factor/epidermal growth factor receptor signaling [74, 75]. In addition, they showed that PKM2 knockdown in gastric cancer cells reduces sphere formation ability, tumorigenesis, and metastasis by reversing the Warburg effect [72]. Interestingly, PKM2 expression is induced by the *H. pylori*-derived CagA oncoprotein through MAPK signaling [72]. This evidence provides new insight concerning carcinogenic activity of CagA and demonstrates that enhanced PKM2 expression plays a pivotal role in gastric carcinogenesis by regulating cancer-specific metabolism.

RNA-binding motif 5 (*RBM5*) is known to be a tumor suppressor gene that regulates cell differentiation, cell proliferation, and apoptosis [76–78]. RBM5 regulates alternative splicing of multiple target genes [79–81]. Especially, p53 transcriptional activity is regulated by RBM5 in the absence and presence of DNA damage [82]. This effect of RBM5 is known to be associated with tumor-suppressive functions [82]. In addition, the expression of RBM5 is reduced in various cancer cells [83, 84]. Kobayashi et al. examined RBM5 expression in tumor tissue specimens obtained from patients with resected gastric cancer and evaluated the relationship between RBM5 protein expression and clinicopathological parameters [85]. This report shows that RBM5 expression is significantly decreased in gastric cancer

specimens [85]. Interestingly, it has been revealed that decreased RBM5 expression is more conspicuous in advanced stages (III and IV) than in early stages (I and II) and is significantly associated with tumor depth, TNM stage, and lymph node metastasis [85]. These results indicate that downregulation of RBM5 is involved in tumor progression rather than carcinogenesis in gastric cancer. In addition, RBM5 expression in undifferentiated gastric cancer tissues is lower than in differentiated tissues, indicating that decreased RBM5 expression also plays a role in the dedifferentiation of gastric tumors [85]. It has also been shown that specific knockdown of RBM5 in gastric cancer cells induces cell proliferation by decreasing the expression of p53 and restoring RBM5 expression reduced their proliferation by recovering expression of p53 [85]. These findings suggest that *RBM5* behaves as a tumor suppressor gene in gastric cancer and that decreased RBM5 expression would be involved in the malignancy of gastric cancer cells.

Recurrence and metastasis after curative operation occur in some patients with advanced gastric cancers. Hence, specific biomarkers for recurrence and metastasis are required. Examination of the expression levels of RBM5 is expected to be a biomarker for assessing the risk of recurrence and metastasis. Recently, Hirata et al. showed that CD44 variant 9 (CD44v9) expression represents a potential predictive marker for recurrence in early gastric cancers [86]. CD44 is one of the cell surface markers associated with cancer stem cells [87, 88]. CD44v9 interacts with and stabilizes xCT, a glutamate-cystine transporter, leading to increased intracellular levels of reduced glutathione (GSH). It has been reported that CD44v9-positive cells demonstrate an enhanced ability to suppress the production of ROS, resulting in subsequent therapeutic resistance, recurrence, and metastasis of tumors [89–91]. Exploration and identification of specific biomarkers for recurrence and metastasis of gastric cancer by Japanese gastric cancer research would provide us a novel therapeutic approach for gastric cancer in the future.

4.4 Epstein–Barr Virus Infection in Gastric Carcinogenesis

Epstein-Barr virus (EBV), also known as human herpes virus 4, is one of the most common human viruses and is well known as a human oncovirus [92]. Infection with EBV occurs in infancy or childhood, and the majority of adults have established lifelong latent infections [93]. EBV infection contributes to the development of gastric cancer, and it accounts for <10% of gastric cancer. EBV-induced gastric cancer is known as inflammation-mediated cancer. The NF-κB activated by inflammation induced by various stimuli, such as bacterial and viral products, cytokines, DNA damage, and oxidative damage, has been demonstrated to contribute to tumorigenesis [94]. Shimizu et al. showed that various viral factors and inflammatory reactions lead to the aberrant expression of activation-induced cytidine deaminase (AID) by NF-κB activation in several epithelial cells [95]. AID is a nucleotide-editing enzyme that is essential for somatic hypermutation and class-switch recombination of the immunoglobulin gene and contributes to the accumulation of genetic alterations in tumor-related genes. It is known that AID as a genomic modulator is aberrantly expressed by

NF-κB in *H. pylori*-associated gastric cancer [96–98]. Mohri et al. examined whether aberrant AID expression as a result of increasing NF-κB expression also applies to EBV-induced gastric cancer [99]. Interestingly, expression of AID and NF-κB was significantly low in EBV-associated gastric cancer compared with that of non-EBV-associated gastric cancer [99]. These results suggest that AID expression may be irrelevant to gastric cancer induced by EBV infection, and genomic modulation by AID is not required in EBV-infected epithelial cells. It is thought that *H. pylori* infection induces atrophic gastritis and intestinal metaplasia with the aberrant expression of AID, leading to the gastric carcinogenesis through the accumulation of genetic mutations [100]. Therefore, it is inferred that the mechanism of gastric carcinogenesis induced by EBV infection is different from that of *H. pylori* infection. Further research is required to clarify how DNA hypermethylation by EBV infection is associated with gastric carcinogenesis.

4.5 Conclusion

The accumulation of multiple abnormalities promotes the development of gastric cancer and confers growth advantages to gastric cancer cells (Fig. 4.1). Understanding the molecular mechanisms associated with the progression of gastric cancer is critical for improving clinical outcomes.

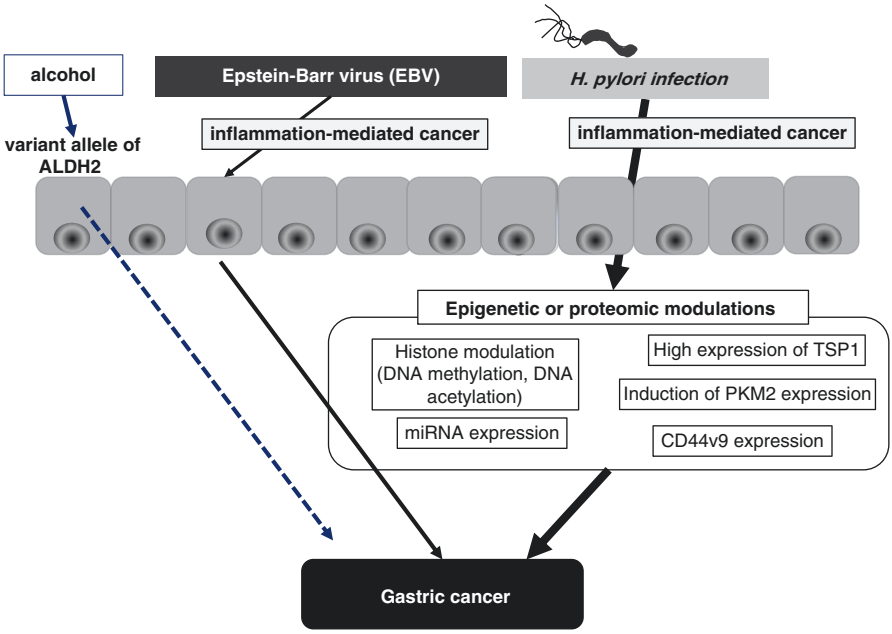

Fig. 4.1 Epigenetic and proteomic modulations in *H. pylori*-infected gastric epithelial cells promoting carcinogenesis and the progression of gastric cancer

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Chapter 5
Pathology of Gastric Cancer

Takashi Yao and Ryo Wada

Abstract Early detection and accurate diagnosis have strong impacts on cancer care; therefore, pathological diagnosis of biopsy specimen is important. It is too late that the lesion is followed up until the carcinoma invades the submucosa or more. For the better quality of life (QOL) of patients, carcinomas should be endoscopically diagnosed and resected before metastasis. To achieve correct histological diagnosis on early-stage gastric carcinomas by biopsy specimen, it is necessary to understand the difference in histological diagnosis between Japanese and Western pathologists and learn the characteristic histological features of noninvasive well-differentiated adenocarcinomas, especially those of low-grade atypia. In addition, for selecting the suitable therapy, it is also necessary to know the clinicopathological features of special types of gastric carcinomas and how to correctly perform a histological evaluation of endoscopically resected specimens.

Keywords Gastric cancer · Histological diagnosis · Adenocarcinoma with enteroblastic differentiation · Adenocarcinoma of fundic gland type · Endoscopic curative resection

5.1 Differences in Histological Diagnosis between Japanese and Western Pathologists

It is well known that there are discrepancies in the diagnosis of gastrointestinal neoplasia between Japanese and Western pathologists [1–6]. In Western countries, the most reliable finding for the diagnosis of carcinoma is the presence of stromal

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invasion (desmoplastic reaction). Accordingly, noninvasive epithelial neoplasia is classified into low-grade dysplasia (LGD) and high-grade dysplasia (HGD) subtypes, by degree of cytological atypia. In contrast, in Japan the diagnosis of carcinoma is made by the combination of cytological and architectural abnormalities, irrespective of stromal invasion. Accordingly, noninvasive epithelial neoplasia is classified into adenoma and adenocarcinoma by cytological features.

In Japan, the five-tiered group classification is widely used for the histological diagnosis of endoscopic biopsy specimens. The updated group classification [7] is similar to the Vienna classification [3]; however, these two classifications are partly different. Between the two classifications, there is no difference between Group 1 and Category 1 (non-neoplasia) and Group 2 and Category 2 (indefinite for neoplasia), and the comparisons between Groups/Categories 3, 4, and 5 between the two classifications are demonstrated in Table 5.1.

In the Vienna classification system, the diagnoses of high-grade adenoma/HGD, noninvasive carcinoma, and suspicious invasive carcinoma are clustered into one category (Category 4), termed as noninvasive high-grade neoplasia. This category is defined as neoplasia with cytological and architectural features of carcinoma but without evidence of stromal invasion. Utilization of the Vienna classification system has improved the percentage of agreement during diagnoses [3, 4]. The different terms, HGD and intramucosal carcinoma, can be explained by simple differences in nomenclature.

However, histological diagnoses based on biopsy specimens using the Vienna classification system may result in the underestimation of the neoplastic grade or depth of invasion [8, 9], and this underestimation has been proven in follow-up studies from Western countries [10–15]. HGD frequently progresses to invasive carcinoma over a short period of time, and incidences of progression are 67–85% over mean intervals of 4 months to 1.5 years [10–15]. It is reasonable that such lesions of HGD could initially have been carcinomas but did not transform into carcinomas. In contrast, only 10% of HGD cases were finally diagnosed as carcinoma in a Japanese follow-up study [16]. At the very least, the term well-differentiated adenocarcinoma should be used for HGD.

On the other hand, the most critical point is that even LGD (Vienna Category 3), as defined by Western pathologists, can be diagnosed as well-differentiated adenocarcinoma of low-grade atypia (WD-AC-LG) (Group 5) by Japanese pathologists (Table 5.1). Incidences of progression from LGD to invasive carcinoma were 0–23%

Table 5.1 Comparison between the Vienna classification and Japanese group classification

			Category		
			3	4	5
Group	3	Adenoma	LG-adenoma/LGD		
	4	Suspicious carcinoma		HG-adenoma/HGD	
	5	Carcinoma	Some of LGD	Most of HGD	
				Noninvasive carcinoma	
				Suspicious invasive carcinoma	Invasive carcinoma

in Western studies [10–15] but only 3% in a Japanese study [16]. These results suggest a difference in diagnostic criteria for LGD (including LG adenoma), and in fact, histological features of LGD that have been demonstrated in some reports [17, 18] should be classified as WD-AC-LG by Japanese diagnostic criteria. In order to solve this discrepancy between biopsy and resected specimens, the differential diagnosis between adenoma and WD-AC-LG is critical.

5.2 Differential Diagnosis Between Adenoma and WD-AC-LG

Japanese pathologists have learned and gained experience from routinely assessing large numbers of biopsy specimens provided by endoscopists and from the subsequent feedback gained by examining resected specimens from the same neoplastic lesions. The Japanese diagnostic criteria for intramucosal carcinoma have been established by comparing the histological features of the mucosal component with that of the submucosal component in the same lesion. The intramucosal component of invasive carcinoma should be termed carcinoma if it shows the same cytological features as the submucosal component, regardless of stromal invasion. The invasive ability of carcinoma has already been acquired at a mucosal stage, and therefore, it is logical to make a carcinoma diagnosis based on the cytological features of the mucosal component.

We have also learned that even WD-AC-LG has invasion abilities [19] and the cytological features of WD-AC-LG are different from those of adenoma. The common histological feature of low-grade adenoma and WD-AC-LG is noninvasive, well-differentiated neoplasia with nuclei located at the basal site and low nucleus-to-cytoplasm (N/C) ratio (less than 50%); however, the difference between them is nuclear morphology (shape and arrangement). Adenomas, except for the pyloric gland type, have spindle-shaped nuclei that are regularly arranged at the basal side (Fig. 5.1a), whereas WD-AC-LG has round-to-oval nuclei arranged at the basal area with or without irregular arrangement (Figs. 5.2a and 5.3a).

In addition to our experience, the reasonableness of the Japanese diagnostic criteria has been supported by the following studies. First, the presence of gastric differentiation suggests adenocarcinoma, rather than adenoma, and a follow-up study of borderline lesions revealed that the presence of gastric differentiation is one of the risk factors for malignant transformation [20, 21]. Second, by using the Japanese criteria, the tendency for cell differentiation is different from typical adenoma and has small intestinal differentiation that is distinguished by presence of goblet cells, brush border, and Paneth cells, and the carcinoma tends to express gastric or gastrointestinal differentiation [22, 23]. Third, the incidence and pattern of adipophilin expression are different between adenoma and carcinoma [24]. Fourth, most adenomas tend to have a band-like proliferating zone near the surface, whereas carcinomas tend to have irregularly or diffusely distributed proliferating cells [22–24]. Fifth, genetic abnormalities that are the same as those observed for advanced gastric

carcinoma were detected even in WD-AC-LG [25]. These findings demonstrate that cytological differentiation and distribution of proliferating cells are important for differential diagnoses, in addition to nuclear findings.

The algorithm for differential diagnosis between adenoma and WD-AC-LG is proposed according to nuclear features and cytological differentiation (Fig. 5.4). This is just an algorithm, and for individual cases, intermediate lesions do exist. When the differential diagnosis is difficult, immunohistochemical stains are useful for evaluating cell differentiation through identifying MUC5AC (foveolar cells), MUC2 (goblet cells), MUC6 (pyloric gland and mucous neck cells), and CD10 (small intestinal brush border). Typical adenomas present small intestinal differentiation that is characterized by the presence of goblet cells, brush border, and Paneth

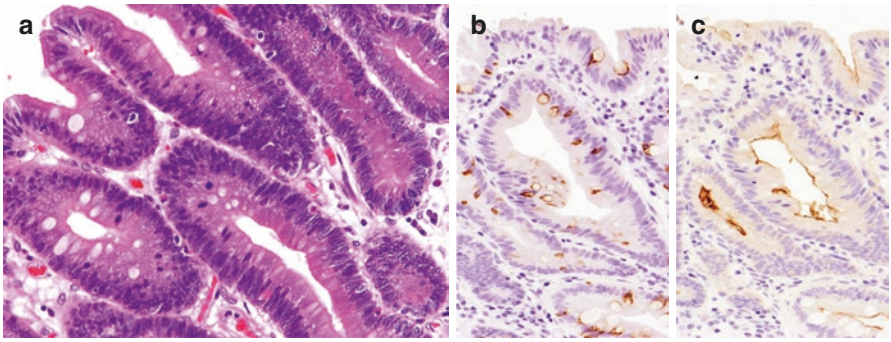

Fig. 5.1 Tubular adenoma. The tumor is composed of columnar epithelium with eosinophilic cytoplasm, admixed with some goblet cells and Paneth cells. The nuclei are spindle-shaped and regularly arranged at the basal side (**a**). Immunohistochemical stains highlight the existence of goblet cells by MUC6 (**b**) and brush border by CD10 (**c**), which indicates small intestinal differentiation

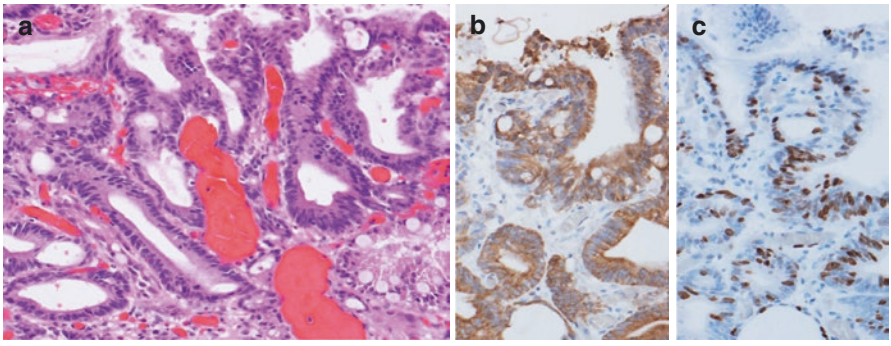

Fig. 5.2 Well-differentiated adenocarcinoma of low-grade atypia. The tumor is composed of columnar epithelium with pale eosinophilic cytoplasm. Neither goblet cell nor Paneth cell is identified. The nuclei are rounded and located at the basal side with an irregular arrangement (**a**). The immunohistochemical stain with MUC5AC reveals gastric foveolar differentiation (**b**). The diffuse distribution f Ki-67 is also characteristic of adenocarcinoma (**c**)

cells (Fig. 5.1b,c), whereas adenocarcinomas tend to express gastric, gastrointestinal, or null phenotypes (Figs. 5.2b and 5.3b). In addition, the evaluation of proliferating cell distributions by Ki-67 is also useful. When Ki-67 positive cells are irregularly or diffusely distributed in the tumor (Figs. 5.2c and 5.3c), it is more likely that the tumor is adenocarcinoma, rather than adenoma [22, 23].

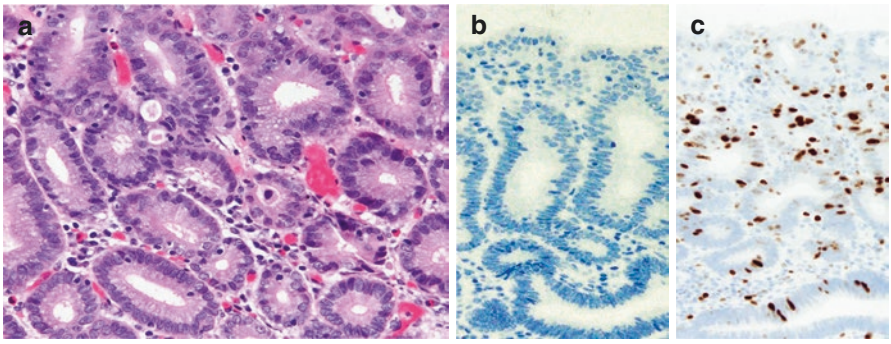

Fig. 5.3 Well-differentiated adenocarcinoma of low-grade atypia. The tumor is composed of columnar epithelium with pale eosinophilic cytoplasm. Neither goblet cell nor Paneth cell is identified. The nuclei are rounded and located at the basal side without an irregular arrangement (**a**). Immunohistochemically, MUC2 (**b**), MUC5AC, MUC6, and CD10 are negative in this tumor, indicating null phenotype. The irregular distribution f Ki-67 is also characteristic of adenocarcinoma (**c**)

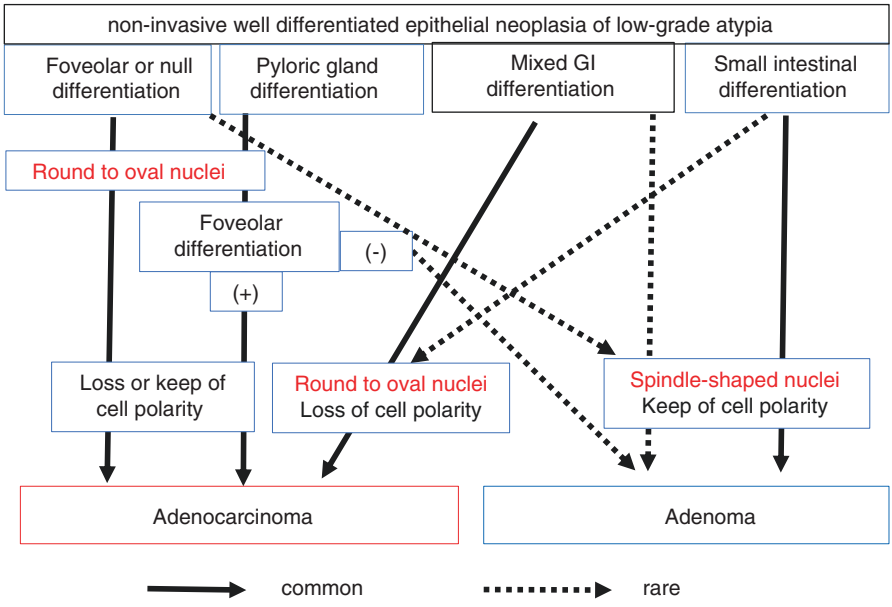

Fig. 5.4 Algorithm of differential diagnosis between adenoma and well-differentiated adenocarcinoma of low-grade atypia

Pyloric gland-type adenomas are a rare and unique variant. Such tumors are mainly composed of mucous cells that are similar to M pyloric gland-type cells (MUC6 positive) and covered by foveolar-type cells (MUC5AC positive). The typical pyloric gland-type adenoma has a proliferating zone near the surface, between foveolar-type cells and pyloric gland-type cells. The irregular or diffuse distribution of proliferating cells and/or diffuse positivity for MUC5AC are characteristics of adenocarcinomas, rather than adenomas.

5.3 Special Types of Gastric Carcinoma in Japanese Classification

The present Japanese histological classification of gastric carcinoma [7] is similar to the WHO classification [26], although there are slight differences. The comparison between Japanese and WHO classifications is shown in Table 5.2. Three histological types of poorly differentiated adenocarcinomas, solid type (por1),

Table 5.2 Comparison between Japanese and WHO classifications

Japanese classification 2017 (15th Ed.)	WHO classification 2010
Common type	
Papillary adenocarcinoma (pap)	Papillary carcinoma
Tubular adenocarcinoma	Tubular carcinoma
Well-differentiated (tub1)	
Moderately differentiated (tub2)	
Poorly differentiated	Poorly cohesive carcinoma
Solid type (por1)	(No description)
Nonsolid type (por2)	
Signet-ring cell carcinoma (sig)	(Included in poorly cohesive carcinoma)
Mucinous carcinoma (muc)	Mucinous carcinoma
(No description)	Mixed adenocarcinoma
Special type	Neuroendocrine neoplasms
Carcinoid tumor	Neuroendocrine tumor (NET), G1 & G2
Endocrine carcinoma	Neuroendocrine carcinoma
(No description)	Mixed adenoneuroendocrine carcinoma
Adenosquamous carcinoma	Adenosquamous carcinoma
Squamous cell carcinoma	Squamous cell carcinoma
Adenocarcinoma with enteroblastic differentiation	(similar to embryonal carcinoma)
Hepatoid adenocarcinoma	Hepatoid adenocarcinoma
Adenocarcinoma of fundic gland type	(No description)
Carcinoma with lymphoid stroma	Carcinoma with lymphoid stroma
Undifferentiated carcinoma	Undifferentiated carcinoma

adenocarcinoma with enteroblastic differentiation (AC-Ent), and adenocarcinoma of fundic gland type (AC-FG), are listed in the Japanese classification but not described in the WHO classification. The clinicopathological characteristics are as follows:

5.3.1 *Poorly Differentiated Solid-Type Adenocarcinoma (por1)*

Adenocarcinoma without glandular formation is classified into poorly differentiated adenocarcinoma, which is further classified into two subtypes: solid type (por1) and nonsolid type (por2). Por1 tends to metastasize through lymphatic channels and disseminate throughout the peritoneum, whereas por1 tend to metastasize through veins. Por1 is frequently accompanied by differentiated components at the tumor periphery [27, 28]. Although por1 can be classified into poorly differentiated-type one, por1 is histogenetically and biologically similar to well-differentiated-type one. Por1 was firstly described in the Japanese classification of gastric carcinoma (12th Ed, 1993), and carcinoma with lymphoid stroma that had previously been categorized as por1 was redefined as special type in the Japanese classification of gastric carcinoma (14th Ed, 2010), because carcinoma with lymphoid stroma is a special type that is associated with EB viral infection. Carcinoma with solid growths, such as hepatoid adenocarcinoma and endocrine carcinoma, should be differentiated by immunohistochemistry.

5.3.2 *Adenocarcinoma with Enteroblastic Differentiation (AC-Ent)*

AC-Ent was first reported by Matsunou [29], and only a few cases have been reported under a different name of clear-cell (glycogen-rich) adenocarcinoma [30, 31]. AC-Ent diagnostic criteria have not been established, and its clinicopathological features have not been clarified, although AC-Ent was introduced in the Japanese classification of gastric carcinoma (14th Ed, 2010) as a miscellaneous carcinoma with a short description.

In 2016, Murakami et al. established the significance of AC-Ent, which has an aggressive biological behavior with high incidence of liver metastasis, and defined AC-Ent as an adenocarcinoma with a clear cytoplasm that resembles fetal gut tissue and displays tubular, papillary, and solid growths with expression of at least one enteroblastic marker (AFP, glypican 3, or SALL4) [32] (Fig. 5.5). Even early-stage AC-Ent has an aggressive biological behavior, which is demonstrated by the high incidence of venous invasion and liver metastasis [32, 33]. Hepatoid adenocarcinoma shares characteristic features with AC-Ent, such as histological features,

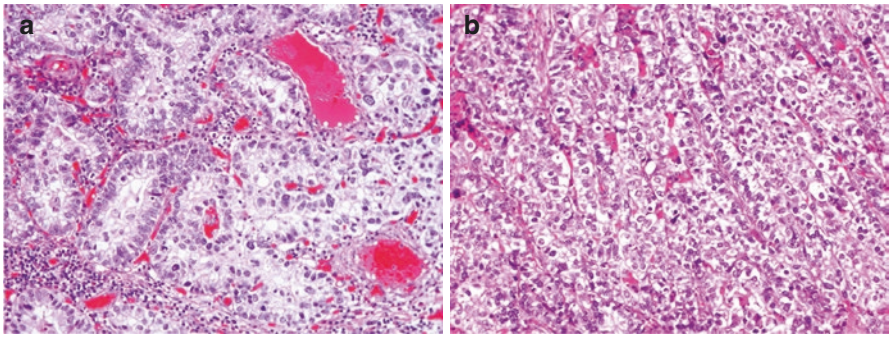

Fig. 5.5 Adenocarcinoma with enteroblastic differentiation. The tumor is composed of clear cytoplasm growing in a tubular structure (**a**) or a solid sheet (**b**)

expression of enteroblastic markers, and high incidence of liver metastasis [34–36]. Therefore, hepatoid adenocarcinoma could be included in the AC-Ent category, as an AC-Ent solid variant.

5.3.3 Adenocarcinoma of Fundic Gland Type (AC-FG)

Adenocarcinoma with chief cell differentiation was first reported by Tsukamoto et al. in 2006 [37], and AC-FG was introduced by Ueyama et al. in 2010 as a new type of gastric adenocarcinoma with distinct clinicopathological characteristics, including tumor location (upper stomach), histological features, phenotypic expression, and low-grade malignancy (low proliferating activity, no lympho-vascular invasion, and good prognosis) [38]. Histologically, AC-FG is defined by epithelial neoplasia that is mainly composed of neoplastic glandular cells that mimic chief and/or parietal cells (Fig. 5.6) and are positive for pepsinogen I and/or H^+/K^+-ATPase. Almost all cases of AC-FG were positive for pepsinogen I and MUC6, which suggests that AC-FG is mainly composed of carcinoma cells with immature differentiation toward chief cells [39]. AC-FG usually shows very low N/C ratio and resembles fundic glands, and therefore, diagnosis using a biopsy specimen is sometimes difficult.

AC-FG, especially when restricted to the mucosa, is recommended by Western pathologists to be classified as an oxyntic gland polyp/adenoma [40]. Although AC-FG is a low-grade malignancy, this mucosal lesion without metastatic potential should be treated as a carcinoma and undergo endoscopic resection before acquiring metastatic potential. Recently, aggressive variants with lympho-vascular invasion or intramural metastasis have been found [41, 42], and more recently, AC-FG was identified to lack association with *Helicobacter pylori* infection [41, 43]. More attention should be paid to AC-FG now and in the future.

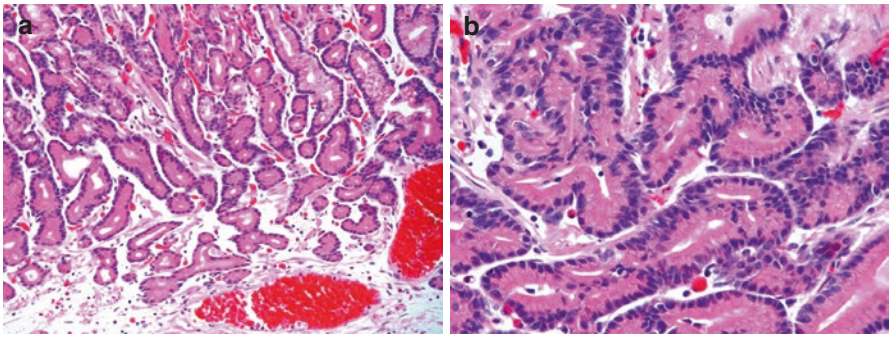

Fig. 5.6 Adenocarcinoma of fundic gland type. The tumor is composed of highly differentiated columnar cells mimicking fundic gland cells, predominantly chief cells, with pale gray-blue, basophilic cytoplasm and mildly enlarged nuclei, growing in an irregular tubular structure (**a**, **b**)

5.4 Histological Evaluation of Endoscopically Resected Specimens

Curative endoscopic resections should be performed for carcinomas with low risk of lymph node metastasis, and the incidence of lymph node metastasis for intramucosal gastric carcinoma has been reported to be approximately 2% [44–47].

The Japanese gastric cancer treatment guidelines (2014, ver.4) have provided indication of curative endoscopic resections [48] (Fig. 5.7). The curability of endoscopic resection is evaluated by histological examination of the status of resected margin, tumor size, histological type, depth of invasion, presence of ulcer (including scar), and lympho-vascular invasion.

In order to evaluate the histological details, the proper treatment of the resected specimen is essential. It should be extended with pins on the board, fixed in 10% formalin solution, and completely cut in stepwise sections 2–3 mm in width. A record of macroscopic pictures before and after sectioning is also recommended [49].

With regard to histological type, the predominant histological type is usually representative of the lesion. Even if the representative type is a differentiated type, the presence of a poorly differentiated component increases the risk of metastasis [44, 47]. As our knowledge of differentiated-type adenocarcinomas that are accompanied with some areas of undifferentiated components is currently insufficient, such tumors are regarded as non-curative for the time being, and additional surgical treatments are recommended.

The significance of papillary adenocarcinoma (pap) has not been described in the Japanese guideline. Although pap is classified as a differentiated-type adenocarcinoma, pap is known to be an adverse prognostic factor by a higher risk of lympho-vascular invasion and metastasis to the lymph nodes and liver, compared with tubular adenocarcinoma [50–52]. This tendency was confirmed by analysis on endoscopically resected gastric cancers [53].

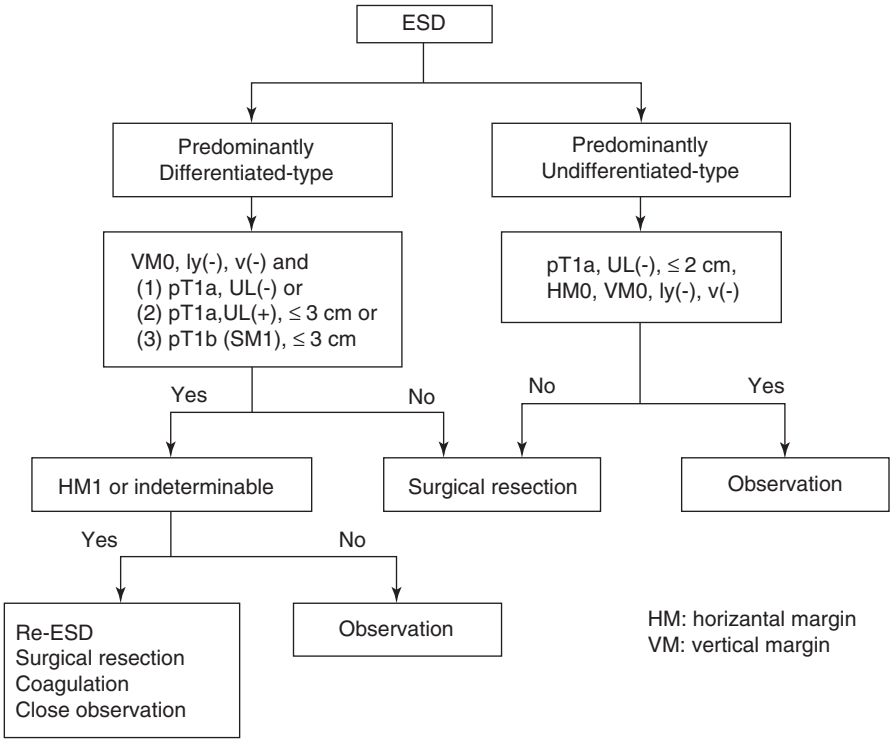

Fig. 5.7 Treatment options after endoscopic resection from Japanese gastric cancer treatment guideline 2014 [4]

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Part III
Risk Clarification and Cancer Screening Before and After Eradication

Chapter 6
Serum Markers

Masanori Ito, Tomoyuki Boda, Takahiro Kotachi, Mariko Kiso, Kazuhiko Masuda, Kosaku Hata, Masaharu Yoshihara, Shinji Tanaka, and Kazuaki Chayama

Abstract The evaluation of *Helicobacter pylori* infection or the presence of chronic gastritis induced by *H. pylori* is regarded as the most important risk factor for gastric cancer development. The serum antibody test against *H. pylori* is the most popular in the clinical practice as well as for a population-based gastric cancer mass survey. However, some *H. pylori*-infected patients had negative titer (called negative-high titer), which may be induced by unexpected or accidental eradication of *H. pylori*. Serum pepsinogen (PG) is another popular serum marker for evaluating the status of gastric inflammation. Miki first established a systematic diagnostic panel with PG-I and PG I/II levels to evaluate gastric cancer risk, which was called the "pepsinogen test." Further, Miki and Inoue created a diagnostic panel by combination of serum anti-*H. pylori* antibody titer and the PG test, which is called the "ABC risk stratification system." However, in this system, contamination of patients with past infection of *H. pylori* into Group A is a crucial problem called the "pseudo A problem." Recently, the Japanese Society for Helicobacter Research have created a new flow chart for diagnosis and treatment by considering gastric cancer risk by *H. pylori* infection. In this panel, morphological (endoscopic) evaluation is included to diagnose *H. pylori*-uninfected patients with serum antibody test.

Keywords *Helicobacter pylori* · Serum antibody · Pepsinogen · Pepsinogen test · ABC risk stratification system · Endoscopy

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6.1 Serum Anti–*Helicobacter pylori* Antibody Titer

Helicobacter pylori infection is a risk factor for gastric carcinogenesis [1]. Gastric cancer development in patients without *H. pylori* infection is rare, especially in Japan [2]. Therefore, the evaluation of *H. pylori* infection or the presence of chronic gastritis induced by *H. pylori* is regarded as the most important risk factor for gastric cancer risk [3]. The Japanese Ministry of Health and Welfare approved six testing methods for *H. pylori* infection including an antibody test. From these methods, the serum antibody test against *H. pylori* is the most popular in the clinical practice as well as for a population-based gastric cancer mass survey in Japan. In particular, a specific type of enzyme-linked immunosorbent assay named the E-plate (Eiken, Japan) is the most popular in Japan [4]. The antigen used in this kit was extracted from the standard *H. pylori* strain derived from a Japanese patient [4].

The cutoff level used for this E-plate is 10 U/mL, but recent studies have demonstrated that a majority of true *H. pylori*-uninfected patients have a titer less than 3 U/mL [5]. This suggests that many *H. pylori*-infected (most are supposed to be past-infected) patients had negative titer from 3.0 to 9.9 U/mL (called negative-high titer) [6]. In Japan, unexpected or accidental eradication of *H. pylori* by antibiotics may be the main reason why some past-infected patients had negative-high titer against *H. pylori*.

6.2 Serum Pepsinogen

Serum pepsinogens (PGs; PG-I and PG-II) are zymogens of pepsin in the gastric mucosa and representative markers for gastric inflammation. Although pepsinogens are produced in gastric glands (PG-I from chief cells in the fundic gland and PG-II from whole gastric/duodenal glands) and secreted into the gastric lumen, approximately 1% have backflow into blood vessels [7]. Samloff first used "serologic biopsy" in 1982 for the clinical application of serum PG levels to evaluate gastric inflammation [8]. In Japan, Miki et al. first reported the usefulness and importance of serum PG levels to evaluate the status of gastric atrophy [9].

Serum PG levels are the most popular and standard serum marker for evaluating the status of gastric inflammation. Naito et al. summarized previous studies and demonstrated the importance of serum PG level in the evaluation of gastric inflammation [10]. The serum level of PG-I increases with inflammation in the gastric corpus and gastric antrum [11]. On the other hand, PG-I level decreases in patients with atrophy in the gastric corpus [12]. These data indicate that PG-I levels first increase with inflammation and then decrease with increasing atrophy in the gastric corpus. On the other hand, the level of PG-II increases with gastric inflammation both in the corpus and antrum [13]. PG-II levels also increase in patients with enlarged-fold gastritis, in which atrophic gastritis must be present [11]. These data suggest that the PG-II level is a marker of active inflammation not only in the corpus but also in the antrum. In cases with corpus atrophy and inflammation, the PG I/II ratio exhibited decreasing levels, which suggests that it is a valid marker of gastric

inflammation in the corpus [14]. Kiyohira et al. demonstrated similar results and made a diagnostic panel with the I/II ratio and PG II levels to diagnose histological features of gastric inflammation [15].

Therefore, serum pepsinogen levels are known to be an excellent marker for evaluation of gastric cancer risk. Yoshihara et al. demonstrated the importance of PGs, especially PG I/II, as a clinical risk factor for the development of gastric neoplasm [16]. Because high PG-II serum titer indicates the presence of active gastritis, Ito et al. demonstrated the importance of serum PG-II level for evaluating gastric cancer risk of diffuse type [17].

By eradication therapy, the degree of activity/inflammation could be improved at an early stage [18]. Although the grades of atrophy and intestinal metaplasia could also be improved in a portion of cases undergoing *H. pylori* eradication, a relatively long period after therapy is required [19]. Previous studies evaluated PG-I, PG I/II, and a decrease in PG II levels after eradication therapy [20–22]. Haneda et al. reported criteria for identifying a high-risk group for gastric cancer using the serum PG I/II ratio (less than 4.5) after successful eradication of *H. pylori* [23].

6.3 The Pepsinogen Test, a Diagnostic Panel Using Serum PG Levels

Serum PG levels are the representative marker for evaluating gastric inflammation, which suggests possible application for gastric cancer assessment. Miki first used a systematic diagnostic panel with PG-I and PG I/II levels to evaluate gastric cancer risk, which was called the "pepsinogen test." [24] In the "pepsinogen test," the cutoff values were set at 70 (50) ng/mL and 3.0 (2.0) for PG-I and PG I/II, respectively. Patients with PG I ≤70 (50) ng/mL and PG I/II ≤3 (2) were positive on the pepsinogen test and judged as having atrophic gastritis in the corpus, which meant at risk for gastric cancer. However, these criteria identify the high-risk group for gastric cancer development but do not diagnose the presence of chronic gastritis induced by *H. pylori* infection. To identify *H. pylori* infection by PG titers, revised criteria are needed. Recently, Kitamura et al. demonstrated new criteria of PGs for diagnosis of chronic gastritis induced by *H. pylori* infection [25]. A revised cutoff value of PG I/II ≤5 had the best sensitivity and accuracy for diagnosing *H. pylori*-induced gastritis. Furthermore, in a mass screening of healthy subjects, a cutoff value of PG I/II ≤4.5 was better for diagnosing the presence of gastritis because of sensitivity and specificity >80% [25]. In addition, Kikuchi et al. reported from a multicenter study that the optimal criterion was a PG-II value of at least 10 (12) ng/mL or a PG I/PG II ratio no more than 5.0 (4.0), which produced 96.3% (95.1%) sensitivity and 82.8% (72.8%) specificity [26]. By evaluation of serum PG levels, we can evaluate gastric cancer risk to patients, but it should be noted that serum PG levels were affected by some factors including gastrectomy, *H. pylori* eradication, the use of proton pump inhibitors (PPI), or renal dysfunction. The number of PPI users is increasing, and a detailed interview is essential prior to the application of the PG test [27].

6.4 ABC Risk Stratification System, a Diagnostic Panel Using Serum Pepsinogens and Serum Anti–*H. pylori* Antibody Titer

H. pylori infection is a key event for the development of gastric cancer and can be diagnosed by serum antibody against *H. pylori* titer. However, this titer may become negative when the degree of atrophic gastritis progresses to a severe grade. Miki and Inoue further established a diagnostic panel by combination of serum anti-*H. pylori* antibody titer and the PG test, which is called the "ABC risk stratification system" (Fig. 6.1) [28, 29]. Subjects with negative results for both the anti-*H. pylori* antibody serology test and the PG test were classified in Group A, anti-*H. pylori* antibody-positive/PG-negative subjects were in Group B, anti-*H. pylori* antibody-positive/PG-positive subjects were in Group C, and anti-*H. pylori* antibody-negative/PG-positive subjects were in Group D. In particular, multivariate meta-analyses suggested that Group A had a lower gastric cancer risk than Group B/C and that Group B had a lower risk compared with Group C/D [30]. In addition, Yanaoka et al. demonstrated the implication of this classification in their cohort study in which gastric cancer risk increases in order A to D [31].

In this system, Group A includes subjects with neither *H. pylori* infection nor chronic atrophic gastritis, but Group A contains *H. pylori*-infected subjects. Boda et al. reported that approximately 10% of patients with gastric tumors (cancer or adenoma) belong to Group A [32]. The cancer risk of these patients may be underappreciated using the current criteria of the ABC classification system. *H. pylori*-infected subjects (including past-infected) in Group A is a crucial problem in ABC risk stratification. Because subjects in Group A are judged extremely low risk for gastric cancer as healthy *H. pylori*-uninfected individuals, they may be excluded from population-based gastric cancer mass surveys. Contamination of patients with past infection of *H. pylori* who may have gastric cancer risk is a crucial problem called the "pseudo A problem." Therefore, a revised ABC risk classification system has been proposed in which the revised cutoff value of serum anti-*H. pylori* antibody titer (3.0 U/mL) is used [33].

	A	B	C	D
Anti-*H. pylori* antibody	−	+	+	−
Pepsinogen test	−	−	+	+

Fig. 6.1 ABC risk stratification system. Anti-*H. pylori* antibody: cut-off, 10 U/mL. Pepsinogen test: cut-off, PG I ≦70 ng/mL and I/II ≦ 3.0

6.5 A New Risk Stratification System Using Serum Markers and Endoscopic Evaluation

Several serum markers are valuable for evaluating gastric cancer risk, but there is no serum marker that is satisfactory for gastric cancer screening. The Japanese Society for Helicobacter Research (JSHR) created a flow chart for diagnosis and treatment by considering gastric cancer risk from *H. pylori* infection [34]. The JSHR conducted a multicenter study to clarify the optimized serum antibody titers against *H. pylori* by E-plate (Eiken, Japan) for evaluation of gastric cancer risk [35]. A total of 2519 cases were registered from 10 institutes or hospitals. According to this multicenter study, the most reliable cutoff level was 3.0 U/mL for evaluating gastric cancer risk by the E-plate. However, the accuracy of risk evaluation by serum antibody was not satisfactory especially in the elderly. Therefore, morphological evaluation (endoscopic evaluation of atrophic gastritis in the corpus) is essential to diagnose *H. pylori*-uninfected patients with this serum antibody test.

Therefore, the JSHR concluded that endoscopic examination should be performed for evaluating gastric cancer risk even in subjects with anti-*H. pylori* antibody titer less than 3 U/mL (evaluated by Eiken E-plate, Fig. 6.2) [34]. These patients have little gastric cancer risk if antibody titer is less than 3 U/mL and show no atrophic gastritis in the corpus (C0 or C1 by Kimura-Takemoto classification) [36]. If patients have gastric atrophy in the corpus (grade C2 or more) or have serum anti-*H. pylori* titer ≥3 U/mL, a patient should receive the *H. pylori* test by urea breath test, stool antigen test, or rapid urease test. Eradication of *H. pylori* should be considered in patients with a positive result in the above tests for the primary prevention of gastric cancer [37, 38].

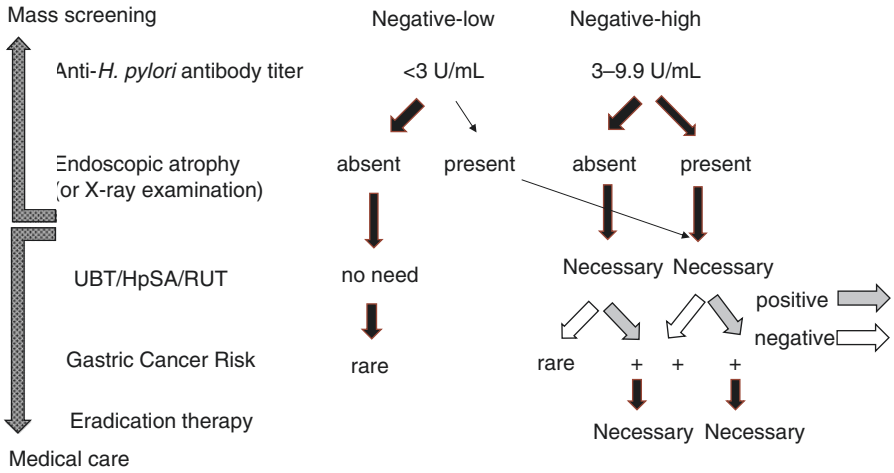

Fig. 6.2 Recommendation for gastric cancer screening and primary prevention of gastric cancer by anti-*H. pylori* antibody. Note: Risk stratification must be applied in patients before eradication therapy

6.6 Gastrin and Other Diagnostic Panels

In Western countries GastroPanel, which is an ELISA-based biomarker panel, includes three markers for mucosal atrophy (PG-I and PG-II for the corpus; gastrin-17 for the antrum) and the *H. pylori* IgG antibody [39]. Serum gastrin level is another important serum marker of gastritis. It should be mentioned that the etiology of atrophic gastritis is not similar between Japan and Scandinavian countries because autoimmune gastritis is more prevalent than in Japan [40]. In Western and Scandinavian countries, gastrin level is a known marker for antral inflammation. On the other hand, in Japan increased serum gastrin level (hypergastrinemia) is a marker for decreased acid output and indicates atrophy in the gastric corpus [41]. In addition, only the total gastrin level, including gastrin-17, can be assessed in the domestic ELISA kit in Japan. Furthermore, the titer of PG evaluated in each country is not always identical between the Western and Eastern method because the ELISA kit is not identical between the two countries [42].

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Chapter 7
Gastric Cancer Risk Prediction Using Epigenetic Alterations Accumulated in Noncancerous Gastric Tissues

Masahiro Maeda, Harumi Yamada, Hiroshi Moro, and Toshikazu Ushijima

Abstract Risk prediction for gastric cancer (GC) is important, especially for *H. pylori*-eradicated individuals whose number is rapidly increasing in Japan. For accurate cancer risk prediction, analysis of epigenetic changes, particularly aberrant DNA methylations, has a great potential. It is induced in the gastric mucosa by *H. pylori* infection, persists for life, and is causally involved in gastric carcinogenesis. The DNA methylation levels in individuals without current *H. pylori* infection correlate with GC risk and have a greater impact than that of accumulated point mutations. A methylation marker is necessary to assess the overall epigenomic damage accumulated in the genome of gastric epithelial cells. Initially, CpG islands methylated in GC cells were used. More informative markers were then isolated by an analysis of the gastric mucosa of gastric cancer patients and healthy individuals. Finally, highly informative markers unaffected by contaminating blood cells have been developed using an advanced technology and a screening algorithm. With an aim of bringing epigenetic cancer risk diagnosis into practice, we first conducted a multicenter prospective cohort study for risk prediction of metachronous GC among GC patients who had undergone endoscopic treatment and achieved the first proof of concept. We are currently conducting a new, nationwide study for risk prediction of primary GC among healthy *H. pylori*-eradicated individuals. Epigenetic cancer risk diagnosis, which was initially developed for GC and potentially applicable to other inflammation-associated cancers, has a great potential to contribute to precision medicine.

Keywords Epigenetics · Cancer risk · DNA methylation · Inflammation · Field cancerization

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7.1 Introduction

Gastric cancer prevention has been tackled in many countries, and, for this, *Helicobacter pylori* (*H. pylori*) eradication is critically important [1]. In Japan, as a national policy, the national health insurance coverage for *H. pylori* eradication therapy was extended to chronic gastritis for prevention of gastric cancer, and this change is now leading to a paradigm shift in the epidemiology of gastric cancer. *Helicobacter pylori* eradication is expected to result in a decreased incidence and an altered spectrum of gastric cancer. At the same time, this new policy has led to a marked increase in the number of individuals who had undergone *H. pylori* eradication therapy, who are recommended to have periodical surveillance because gastric cancer can develop even after *H. pylori* eradication [2]. Therefore, such individuals may suffer from anxiety and have to bear the cost of X-ray or endoscopic examinations, which is also a social burden. Thus, a risk stratification system is necessary.

However, to date, there are no established biomarkers for gastric cancer risk prediction among healthy individuals after *H. pylori* eradication therapy. Genetic polymorphisms associated with enhanced host inflammatory response to *H. pylori* infection [3] or cell proliferation [4] have been well studied to predict gastric cancer risk. Nevertheless, the predictive power of these markers has been shown to be insufficient so far. *Helicobacter pylori*-associated mucosal changes, such as gastric atrophy, have also been known to be associated with the development of gastric cancer [5]. However, most individuals infected with *H. pylori* suffer from gastric atrophy, and endoscopic examinations offer insufficient screening. Further, pepsinogen test combined with anti-*H. pylori* antibody test has a great advantage of being noninvasive, but its validity in healthy individuals that have undergone *H. pylori* eradication therapy is unknown at present.

As a potential biomarker to overcome these shortcomings, epigenetic alteration, namely, aberrant DNA methylation, accumulated in gastric mucosa has been highlighted. Cross-sectional studies showed close correlations between levels of DNA methylation and gastric cancer risk in individuals without current *H. pylori* infection [6, 7]. To assess the levels of DNA methylation in gastric mucosa, the use of appropriate epigenetic markers reflecting overall epigenomic damage is important [8]. In a multicenter prospective cohort study using such markers [9, 10], the utility of epigenetic cancer risk prediction was successfully demonstrated for metachronous gastric cancer in gastric cancer patients who had undergone endoscopic treatment [11, 12]. Also, using more informative methylation markers [13], we have launched a novel nationwide multicenter prospective cohort study (UMIN000016894) for predicting a risk of developing a primary gastric cancer in healthy individuals that have undergone *H. pylori* eradication therapy.

In this chapter, we first briefly explain DNA methylation. We then introduce risk prediction for gastric cancer using epigenetic alterations accumulated in the gastric mucosa, emphasizing on isolation of informative epigenetic markers associated with risk and two multicenter prospective cohort studies regarding risk prediction for gastric cancer.

7.2 Aberrant DNA Methylation and Gastric Cancer

Epigenetics refers to the study of the gene expression and genome structure maintained through multiple somatic cell divisions without changes in gene sequences. Epigenetics plays a central role in differentiation, in which diverse and stable cell types are produced, and also in reprogramming [14]. DNA methylation, the covalent addition of a methyl group to the five position of a cytosine in a CpG site (Fig. 7.1a), is a key epigenetic modification. The methylated status of a CpG site is

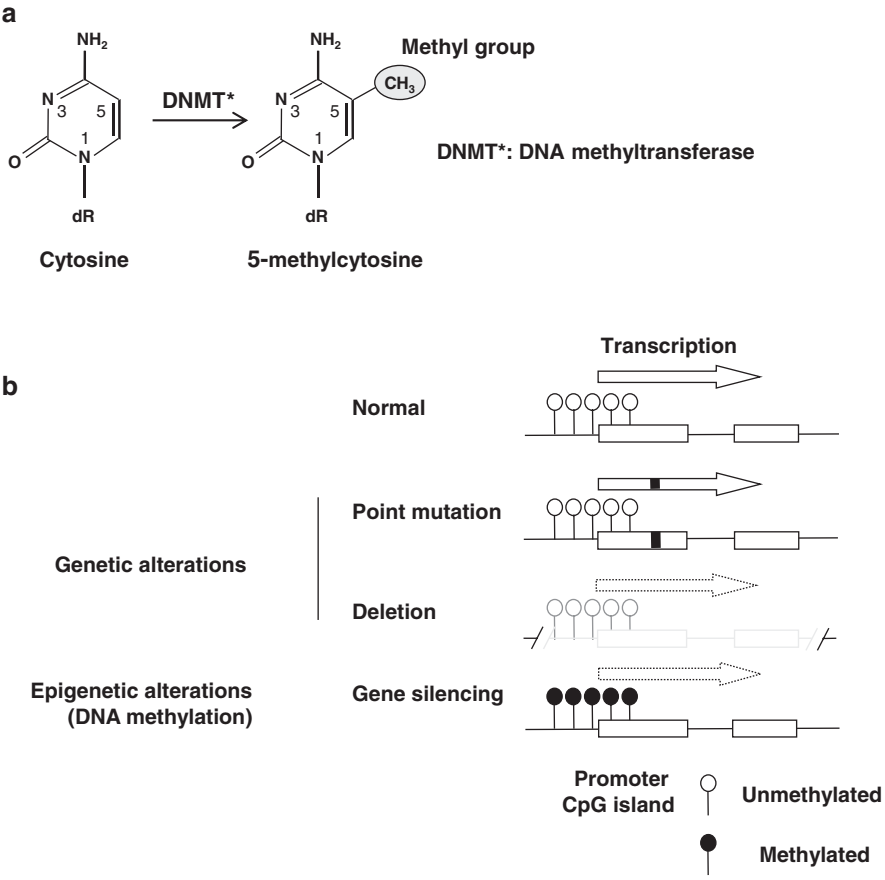

Fig. 7.1 DNA methylation and its biological significance. (**a**) DNA methylation in epigenetics refers to the covalent addition of a methyl group to the five position of a cytosine. DNA methylation takes place at two cytosine residues (both strands) in a CpG site, and the methylated status is maintained by DNA methyltransferases (DNMTs) through DNA synthesis during cell division. (**b**) Three modes of inactivation of a gene with a promoter CpG island. Genetic alterations, such as a point mutation and genomic deletion, can inactivate a gene by altering the protein function or elimination of its transcript. At the same time, aberrant DNA methylation of the promoter CpG island causes the loss of transcription of the downstream gene (gene silencing)

maintained by DNA methyltransferases (DNMTs), even after DNA synthesis during cell division. DNA methylation at a promoter CpG island can strongly repress transcription of the downstream gene (gene silencing) (Fig. 7.1b).

Epigenetic abnormalities, especially aberrant DNA methylation of promoter CpG islands, are involved in diverse types of cancers and play a crucial role in gastric cancer [15]. This crucial role was proposed based on the higher frequencies of inactivation of tumor-suppressor genes by aberrant DNA methylation than those by mutations [16]. A recent integrated analysis of genetic and epigenetic alterations in gastric cancer supported this [17]. Specifically, inactivation of tumor-suppressor genes and activation of the WNT pathway were caused by aberrant DNA methylation more frequently than by mutations. In addition, other comprehensive studies involving exome and whole-genome sequencing could identify only a limited number of driver mutations besides *TP53* and *CDH1* [18, 19]. These studies indicated the major role of aberrant DNA methylation in gastric carcinogenesis.

Aberrant DNA methylation in gastric mucosa is induced by *H. pylori* infection [20]. Animal studies showed that not *H. pylori* itself but the resultant inflammation is critical for the induction of methylation [20] and that a specific type of inflammation characterized by persistent infiltration of monocytes and macrophages with residual neutrophils is important [21]. Notably, administration of a DNA demethylating agent, 5-aza-2′-deoxycytidine (5-aza-dC), to *H. pylori*-infected animals treated with a mutagen, *N*-methyl-*N*-nitrosourea (MNU), reduced the incidence of gastric cancers by half, coupled with decreased methylation levels. This clearly showed that aberrant DNA methylation is causally involved in gastric carcinogenesis and indicated that a DNA demethylating agent has a preventive effect against gastric cancer [22]. Taken together, aberrant DNA methylation is induced in the gastric mucosa by chronic inflammation triggered by *H. pylori* infection and is causally involved in gastric carcinogenesis.

7.3 Close Correlation Between DNA Methylation in the Gastric Mucosa and Gastric Cancer Risk

Aberrant DNA methylation is one of the important causes of gastric cancer, and as expected, its accumulation levels in the gastric mucosa are correlated with cancer risk [6, 7, 23, 24] (Fig. 7.2). The extent of aberrant DNA methylation in the gastric mucosa is determined by duration of infection [25], *H. pylori* strains [26], and, possibly, host inflammatory responses [27]. After *H. pylori* eradication therapy, DNA methylation levels decrease in a gene-specific manner, and residual methylation persists for a long time [28–30]. Importantly, cross-sectional studies showed that, among individuals without current *H. pylori* infection, gastric cancer patients, who have a high risk of a subsequent metachronous gastric cancer [31], have higher residual methylation levels than those in healthy individuals [6, 23, 24]. Additionally, patients with multiple gastric cancers, who are considered to have a very high risk of a subsequent metachronous gastric cancer [32], showed higher residual DNA

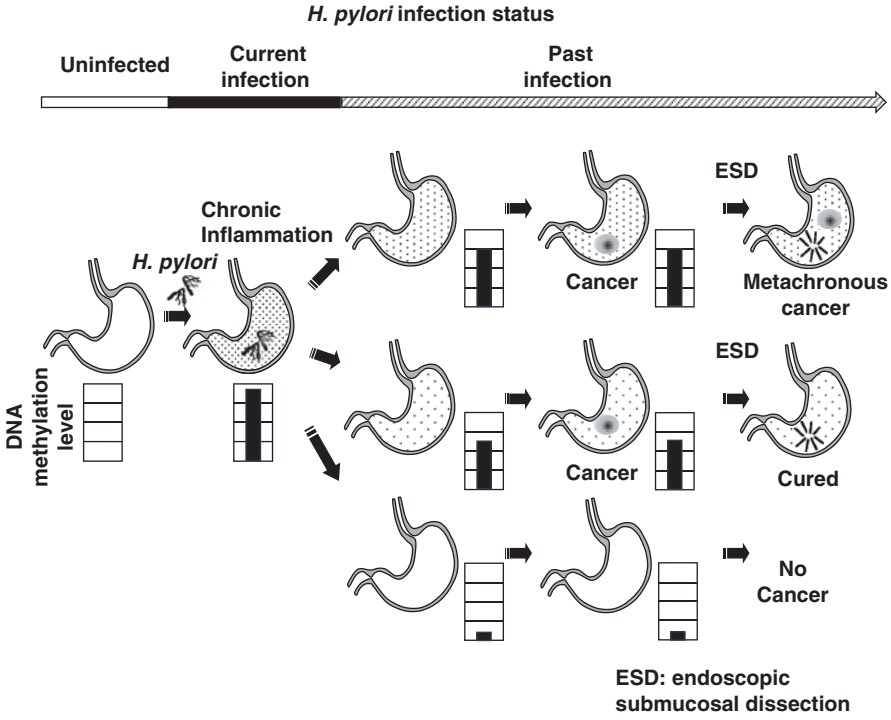

Fig. 7.2 DNA methylation levels in the gastric mucosa and gastric cancer risk, in relation to a clinical course of *H. pylori* infection. After establishment of *H. pylori* infection in early childhood, *H. pylori*-triggered chronic inflammation induces aberrant DNA methylation in gastric mucosa. Once *H. pylori* is eradicated, DNA methylation level decreases in a gene-specific manner. Residual levels of aberrant DNA methylation after inflammation subsides are correlated with gastric cancer risk. For example, individuals with the highest residual methylation levels tend to develop a gastric cancer and to develop a metachronous gastric cancer even after curative treatment of the initial cancer by an endoscopy [43]

methylation levels than those in patients with a single cancer [7] (Fig. 7.2). These studies strongly indicated that accumulation of aberrant DNA methylation in the gastric mucosa is closely associated with gastric cancer risk, producing "an epigenetic field defect" or "epigenetic field for cancerization" [33].

Even when compared with point mutations accumulated in the gastric mucosa, epigenetic alterations have recently been shown to have a higher impact on gastric cancer risk [34]. To quantify rare point mutations in normal tissues, such as those at the level of 10^{-5} per base pair, we developed a novel method [35]. Using this method, we quantified point mutation frequencies and degree of aberrant DNA methylation in the gastric mucosa of individuals at different risk levels and analyzed their relative impact on cancer risk. Surprisingly, gastric cancer patients showed no significant increase in point mutation frequencies compared with those in healthy individuals. In contrast, DNA methylation levels were much higher in gastric cancer patients than those in healthy individuals. As a result, aberrant DNA methylation

was considered to have a 2.3-fold higher impact on gastric cancer risk [34]. To support the validity of the analysis, individuals with a history of *H. pylori* infection had a mutation signature of activation-induced cytidine deaminase (AID) [36], and the impacts of point mutations and aberrant DNA methylation were similar on the risk of esophageal squamous cell carcinomas [34].

7.4 Identification of Methylation Markers for Cancer Risk

Informative markers that reflect overall epigenomic damage are required for precise estimation of cancer risk. In 2006, we utilized CpG islands methylated in gastric cancers [37] to find a correlation between DNA methylation levels and gastric cancer risk [6], albeit mostly with relatively low odds ratios. In 2010, we enhanced the marker isolation strategy by directly comparing noncancerous mucosa of healthy individuals and gastric cancer patients. We identified DNA methylation markers with sufficiently high odds ratios (12.7–36.0) by using MeDIP-on-chip [9], along with a promising one isolated by a traditional method [10]. These DNA methylation markers were used in the multicenter prospective cohort study for risk prediction of metachronous gastric cancer [11, 12].

Recently, we further enhanced the isolation strategy by (1) adopting a state-of-the-art technology for epigenetic analysis, namely, Infinium bead array; (2) using a larger number of samples; (3) adopting a novel statistical algorithm, iEVORA [38, 39]; and (4) paying attention to contaminating cell types other than gastric epithelial cells. We have successfully isolated nine additional DNA methylation markers optimized for risk prediction of gastric cancer in healthy individuals who has undergone *H. pylori* eradication therapy [13]. The novel markers were unlikely to be affected by contaminating blood cells and showed sufficient performances (AUC, 0.70–0.80) with high odds ratios (5.43–23.41). Some of the markers were superior to a previously used marker, *miR-124a-3*, for the multicenter prospective cohort study for risk prediction of metachronous gastric cancer (Fig. 7.3). Additionally, subgroup analysis showed that DNA methylation levels of the novel markers were associated with gastric cancer risk independent of gastric atrophy, gender, or age.

All the above DNA methylation markers proved to be informative for estimation of gastric cancer risk to varying degrees. Also, their methylation levels were highly correlated with each other. In addition, almost all of the newly identified marker genes had very low expression levels in the normal gastric mucosa irrespective of *H. pylori* infection. Generally, genes having low expression levels are known to be susceptible to aberrant DNA methylation [40–42]. All of these indicated that aberrant DNA methylation of the novel marker genes was unlikely to be involved in gastric carcinogenesis but to be passengers [13].Their good performance in risk prediction could be explained by their high susceptibility to aberrant DNA methylation and the resultant association with overall epigenomic damage caused by *H. pylori* infection, rather than their gene functions in gastric carcinogenesis.

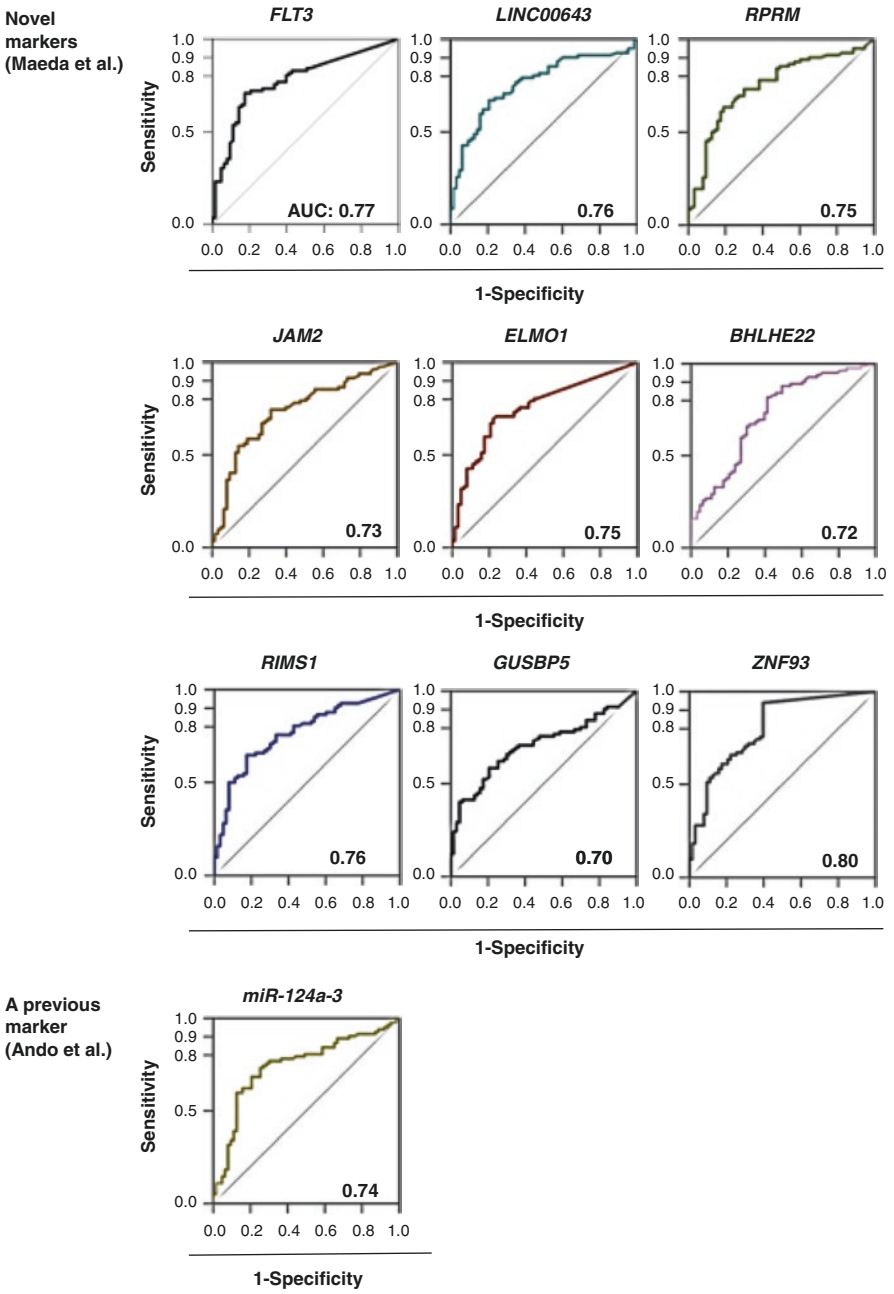

Fig. 7.3 A high predictive power of methylation markers for gastric cancer risk prediction. ROC curves of nine novel methylation markers and a previous marker, *miR-124a-3*, are shown. AUC values are also shown in each panel. Some of the novel methylation markers showed higher AUCs than *miR-124a-3*. *ROC* receiver operating characteristic, *AUC* area under the curve [13]

7.5 Two Multicenter Prospective Cohort Studies for the Cancer Risk Prediction

To bring a novel system for cancer risk prediction using accumulation of aberrant DNA methylation into practice, we planned a multicenter prospective cohort study in 2007. At that time, two study designs were considered: (1) risk prediction of metachronous gastric cancer in gastric cancer patients curatively treated with endoscopy and (2) risk prediction of primary gastric cancer in *H. pylori*-eradicated healthy individuals.

7.5.1 A Prospective Cohort Study for Risk Prediction of Metachronous Gastric Cancer

Risk prediction of metachronous gastric cancer in gastric cancer patients was considered to be a most feasible choice in 2007. To obtain a significant result based on the odds ratios calculated from our cross-sectional studies, a sufficient number of events (development of metachronous gastric cancer) within a 5-year follow-up were likely to be achieved owing to the high incidence (approximately 2% per year) of metachronous gastric cancer [31]. Although risk prediction among cancer patients is a very difficult task and a positive result may not change clinical practice, we believed that clinical demonstration of the utility of epigenetic cancer risk diagnosis would have great scientific and future clinical value.

In the study, 826 gastric cancer patients without current *H. pylori* infection and after endoscopic submucosal dissection (ESD) were enrolled. An endoscopic biopsy was taken from a fixed point in the antrum of the stomach, and methylation levels of three preselected marker genes (*miR-124a-3*, *EMX1*, and *NKX6-1*) [9, 10] were assessed. Annual follow-up to detect a metachronous gastric cancer was conducted as practice by trained endoscopists blind to methylation information. A total of 795 patients who received at least one follow-up (median observation period, 5.46 years) were classified into quartiles according to the methylation levels of individual markers at the time point of enrollment, and cumulative incidences of metachronous gastric cancer and relative risk were analyzed in the quartiles. Kaplan-Meier analysis showed much higher cumulative incidence of metachronous gastric cancer in the highest quartile (Q4) compared with that in the lowest quartile (Q1) (Fig. 7.4). Multivariate analysis adjusting for hospital, gender, age, *H. pylori* infection before enrollment, pepsinogen index, past history of endoscopic resection, smoking, and green vegetable intake showed that the highest quartile of the methylation level of *miR-124a-3* showed a threefold increase of hazard ratio in developing a metachronous gastric cancer compared with that of the lowest quartile [11, 12].

This was the first multicenter prospective cohort study to achieve the proof of concept of cancer risk prediction using epigenetic markers. However, even the quartile with the lowest methylation levels showed approximately 10% of 5-year cumu-

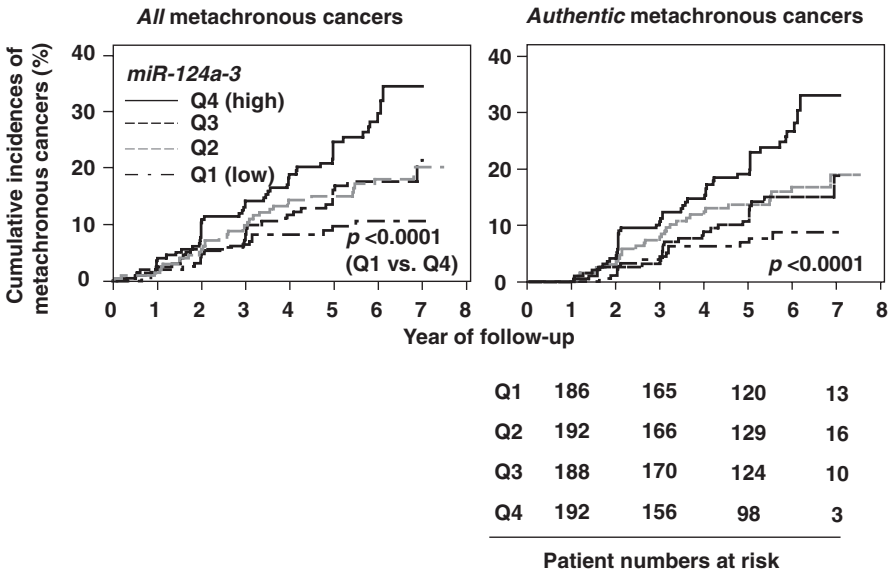

Fig. 7.4 Cancer risk stratification using an epigenetic marker shown by a multicenter prospective cohort study. Cumulative incidences of metachronous gastric cancers are plotted for quartiles of methylation levels (Q1–Q4). Authentic metachronous cancers were defined as those that developed 1 year after the enrollment to exclude the overlooked cases. The Q4 (highest) had a much higher cumulative incidence than the Q1 (lowest) [12]

lative incidence of metachronous gastric cancer (Fig. 7.4). Therefore, this high risk inherent to the cohort impeded a change in the current clinical practice.

7.5.2 A Multicenter Prospective Cohort Study for the Risk Prediction of Primary Gastric Cancer

In 2013, national health insurance coverage for *H. pylori* eradication therapy to treat *H. pylori*-associated chronic gastritis, particularly for prevention of gastric cancer, became available in Japan. This enabled us to recruit a large number of individuals that have undergone *H. pylori* eradication therapy to a multicenter prospective cohort study for risk prediction of primary gastric cancer. Thus, using the highly informative novel markers described above [13], we have launched a new nationwide multicenter prospective cohort study for risk prediction of primary gastric cancer in *H. pylori*-eradicated healthy individuals (UMIN000016894). In this study, 2000 individuals that have undergone *H. pylori* eradication therapy with open-type gastric mucosal atrophy will be enrolled. Most of the participants have very low methylation levels in the gastric mucosa and are expected to have a low risk of gastric cancer despite the presence of gastric atrophy. This study is expected to identify individuals who have

a high risk of developing gastric cancer and personalize surveillance intervals for screening of gastric cancer. This will lead to a reduction in the burdens of both the individual and the society and to a contribution to the national health insurance.

7.6 Conclusions

Epigenetic cancer risk prediction will soon be a clinical practice. The research on the epigenetic cancer risk prediction is primarily focused on the gastric cancer owing to its epigenetic nature. However, the risk prediction methods can be applicable to other types of cancers, particularly to inflammation-associated cancers. Epigenetic changes are imprinted in a normal tissue as a memory of past irreversible damage to the tissue and thus can contribute greatly to a precise cancer risk prediction technique.

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Compliance with Ethical Standards

Conflict of Interest: The authors (MM and TU) made a joint patent application with Sysmex Corporation for identified epigenetic markers.

Ethical Standards: This article does not contain any studies with human or animal subjects performed by any of the authors.

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Chapter 8
Gastric Cancer Screening in Japan

Shigemi Nakajima

Abstract The past, present, and future of gastric cancer screening in Japan are introduced. Gastric cancer screening was started with barium X-ray examination (upper gastrointestinal series, UGIS) in 1950s. The main characteristic is the double-contrast method with barium sulfate and carbon dioxide gas. Those who are suspected of having gastric cancer are recommended further examination with endoscopy. The effect of UGIS screening on the death of gastric cancer is significant. Recently those who are suspected with *H. pylori* infection are diagnosed with UGIS and recommended endoscopic examination followed by eradication therapy. Because UGIS has problems such as X-ray exposure, it should be updated with risk evaluation to reduce X-ray exposure and the cost for low-risk subjects. On the other hand, endoscopic screening was started in 1980s. The effect of endoscopic screening on the death of gastric cancer was also significant, and the effect was superior to that of UGIS screening. However, endoscopic screening has some problems to perform in a large population such as cost and capacity. Since *H. pylori* infection rate and gastric cancer death rate are decreasing, gastric cancer screening with an image test (UGIS or endoscopy) should be updated to include gastric cancer risk evaluation to be more efficient. Serum tests have merits to screen high-risk subjects for gastric cancer, but the tests are not perfect to rule out 100% gastric cancers. We have to know the limitations. A combination of an image test and serum tests may be useful for covering the weakness of each other.

Keywords Gastric cancer · Screening · Barium · X-ray · UGIS · Endoscopy · *Helicobacter pylori* · Risk evaluation · Serum test

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8.1 Introduction

Gastric cancer was first among all neoplasms as to the cause of cancer death and one of the main social problems in Japanese community; gastric cancer screening started from the 1950s in Japan [1]. Since then gastric cancer screening has been widely performed in Japan. At first, the method of gastric cancer screening was barium X-ray examination (upper gastrointestinal series, UGIS). Endoscopic examination was adopted in the 1980s, and the number of participants gradually increased. However, the main method is still UGIS in population-based screening even now. One of the main characteristics of this method of UGIS is called the double-contrast method with barium sulfate and carbon dioxide gas [2, 3]. This method has been established in Japan and is still used today. Those who are suspected of having gastric cancer with UGIS are recommended to undergo further examination with endoscopy. Because population-adjusted gastric cancer death rate has been decreasing in Japan (http://ganjoho.jp/reg_stat/statistics/stat/annual.html), gastric cancer screenings may have contributed partly to the decrease. Recently, *Helicobacter pylori* infection, one of the main causes of gastric cancer, is considered in gastric cancer screening [4]. In this chapter, I will explain Japanese gastric cancer screening until now and in the near future.

8.2 A Brief History of Gastric Cancer Screening in Japan

In the beginning, gastric cancer screening was started with UGIS in some facilities in Japan in 1950s [1]. Automobiles or buses equipped with an indirect X-ray photography machine were used in rural area in 1960s. Since the double-contrast method has been established in 1963 by Shirakabe [2] and Ichikawa [3], this method has gradually permeated Japan. In 1983, the Elderly Health Law was enacted, and local governments had to perform gastric cancer screening for elderly people (40 years or older) in the communities with the cost supported by health insurance funds. Since then UGIS has been widely adopted all over Japan for screening gastric cancer in medical facilities or buses equipped with X-ray photofluorography machine. Progresses in photofluorography machines have contributed to the spreading of gastric cancer screening with UGIS.

Although gastric cancer screening began without enough evidence to reduce gastric cancer death, the efficacy was later approved in the Japanese guidelines for gastric cancer screening in 2005 by a research group funded by the government [5]. In 2014 the evidence was further confirmed with meta-analyses of case-control studies and cohort studies (Table 8.1) [6]. Endoscopic examination was also approved as an effective gastric cancer screening method to decrease gastric cancer death (Table 8.2) [6, 7]. Because Japan has one of the world's leading gastric cancer death rate, gastric cancer screening with either UGIS or endoscopy is effective to reduce gastric cancer death. However, gastric cancer screening may not be always

Table 8.1 Effect of UGIS screening on the death of gastric cancer, meta-analyses

	Index	value	95% CI
Case-control studies	Odds ratio	0.52	0.35–0.76
Cohort studies	Hazard ratio	0.60	0.50–0.73

From: Gastric Cancer Screening Evidence Report 2014 *(in Japanese)*. http://canscreen.ncc.go.jp/pdf/iganguide1501.pdf

Table 8.2 Mortality reduction with endoscopic screening in Japan

Year	Author	Indicators	Mortality reduction	95%CI	Cities
2007	Matsumoto et al.	SMR	Male 0.71	0.33–1.10	
			Female 0.62	0.19–1.05	
2011	Hosokawa et al.	Adjusted HR	0.23	0.07–0.76	Fukui
2015	Hamashima et al.	SMR	0.43	0.30–0.57	Niigata
2015	Hamashima et al.	RR	0.327	0.118–0.908	Tottori and Yonago

SMR standard mortality ratio, *HR* hazard ratio compared with UGIS, *RR* relative risk compared with the subjects screened by radiography, adjusted by sex, age group, and resident city
Data from: Hamashima C. World J Gastroenterol 2016 [7]

efficient if gastric cancer death rate is not as high as in Japan. Recently *H. pylori* has been recognized as one of the main causes of gastric cancer [4]. Fortunately *H. pylori* infection rate has already been decreasing in Japan [8], and along with this the adjusted death rate of gastric cancer is decreasing. We should modify gastric cancer screening method to be more efficient in the very near future when *H. pylori* infection has further decreased.

8.3 Facts on Gastric Cancer Screening in Japan

In the recent report from the Japanese Society of Gastrointestinal Cancer Screening [9], the facts on gastric cancer screening were summarized. Gastric cancer detection rate with UGIS and endoscopy was 0.075% and 0.19%, respectively (Tables 8.3 and 8.4). The rate of early gastric cancer was 74.2% and 63.2% with UGIS and endoscopy, respectively (Tables 8.4 and 8.5). The accuracy data on UGIS and endoscopy are summarized in Table 8.6 [5, 10]. There is no significant difference in sensitivity and specificity between UGIS and endoscopy so far [10]. The efficacy of gastric cancer screening with UGIS on the reduction of gastric cancer death was significant by two meta-analyses (Table 8.1) [6]. The efficacy of gastric cancer screening with endoscopy on the reduction of gastric cancer death was summarized by Hamashima (Table 8.2) [7]. Recent three studies on endoscopic screening showed the significant efficacy on the death of gastric cancer. In addition two of them showed significant superiority of endoscopy to UGIS. Thus endoscopic gastric cancer screening is promising to reduce gastric cancer death.

Table 8.3 Facts on gastric cancer screening with UGIS in Japan (2014)

		Formula	Total	Male	Female
A	Number of subjects	A	6,682,592	3,353,273	2,511,028
B	Required further examination	B	428,083	243,986	138,980
C	Recall rate (%)	B/A	6.41	7.28	5.53
D	Underwent further examination	D	269,622	150,981	105,272
E	Rate of further examinees (%)	D/B	63.0	61.9	75.8
F	Subjects with gastric cancer	F	5041	3613	1245
G	Detecting rate of gastric cancer (%)	F/A	0.075	0.108	0.050

Data from: Annual Report of Gastrointestinal Cancer Screening 2014. The Japanese Society of Gastrointestinal Cancer Screening [9]

Table 8.4 Facts on gastric cancer screening with endoscopy in Japan (2014)

		Formula	Total	Male	Female
A	Number of subjects	A	541,243	305,067	236,176
B	Subjects with gastric cancer	B	1039		
C	Cancer detecting rate (%)	B/A	0.19		
D	Early gastric cancer	D	657		
E	Rate of early cancer (%)	D/B	63.2%		

Data from: Annual Report of Gastrointestinal Cancer Screening 2014. The Japanese Society of Gastrointestinal Cancer Screening [9]

Table 8.5 Depth of invasion of UGIS-detected gastric cancer (2014)

Depth of invasion	M	SM	MP	SS	SE	SI	total
Number of subjects	1731	864	301	323	253	29	3501
Rate of subjects (%)	49.5	24.7	8.6	9.2	7.2	0.8	100
Early or advanced	M + SM		MP + SS + SE + SI				Total
Number of subjects	2595		906				3501
Rate of subjects (%)	74.2		25.8				100

M mucosa, *SM* submucosa, *MP* muscularis propria, *SS* subserosa, *SE* serosa, *SI* invasion to the adjacent structures
Data from: Annual Report of Gastrointestinal Cancer Screening 2014. The Japanese Society of Gastrointestinal Cancer Screening [9]

However, endoscopic screening has some demerits such as discomfort, adverse effects, infection, false-positive and negative results, overdiagnosis, shortness of medical resources, and higher cost [6, 7, 11]. On the other hand, demerits of UGIS include X-ray exposure, adverse events, misdiagnosis, decrease in medical doctors who can diagnose UGIS images, and necessity of endoscopy when gastric cancer was suspected [6, 11]. Since *H. pylori* prevalence and adjusted gastric cancer death rate have been decreasing now in Japan, indiscriminate gastric cancer screening may not be always efficient. The methods of gastric cancer screening should be updated to be more efficient according to the risk of gastric cancer such as *H. pylori* infection.

Table 8.6 Accuracy of gastric cancer screening

Accuracy of UGIS screening[a]	Percent
Sensitivity	56.8–88.5
Specificity	81.3–92.0
Positive predictive value	0.78–2.00

Subject group[b]	Method	Sensitivity	95% CI	Specificity	95% CI
Prevalence screening	UGIS	0.893	0.718–0.977	0.856	0.846–0.865
	Endoscopy	0.955	0.875–0.991	0.851	0.843–0.859
Incidence screening	UGIS	0.885	0.664–0.972	0.891	0.885–0.896
	Endoscopy	0.977	0.919–0.997	0.888	0.883–0.892

[a]Accuracy indices of UGIS form the seven studies summarized in the previous Japanese guideline: Hamashima C, et al. Jpn J Clin Oncol 2008 [5]

[b]Accuracy indices were compared between UGIS and endoscopy in Yonago, Japan. The prevalence screening group was defined as including persons who had no screening over 2 years earlier and those who were being screened for the first time. The incidence screening group was defined as including persons who were screened by the same method 1 year earlier. In both groups, the sensitivity seems greater in endoscopy than in UGIS, but there was not significant difference. Specificity was not different. Data from Hamashima C, et al. Int J Cancer, 2013 [10]

8.4 Double-Contrast Methods in UGIS

Double-contrast method in UGIS was established by Shirakabe [2] and Ichikawa [3]. The principle of this method is a simultaneous use of two different contrast media: one is barium sulfate as a positive-contrast medium and the other is carbon dioxide gas as a negative-contrast medium. A thin barium layer on the surface of the mucosa is visualized into an X-ray image of the surface of the stomach which is inflated with carbon dioxide gas (Fig. 8.1). Using a single-contrast medium only visualizes silhouettes of the stomach, but using the two contrast media visualizes not only silhouettes but also surface patterns of the mucosa and folds of the stomach. Thus the double-contrast method visualizes gastric cancers more clearly than single-contrast methods (Figs. 8.2 and 8.3). This method also visualizes cancers which were not detected with silhouettes only (Figs. 8.4 and 8.5). In addition, it also enables us to diagnose nonmalignant diseases such as chronic gastritis, polyps, peptic ulcers, submucosal tumors, etc. Recently gastric cancer risk can be evaluated with double-contrast UGIS through diagnosis of background gastric mucosa as described later.

Barium sulfate is not a uniform material and varies among suppliers. Barium sulfate products with low viscosity are commonly used with high concentrations near 200% (w/v) in recent Japanese gastric cancer screening. Sodium bicarbonate powder 3.5–5 g is used as a producer of carbon dioxide gas. Barium sulfate and sodium bicarbonate are safe and cheap, but very rarely the additives may be allergenic. Those who have stenosis in the intestine or diverticulum in the colon should be avoided or carefully be indicated.

X-ray photography was used in the early years, but later most of facilities were equipped with X-ray fluoroscopy/photography machine. The latter greatly contrib-

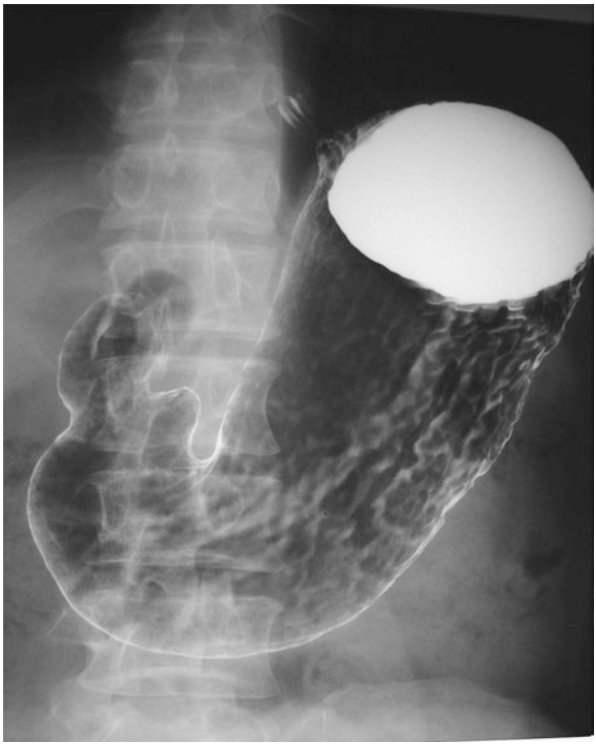

Fig. 8.1 A picture of *H. pylori-negative* stomach with double-contrast method

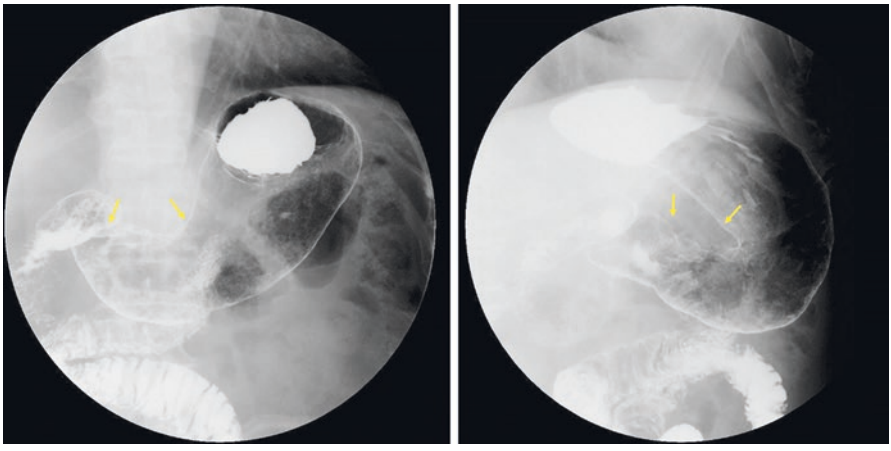

Fig. 8.2 Pictures of a case with advanced gastric cancer, double-contrast method. The wall of lesser curvature of the antrum shows irregular surface indicating invasive tumors at the part. Wall irregularity shows tumor invasion at least from the lower corpus to mid-antrum (between arrows). Resected stomach revealed poorly differentiated adenocarcinoma invading to subserosa

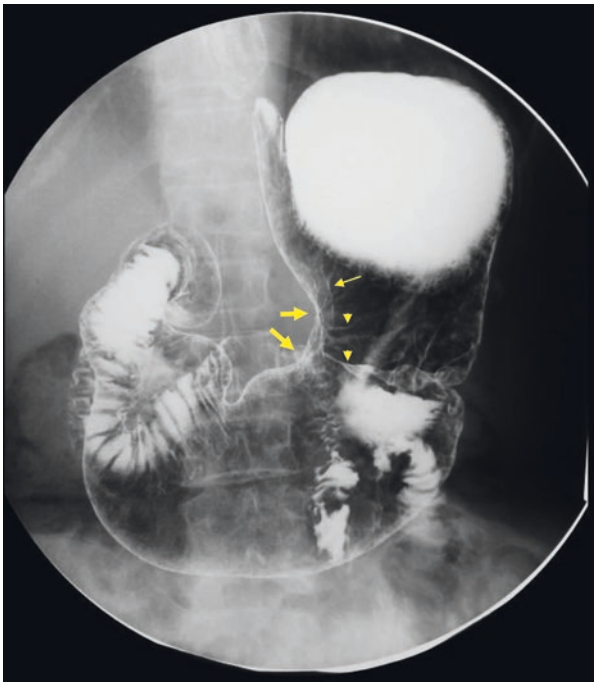

Fig. 8.3 A picture of a case with advanced gastric cancer, double-contrast method. The wall of lesser curvature of the middle to lower corpus protrudes into the lumen showing a tumor in the part (thick arrows). Parts of the surface of the tumor (thin arrow) and converging folds (arrowheads) are demonstrated with double-contrast method. Resected stomach showed poorly differentiated adenocarcinoma invading to subserosa

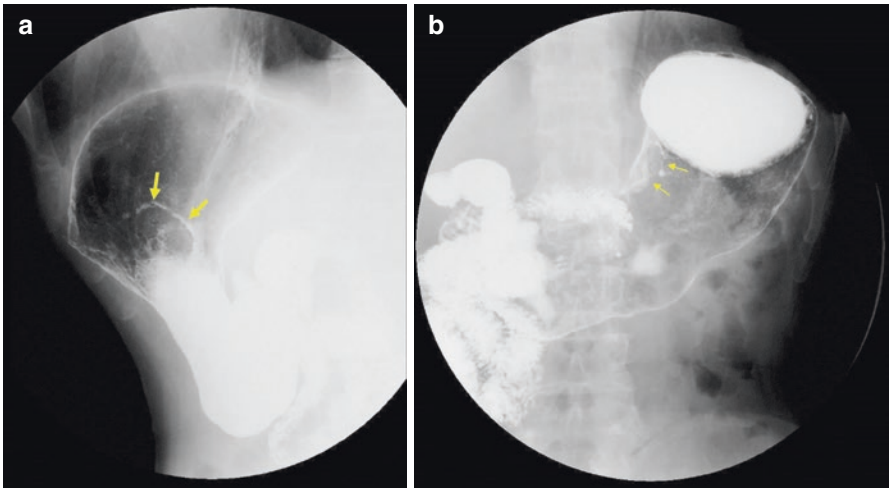

Fig. 8.4 A picture of a case with advanced gastric cancer with double-contrast method. A tumor is clearly demonstrated at the upper corpus of the stomach (**a**). Parts of the surface of the tumor are observed in the lessor curvature of the corpus (**b**). This tumor could not be demonstrated with the silhouettes only but demonstrated with double-contrast method. Resected stomach showed well differentiated tubular adenocarcinoma invading to muscularis propria

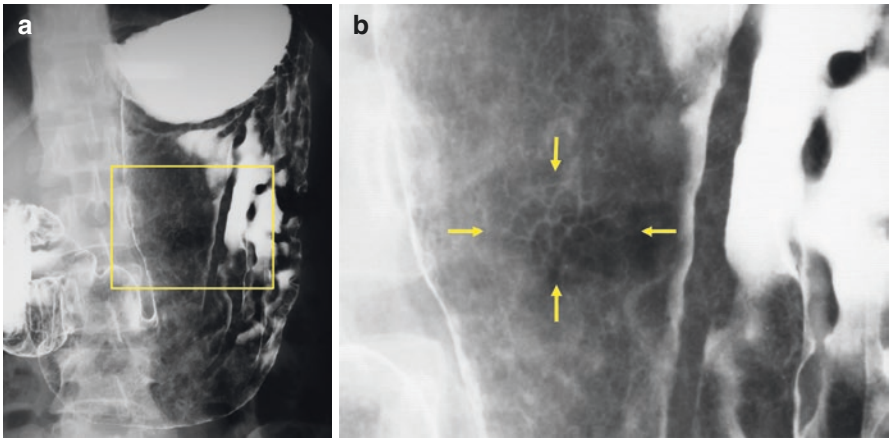

Fig. 8.5 A case of early gastric cancer in the lower corpus of the stomach, double-contrast method. The area enclosed in the square in the left picture (**a**) is enlarged into the right picture (**b**). A small mucosal area with cobblestone-like appearance is visible in the lower corpus of the stomach (arrows indicated). The lesion was resected with endoscopy (endoscopic submucosal resection, ESD) and revealed moderately differentiated tubular adenocarcinoma localized in the mucosa (T1a)

uted in the progress of gastric cancer screening in Japan. Recently most facilities are equipped with digital radiography system with or without flat panel image intensifier. These systems have been already used for telediagnosis where radiologists are lacking and may be used for automatic diagnosis in the near future.

8.5 Diagnosis of Background Gastric Mucosa with UGIS

Since *H. pylori* was discovered in 1983 as the main cause of chronic active gastritis [12], it has been considered carcinogenic [4]. In 1999 authors discovered the relationship between *H. pylori* infection and the images of the stomach in double-contrast UGIS [13, 14]. The essence and methods of diagnosing background gastric mucosa are abstracted in Figs. 8.6, 8.7, 8.8, 8.9, 8.10, 8.11, 8.12, 8.13, and 8.14 and Table 8.7 [14–17]. *H. pylori*-infected stomach is diagnosed as chronic gastritis with >95% sensitivity and specificity [18]. Thus UGIS can be used for not only gastric cancer screening but also gastric cancer risk evaluation. Yamamichi et al. [19] and Itoh et al. [20] reported that gastric cancer risk could be predicted with UGIS. In 2016 the Japanese Society of Gastrointestinal Cancer Screening have made a consensus that chronic gastritis should be diagnosed as a risk for gastric cancer when diagnosing UGIS images [21]. Because the majority of population is becoming *H. pylori*-negative in recent years in Japan [8], we have to discriminate subjects at high risk. Thus risk evaluation with UGIS may be useful to make gastric cancer screening more efficient. For example, we diagnose background gastric mucosa with UGIS and classify subjects into three groups: present, past, and *naïve H. pylori*

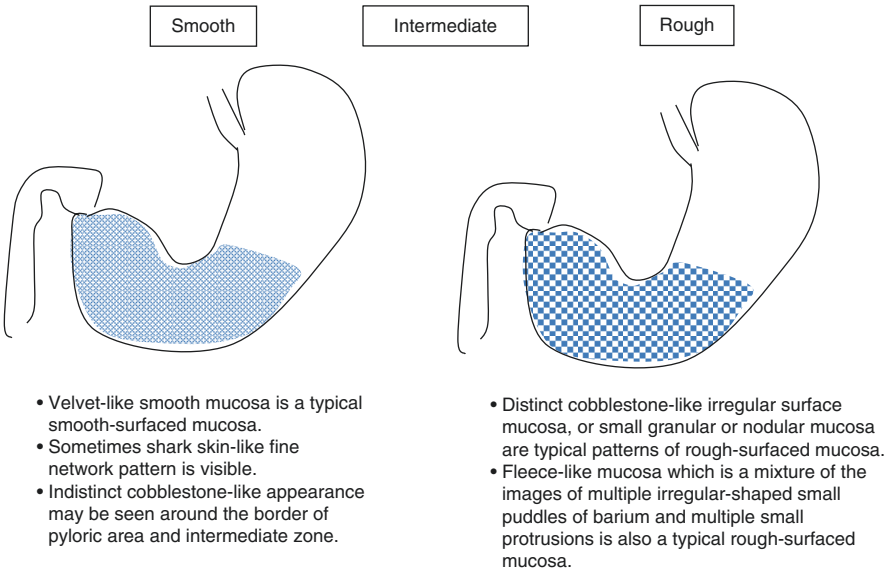

Fig. 8.6 Classification of gastric surface images. Modified from Nakajima S. In: Asaka M. (ed), Peptic Ulcer, Second Edition. Saishin Igaku Supplement, 2012 [15]

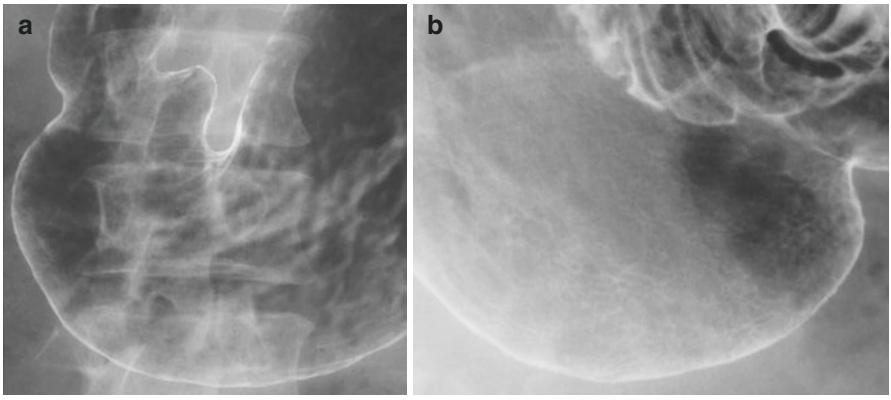

Fig. 8.7 Smooth-type gastric mucosa. (**a**) Velvet-like smooth-type gastric mucosa. Mucosa is non-structural at a glance, but a fine regular uniform pattern is visible in enlarged images. This is the typical smooth-type mucosa. (**b**) Shark skin-like gastric mucosa. A fine network-like pattern is seen. This pattern is sometimes seen in the antrum to angle of the stomach with velvet-like mucosa. This is one of the smooth-type mucosa. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

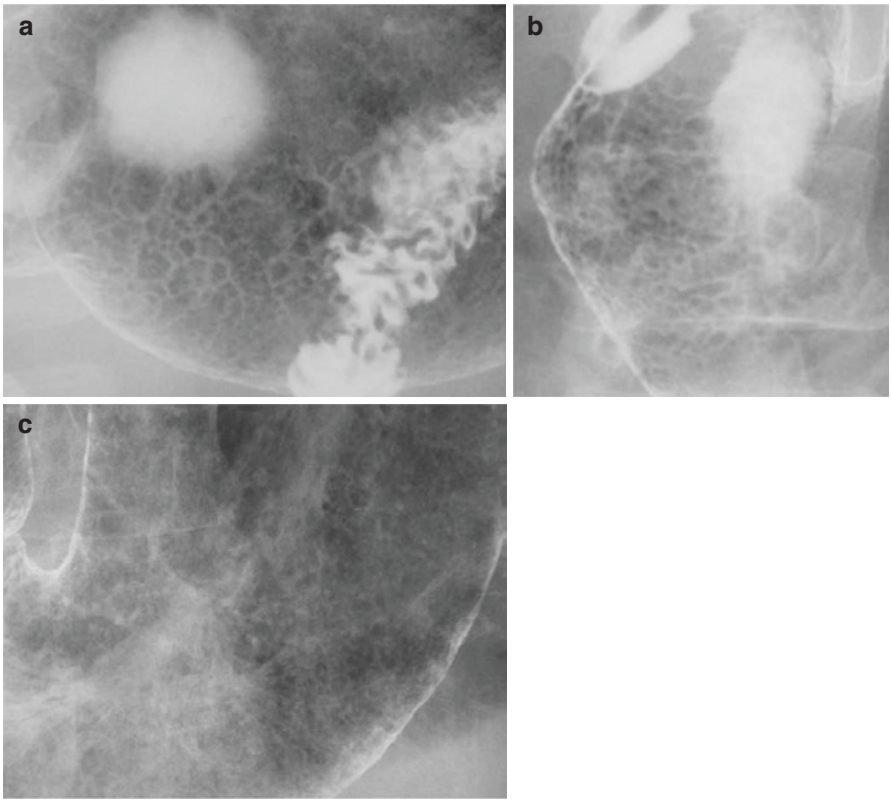

Fig. 8.8 Rough-surfaced mucosa. (**a**) Cobblestone-like mucosa. A distinct cobblestone-like mucosa is shown. This is one of the typical rough-surfaced mucosa in *H. pylori-infected* stomach. (**b**) Granular mucosa. Multiple irregular-shaped granular or nodular mucosa is seen. This is one of the typical rough-surfaced mucosa in *H. pylori-infected* stomach. (**c**) Fleece-like mucosa. This image consists of a mixture of multiple irregular-shaped small puddles of barium and multiple small protrusions. The images like this are called fleece-like mucosa. This is one of the typical rough-surfaced mucosa. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

infection. *H. pylori-naïve* subjects do not need further endoscopy nor next year screening, but those who have present infection need further evaluation with endoscopy and eradication therapy. Those who are diagnosed as past *H. pylori* infection are recommended annual gastric cancer screening. Thus the risk evaluation with UGIS enables us not only to select subjects who need endoscopy or annual surveillance but also to protect low-risk subjects from annual X-ray exposure and save money. Combination with UGIS and serum anti-*H. pylori* antibody test with or without pepsinogen test is considered as a more accurate risk evaluation, and the efficacy of the combination is now under the verification with a prospective study.

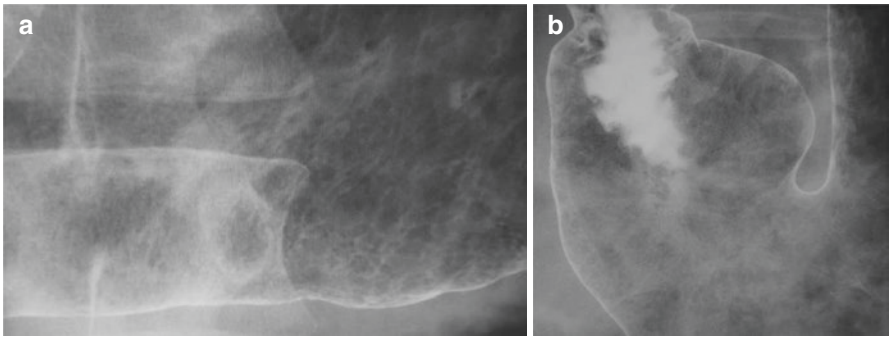

Fig. 8.9 Intermediate-type surface mucosa. (**a**) One of the intermediate-type mucosas. This image is from *H. pylori-eradicated* stomach. The image is not velvet-like nor typical rough-type. (**b**) Ground glass-like appearance. This image is from *H. pylori-eradicated* stomach. The mucosa is not velvet-like nor typical rough type. It looks like ground glass or as if it is covered with mist. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

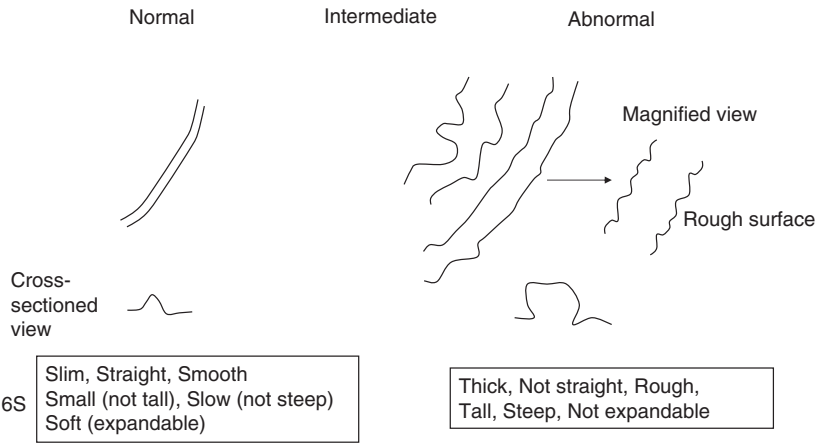

Fig. 8.10 Types of fold shape. Modified from: Nakajima S, et al. Jpn J Helicobacter Res, 2007 [14]

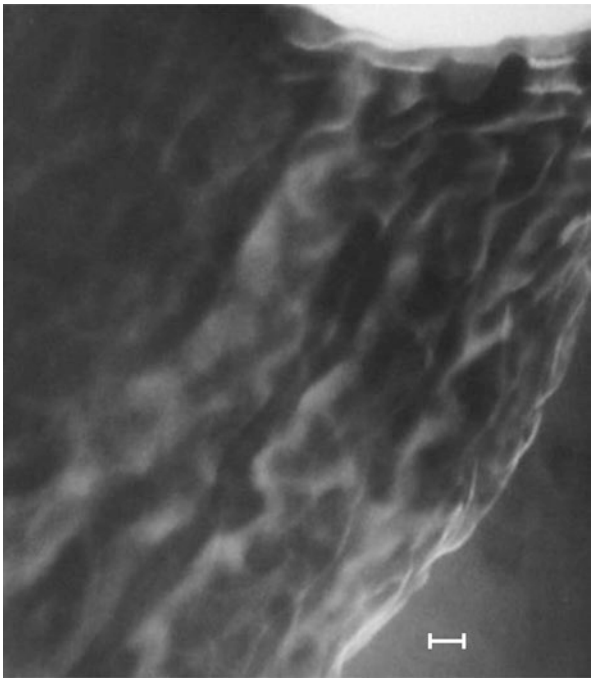

Fig. 8.11 Normal gastric folds. A case with normal folds. This is a picture *of H. pylori-negative* stomach. The folds are slim, straight, smooth, soft, small in height, and slow sloping. The findings satisfy 6S. The scale indicates 5 mm. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

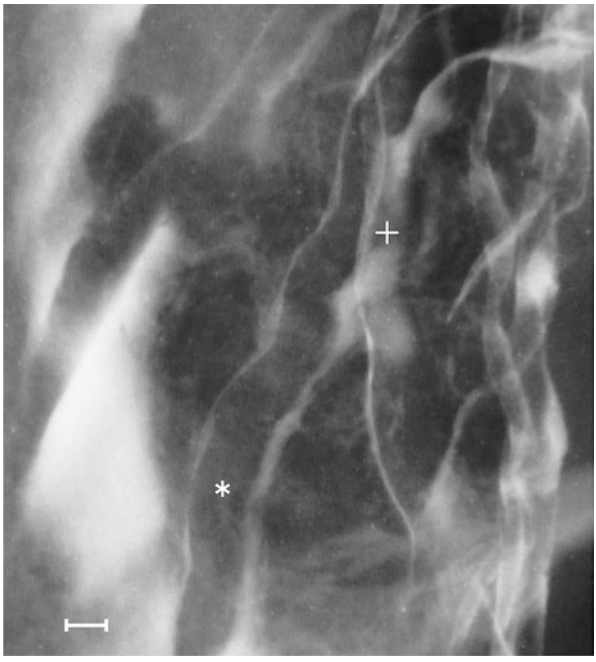

Fig. 8.12 Abnormal gastric folds. This is a picture of gastric folds of *H. pylori-positive* stomach. Folds are thick, tall, steep, not smooth on the surface, not soft, and run windingly. A fold on the posterior wall (asterisk) and a fold on anterior wall (plus) are indicated. The scale shows 5 mm. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

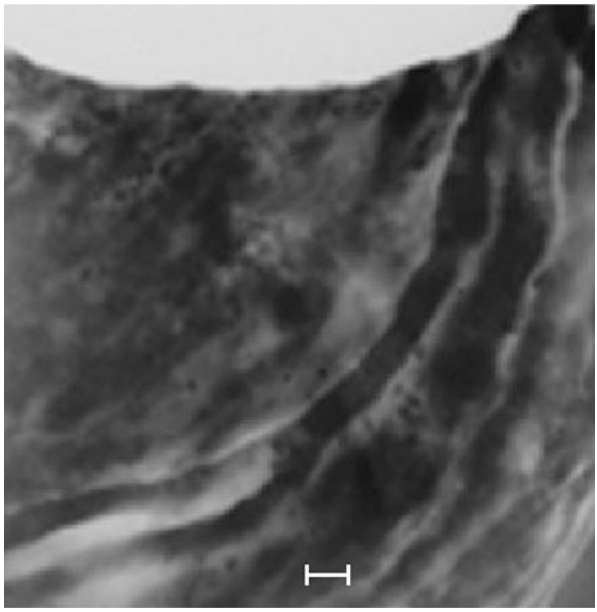

Fig. 8.13 Intermediate-type gastric folds. This is a picture of gastric folds of *H. pylori-eradicated* stomach. Some folds are thick, tall, and steep, but other folds are not. Folds consisting of both findings of normal and abnormal types are classified intermediate-type. The scale shows 5 mm. From: Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with Barium X-ray Examination [16]

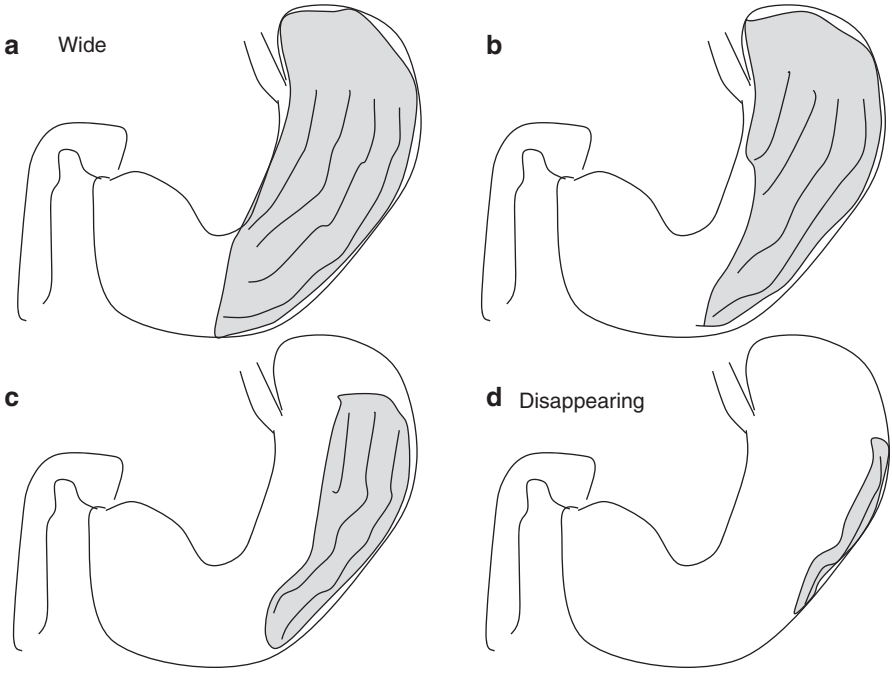

Fig. 8.14 (**a**) Wide distribution folds (no atrophy), (**b**) Mildly disappeared folds (mild atrophy), (**c**) Moderately disappeared folds (moderate atrophy), (**d**) Markedly disappeared fold (wide atrophy). Types of fold distribution. Modified from: Nakajima S, et al. Jpn J Helicobacter Res, 2007 [14]

Table 8.7 A standard for diagnosis of background gastric mucosa with UGIS

		Fold types and distribution			
		Normal type and wide	Intermediate type	Abnormal type	Folds disappeared
Mucosal surface pattern	**Smooth**	Non-infected	Past infection suspected	Present infection suspected	Past infection suspected
	Intermediate				
	Rough	Present infection suspected		Present infection	Present infection suspected

H. pylori-naïve normal stomach has smooth mucosal surface and normal folds with wide distribution. Otherwise either present or past *H. pylori* infection is suspected. This standard is not 100% accurate. The precise diagnosis should be made with other *H. pylori* tests and past history of eradication therapy

Modified from Kansai GI Imaging Research Group. Atlas for Diagnosing *H. pylori* Infection with barium X-ray examination [16]

8.6 Endoscopic Gastric Cancer Screening

Endoscopic gastric cancer screening was approved by Japanese government in 2016 according to the Committee Report in 2014 [6]. Subjects of the screening are 50 years old and older, and the interval of the screening is 2 years. Endoscopic gastric cancer screening is effective in reducing gastric cancer death (Table 8.2). However, it has a lot of problems as described above. One of the biggest problems is the capacity of endoscopic screening. In recent years endoscopists in hospitals have a lot of time-consuming procedures to do such as total colonoscopy, endoscopic submucosal dissection (ESD), endoscopic biliary tract therapeutic procedures, etc. Most endoscopists in hospitals do not have enough time to add screening upper GI endoscopies. In addition, the gastric cancer detecting rate in endoscopic gastric cancer screening is only 0.19% (Table 8.4), and *H. pylori*-negative subjects are increasing; more than 99% of subjects do not have gastric cancer in endoscopy screening subjects. Thus indiscriminate endoscopic screening may not be efficient or cost-effective. Because most endoscopists in hospitals are too busy, endoscopic screening should be performed by primary care physicians or in healthcare facilities. The Japanese government should evaluate the efficiency of indiscriminate endoscopic screening.

In 2014 Kyoto Classification of Gastritis was published (see English version published in 2017) [22]. The classification is based on the visible endoscopic diagnosis in view of *H. pylori* infection: present infection, past infection, and no infection. The endoscopic classification corresponds to the worldwide pathological classification of gastritis: updated Sydney system [23]. Evaluation of atrophy is also recommended because gastric mucosal atrophy is a risk factor for gastric cancer. Recently the Updated Kimura-Takemoto Classification (Fig. 8.15) [24] is used for endoscopic grading of gastric atrophy. Using these classifications, endoscopic gastric cancer risk evaluation may be possible to do (Table 8.8) [25], and it may lead endoscopic gastric cancer screening more efficient. Screening of high-risk subjects with serum tests will make endoscopic gastric cancer screening more efficient as described in the next section.

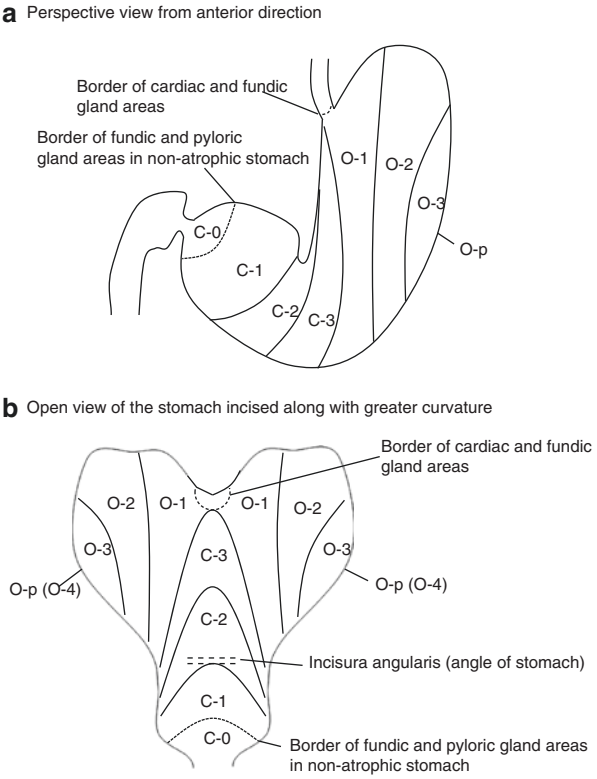

Fig. 8.15 Grading of endoscopic atrophic border. (**a**) Perspective view from anterior direction. (**b**) Open view of the stomach incised along with greater curvature. Each zone shows a range of atrophic border of fundic and pyloric gland areas. "C" and "O" represent closed and open-type atrophy, respectively. C-0 means no findings of atrophy. The dotted line between C-0 and C-1 is an imaginary line which is invisible with usual white-light endoscopy. O-p means pan-atrophy in which whole body of the stomach shows atrophic mucosa. Incisura angularis (angle of stomach) is demonstrated with the two dotted parallel lines. Modified from Nakajima S, et al. Helicobacter Research 2009 [23]

Table 8.8 Endoscopic risk classification with Kyoto classification of gastritis and updated Kimura-Takemoto classification for endoscopic atrophy

Gastritis (Kyoto classification)	Atrophy (updated Kimura-Takemoto classification)							
	C-0	C-1	C-2	C-3	O-1	O-2	O-3	O-p
CAG								
CIG								
N, NG								

CAG chronic active gastritis, *CIG* chronic inactive gastritis, *N* normal, *NG* non-gastritis
C-0 to O-p are explained in Fig. 8.15
From Nakajima S, et al. Medical Practice 2018 [25]

8.7 Screening with Serum Tests: So-Called ABC Method

Since *H. pylori* infection and gastric mucosal atrophy were considered the main risks for gastric cancer, serum anti-*H. pylori* antibody test and serum pepsinogen test, the latter of which is a marker of gastric mucosal atrophy, have been proposed for the screening of high-risk subjects for gastric cancer [26]. Details are described in the other chapter. Because serum tests are very easy, cheap, and almost noninvasive, higher screening rate (participation rate) is expected in the screening with serum tests than previous gastric cancer screening with UGIS. The combination of serum anti-*H. pylori* antibody test and serum pepsinogen test is called "ABC method," because the participants are divided into three groups as in Table 8.9 [26]. According to Otsu city, Shiga prefecture, where ABC method was adopted in 2012, the participation rate of ABC method was 4.5-fold higher than that with UGIS (12.6% vs. 2.8%, Table 8.10), although these two rates are not strictly comparable because the method of recruitment and eligible ages are different. However, the same numbers of gastric cancer were diagnosed from both UGIS and ABC method. It was revealed that almost half of those who underwent ABC method had never undergone previous gastric cancer screening with UGIS [26]. Thus the screening with serum tests would be a good tool to recruit subjects who had never undergone gastric cancer screening.

Table 8.9 Serum gastric cancer risk evaluation, the so-called ABC method

		Pepsinogen test	
		−	+
***H. pylori* antibody test**	−	A	C
	+	B	

From: A manual for gastric cancer risk screening, ABC method, Second edition. Nanzando, Tokyo, Japan, 2014 [26]

Table 8.10 The facts on UGIS and ABC method in Otsu city, 2014

	UGIS	Population adjusted	ABC method
Number of eligible subjects	118,889	83,222	23,000
Number of participant	1993	1993	2902
Screening rate	1.7%	2.4%	12.6%
Number of gastric cancer	4	4	4
Gastric cancer detection rate	0.20%	0.20%	0.14%
Number of endoscopy-tested subjects	270	270	569
Positive predictive value	1.48%	1.48%	0.70%

Although 118,889 persons (40 years old or older) are eligible for UGIS screening in Otsu city, about 30–40% persons undergo gastric cancer screening offered by their health insurance or other opportunities every year by the Annual Health, Labour, and Welfare Report 2013–2014. So I estimated at least 60% people had not undergone the screening tests and adjusted the number of eligible subjects for UGIS as in the middle column. ABC method was recommended every 5 years between 40–60 year old in Otsu city

Modified from: A manual for gastric cancer risk screening, ABC method, Second edition. Nanzando, Tokyo, Japan, 2014 [26]

Although serum tests have many merits to screen high-risk subjects, the ABC method has some limitations [27, 28]. One of the biggest demerits is that those who have both negative in *H. pylori* antibody and pepsinogen tests (group A) are not always gastric cancer-negative. About 10% gastric cancer patients belong to group A, if all gastric cancer patients underwent ABC method after the diagnoses [27, 28]. The main reason is from the cut-off value of the tests. Because the sensitivity and specificity are not 100% with the serum tests, some gastric cancer patients show negative results in both tests. Even if cut-off values are changed, the problem will not disappear completely. Thus we have to know the limitation before we use serum screening. One of the solutions of the problem is a combination of serum tests and image tests as in the next section.

8.8 Combination of the Methods

Because every single method is not enough to screen gastric cancer perfectly and efficiently, combination of the different methods may be useful. For example, simultaneous gastric cancer screening with UGIS and serum *H. pylori* antibody test may be more precise to evaluate gastric cancer risk because the image diagnosis with UGIS covers the pseudo-negative results of serum test and vice versa. We have to show evidence to prove the efficiency of the combination. A prospective cohort study has started in Japan. Other combinations may be tried to find more efficient screening methods. These experiences in Japan may be useful in other countries or communities in the world [29].

8.9 Summary

The past, present, and future of gastric cancer screening in Japan are introduced in this chapter. Although endoscopic screening is effective to reduce gastric cancer death, it has some problems to perform. Endoscopic gastric cancer screening should be updated to include gastric cancer risk evaluation to be a more efficient method in view of cost performance, especially in the communities where *H. pylori* infection rate or gastric cancer death rate is not high. Double-contrast UGIS is also effective in reducing gastric cancer death. Because UGIS also has problems such as X-ray exposure, it should be updated with risk evaluation to reduce X-ray exposure for low-risk subjects and reduce the cost. For those who are suspected *H. pylori* infection, endoscopic examination and eradication therapy should be recommended. Serum tests are useful to screen high risk subjects for gastric cancer, but they are not perfect to rule out patients with gastric cancer. We have to know the limitations. Combination of UGIS and serum tests may be useful for covering the weakness of serum tests or UGIS with each other.

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Chapter 9
Endoscopic Diagnosis

Takashi Nagahama, Noriya Uedo, and Kenshi Yao

Abstract The strategy of early detection and early treatment of gastric cancer to reduce the mortality rate has been widely implemented in Japan. To detect early-stage gastric cancer, considerable education regarding endoscopic methods for systematic screening is carried out. If suspicious lesions for gastric cancer are detected by conventional white-light imaging, a detailed differential diagnosis of cancerous and non-cancerous lesions by image-enhancement endoscopy with/without magnification, followed by biopsy, is performed as a usual subsequent approach. In the case of lesions diagnosed as cancer by histology, a endoscopist performs a detailed examination before treatment to define the (1) histological type, (2) tumor size, (3) presence and absence of ulceration or scar, and (4) depth of invasion to determine the treatment indication, i.e., endoscopic or surgical. Regarding the tumor size, the boundary of the lesion should be precisely identified in order to determine the excision line. Recently, the ABC method using a combination of serum *Helicobacter pylori* antibody and pepsinogen tests, which involves an overview of risk stratification of gastric cancer, is recommended as a part of gastric cancer screening.

Keywords Endoscopy · Diagnosis · Stomach cancer · Early gastric cancer · Chromoendoscopy · Narrow-band imaging

9.1 Introduction

Although the incidence of gastric cancer has been consistently declining in recent years, it remains the fifth most common cancer and the third highest cause of cancer-related deaths worldwide. Japan has one of the highest incidence rates with age-standardized rates of 45.7 and 16.5 per 100,000 in men and women,

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respectively [1]. Therefore, in Japan, tremendous effort has been taken to make diagnosis of early-stage gastric cancer over the years. In 1962, the Japan Gastroenterological Endoscopy Society defined “early gastric cancer (EGC)” as cancer confined to the mucosa or submucosa irrespective of the presence or absence of lymph node metastases [2]. This definition was based on the fact that such early-stage gastric cancer has a favorable prognosis, with a 5-year postoperative survival rate ≥95% [3]. The one of the reasons for the good outcome for EGC patients is explained by low prevalence of lymph node (LN) metastases. The LN metastasis is found in 10–20% of cases of EGC, but 70% of these metastases are confined to regional LNs [4]; thus they are radically removed by gastric resection and LN dissection.

To reduce the gastric cancer mortality in Japan, population-based screening program using barium contrast radiography was introduced in the 1960s and was distributed throughout the country. Endoscopic examination has not been recommended as a method of population-based screening for many years because of concerns for overdiagnosis, as well as herms related to procedures such as perforation or hemorrhage [5]. However, results of Japanese and Korean cohort studies verified the effectiveness of endoscopic screening to reduce gastric cancer mortality [6–8]; therefore, the Japanese Guideline for Gastric Cancer Screening (2014 Edition) finally recommends endoscopic screening examination for individuals over 50 years of age every 2–3 years [9]. Throughout the period, many studies had been conducted to analyze morphological characteristics of EGC in radiographic images in comparison to histological findings of the surgically resected specimens. Recently, with the introduction of high-resolution videoendoscopy, more detailed investigation for association between endoscopic morphological characteristics and histological findings has been performed in similar ways. As the concept of evidence-based medicine was not well established in the past, levels of evidence for these investigations were not high. However, the studies were conducted with great enthusiasms of Japanese endoscopists, and such knowledge and techniques have been integrated in Japanese endoscopic practice for a long time. Although the population-based screening is being conducted in Japan and it detects gastric cancer in around 5,000 patients, this number accounted for only 4% of gastric cancer patients all over Japan [10]. Despite population screening, >80% of patients with EGC present through such clinical practice: routine endoscopic examinations in hospitals, outpatient clinics or individual health checks [11].

Moreover, development of endoscopic submucosal dissection (ESD) technique has changed endoscopic diagnosis of EGC in some aspects. In the past, only small lesions without ulcer or scar were removable with endoscopic mucosal resection (EMR) method. However, ESD enabled us to resect large lesions or lesions with ulcer scars and expanded indication of endoscopic resection to such lesions [12, 13]. As a result, endoscopic resection is established as one of standard treatments for EGC [14, 15], and early detection became the major importance from the viewpoint of quality of life. Moreover, for pretreatment evaluation for ESD, detailed examination is necessary to determine tumor extent (size), histological type and depth, and

presence or absence of ulcers or scars. As well as dye-based image-enhanced endoscopy (IEE, chromoendoscopy), equipment-based IEE such as narrow-band imaging (NBI), blue laser imaging (BLI), iScan, etc., and magnifying endoscopy, are implemented in clinical practice nowadays and have improved endoscopic diagnosis of EGC.

The present chapter illustrates the current practice of endoscopic diagnosis of EGC, in relation to the accumulated evidence and the consensus among Japanese endoscopists.

9.2 Detection of EGC

When a case of missed gastric cancer within 1 year after screening examination is defined as false-negative results, diagnostic accuracy of endoscopic screening in Japanese practice shows the sensitivity of around 85–95%; if a case of gastric cancer detected within 3 years is defined as the false-negative, the sensitivity is 75–90% [16, 17]. The reasons for missed gastric cancer may include the endoscopist's insufficient observation method (technique) and experience (knowledge) [18]. Therefore, in order to detect EGC effectively, it is essential to perform systematic screening examinations under adequate preparation using mucolytics and defoaming agents and to understand suggestive findings for gastric cancer [19, 20].

9.2.1 Preparation

Froth and mucus adhering to the mucosal surface can obstruct endoscopic observation, leading to minor mucosal changes being overlooked. To increase the visibility of the mucosa, a mixture of water and mucolytic and antifoaming agents, consisting of 100 mL of water, 20,000 U of pronase (Pronase MS; Kaken Pharmaceutical, Tokyo, Japan), 1 g of sodium bicarbonate, and 10 mL of 20 mg/mL dimethylpolysiloxane (Gascon; Horii Pharmaceutical Industries, Osaka, Japan), is administered to a patient before endoscopy. In two randomized controlled studies, pronase with defoaming agents was found to improve mucosal visibility [21, 22]. *N*-acetylcysteine can be used where the pronase is unavailable [23].

Active peristalsis interferes endoscopic examination of the stomach. Therefore, an anticholinergic agent, either 10–20 mg of scopolamine butylbromide (Buscopan; Nippon Boehringer Ingelheim, Tokyo, Japan) or 1 mg of glucagon (Glucagon G Novo; Eisai, Tokyo, Japan), is administered by intramuscular or intravenous injection. A topical application of peppermint oil or formulations of its principal component, 0.8% l-menthol (Mincrea; Nihon Pharmaceutical Co. Ltd., Tokyo, Japan), has been reported to be effective and safe antiperistaltics [24, 25].

9.2.2 *Screening Examination Methods*

Even nowadays, standard endoscopi screening method for gastric cancer is white-light endoscopy (WLE), and there is no clear evidence that IEE such as NBI, BLI, linked-color imaging, autofluorescence imaging, etc. is advantageous over WLE.

In Japanese usual practice, the gastric mucosa is observed according to the following sequence [26]: (1) from the cardia to the pyloric ring with forward observation and by the same route with retroflex observation to the cardia (Fig. 9.1a) or (2)

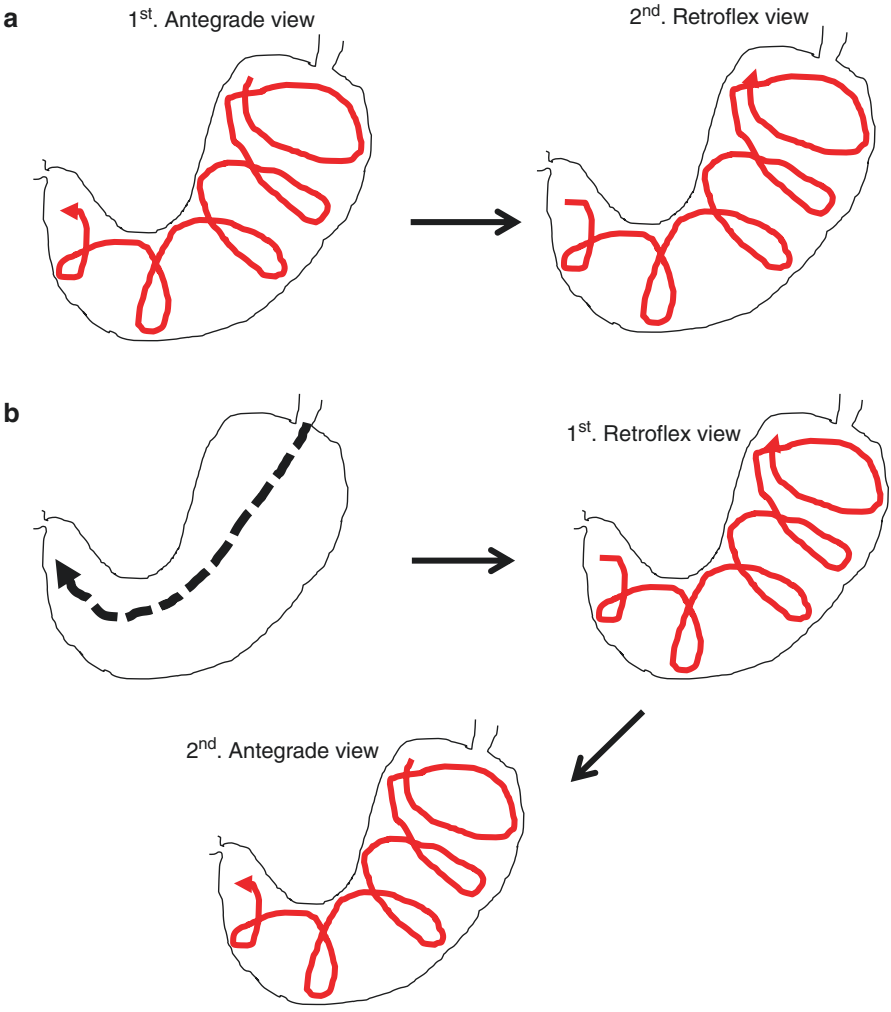


Fig. 9.1 Observation method for gastroscopy. Observation of the stomach with antegrade view followed by retroflex view (**a**) and with retroflex view followed by antegrade view (**b**)

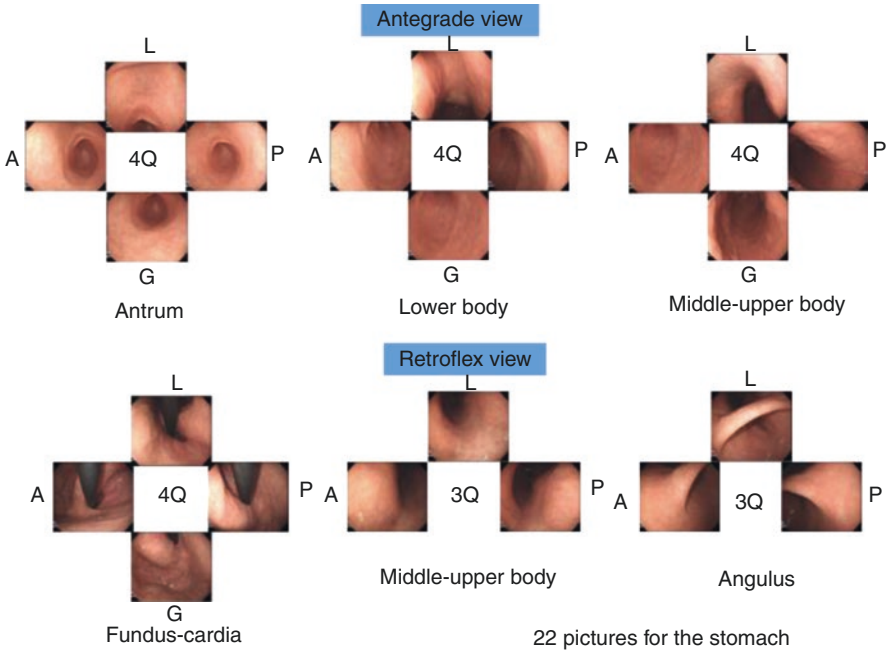


Fig. 9.2 The systematic screening protocol for the stomach (SSS)

initial insertion to the pyloric ring without observation and retroflex observation by the same route to the cardia, then forward observation to the pylorus again (Fig. 9.1b). Yao unified and simplified the abovementioned different routes of observation and has proposed the systematic screening protocol for the stomach (SSS, Fig. 9.2) [18]. In this method, all four to three quadrant directions (the anterior wall, posterior wall, greater curvature, and lesser curvature) at the middle-upper corpus, lower corpus, and the antrum are examined and recorded.

EGC is sometimes overlooked by the pooled gastric fluid and adhered mucus on the mucosal surface. In addition, if the air insufflation is not enough, lesions in the corpus greater curvature are concealed by the folds and are overlooked [18, 19]. Accordingly, vigorous cleansing of the gastric mucosa, complete suctioning of gastric fluid, and sufficient distention of the gastric lumen by adequate air insufflation are important to avoid missing EGCs (Fig. 9.3).

9.2.3 Criteria for Lesions Suspicious for EGC

For identification of suspicious lesions for EGC, it is necessary to understand the characteristic findings of EGC. The principal characteristics of epithelial neoplasm are (1) abnormal growth and (2) adherence of tumor cells. Tumor tissues generally

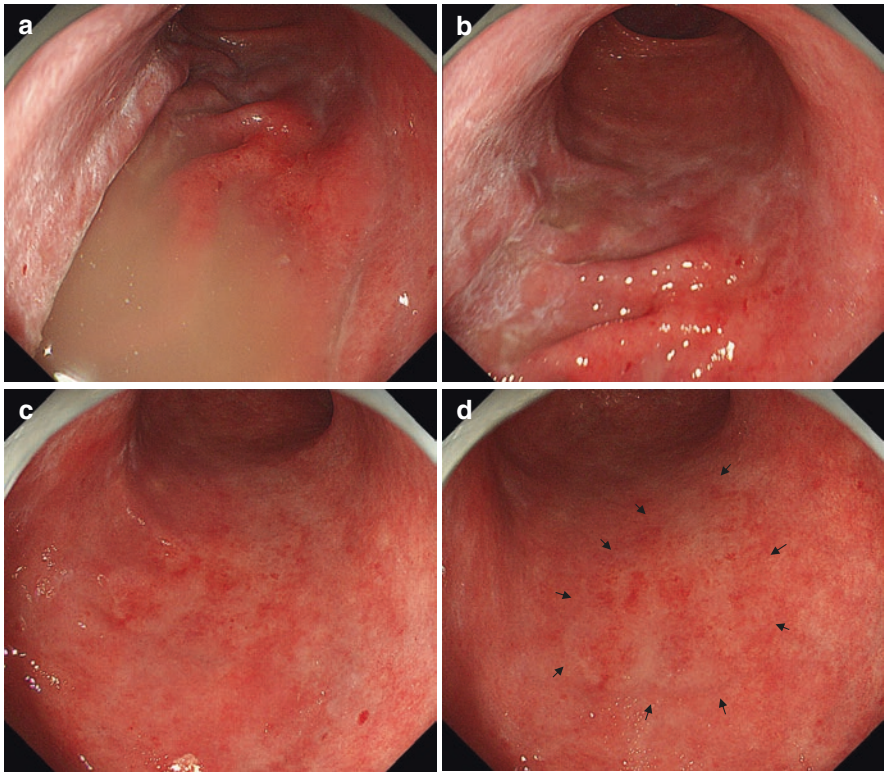


Fig. 9.3 After entrance to the stomach (**a**), fluid is aspirated (**b**), mucous on the surface is washed (**c**), and with enough air insufflation, a flat undifferentiated-type early gastric cancer was observed adequately (black arrows, **d**)

consist of the epithelium and the stroma. Abnormal growth of the stroma, which includes vessels, is represented as irregularity in color (whitish or reddish), and abnormal growth of the epithelium is represented as irregularity of surface of the lesions (elevated or depressed) (Fig. 9.4a). A demarcation line is formed between the lesion and the surrounding mucosa because of continuous growth of the adhered cancer cells (Fig. 9.4b).

Accordingly, the diagnostic criteria of EGC by WLE are (1) irregularity in color (whitish or reddish), (2) irregularity in surface (elevated or depressed), and presence of demarcation line (demarcated lesion) [27, 28]. If the lesion fulfilled the criterion of (1) and (3), or (2) and (3), the diagnosis of EGC is made (Fig. 9.4c). In addition, if lesions show the similar color and surface to the surrounding mucosa, the loss of vascular network in the background mucosa can be a suspicious finding for EGC (Fig. 9.5a). Spontaneous mucosal bleeding can be also a clue for detection of EGC (Fig. 9.5b). Although these findings are sometimes found in benign diseases, cancer

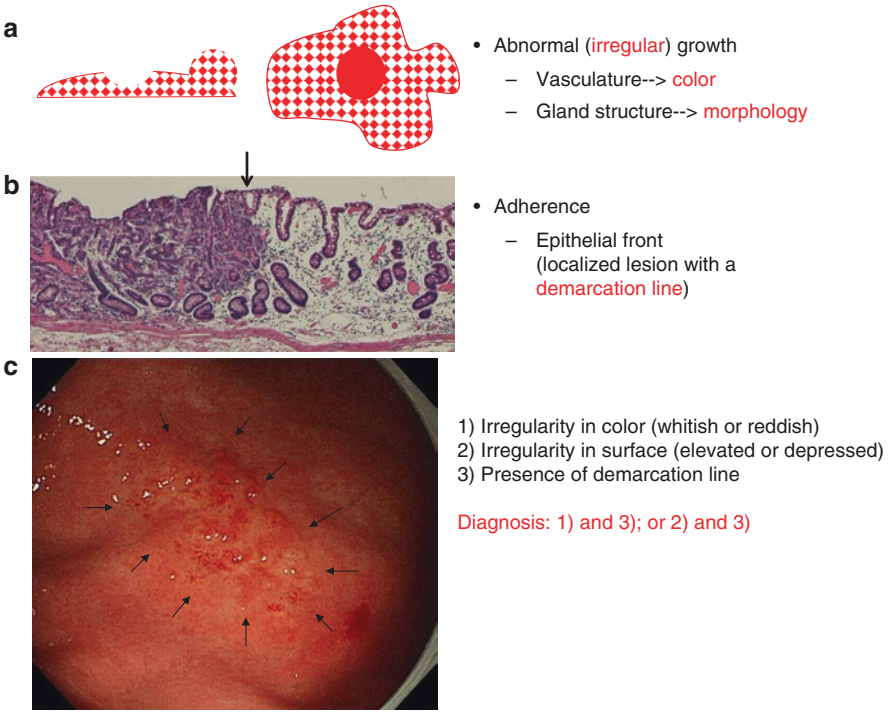


Fig. 9.4 Characteristic finding of suspicious lesion. Neoplastic tissue grows irregularly, so irregular growth of the epithelium represents as irregularity in morphology and that of stromal tissue represents as irregularity in color (**a**, **c**). Epithelial neoplastic cells adhere to each other, so it forms a demarcation line (epithelial front, black arrows in **b**, **c**) between the surrounding mucosa (**b**, **c**)

is strongly suspected if it is found as a single isolated lesion, whereas it is less suspected if they are multiple or have symmetrical distribution (Fig. 9.6).

9.3 Differential Diagnosis of Cancerous and Non-cancerous Lesions

After detection of suspicious lesions for EGC by WLE, a differential diagnosis between cancerous and non-cancerous lesions is made to determine the need for biopsy. Biopsies are usually not carried out for lesions diagnosed confidently as non-cancerous according to endoscopic finding. In Japan, the dye-based and equipment-based IEE is commonly used for differential diagnosis of the gastric lesions.

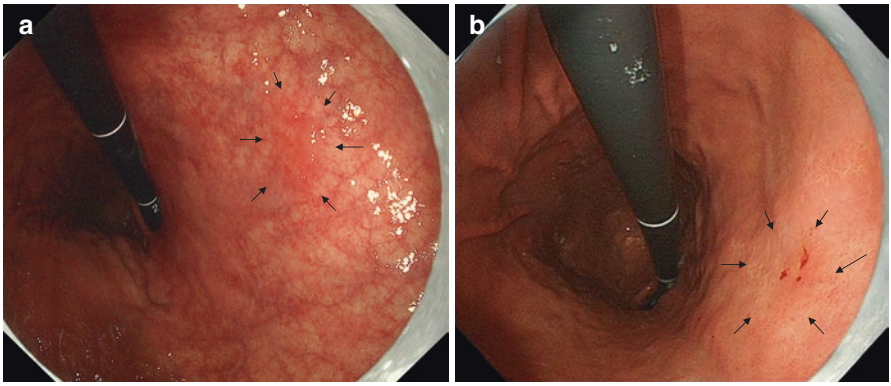


Fig. 9.5 When the lesion has similar color and morphology to the surrounding mucosa, disappearance of the background vascular network (black arrows in **a**) or spontaneous hemorrhage (black arrows in **b**) can be a clue to detect suspicious lesions

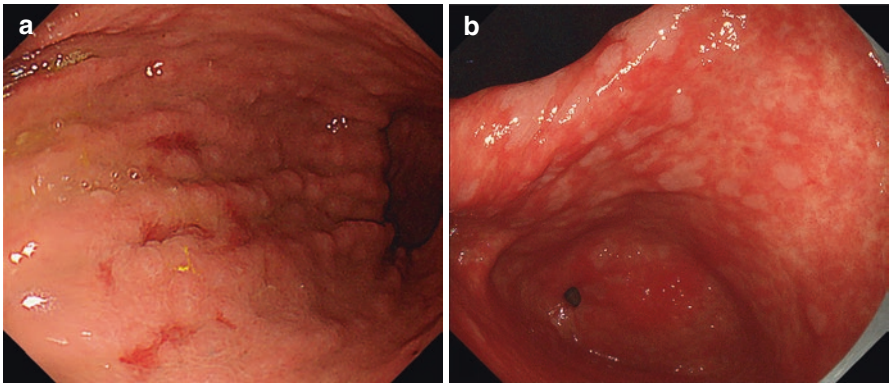


Fig. 9.6 A multiplicity of similar findings is indicative for benign lesion. Multiple reddish depressions are gastric erosions (**a**), and multiple whitish patches are intestinal metaplasia (**b**)

9.3.1 Dye-Based IEE (Chromoendoscopy)

In chromoendoscopy, 0.05–0.2% indigo-carmine solution is applied by using direct syringe flush through working channel or by using a spraying catheter (PW-5L-1, PW-6P-1, or PW-205V, Olympus Medical Systems Corp., Tokyo, Japan) [29]. Contrasting the topography and color of the mucosa, the indigo carmine facilitates evaluation of characteristics of the lesion and the surrounding mucosa (Fig. 9.7). The diagnostic criteria of EGC by chromoendoscopy are the same to those of WLE: (1) irregularity in color (whitish or reddish), (2) irregularity in surface (elevated or depressed), and presence of demarcation line (demarcated lesion). Chromoendoscopy, especially, improve visualization of demarcation line. If the margin (demarcation line) of the lesion appears smooth and the lesion is

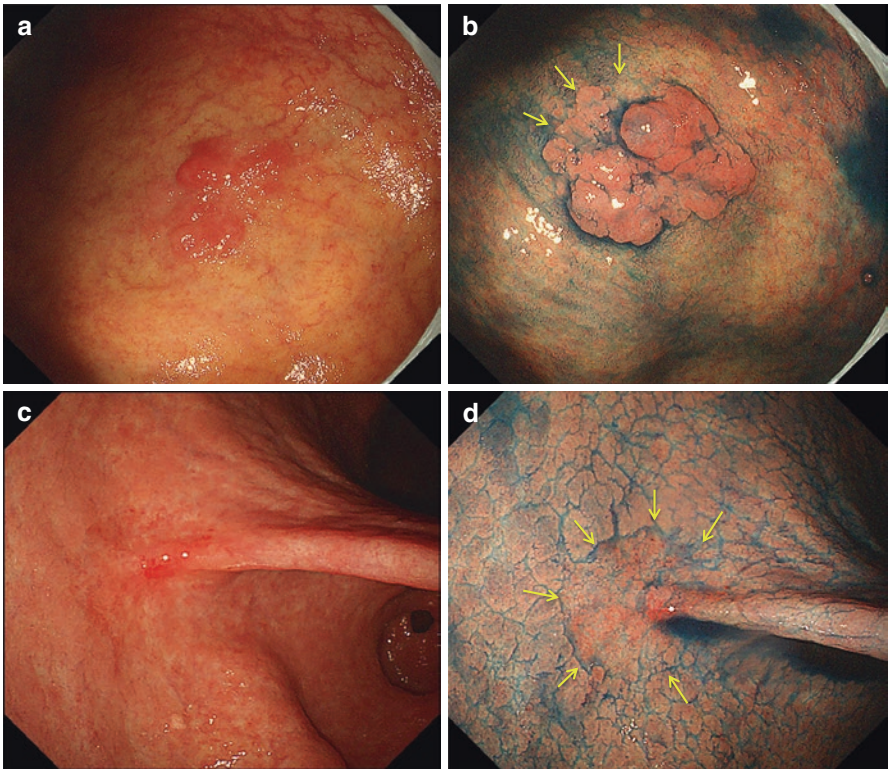


Fig. 9.7 Chromoendoscopy using indigo carmine for early gastric cancer (EGC). A reddish elevated lesion is observed in the gastric corpus (**a**). With indigo-carmine chromoendoscopy, morphological characteristic of the lesion becomes apparent (**b**). The lesion had flat extension on the oral side (yellow arrow in **b**). A small reddish area is seen at the anterior wall of the incisura angularis (**c**). Chromoendoscopy revealed shallow depressed area around the reddish area (yellow arrow in **d**)

symmetrically circular or oval in shape, it is likely to be non-cancerous. Cancerous lesion has irregular demarcation line that often shows a "moth-eaten" appearance and/or encroachment (Fig. 9.8) [40], and the surface pattern is morphologically irregular.

9.3.2 NBI with Magnifying Endoscopy (M-NBI)

NBI with magnifying endoscopy (M-NBI) enables to examine the microvessel pattern and micro-surface patterns of the mucosa. Yao et al. proposed the vessel and surface (VS) classification system for M-NBI [30]. The VS classification system includes anatomical terms to correlate endoscopic appearance with histological findings (Table 9.1). In this system, both the microvessel and micro-surface patterns

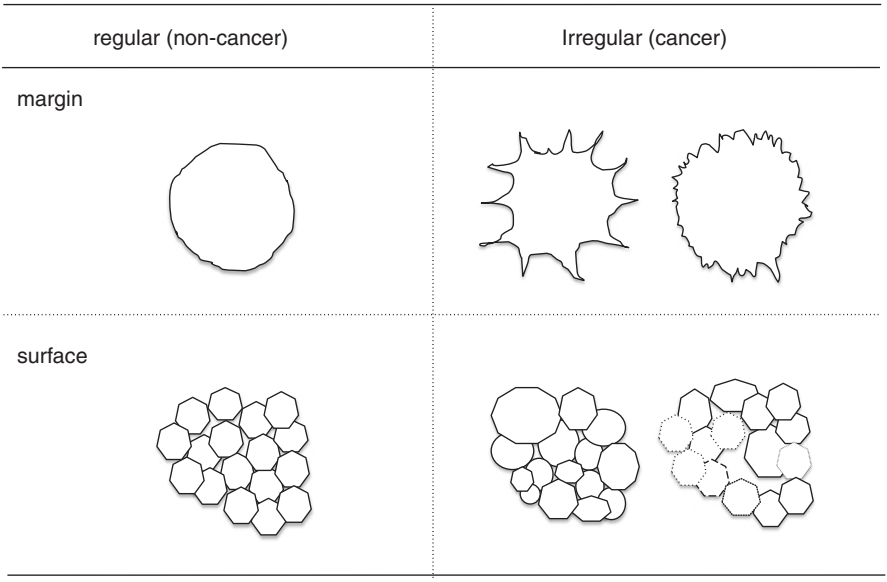


Fig. 9.8 Characteristic finding of cancerous and non-cancerous lesions in chromoendoscopy. A non-cancerous lesion has regular margin, and its surface structure is regular and uniform, whereas a cancerous lesion usually has irregular speculated margin, and the surface structure is irregular and uneven

Table 9.1 Terminology for VS classification system

V, microvascular (MV) pattern
Subepithelial capillary network (SECN)
Collecting venule (CV)
Pathological microvessels (MV)
S, micro-surface (MS) pattern
Marginal crypt epithelium (MCE)
Crypt-opening (CO)
Intervening part (IP)
Light blue crest (LBC): Brush border
White opaque substance (WOS): Lipid droplets

are classified as regular, irregular, or absent (Fig. 9.9). The criteria for high-grade dysplasia or EGC in the VS classification system are (1) irregular microvessel pattern, (2) irregular micro-surface pattern, and (3) presence of a demarcation line. The diagnosis of EGC is made when the lesion has irregular microvessel pattern with demarcation line or irregular micro-surface pattern with demarcation line. Besides, Yao et al. described the finding of "vessel and surface discordance" in which irregular vessels extended irrespective of the epithelial structure (Fig. 9.10) were a specific sign of adenocarcinoma [31]. Doyama et al. coined a term "white-globe appearance"

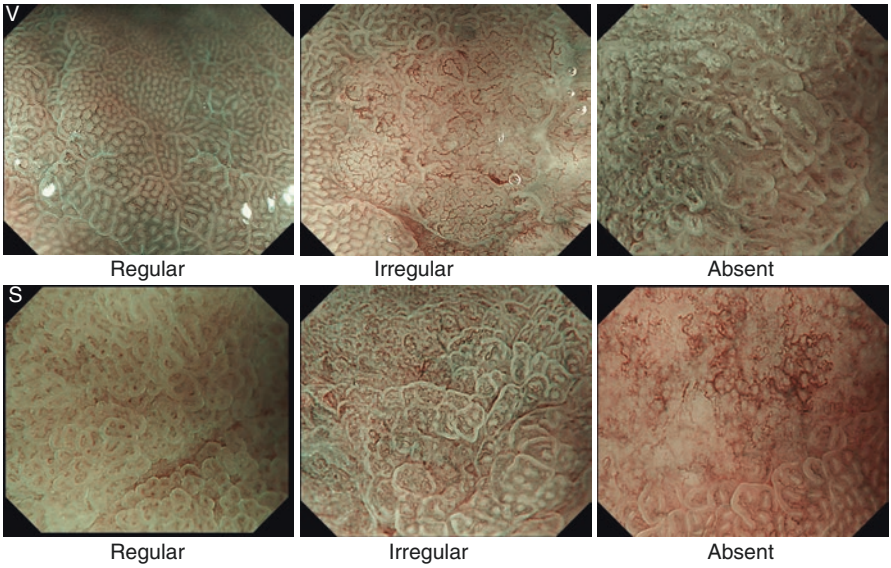


Fig. 9.9 The vessel plus surface (VS) classification system. The microvessel pattern and the micro-surface pattern are evaluated independently in magnifying NBI images. Both patterns are classified as regular, irregular, or absent

for small white globular lesion underneath cancerous eithelium, and indicated its high specificity (97.5%) for making diagnosis of EGC (Fig. 9.11) [32, 33].

In patients at high risk for EGC, the diagnostic accuracy of M-NBI for small (≦10 mm) depressed lesions was significantly superior to that of WLE (90% vs. 65%, $p < 0.001$). Moreover, when M-NBI was performed subsequently to WLI, the diagnostic accuracy, sensitivity, and specificity were 97%, 95%, and 97%, respectively [34]. After that the same investigators conducted a multicenter prospective cohort study in patients undergoing screening gastroscopy, and validated the VS classification system showed similar diagnostic accuracy (sensitivity, specificity, and accuracy of 60%, 98%, and 96%, respectively) for lesions with any macroscopic types. In addition, the study suggested possibility of M-NBI to reduce the number of biopsies performed, as it worked as an optical biopsy for suspicious lesions [35]. Taking these research results into account, the Japanese Gastroenterological Association issued the magnifying endoscopy simple diagnostic algorithm for gastric cancer (MESDA-G) in collaboration with the Japan Gastroenterological Endoscopy Society, Japan Society of Gastroenterology, and Japanese Gastric Cancer Association [36]. The MESDA-G proposed the algorithm in which presence or absence of demarcation line is evaluated first, and then, in lesions with clear demarcation line, presence or absence of irregular microvessel patterns or micro-surface patterns is evaluated (Fig. 9.12). Usefulness of M-NBI for

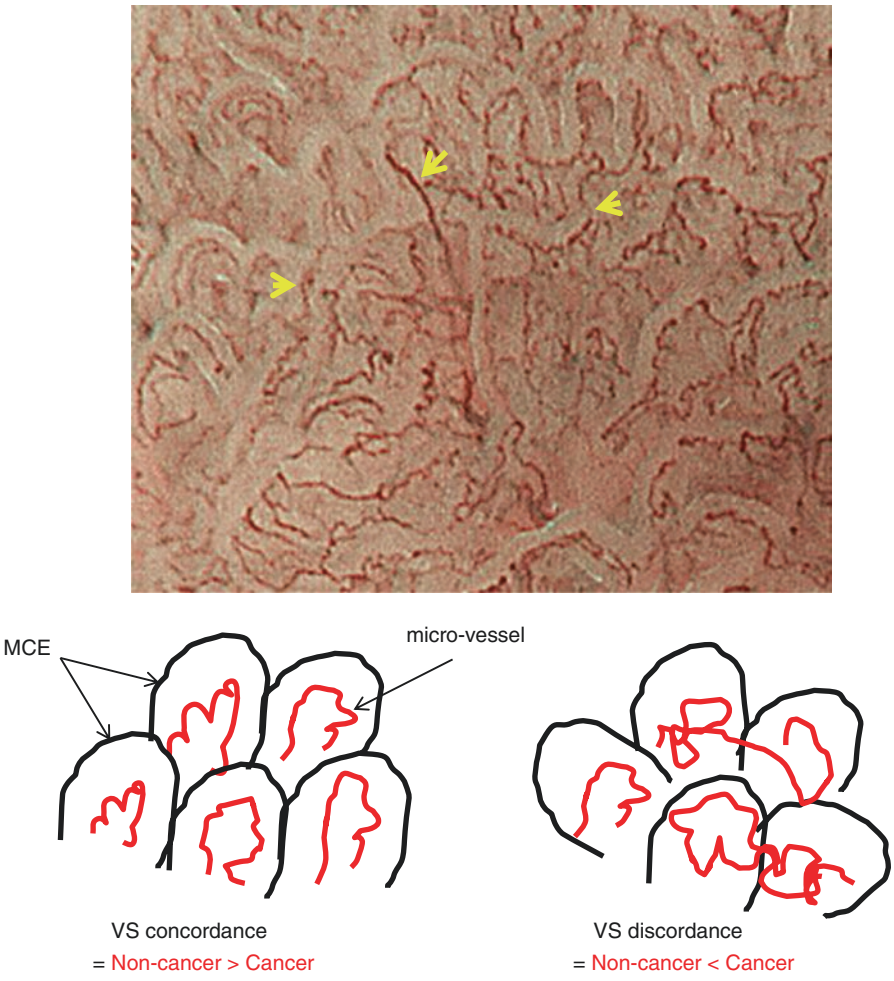


Fig. 9.10 A magnifying NBI image of the vessel plus surface (VS) discordance (yellow arrows). In a non-cancerous lesion, microvessels are situated inside the marginal crypt epithelium, while in a cancerous lesion, irregular microvessels extend irrespective of micro-surface structure. *MCE* marginal crypt epithelium

diagnosis of minute (≤5 mm) EGC [37] and for differential diagnosis of elevated EGC from adenoma is also reported [38, 39].

In recent years, BLI with magnifying endoscopy has been reported to show the similar diagnostic performance to M-NBI [40].

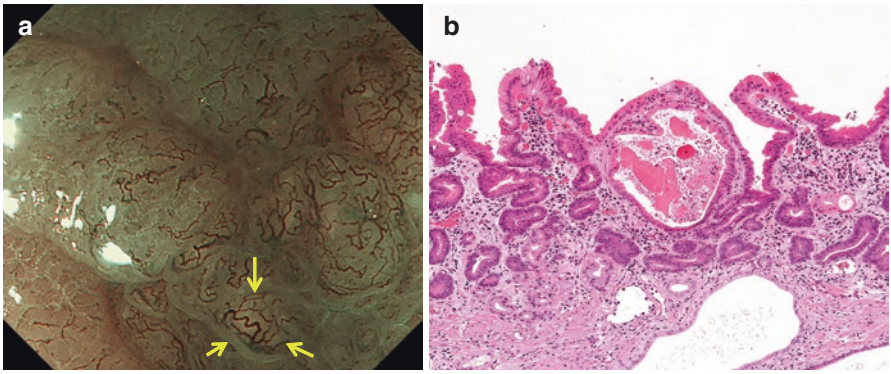


Fig. 9.11 A magnifying NBI image of the white globe appearance (WGA). A white globular lesion is observed underneath cancerous epithelium (**a**). The WGA is a highly specific finding for making diagnosis of early gastric cancer, and it corresponds with a histological finding of intraglandular necrotic debris (**b**)

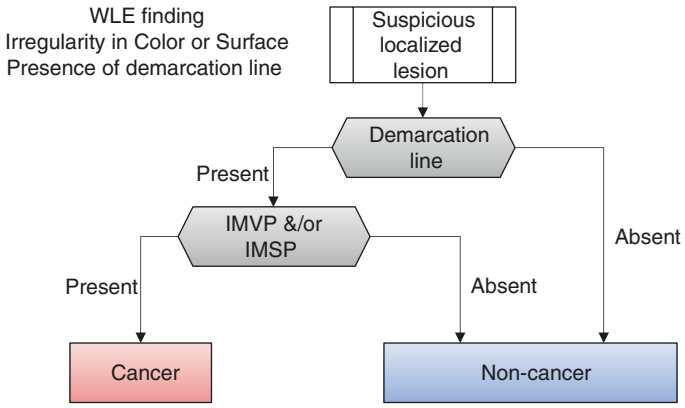


Fig. 9.12 The magnifying endoscopy simple diagnostic algorithm for gastric cancer (MESDA-G). First, presence or absence of demarcation line is evaluated for a suspicious lesion. If the demarcation line is absent, the lesion is diagnosed as non-cancer. If the lesion has demarcation line, presence or absence of irregular microvessel patters or micro-surface patterns is evaluated. If both findings are regular, the lesion is diagnosed as non-cancer, while if any of the findings are irregular, the lesion is diagnosed as cancer. (The figure is cited from reference 36 and modifie) Abbreviations: *IMVP* irregular microvascular pattern, *IMSP* irregular microsurface pattern

9.4 Risk Stratification

Assessment of patients' gastric cancer risk is important in terms of increasing index of suspicion of cancerous lesion for abnormal findings found at endoscopic examination. Moreover, surveillance examination is recommended for patients at high risk for gastric cancer.

9.4.1 Helicobacter pylori (H. pylori) Infection and Serum Pepsinogen

H. pylori infection is a definitive (class 1) carcinogen for developing gastric cancer. The results of cohort studies [41, 42] and meta-analysis of cohort studies [43] support the evidence for usefulness of risk stratification using combination of serum pepsinogen and *H. pylori* antibody tests. The ABC method combines the pepsinogen I level and pepsinogen I/II ratio with serum *H. pylori* antibody titer, and categorizes patients into the following four groups: Group A, *H. pylori*-negative and non-atrophic; Group B, *H. pylori*-positive but non-atrophic; Group C, *H. pylori*-positive and atrophic; and Group D, *H. pylori*-negative but atrophic. A prospective cohort study showed increased risks of gastric cancer from groups A to D: hazard ratios of 1, 1.1, 6.0, and 8.2, respectively [42]. Patients in Group D have extensive atrophy, and *H. pylori* can colonize no longer. Limitations of the ABC methods are the facts that it is not applicable to a highly endemic population for *H. pylori* as all subjects were determined at high risk; and that contamination of subjects showing *H. pylori* antibody low titer (previous or current *H. pylori* infection) who have a risk for developing gastric cancer into the group A [44, 45].

9.4.2 Evaluation of Gastric Cancer Risk on the Basis of Endoscopic Findings

Several endoscopic findings are reported to be associated with gastric cancer risk; thus the patients' risk can be estimated on the basis of the endoscopic findings during endoscopy. The most common endoscopic finding for assessment of gastric cancer risk in Japan is endoscopic mucosal atrophy, which is characterized by (1) lost gastric folds, (2) pale mucosal color, and (3) increased vessel visibility of background mucosa [46]. The findings of endoscopic mucosal atrophy predict the presence of histological atrophy with sensitivity/specificity of 46%/86% (lost gastric folds) and 79%/68% (increased vessel visibility), respectively [47]. Kimura and Takemoto classified the extent of endoscopic mucosal atrophy into 6 types: from C-1 to O-3, and it is recently merged into the following three types: (1) none-mild, the endoscopic atrophic mucosa confine to the lower part of the corpus lesser curvature; (2) moderate, the endoscopic atrophic mucosa confine to the corpus lesser curvature but extend to the cardia; and (3) severe, the endoscopic atrophic mucosa extend to the anterior/posterior walls or the greater curvature of the corpus (Fig. 9.13). A cohort study by Uemura et al., in which 1526 subjects were enrolled, demonstrated that patients with severe endoscopic mucosal atrophy had 4.9 times higher risk of gastric cancer compared to those with none or mild endoscopic mucosal atrophy [76]. Masuyama et al. evaluated association between extent of the endoscopic mucosal atrophy and gastric cancer and found the gastric cancer prevalence significantly increased as the extent of atrophic gastritis widened [48].

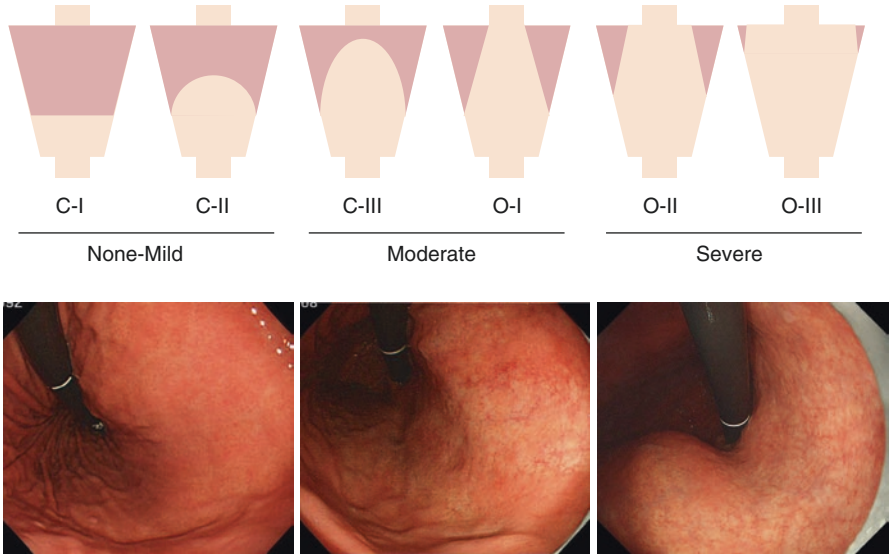


Fig. 9.13 The Kimura-Takemoto classification for atrophic gastritis. Extent of atrophic mucosa characterized by lost gastric folds, pale color, and increased vessel visibility is classified into from C-I to O-III. When the atrophic mucosa confines to the lower part of the corpus lesser curvature, it is classified as "none-mild"; when it extends to the cardia, it is classified as "moderate"; and if it extends to the anterior/posterior walls or the greater curvature of the corpus, it is classified as "severe"

Intestinal metaplasia can be diagnosed according to WLE findings of villous micro-surface appearance, whitish mucosa, and rough mucosal surface with sensitivity/specificity of 94.6%/69.1% in the antrum and 86.1%/65.9% in the corpus [49]. Sugimoto et al. reported that scores for endoscopic finding of atrophy and intestinal metaplasia in the background mucosa according to the Kyoto classification were significantly higher in EGC patients than that in patients with *H. pylori*-associated gastritis. A multivariate analysis revealed that endoscopic findings of intestinal metaplasia (OR 4.5; 95% CI, 3.3–6.0; $p < 0.001$) and male sex (OR 1.7, 1.1–2.7, $p = 0.017$) [50] were independent risk factors for gastric cancer.

Kamada et al. conducted a case-control study to assess gastric cancer risk in young patients with nodular gastritis. In patients with nodular gastritis up to 29 years of age, the gastric cancer risk was significantly higher (odds ratio of 64.2; 95% CI, 16.4–250.9%) than that of age- and sex-matched patients with non-nodular *H. pylori*-associated gastritis, suggesting strong association between nodular gastritis and development of undifferentiated type gastric cancer in young patients [51].

In a cohort study, Watanabe et al. investigated association between endoscopic finding and risk of gastric cancer in patients with positive *H. pylori*-infection but non-atrophic gastritis and indicated that the rugal hypertrophic gastritis (severely

enlarged tortuous folds in the gastric body) was an independent predictor factor for developing gastric cancer (hazard ratio of 43.3, 95% CI of 5.16–363) [52].

Sekikawa et al. indicated that patients with gastric xanthoma had higher incidence of gastric cancer than those without gastric xanthoma in a cohort study. Multivariate analysis indicated that open-type endoscopic atrophy (odds ratio of 7.2; 95% CI, 2.5–21; $P < 0.0001$) and gastric xanthoma (odds ratio of 5.9; 95% CI, 2.7–13; $P < 0.0001$) were independent risk factors for the development of gastric cancer [53].

9.5 Diagnosis to Determine the Indications for Endoscopic Resection

Indications, whether to perform or not to perform endoscopic resection for EGC, are established by endoscopic findings. It is therefore essential to determine the following findings of EGC before treatment: (1) size (extent of a lesion), (2) histological type, (3) depth of tumor invasion, and (4) presence/absence of ulcer or scar [14]. In the latest version of the Gastric Cancer Treatment Guideline 2018 issued by the Japanese Gastric Cancer Association, the following lesions are defined as absolute indication for endoscopic resection: (1) ≤2 cm, differentiated-type, intramucosal cancer (cT1a), without ulcer or scar (UL0); (2) >2 cm, differentiated-type, intramucosal cancer (cT1a), without ulcer or scar (UL0); and (3) ≤3 cm, differentiated-type, intramucosal cancer (cT1a), with ulcer or scar (UL1). A lesion ≤2 cm, undifferentiated type, cT1a, and UL0 is regarded as expanded indication lesion.

After the endoscopic resection, the specimen is fixed with formalin, is cut into every 2–3 mm width strips and their histological finding is evaluated for curativity. Curative resection criteria include (1) size, (2) histological type, (3) depth of tumor invasion, (4) presence/absence of ulcer or scar, (5) presence/absence of lymphovascular involvement, and (6) horizontal/vertical resection margin. If the histological findings of resected specimen did not fulfill curative resection criteria, the patients are recommended for surgery (gastrectomy and lymph node dissection).

9.5.1 Determination of Lateral Margin and Size

Diagnosis of lateral extent of EGC before treatment is crucial to decide indication of endoscopic resection and to achieve complete removal of the lesion. The indigocarmine chromoendoscopy is effective for determining the boundary between cancerous and non-cancerous mucosa according to difference of surface structure of the mucosa associated with lateral extension of cancer tissue (Fig. 9.14) [29]. Diagnostic accuracy of chromoendoscopy for delineation of EGC is reported to be around 80% [54].

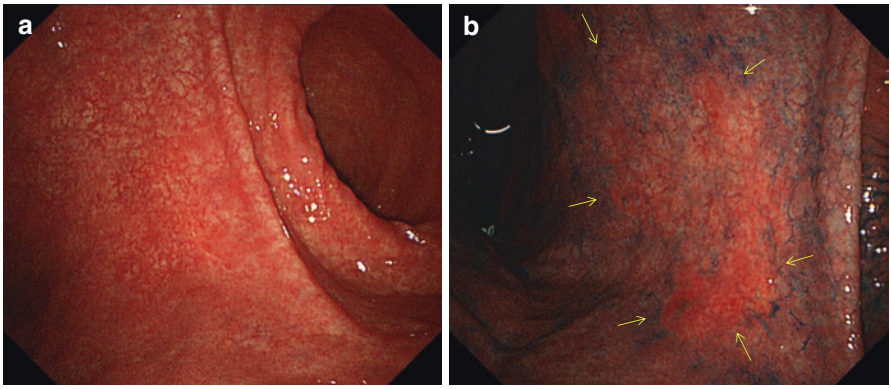


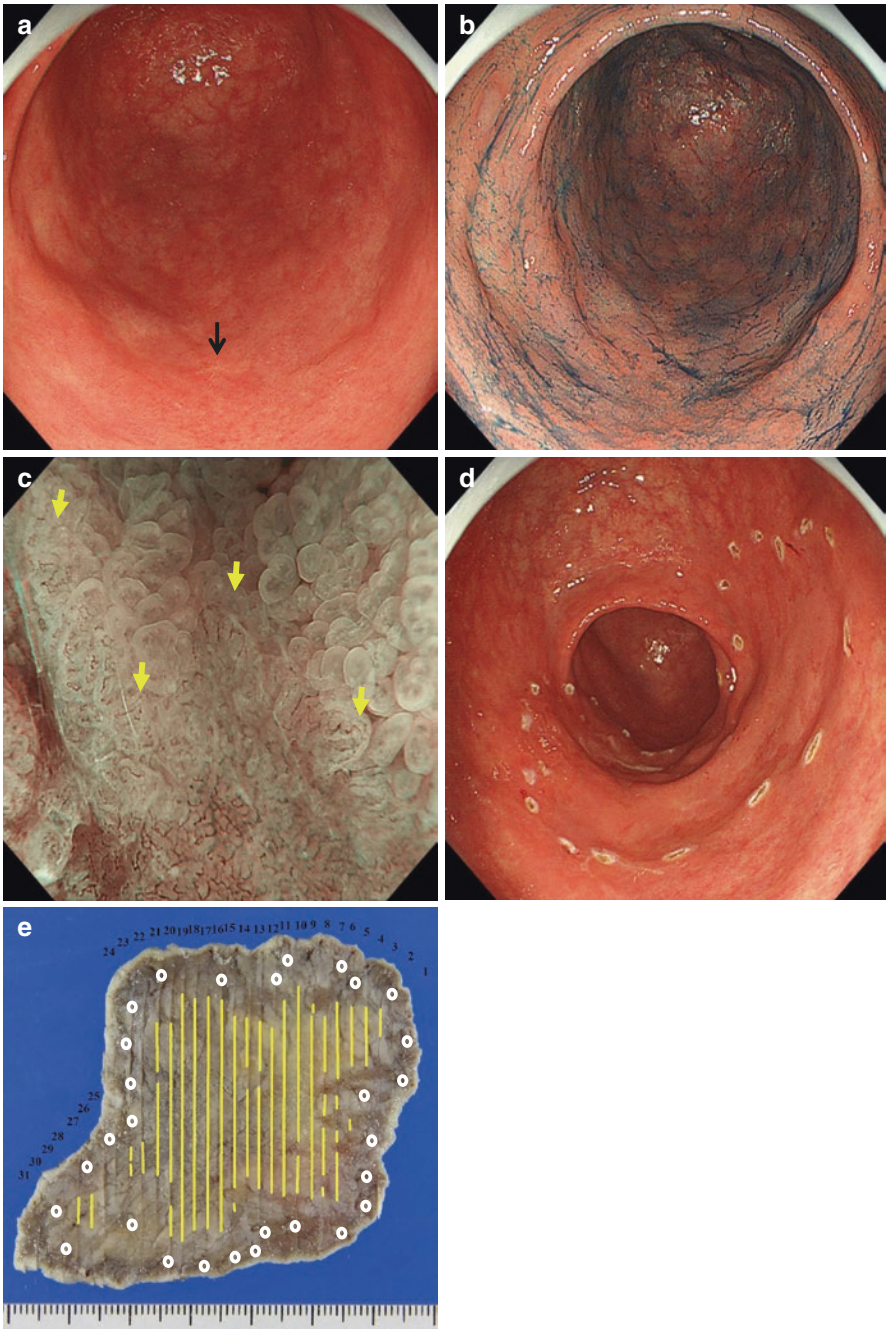
Fig. 9.14 A flat (Type 0-IIb) lesion in the lesser curvature of the corpus. The lesion may be recognized as irregularity of the background vascular network in a white-light endoscopic image (**a**). After application of indigo-carmine chromoendoscopy, it is observed as a large dye-shedding area (yellow arrows in **b**)

Recently, usefulness of M-NBI for delineation of EGC is reported [54–56]. In a retrospective observational study, Nagahama et al. indicated that M-NBI increased accurate delineation rate from 81 to 95% (Fig. 9.15) [54]. A single-center, comparative study showed superiority of M-NBI over chromoendoscopy for delineation of EGC in patients undergoing ESD, with diagnosis accuracy of 89% vs. 76% (P = 0.007) [56]. Recent, multicenter, randomized controlled study including patients who received both ESD and surgery demonstrated similar diagnostic accuracies of M-NBI and chromoendoscopy at 88% and 86%, respectively (P = 0.63) [77]. In this study, the positive resection margin rate after treatment was 0% in both M-NBI and chromoendoscopy groups, suggesting that, even in situation where M-NBI is unavailable, similar clinical outcomes for margin delineation can be achieved by chromoendoscopy.

Usually lesion size is roughly estimated by gross endoscopic finding. In practice, the lesion size can be objectively measured in comparison with an endoscope diameter, opened biopsy forceps, measuring rubber disk [57], or measuring forceps (M2-1C, -2C, Olympus medical systems, Corp, Tokyo, Japan). Although indication to perform endoscopic resection is determined by the size in endoscopic images, curativity of resection is judged by histological size because the background data for risk of lymph node metastases is obtained on the basis of histological findings.

9.5.2 Histological Types

Histological type is an important factor to determine the indication of endoscopic resection in patients with EGC [14, 15]. Morphological type is useful for estimation of histological type of EGC in clinical practice (Fig. 9.16). Protruding (0-I) or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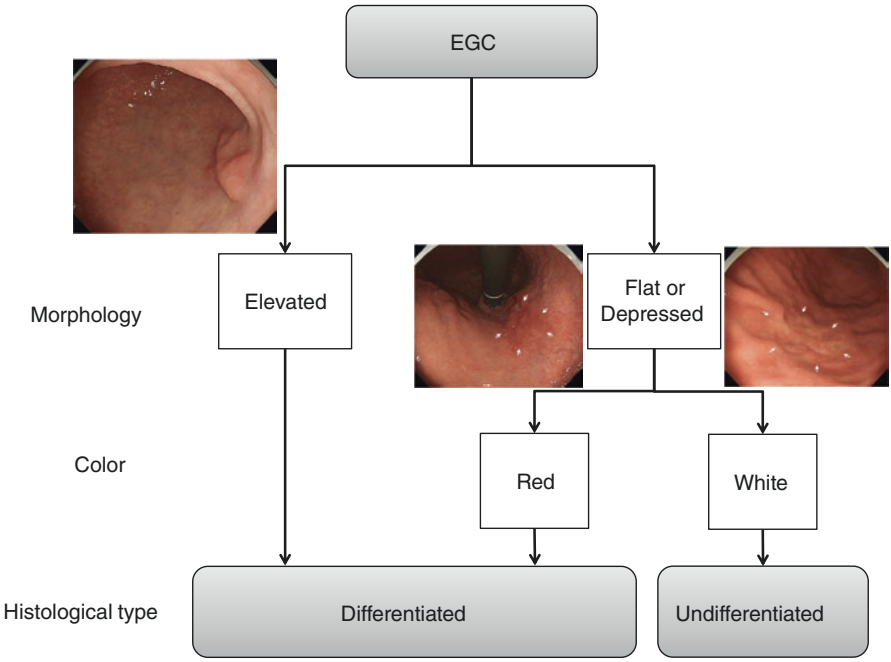

Fig. 9.16 Diagnostic algorithm for histological type of early gastric cancer in white-light endoscopy. Elevated lesion is usually a differentiated type. For a flat or depressed lesion, differentiated type is likely to be reddish, whereas undifferentiated types tend to be whitish

superficially elevated-type (0-IIa) EGC is likely to be a differentiated type and is rarely an undifferentiated type (sensitivity of 24%, specificity of 99%, and positive and negative likelihood ratios of 15.7 and 0.77, respectively) [58]. For EGC with depressed-type morphology, differentiated type usually looks reddish, whereas undifferentiated type looked whitish [59]. In more detailed morphological characteristic, a differentiated-type depressed EGC has the uniform surface and spiculated margin at the depressed area and is often accompanied by marginal elevation (Fig. 9.17a, b), while an undifferentiated-type depressed EGC has depressed area, which is often accompanied by nodules of regenerative mucosa inside, with sharp precipitous margin (Fig. 9.17c, d) [60]. Assessment of the findings of the

Fig. 9.15 A lesion in that magnifying NBI was useful for delineation of the boundary. A tiny depression was observed at the greater curvature of the incisura angularis (**a**). Indigo-carmine chromoendoscopy did not delineate the lesion boundary well (**b**). Magnifying NBI revealed boundary of the lesion according to changes of microvessel and micro-surface patterns (yellow arrows in **c**). The marking was made according to the magnifying NBI findings (**d**). The lesion was removed by endoscopic submucosal dissection technique with clear resection margins (**e**, yellow bars indicated histological extent of the lesion)

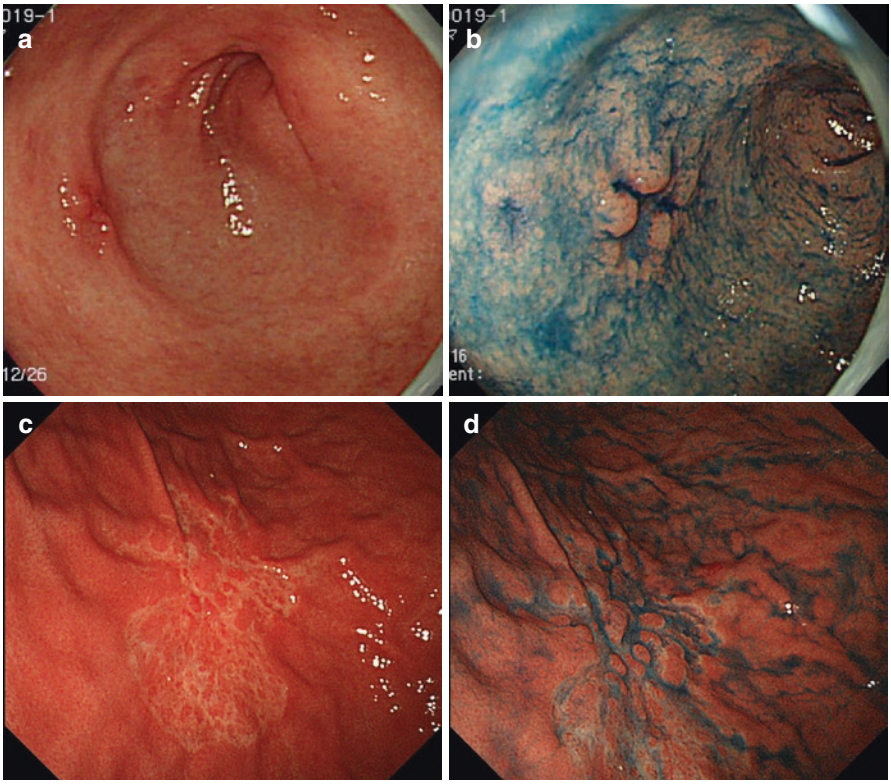

Fig. 9.17 Representative endoscopic images of a differentiated type early gastric cancer (EGC). A reddish depressed lesion exists in the antrum (**a**), and the lesion has spiculate margin in chromoendoscopic image (**b**). Representative endoscopic images of an undifferentiated type EGC. A whitish depressed lesion with multiple reddish granules is seen in the lower corpus (**c**), and chromoendoscopy revealed sharp precipitous margin (**d**)

background mucosa is also helpful to predict histological type of EGC. A differentiated type lesion develops more frequently in the mucosa with atrophy and/or intestinal metaplasia, whereas an undifferentiated type lesion tends to develop on mucosa with few atrophic changes [60].

Recently, usefulness of M-NBI for differentiation of histological types of EGC is reported. In the differentiated type, the lesion has a clear demarcation line, and irregular microvessels show fine network pattern (sensitivity of 66% and specificity of 96%, Fig. 9.18a) [61, 62]. In contrast, in the undifferentiated type, the regular surface pattern of the background mucosa is lost [63], and irregular microvessels show corkscrew patterns inside the demarcation line (sensitivity of 86% and specificity of 96%; Fig. 9.18b) [61, 62].

Basically, histological type to determine indication of endoscopic resection is referred to histological finding of biopsy specimens. However, point diagnosis by forceps biopsy may not accurately reflect entire histological finding because

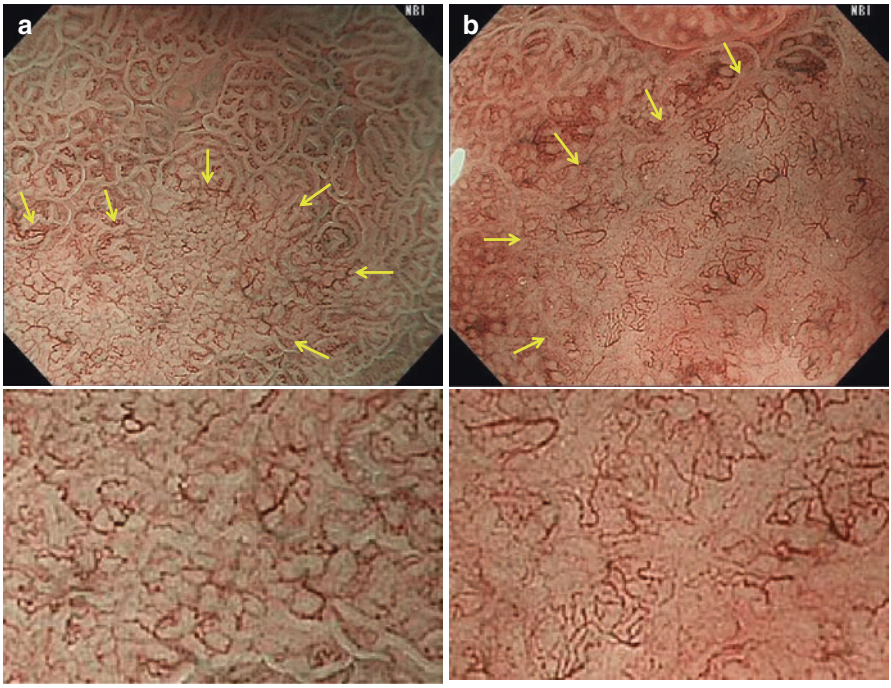

Fig. 9.18 Magnifying NBI images of differentiated type (**a**) and undifferentiated type (**b**) early gastric cancers. The former has fine network patterns of irregular microvessels, while the latter shows corkscrew pattern of irregular microvessels

histological type of EGC is often ununiform. One of advantages of endoscopic diagnosis of histological type is capability of examining a whole area of the lesion. Endoscopic diagnosis of histological type would improve diagnostic accuracy by targeted forceps biopsy and offers comprehensive consideration to decide treatment indication of endoscopic resection.

9.5.3 Invasion Depth

Indication of endoscopic resection is cT1a (intramucosal), and there is no definitive diagnostic criteria for cT1b1 (shallow, ≤500 μm, submucosal invasion); therefore, differential diagnosis of cT1a from cT1b (submucosal) EGC is important to decide treatment indication. Morphological evaluation by conventional WLE is currently the most commonly used method for diagnosis of tumor depth in Japan. The indicative findings for T1b2 (deep, >500 μm, submucosal invasion) EGC in conventional endoscopy include enlargement of converged fold, fusion of converged folds [64, 65], size 30 mm or more [66], marked redness [65, 66] surface irregularities [65–67], marginal protrusions [68], and submucosal tumor-like marginal elevation [66, 67].

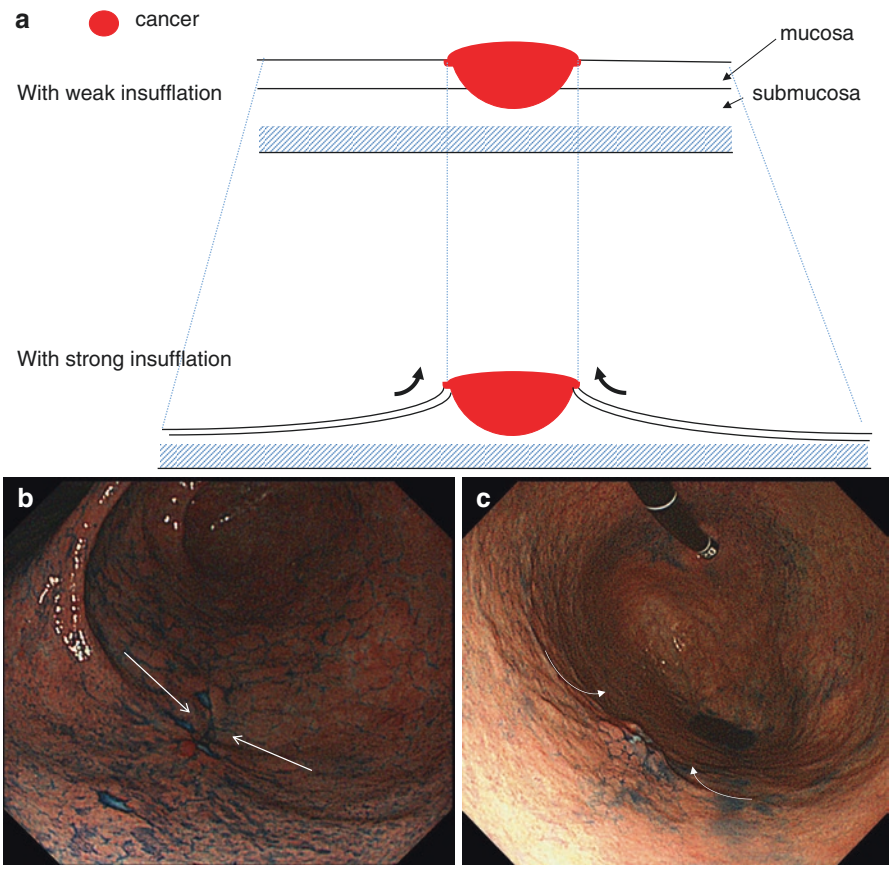

Fig. 9.19 Schematic images of the non-extension sign (**a**). With large amount of air insufflation, an intramucosal early gastric cancer (EGC) appears flattened (**b**). In contrast, with strong extension of the gastric wall, a submucosal invasive EGC protrudes toward the lumen with lifting of the surrounding non-cancerous mucosa because of the fibrotic cancerous tissue in the submucosa (**c**)

The positive predictive values for T1b2 using these indices are reported to be 63–89%; however, the use of the multiple indices makes confusion among evaluaters and may increase variability of diagnosis. Recently, Nagahama et al. demonstrated the efficacy of the non-extension sign as a predictor for deep submucosal invasion of EGC [68]. In this endoscopic finding, fibrosis in the stroma of the submucosal cancerous glands (desmoplastic reaction) causes submucosal tumor-like marginal elevation when the gastric lumen is distended by sufficient air insufflation (Fig. 9.19). Evaluation of only this finding provides excellent diagnostic performance (sensitivity of 92.0% and specificity of 97.7%) for depth prediction; thus further validation by multicenter prospective study is warranted.

Many reports have described the usefulness of endoscopic ultrasound (EUS) for diagnosis of tumor depth of EGC [69–72]. However, some comparative studies suggest that expert endoscopists' conventional endoscopic diagnosis is almost similar (71% vs. 63%) [72] to or even better (73.7% vs. 67.4%, $p < 0.001$) [73] than EUS. In particular, Tsujii et al. indicated that, for lesions diagnosed as cT1a by conventional WLE, EUS did not change the diagnosis in 82% of cases; however, for lesion diagnosed as cT1b by WLE, EUS downstaged the tumor depth in 42%, enabling the judgment on indication for endoscopic resection [67]. Therefore, for lesions appear cT1a in WLE, ESD can be carried out directly, whereas EUS for lesions diagnosed as cT1b by WLE may reduce over surgery.

9.5.4 Ulceration and Scar

For EGC with ulceration or scar, endoscopic resection is indicated for only lesion ≤3 cm with differentiated histological type, and endoscopic resection is contraindication for lesions with undifferentiated histology. In principle, the presence of ulceration is determined on the basis of mucosal defect or mucosal convergence in WLE. Sometimes, an intramucosal depressed type EGC is covered by whitish mucous or exudate, so it is important to distinguish it from open ulcer. Sole fold convergence is not a sign of deep submucosal invasion. The converged folds by ulcer scar in an intramucosal EGC are straight or tapered lines toward a single point [68]. Enlarged disrupted folds on the margin of the lesion arise a suspicion of deep submucosal invasion. For EGC with ulcer scar, depth and amount of fibrosis in the gastric wall are associated with difficulty of ESD. EUS can predict not only presence of ulcer scar but also difficulty of ESD procedure by assessing ulcer depth [74].

9.6 Summary

The current Japanese practice of endoscopic diagnosis of EGC, in relation to the available evidence and the common consensus among Japanese endoscopists was presented. Endoscopic resection cannot be performed unless the intramucosal EGC is detected. The good clinical outcome is achieved by proper treatment indication based on accurate diagnosis. We hope the information in this chapter help improving endoscopic diagnosis, management and outcome in patients with EGC.

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Part IV
Therapy

Chapter 10
Endoscopic Treatment

Takuji Gotoda

Abstract In the past, gastrectomy with lymph node dissection was the gold standard treatment for all patients with operable gastric cancer including early cancer. It has been well known that the incidence of lymph node metastasis from early gastric cancer is not so high. If the group with very low risk of lymph node metastasis is identified, cure can be accomplished by endoscopic resection as local control.

EMR techniques could not be used to remove lesions en bloc larger than 2 cm. Piecemeal resections in lesions larger than 2 cm lead to a high risk for local cancer recurrence and inadequate pathological staging. Thus, the indication for endoscopic resection has been very strict. ESD which can allow en bloc resection regardless of the tumor size is now standard option. From revised Gastric Cancer Treatment Guideline, the case now classified into expanded criteria is modified as absolute criteria, if the lesion is removed by ESD.

When the tumor does not meet several pathological factors, the resection is finally valued as "non-curative" resection, then recommended to undergo surgery. However, only 5–10% lymph node metastasis is found in patients who underwent surgery. Recently, a simple scoring system called as "eCura system" for decision-making in patients with non-curative ESD has been established using large-scale retrospective study. This scoring system predicted cancer-specific survival in patients who did not meet the curative criteria. ESD without additional treatment may be an acceptable option for patients at low risk, especially elderly patients.

In the medical care, the duty of medical professionals should be to alleviate the concern of patients as much as possible by providing them with detailed information on postoperative outcomes and potential risks estimated based on outcome assessment. Moreover, medical professionals must continuously consider whether complete treatment attempted by physicians is beneficial for patients and whether treatment that is not the best but more tolerable to the patients is an option.

Keywords Early gastric cancer · Japanese Gastric Cancer Treatment Guideline · eCura system · Endoscopic submucosal dissection · Clutch Cutter

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Abbreviation

DFC	Dental floss and a hemoclip
EGC	Early gastric cancer
EMR	Endoscopic mucosal resection
EMRC	EMR with cap-fitted panendoscope method
EMRL	EMR using multiband ligation
ESD	Endoscopic submucosal dissection
IT knife	Insulated-tip diathermic knife
LNM	Lymph node metastasis
QOL	Quality of life

10.1 Introduction

In the history of gastric cancer treatment, many of the cases with gastric cancer discovered in the 1970s were in the advanced stage. As represented by the Appleby operation, extended radical surgery with lymph node metastasis (LNM) was globally accepted as a mainstream approach to gastric cancer, even in the early gastric cancer (EGC). With the widespread adoption of nationwide screening in Japan [1], and the advancement of endoscope technology in the 1980s, the number of patients diagnosed with early gastric cancer has increased.

In cancer treatment, completely curing the illness is extremely important. However, if quality of life (QOL) is impaired by procedures that are superior only in terms of reducing marginal risks, patients may have difficulties in daily life and social rehabilitation after treatment. The stomach not only serves as a storage compartment but also plays a role in external secretion for digestion and absorption as well as in internal secretion. Therefore, if there is no difference of curability among different treatment methods, long-term QOL should be considered seriously when we select a treatment method, especially in elderly patients.

Medical care will always be provided with consideration of the following points: whether treatment is really minimally invasive, whether "complete" treatment attempted by physicians is beneficial for patients, and whether treatment that is not the best but more tolerable to the patients is an option [2].

10.2 Overview of Endoscopic Resection for EGC

Endoscopic resection to treat cancer is perhaps the most gratifying endoscopy to perform because of its minimally invasive curative potentials [3]. Endoscopic resection allows complete pathological staging of the cancer, which is critical for potential of metastasis [4]. Patients who are stratified to have no or lower risk for LNM than the risk of mortality from surgery are ideal candidates for endoscopic resection

[5, 6]. The optimal staging method of early gastrointestinal cancer is to evaluate the pathology of en bloc resected material [7, 8]. In addition to allow pathological staging, en bloc resection with negative vertical and horizontal margins is to protect the patient from the risk of local recurrence.

The first endoscopic resection was reported in colorectal polypectomy using high-frequency electric surgical unit [9]. Indeed the first endoscopic polypectomy used to treat pedunculated or semipedunculated EGC was first described in Japan in 1974 [10].

The "strip biopsy" technique, an early method of endoscopic mucosal resection (EMR) technique, was devised in 1984 as an application of endoscopic snare polypectomy [11]. To obtain resected material with less tissue damage causing adequate pathological staging, a technique called ERHSE (endoscopic resection with local injection of hypertonic saline epinephrine solution) was developed in 1988 [12].

EMR with cap-fitted panendoscope method (EMRC) was developed in 1992 for the resection of early esophageal cancer and directly applicable for the resection of EGC [13, 14]. The technique of EMR using ligation, which subsequently was extended to EMR using multiband ligation (EMRL), utilizes band ligation to create a "pseudopolyp" by suctioning the lesion into the banding cap and deploying a band underneath it [15, 16]. The EMRC and EMRL techniques have the advantage of being relatively simple. However, these techniques cannot be used to remove lesions en bloc larger than 2 cm [17, 18]. Piecemeal resections in lesions larger than 2 cm lead to a high risk for local cancer recurrence and inadequate pathological staging [19, 20].

Insulated-tip diathermic knife (IT knife) was devised in late 1990s at the National Cancer Center Hospital Japan in order to resolve problems observed from use of the EMR techniques for the resection of EGC. IT knife has a ceramic ball tip, thus preventing it from puncturing the wall during the application of cautery and causing perforation. The knife can also be used to dissect the submucosa—leading to the name of the technique: endoscopic submucosal dissection (ESD) technique [21–23]. Subsequent studies have proven that ESD, using standard single channel endoscope, can be used for resection of large lesions "en bloc" allowing a precise pathological staging. Complete en bloc resection regardless of tumor size, location, and/or submucosal fibrosis can now be possible [24]. Very recently, ESD has been tried to improve an easier procedure [25, 26].

10.3 Procedure of ESD for Stomach

ESD has higher risk of complications such as severe bleeding or perforation and still requires high endoscopic skills. In order to standardize ESD procedure worldwide, more innovation and modification should be demanded. The traction method using dental floss and a hemoclip (DFC, any hemoclip available) for gastric ESD can make submucosal dissection easier and safer because of good visualization and tension whenever we dissect submucosal layer by any ESD devices (Fig. 10.1) [25,

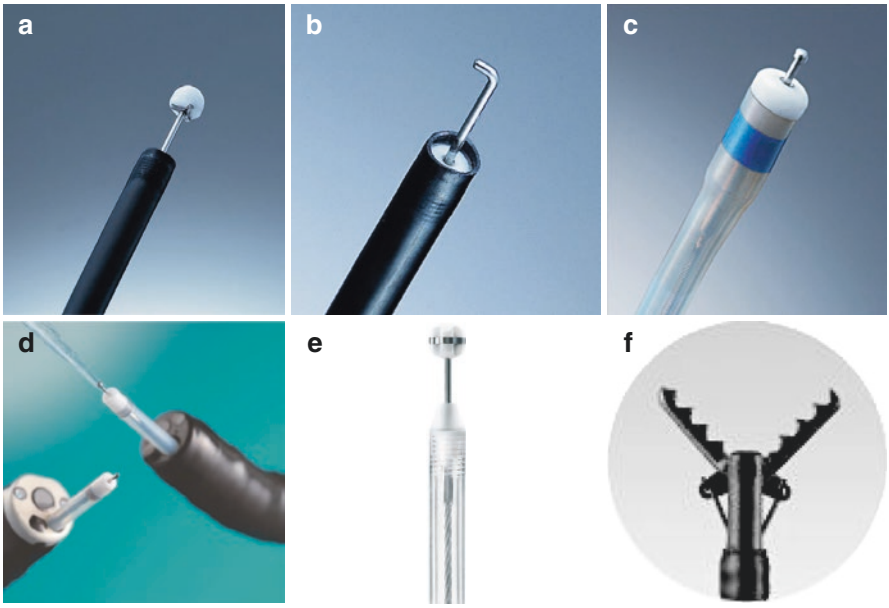

Fig. 10.1 (**a**) IT knife-2 (KD-611L, Olympus Medical Systems). (**b**) Hook knife (KD-620LR, Olympus Medical Systems). (**c**) Dual knife (KD-650L, Olympus Medical Systems). (**d**) Flash knife BT (FUJIFILM Medical Co, Ltd). (**e**) Safe knife (DK2518DV1, FUJIFILM Medical Co, Ltd). (**f**) Clutch Cutter (DP2618DT-50-, FUJIFILM Medical Co, Ltd)

26]. It has been standard that several steps for ESD (marking, injecting fluid, circumferentially mucosal cutting, and submucosal dissection) are carried out by IT knife and needle-type devices in Japan [27].

It is widely accepted that ESD has big advantage to achieve en bloc resection for EGC. However, ESD using conventional devices is technically difficult and requires intensive training under an expert because these knives lack the ability to grasp the targeted tissue which means difficult maneuverability under instability condition (like single-hand surgery). Comparing those devices, Clutch Cutter is technically easier and simpler to perform without any skillful tips. Thus, gastric ESD using Clutch Cutter (DP2618DT-50-, FUJIFILM Medical Co, Ltd) is maybe acceptable in the countries with less incidence of EGC. Thus, in order to standardize gastric ESD procedure, simple ESD with Clutch Cutter under the traction method using DFC as non-tips method is demonstrated in this chapter [28, 29].

10.3.1 Settings

Clutch Cutter used for gastric ESD has a 0.4-mm-wide and 5-mm-long serrated cutting edge well grasping function. The outer side of the forceps is insulated so that electrosurgical current energy is concentrated at the closed blade. Forced coagulation mode (VIO 300D; Erbe, Tübingen, Germany) 30 W (effect 3) is used for

marking, ENDO-CUT Q mode (effect 1, duration 3, interval 1) is used for mucosal incision and submucosal dissection, and soft coagulation mode 100 W (effect 5) is recommended for hemostatic treatment.

A soft transparent hood (JMDN 38819001, Top Corp, Tokyo, Japan) or a small-caliber-tip transparent hood (ST hood, FUJIFILM Medical Co, Ltd) is sometimes useful to stabilize the operating field and to create counter-traction for exfoliating the submucosal tissue [30].

10.3.2 Mucosal Incision

The EGC with 2 cm in size is found on lesser curvature of the gastric angular. Mucosal incision on the peripheral side of the marking dots is smoothly carried out under submucosal injection by normal saline with indigo carmine dye because Clutch Cutter is rotatable to the desired orientation (Fig. 10.2a). Indigo carmine is added to the submucosal injection fluid in order to better identify the blue-colored submucosal layer (any injection needle available). Sodium hyaluronate (MucoUp, Boston Scientific Japan, Tokyo) is also often used because of longer-lasting submucosal cushion in order to prevent perforation [31].

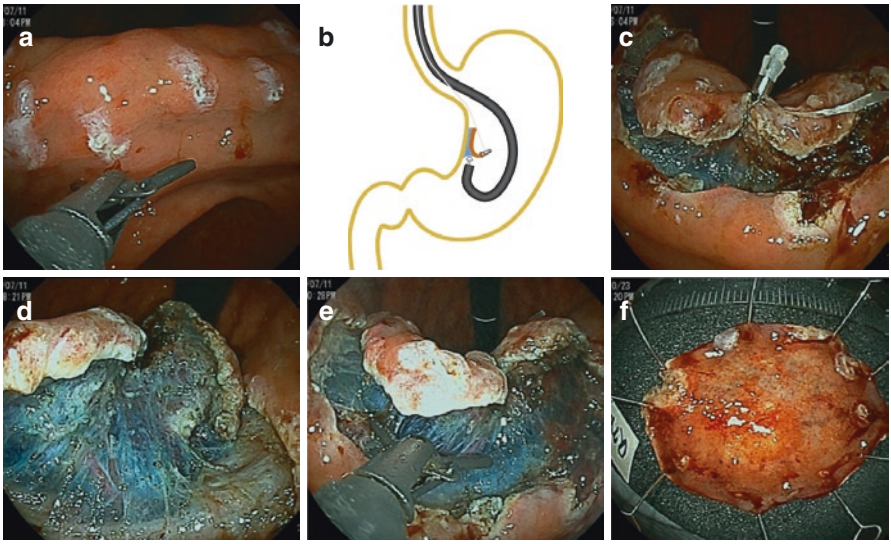

Fig. 10.2 (**a**) Mucosal incision using Clutch Cutter-like scissors. (**b**) Schema and endoscopic view of ESD with traction method using dental floss and a hemoclip, involving an approach from the retroflex endoscopic position: In lesions located in the lesser curvature of the gastric angular, the anal side of the resected mucosa is elevated by pulling the dental floss out through the mouth. (**c**) Hemoclip—tied by dental floss—as an anchor for traction. (**d**) Good visualization and tension of the submucosa by oral traction. (**e**) Combination with soft coagulation mode and ENDO-CUT Q mode for submucosal layer with vessels. (**f**) The resected material should be orientated and pinned at its periphery onto a backing with thin needles immediately after its resection

10.3.3 Submucosal Dissection

After completing the circumferential cutting, the submucosal layer underneath the lesion is directly dissected. At this step, traction method is very useful and makes dissection easy, safe, and rapid because of good visualization. The DFC is anchored to a suitable site of the lesion for oral traction (Fig. 10.2b). The clip varies according to the location of the lesion. In lesions approached from the retroflex endoscopy position, the clip is anchored at the anal side edge of the resected mucosa (Fig. 10.2c). During submucosal dissection, the anchored suture material located outside of the patient is pulled to the oral side with gentle manual traction by the operator or an assistant (Fig. 10.2d, e). Good visualization and tension of the submucosa are obtained by the resected mucosa that is turned over.

When a small artery and/or vein in submucosal layer is found, Clutch Cutter can first control with soft coagulation mode and after that cut it with ENDO-CUT Q mode. However, do not hesitate to change Clutch Cutter to Coagrasper G (Olympus Medical Systems) which is much effective in grasping the bleeding vessel and controlling it.

10.3.4 Treatment of Resected Material for Pathological Assessment

The importance of meticulous pathological staging after endoscopic resection cannot be overemphasized. Accurate staging can only be achieved when the specimen is properly oriented by the endoscopist or their assistant immediately after excision in the endoscopy unit prior to be immersed in formaldehyde.

Orientation of the specimen is best performed by fixing its periphery with thin needles inserted into an underlying plate of rubber or wood (Fig. 10.2f). The submucosa side of the specimen is placed in contact with the plate. After fixation, the specimen is sectioned serially at 2 mm intervals parallel to a line that includes the closest resection margin of the specimen so that both lateral and vertical margins are assessed. The depth of tumor invasion (T) is then evaluated along with the degree of differentiation and lymphovascular invasion, if any. The report must include histological type, tumor depth, size, location, and macroscopic appearance. The presence of ulceration and lymphatic and/or venous invasion and the status of the margins of resection should be reported in detail to determine the curability.

10.4 Surveillance After Gastric ESD

According to the Japanese guidelines, the curability after ESD/EMR for EGC is classified into three groups: curative resection, curative resection for expanded indication, and non-curative resection (Fig. 10.3) [3, 32–35]. En bloc resection with no

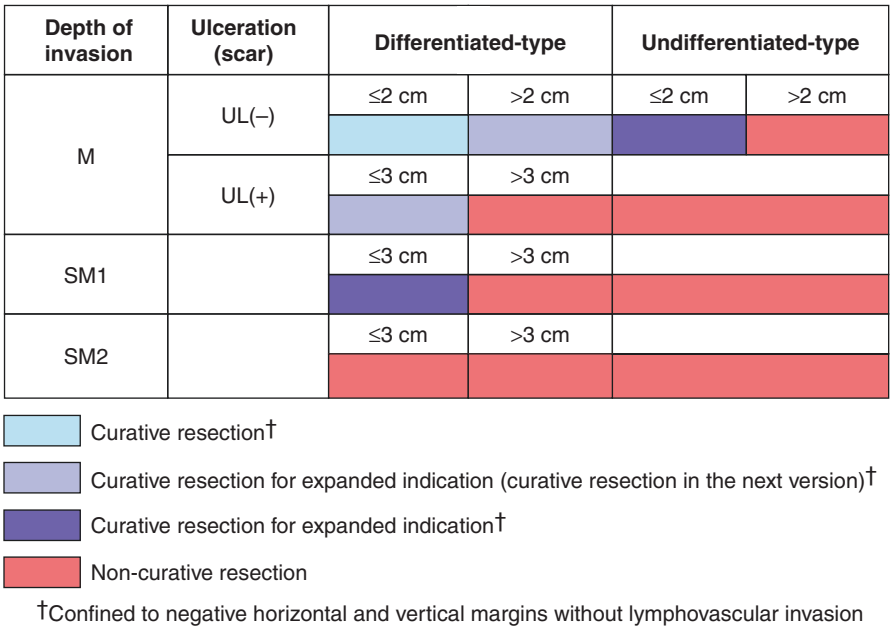

Fig. 10.3 The therapeutic flowchart after gastric ESD/EMR in the Japanese guidelines

lymphovascular invasion and a negative surgical margin are required for curative resection or for expanded indication. No additional treatment is needed in patients with curative resection.

According to the European guidelines (European Society of Gastrointestinal Endoscopy) [36], additional treatment is also not necessary after curative resection, which is the same as in the Japanese guidelines. In the USA, the National Comprehensive Cancer Network Clinical Guidelines (NCCN guidelines) regard EMR and ESD as having the potential of being therapeutic and one of the treatment options for Tis or T1a cancer ≤2 cm [37].

After gastric ESD, we have to pay attention to the development of metachronous gastric cancers. The 5-year and 10-year cumulative incidences were 9.5% and 22.7%, respectively. [38] Almost all secondary gastric cancers were treatable by ESD by the scheduled endoscopic surveillance (6–12 months) [39]. The Japanese guidelines also recommend endoscopic surveillance at intervals of 6–12 months, whereas ESGE and NCCN guidelines recommend annual endoscopy from 1 year after ESD/EMR. Thus, when complete resection could be achieved for the initial EGC, the following endoscopic surveillance is recommended after ESD/EMR (Fig. 10.4) [40].

When the histopathological findings meet the expanded criteria, no additional treatment is needed in the Japanese guidelines (Fig. 10.3). Recently, a multicenter retrospective analysis in Japan clarified that 0.14% (6/4202) of such patients had metastatic recurrence during the median follow-up duration of 56 months after ESD

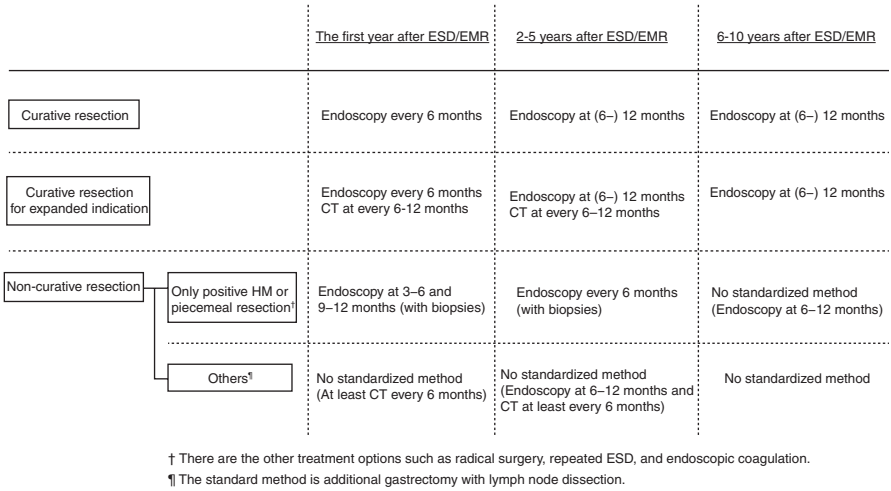

Fig. 10.4 The flowchart of follow-up after gastric ESD/EMR

[41]. Surveillance for metastatic recurrence as well as metachronous gastric cancer is recommended, although the risk of the former is very small. In addition to endoscopic surveillance at every 6 months in the first year and at intervals of 6–12 months for at least 10 years after ESD/EMR, follow-up with computed tomography (CT) (or ultrasonography) is desirable at intervals of 6–12 months. Anyway, we have to carefully explain that these patients have a negligible but not zero risk of metastatic recurrence after gastric ESD/ESD.

It is controversial whether the expanded criteria are applicable for European patients. For differentiated-type EGC, the ESGE recommends ESD for EGCs that meet the expanded criteria, whereas ESMO and the German Society of Gastroenterology give restrictive recommendations [42, 43], which recommend gastrectomy for cases meeting the expanded criteria. Regarding undifferentiated-type EGC, the ESGE guidelines regard ESD for the expanded criteria as an option. In such patients, the ESGE guidelines recommend that gastrectomy is always considered with the decision made on an individual basis. There has been no report about the expanded criteria for gastric ESD/EMR in the USA. As described previously, the NCCN guidelines regard EMR or ESD as one of the treatment options only for Tis or T1a cancer $\leq$2 cm. However, a report based on the Surveillance, Epidemiology, and End Results (SEER) database of the USA suggests the existence of different biological aggressiveness in T1a gastric cancer among racial/ethnic groups [44].

When the lesion does not meet the curative criteria, the lesion is regarded as non-curative resection. In cases of differentiated-type EGC with the only unsatisfactory curative factor of piecemeal resection or resection en bloc with a positive horizontal margin, surgical resection is not the only option because such cases have a very low risk for harboring LNM. Repeated ESD, endoscopic coagulation using a laser or

argon-plasma coagulator, or close observation expecting a burn effect of the initial endoscopic resection could be proposed as an alternative in such cases, with the patient's informed consent.

In the other type of non-curative resection, additional gastrectomy with lymph node dissection is recommended in the ESGE and Japanese guidelines because such lesions have the potential for LNM. When gastric ESD/EMR is performed, 17–29% of the patients do not meet the curative criteria. However, LNM is found in only 5–10% of patients with such lesions [45]. In the clinical setting, nearly half of such patients are followed up with no additional treatment after ESD in Japan, due to the age, underlying disease, and patients' preference. Also, in Germany, 69% (27/39) of such patients were followed up with no additional treatment after non-curative resection for EGC [46].

A randomized controlled trial clarified that prophylactic eradication of *Helicobacter pylori* after ESD/EMR for EGC reduced the risk of metachronous gastric cancer to about one-third [47]. However, some studies including one randomized controlled trial revealed conflicting results [48]. Although eradication therapy is recommended in *Helicobacter pylori*-infected patients, further investigation about this issue is needed.

10.5 Future Perspective

Patients who are stratified to have no or lower risk for LNM than the risk of mortality from surgery are ideal candidates for endoscopic resection. Endoscopic resection allows complete pathological staging of the cancer, which is critical for potential of metastasis. The optimal staging method of EGC is to evaluate the pathology of en bloc resected material [7, 8].

In cancer treatment, completely curing the illness is extremely important. However, if QOL is impaired by procedures that are superior only in terms of reducing marginal risks, patients may have difficulties in daily life and social rehabilitation after treatment [49, 50]. The stomach not only serves as a storage compartment but also plays a role in external secretion for digestion and absorption as well as in internal secretion. Therefore, if there is no difference of curability among different treatment methods, long-term QOL should be considered seriously when we select a treatment method, especially in elderly patients.

Recently, a simple risk-scoring system, named eCura system, was established for stratifying the risk for LNM in such patients [51]. This is a seven-point scoring system with three risk categories based on five clinicopathological factors in order to predict LNM. In this system, three points is assigned for positive lymphatic invasion, and one point is assigned for tumor size of >30 mm, SM2 invasion, positive venous invasion, and positive vertical margin. The rate of LNM in the low (0–1 point), intermediate (2–4 points), and high-risk (5–7 points) categories were 2.5%, 6.7%, and 22.7%, respectively. In addition, when the patients were followed up with no additional treatment after non-curative resection for EGC, 5-year CSS in each

risk category was 99.6%, 96.1%, and 90.1%, respectively. A Japanese multicenter evaluation of laparoscopic gastrectomy (mainly distal gastrectomy) for EGC reported 5-year CSS rates of 99.8% for stage T1a disease and 98.7% for stage T1b disease [52]. Thus, although radical surgery is the standard therapy for patients with non-curative resection for EGC, eCura system provides useful information for deciding the treatment strategy after non-curative resection for EGC, especially in elderly patients and/or those with severe comorbidities.

10.6 Conclusion

The major advantage of endoscopic resection which is local treatment is the ability to provide an accurate pathological staging without precluding future surgical therapy. After endoscopic resection, depth of cancer invasion, degree of cancer differentiation, and involvement of lymphatics or vessels should be carefully assessed for predicting the curability and the risk of LNM. The risk of LNM is then weighted against the risk of surgery.

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Chapter 11
Surgical Treatment: Evidence in Gastric Cancer Surgery Based on Japanese Clinical Trials

Hideaki Shimada

Abstract Obtaining evidence in surgical oncology requires the time and effort of several surgeons. More than 15 years were needed even for D2 lymph node dissection to be generally approved in the Western world after completion of phase III studies. The Japanese Gastric Cancer Society, with a history of more than 50 years, has contributed to many clinical trials based on the Japanese Clinical Oncology Group during the past 30 years. During the past 10 years, the clinical significance of D2 lymphadenectomy, the left thoracotomy approach, para-aortic node dissection, splenectomy, and bursectomy has been evaluated in phase III studies. Although only a few studies have been completed, the laparoscopic approach and robotic surgery have been increasingly performed. Here we review recent evidences for surgical treatment of gastric carcinoma, focusing on Japanese clinical trials.

Keywords Gastric cancer · Lymphadenectomy · Splenectomy · Randomized trial

11.1 Introduction

Surgical treatment of gastric cancer has mainly focused on management of localized disease, with or without appropriate adjuvant chemotherapy. In the early 1970s in Japan, a general consensus about extended lymphadenectomy was developed, which has shown consistent overall survival rates of more than 50% for locally advanced gastric cancer after surgery. Although several procedures combined with extended dissection were performed, there was little evidence from randomized trials before the 2000s. Since the beginning of the twenty-first century, cutting-edge evidence has been established from randomized clinical trials in Japan. This review focuses on changes in the surgical paradigm for gastric cancer. The main topics are D2 lymphadenectomy, para-aortic lymphadenectomy, left thoracotomy, splenectomy, and bursectomy. Recent evidence for laparoscopic gastrectomy and robotic surgery is also reviewed (Table 11.1).

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Table 11.1 Randomized phase III trials of gastric cancer surgery

References	Trials	Clinical questions	No. of patients	Results	Conclusions
Bonenkamp JJ et al. 1999 [1]; Songun I et al. 2010 [5]	Dutch D1D2	D1 vs D2	1078	15-year OS rate was D1 group (21%) vs D2 group (29%) ($p = 0.34$). Gastric cancer-related death rate was D1 group (48%) vs D2 group (37%). Local recurrence was D1 group (22%) vs D2 (12%). Operative mortality rate was D1 (10%) vs D2 (4%) $p = 0.004$)	D2 lymphadenectomy is associated with lower locoregional recurrence and gastric cancer-related death rates than D1 surgery. The D2 procedure was also associated with significantly higher postoperative mortality, morbidity, and reoperation rates
Terashima et al. 2017 [8]	JCOG1001	Brusectomy	1204	3-year OS were non-bursectomy (86.0%) vs bursectomy arm (83.3%)	Although bursectomy can be safely performed without increasing morbidity and mortality, bursectomy was not recommended as a standard treatment for cT3 or cT4 gastric cancer
Sasako et al. 2006 [10]; Kurokawa et al. 2015 [11]	JCOG9502	Left thoracoabdominal approach (LTA)	167	5-year OS rate was TH (52.3%) vs 37.9% vs LTA (37.9%)	LTA does not improve survival after TH and leads to increased morbidity. LTA resections should be avoided in the treatment of adenocarcinoma of the EGJ or gastric cardia

Table 11.1 (continued)

References	Trials	Clinical questions	No. of patients	Results	Conclusions
Sano et al. 2017 [12]	JCOG0110	Splenectomy	505	5-year OS were splenectomy (75.1%) vs spleen preservation (76.4%) noninferiority of spleen preservation was confirmed ($p = 0.025$)	In total gastrectomy for proximal gastric cancer that does not invade the greater curvature, splenectomy should be avoided as it increases operative morbidity without improving survival
Sano et al. 2004 [13]; Sasako et al. 2008 [14]	JCOG9501	Para-aortic nodal dissection (PAND)	523	5-year OS rate was D2 (69.2%) vs D2 lymphadenectomy plus PAND (70.3%)	D2 lymphadenectomy plus PAND does not improve the survival rate
Fujitani et al. 2016 [15]	JCOG0705 REGATTA	Reductive gastrectomy	175	2 years OS was chemotherapy alone (31·7%) vs gastrectomy plus chemotherapy (25·1%). Median overall survival was chemotherapy alone (16·6 months) vs gastrectomy plus chemotherapy (14·3 months)	Since gastrectomy followed by chemotherapy did not show any survival benefit compared with chemotherapy alone in advanced gastric cancer with a single non-curable factor, gastrectomy cannot be justified for treatment of patients with these tumors
Sakuramoto et al. 2007 [4]; Sasako et al. 2011 [6]	ACTS-GC	Adjuvant with S-1	530	5 years OS was S-1 (71.7%) vs surgery only (61.1%). 5 years RFS was S-1 (65.4%) vs surgery only (53.1%)	On the basis of 5-year follow-up data, postoperative adjuvant therapy with S-1 was confirmed to improve overall survival and relapse-free survival in patients with stage II or III gastric cancer who had undergone D2 gastrectomy

11.2 Surgery for Gastric Cancer

11.2.1 Standard Gastrectomy with D2 Lymphadenectomy

Because there has been a strict consensus among Japanese surgeons about the use of D2 lymphadenectomy as a minimum requirement for locally advanced gastric cancer, no prospective randomized studies comparing D1 versus D2 lymphadenectomy have been planned in Japan. In the 1990s, prospective trials were conducted in Europe under the control of proficient Japanese surgeons [1, 2]. Unfortunately, early results showed that D2 lymphadenectomy had no significant benefit for either overall or disease-free survival, possibly due to high morbidity and mortality in the D2 lymphadenectomy group [1, 2]. The increase in morbidity and mortality may have affected the final results of the Dutch study [3], because subgroup analysis excluding patients who underwent pancreaticosplenectomy showed a significant survival advantage for D2 lymphadenectomy. In the 15-year follow-up analysis, it was shown that D2 lymphadenectomy was associated with lower rates of locoregional recurrence and gastric cancer-related death than D1 lymphadenectomy [4].

Gastrectomy is performed for tumors with a risk of nodal metastases. Endoscopic resection is indicated only for tumors of differentiated type, less than 2 cm in diameter, and without ulcer formation. Tumors not indicated for endoscopic resection should be treated with radical gastrectomy (Fig. 11.1). Standard gastrectomy

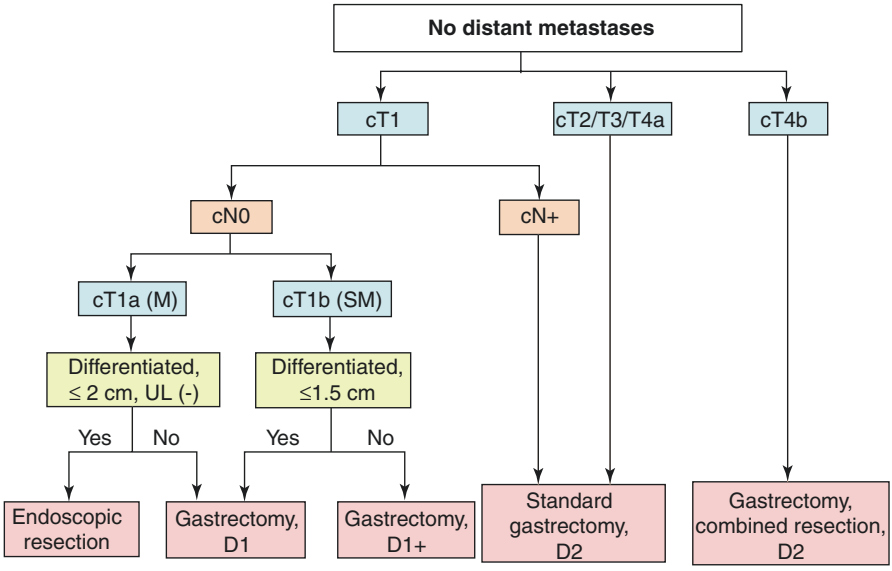

Fig. 11.1 Treatment algorithm for gastric cancer [5]. Japanese Gastric Cancer Association Japanese gastric cancer treatment guidelines 2014 (ver. 4)

involves resection of at least two-thirds of the stomach with D2 lymphadenectomy, combined with removal of the greater omentum for clinically node-positive advanced gastric cancer. In modified surgery, the extent of gastric resection and/or lymphadenectomy (D1, D1+, etc.) is reduced compared with standard surgery [7]. The standard procedures of radical gastrectomy include total gastrectomy (Fig. 11.2a), distal gastrectomy (Fig. 11.2b), pylorus-preserving gastrectomy (Fig. 11.2c), and proximal gastrectomy (Fig. 11.2d). Removal of the greater omentum is usually indicated for T3 or deeper tumors [7]. There is limited evidence that bursectomy reduces peritoneal or local recurrence. The most recent randomized, controlled trial found no survival benefit of bursectomy and a high risk of morbidity for bursectomy in T3/T4a tumors [6].

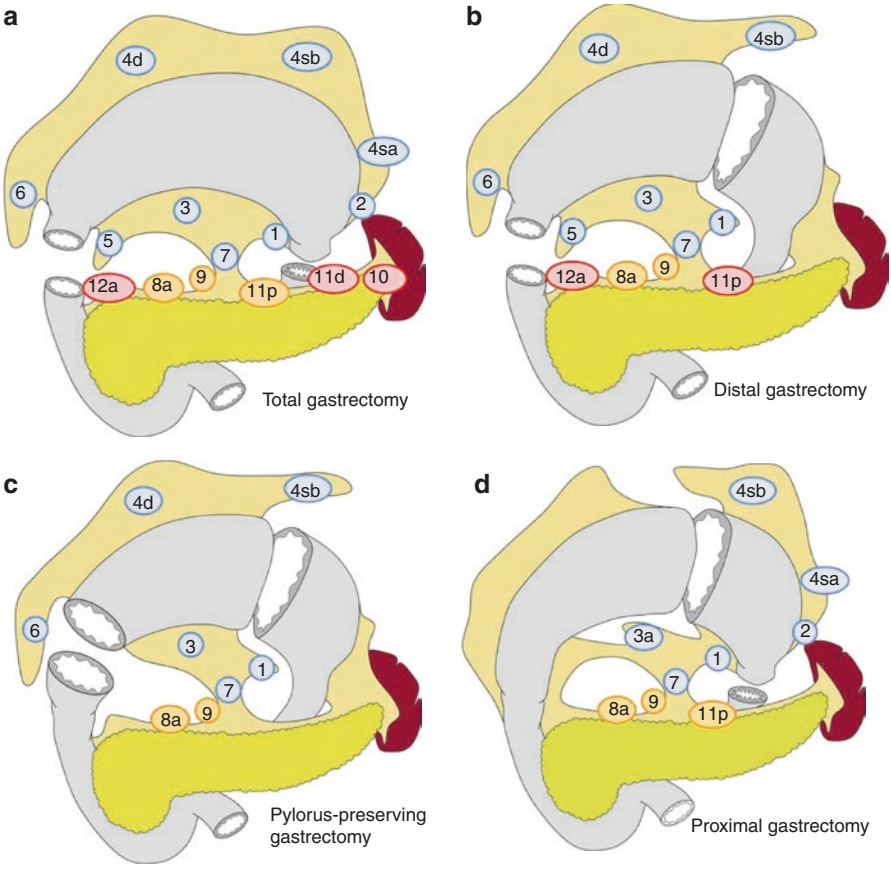

Fig. 11.2 Standard gastrectomy [5]. (**a**) Total gastrectomy, (**b**) distal gastrectomy, (**c**) pylorus-preserving gastrectomy, and (**d**) proximal gastrectomy. Japanese Gastric Cancer Association Japanese gastric cancer treatment guidelines 2014 (ver. 4)

11.2.2 Left Thoracotomy for Adenocarcinoma of the Esophagogastric Junction or Gastric Cardia

Although esophagogastric junction (EGJ) carcinoma has shown a marked increase in incidence globally, the optimal extent of esophagogastric resection for this tumor entity remains controversial. To determine the optimal extent of lymph node dissection for EGJ cancer, an all-Japan questionnaire-based retrospective study was performed [9]. Medical records of 2807 patients with EGJ carcinomas less than 40 mm in diameter who underwent R0 resection between January 2001 and December 2010 were reviewed. Nodal metastases frequently involved the abdominal nodes, particularly those at the right and left cardia, at the lesser curvature, and along the left gastric artery. Nodes along the distal portion of the stomach were much less likely to metastasize, and their dissection seemed unlikely to be beneficial. Although lower mediastinal node dissection may improve survival of patients with esophagus-predominant EGJ cancer, no conclusive result was obtained regarding the optimal extent of nodal dissection in this region because of low dissection rates for nodes of the middle and upper mediastinum.

Because of the inaccessibility of mediastinal nodal metastases, the left thoracoabdominal approach has often been used to treat advanced gastric cancer of the cardia or subcardia. Sasako et al. conducted a randomized phase III study to compare the left thoracoabdominal approach with the abdominal–transhiatal approach for the treatment of EGJ cancer (JCOG9502) [10]. Unexpectedly, the 5-year overall survival in the group treated with the left thoracoabdominal approach was significantly worse than that in the group treated with the transhiatal approach (37.9% vs. 52.3%). Moreover, morbidity was worse in the group treated with the left thoracoabdominal approach. The authors concluded that the left thoracoabdominal approach cannot be justified to treat these tumors because it does not improve survival compared with the transhiatal approach and leads to increased morbidity in patients with cancer of the cardia or subcardia. Based on complete 10-year follow-up data of this phase III study (JCOG9502), Kurokawa et al. reported that the 10-year overall survival rate was 37% for patients treated by the transhiatal approach and 24% for patients treated by the left thoracoabdominal approach ($p = 0.060$) [11]. Subgroup analysis based on the Siewert classification indicated nonsignificant survival advantages in favor of the transhiatal approach. The authors concluded that the left thoracoabdominal approach should be avoided for treatment of adenocarcinoma of the EGJ or gastric cardia.

11.2.3 Splenectomy for Proximal Gastric Carcinoma

Patients with clinically positive metastases in the splenic hilum, which would mandate splenectomy, had a poor overall outcome. Based on the results of a randomized trial of D2 lymphadenectomy, the increased morbidity without clear evidence of survival benefit suggested by some of the studies indicates that splenectomy cannot

be recommended [2, 3]. To clarify the role of splenectomy in total gastrectomy for proximal gastric cancer without invasion of the greater curvature, Sano et al. conducted a multi-institutional, randomized, and controlled trial [12]. A total of 505 patients (254 undergoing splenectomy and 251 undergoing spleen preservation) were enrolled from 36 institutions from all parts of Japan. Splenectomy was associated with higher morbidity and greater blood loss. The 5-year survival was 75.1% in the splenectomy group and 76.4% in the spleen preservation group. The hazard ratio was 0.88 (90.7% confidence interval, 0.67–1.16); thus, the noninferiority of spleen preservation was confirmed ($p = 0.025$). The authors concluded that splenectomy should be avoided in patients undergoing total gastrectomy for proximal gastric cancer that does not invade the greater curvature, because it increases operative morbidity without improving survival.

11.2.4 Para-aortic Nodal Dissection

Para-aortic nodal dissection can be the ultimate local control surgical technique. Based on favorable data from a pilot study, Sano et al. conducted a randomized, controlled trial to compare Japanese standard D2 lymphadenectomy versus D2 lymphadenectomy plus para-aortic nodal dissection (JCOG9501) [13]. A total of 523 patients with potentially curable gastric adenocarcinoma (T2-subserosa, T3, or T4), who were surgically fit, were intraoperatively randomized. Although morbidity in the extended surgery group (28.1%) was slightly higher than that in the standard group (20.9%), there were no differences between the two groups in the incidences of four major complications (anastomotic leak, pancreatic fistula, abdominal abscess, and pneumonia). Unfortunately, although specialized surgeons could safely perform gastrectomy with D2 lymphadenectomy in patients with low operative risk, there was no significant improvement in the 5-year overall survival rate of patients undergoing D2 lymphadenectomy plus para-aortic nodal dissection compared with patients undergoing D2 lymphadenectomy alone (70.3% vs. 69.2%, respectively) [14]. Therefore, at this time, D2 lymphadenectomy plus para-aortic nodal dissection should not be performed in patients with curable gastric cancer.

11.2.5 Volume Reduction Surgery for Advanced Gastric Cancer with a Single Noncurable Factor

Although chemotherapy is considered the standard care for incurable advanced gastric cancer, whether the addition of gastrectomy to chemotherapy improves survival for patients with advanced gastric cancer with a single noncurable factor remains controversial. Fujitani et al. conducted a randomized phase III trial to investigate the superiority of gastrectomy followed by chemotherapy versus chemotherapy alone with respect to overall survival in these patients (REGATTA) [15]. A total of

175 patients with advanced gastric cancer combined with a single noncurable factor confined to either the liver (H1), the peritoneum (P1), or the para-aortic lymph nodes (16a1/b2) were randomly assigned to chemotherapy alone or to gastrectomy followed by chemotherapy. Chemotherapy consisted of oral S-1 80 mg/m^2 per day on days 1–21 and cisplatin 60 mg/m^2 on day 8 of every 5-week cycle. Overall survival at 2 years was 31.7% in the group receiving chemotherapy alone (86 patients) compared with 25.1% in the group receiving gastrectomy plus chemotherapy (89 patients). The median overall survival was 16.6 months in the group receiving chemotherapy alone and 14.3 months in the group receiving gastrectomy plus chemotherapy. The incidence of grade 3 or 4 chemotherapy-associated adverse events was higher in the group receiving gastrectomy plus chemotherapy than in the group receiving chemotherapy alone. The authors concluded that gastrectomy cannot be justified for patients with a single noncurable factor [15].

11.2.6 Laparoscopic Distal Gastrectomy and Robotic Gastrectomy

Although the number of patients undergoing laparoscopic distal gastrectomy is increasing, a prospective study with a sample size sufficient to investigate the benefit of laparoscopic distal gastrectomy has never been reported. Katai et al. conducted a multi-institutional phase II trial (JCOG0703) to evaluate the safety of laparoscopic distal gastrectomy for clinical stage I gastric cancer [16]. Laparoscopic distal gastrectomy with D1 lymphadenectomy plus suprapancreatic node dissection was performed. Among a total of 176 eligible patients, the proportion of patients who developed anastomotic leakage or a pancreatic fistula was 1.7%. The overall proportion of inhospital grade 3 or 4 adverse events was 5.1%. The short-term clinical outcomes were as follows: 43.2% of the patients requested an analgesic on postoperative days 5–10, and the median time from surgery until the first episode of flatus was 2 days. This trial confirmed the safety of laparoscopic distal gastrectomy performed by credentialed surgeons in terms of the incidence of anastomotic leakage or pancreatic fistula formation.

Based on real-world data from 5288 patients from NCD, Hiki et al. reported that there were no significant differences between patients undergoing open distal gastrectomy and those undergoing laparoscopic distal gastrectomy in the number of inhospital deaths (3/1067 vs. 6/1067, $p = 0.51$) or the number of reoperations (20/1067 vs. 29/1067, $p = 0.19$) [17]. Wound infection and dehiscence were more common in patients undergoing open distal gastrectomy. On the other hand, grade B or higher pancreatic fistulas were more frequent in patients undergoing laparoscopic distal gastrectomy. Care must be taken to prevent the formation of pancreatic fistulas in patients undergoing laparoscopic distal gastrectomy, and further improvements in surgical quality are warranted in this regard. Data regarding the long-term outcomes are not yet available, and the results of pivotal phase III studies conducted in Japan (JCOG0912) and Korea (KLASS01) are awaited.

Laparoscopic pylorus-preserving gastrectomy for early gastric cancer has been introduced as a minimally invasive procedure that preserves the function of the pylorus and the capacity of the remnant stomach to maintain a functional reservoir. Tsujiura et al. investigated the surgical and prognostic outcomes in 465 patients who underwent laparoscopic pylorus-preserving gastrectomy for cT1 N0 gastric cancer located in the middle part of the stomach [18]. Regarding short-term surgical results, 14 (3%) of the 465 patients had severe complications classified as Clavien–Dindo grade 3a or above, and no deaths occurred. The 5-year overall survival and relapse-free survival rates were 98% and 98%, respectively. Only two cases of postoperative recurrence were confirmed, and the sites of recurrence were not in the remnant stomach or the regional lymph nodes. Postoperative nutritional status in terms of serum total protein, albumin, and hemoglobin levels was well maintained, and the mean relative body weight (postoperative/preoperative) was 93.24% ± 7.29% after laparoscopic pylorus-preserving gastrectomy. The authors concluded that laparoscopic pylorus-preserving gastrectomy was an acceptable and favorable operative method for clinically diagnosed early-stage gastric cancer in terms of long-term survival and postoperative nutrition.

Although there are a number of single-arm and comparative studies showing the feasibility of robotic gastrectomy, there is no solid evidence from multicenter, randomized clinical trials. Tokunaga et al. conducted a phase II study to assess the feasibility of robot-assisted gastrectomy [19]. A total of 120 patients were recruited between December 2012 and April 2015. The incidence of intra-abdominal infectious complications was 3.3%, and all complications were successfully treated conservatively without reoperation. The data showed that robot-assisted gastrectomy was safe in terms of the incidence and severity of postoperative complications. With articulated devices of robotic gastrectomy, surgeons are able to perform each procedure more meticulously, which can result in less bleeding and damage to organs. Considering the higher medical expenses associated with robot-assisted gastrectomy, its superiority in terms of long-term survival outcomes needs to be confirmed in future studies for it to be accepted more widely.

11.2.7 Function-Preserving Gastrectomy Based on the Sentinel Node Concept in Early Gastric Cancer

Recent meta-analyses and a prospective multicenter trial of sentinel node mapping in early gastric cancer have demonstrated acceptable sentinel node detection rates and accuracy of determination of lymph node status. Sentinel node mapping also allows modification of surgical procedures, including function-preserving gastrectomy in patients with early gastric cancer [20]. A dual-tracer method that uses radioactive colloids and blue dye is currently considered the most reliable method for the stable detection of sentinel nodes in patients with early gastric cancer. New technologies, such as indocyanine green infrared or fluorescence imaging, are also useful for accurate sentinel node mapping in gastric cancer. Theoretically, laparoscopic

function-preserving gastrectomy, including partial resection, proximal gastrectomy, segmental gastrectomy, and pylorus-preserving gastrectomy, is feasible in early gastric cancer when the sentinel nodes are negative for metastases. Takeuchi et al. conducted a multicenter prospective trial in Japan to evaluate function-preserving gastrectomy with sentinel node mapping for long-term survival and quality of life of patients [20]. Nonexposed endoscopic wall-inversion surgery is a new technique for treating gastric cancer with partial resection involving full-thickness resection with endoscopy and laparoscopic surgery without transluminal access. The combination of nonexposed endoscopic wall-inversion surgery and sentinel node biopsy is expected to be a promising, minimally invasive, and function-preserving surgery that is ideal for cN0 early gastric cancer cases. In addition to visualization of the sentinel node, it is essential to accurately assess the presence or absence of lymph node metastasis in the intraoperative management of sentinel node navigation surgery. Reverse transcription-polymerase chain reaction is one of the representative assays used to identify lymph node micrometastases [21]. When performing sentinel node navigation surgery as a minimally invasive surgery, it is important to consider the balance between postsurgical quality of life and curability.

11.3 Future Perspectives

Almost all evidence based on lymph node dissection and/or extent of resection was established only for the patients who were not treated with adjuvant chemotherapy. Although postoperative adjuvant chemotherapy is considered to be standard [4, 6], there is only a few evidences for survival benefits of neoadjuvant therapy. Moreover, the most preferable combination of adjuvant chemotherapy [22] and extended lymphadenectomy is controversial. Along with minimization of postoperative morbidity, the combination of neoadjuvant therapy and extended lymph node dissection will be one of the mainstreams of gastric cancer surgery.

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Chapter 12
Gastric Cancer: Chemotherapy for Advanced Disease with Special Focus on Studies from Japan

Taroh Satoh

Abstract Unresectable or metastatic advanced gastric cancer (AGC) is non-curable, and median survival time (MST) is about 3 months when best supportive care (BSC) is performed. By chemotherapy, MST has been extended to about 13–14 months, and symptomatic relief can be expected by high tumor shrinkage effect (Murad et al., Cancer 72:37–41, 1993).

In the primary treatment, the standard treatment was decided internationally as a combination therapy of pushed pyrimidine and platinum, and treatment strategy was individualized by expression of HER2 protein. In second-line treatment, weekly paclitaxel (PTX) + ramucirumab (RAM) therapy is considered as standard. For third-line therapy, the survival prolonging effect of immune checkpoint inhibitors, which is attracting attention in many types of cancer in recent years, was also observed in gastric cancer. Nivolumab has shown significant survival extension effect in salvage line study. Thus, in recent years, standardization of treatment strategies and individualization based on biomarkers have been in progress, and further approval of new agents is expected to extend overall survival. On the other hand, treatment for the elderly and patients with severe peritoneal metastasis, which are not eligible to receive standard treatment, has not been established and is a future clinical problem. In this chapter, we discuss about treatment strategies and prospects for unresectable/recurrent gastric cancer.

Keywords Chemotherapy for advanced gastric cancer · S-1 · Capecitabine · Ramcirumab · Nivolumab

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12.1 Conventional Chemotherapy

Unresectable or metastatic advanced gastric cancer (AGC) is non-curable, and median survival time (MST) is about 3 months when best supportive care (BSC) is performed. By chemotherapy, MST has been extended to about 13–14 months, and symptomatic relief can be expected by high tumor shrinkage effect [1, 2]. There was no consensus for standard therapy until the 1990s, although there were a lot of randomized clinical trials comparing 5-FU monotherapy with other combination therapies. Neither regimen showed obvious survival benefit over 5-FU monotherapy [3–5]. In the 2000s, JCOG 9912 was conducted to confirm superiority of irinotecan (IRI) + cisplatin (CDDP) combination and non-inferiority of S-1 monotherapy to 5-FU continuous infusion as reference. Although the superiority of IRI + CDDP therapy was not proven in the primary endpoint of overall survival (OS), non-inferiority of S-1 monotherapy has been demonstrated [6]. Furthermore, in the SPIRITS study, S-1 + CDDP therapy (SP therapy) showed superiority in OS to S-1 monotherapy (MST, 13 months vs 11 months; hazard ratio, 0.77; $P = 0.049$) [7]. From the results of these two Phase III trials, SP therapy was established as a standard therapy in Japan.

Capecitabine/cisplatin could be an option, given the previous data from ML17032 [8] ToGA trial [9] and AVAGAST [10] suggesting that CP regimen offers a higher dose of cisplatin compared with the SP (80 mg/m^2 every 3 weeks versus 60 mg/m^2 every 5 weeks [9].

Efficacy of conventional chemotherapy regimen is summarized in Table 12.1. S-1 + IRI [12] and S-1 + docetaxel (DTX) [13] failed to show superiority in OS to S-1 monotherapy. However, S-1 + DTX therapy suggested an extension of OS in follow-up analysis therefore considered as optional for patients with peritoneal metastasis. Subsequently, G-SOX trial was conducted in Japan to prove non-inferiority of S-1 + oxaliplatin (I-OHP) therapy (SOX therapy) to SP. Non-inferiority in progression-free survival (PFS) as co-primary endpoint was proved (median PFS median 5.5 months vs. 5.4 months; hazard ratio, 1.004; 95% confidence interval, 0.540–1.199; non-inferior margin, 1.30; $P = 0.0044$). However statistically non-inferiority was not proven in OS (MST, 14.1 months vs. 13.1 months; hazard ratio, 0.969; 95% confidence interval, 0.812–1.157; non-inferior margin, 1.15; $P = 0.0583$). The Kaplan-Meier curves of both groups almost overlapped in OS and PFS, and in terms of efficacy and safety, SOX was not clearly inferior to SP. Therefore

Table 12.1 Current key clinical trials of conventional chemotherapy

Trial (reference no)	ARM	RR (%)	PFS (M)	OS (M)	HR
JCOG9912 [6]	5-FU	9	2.9	10.8	0.083
	S-1	28	4.2	11.4	($P < 0.001$)
SPIRITS [7]	S-1	31	4	11	0.774
	SP	54	6	13	($P = 0.0366$)
ML17032 [8]	FP	32	5	9.3	0.85
	CP	46	5.6	10.5	($P < 0.008$)
ToGA [9, 11]	CP	34.5	5.5	11.1	
AVAGAST [9, 10]	CP	37	5.3	10.1	

RR response rate, *M* months, *PFS* progression-free survival, *OS* overall survival, *HR* hazard ratio, *5-FU* 5-fluorouracil, *SP* S-1+cisplatin, *FP* 5-fluorouracil+cisplatin, *CP* capecitabine+cisplatin

SOX is considered as one of standard treatment options in Japan [14]. Table 12.1 is the summary of CP vs XP in key clinical Phase III study.

The significance of second-line treatment is established in three Phase III trials [15–17] in which DTX or IRI showed survival benefit over BSC. The WJOG 4007 trial was conducted as a superiority trial of IRI to weekly paclitaxel (wPTX) for a refractory example to a combination therapy of pushed pyrimidine and platinum. As a result, the superiority of IRI in the OS was not shown (hazard ratio, 1.13; 95% confidence interval, 0.86–1.49; $P = 0.38$), but MST was good in both groups (IRI group 8.4 months, wPTX group 9.5 months) with tolerable toxicity, both of which are considered as second line treatment [18].

12.2 Molecular Targeted Agents

Recently many targeted agents had been tested in gastric cancer (Table 12.2).

In ST03 trial, clinical efficacy using bevacizumab in perioperativesetting and setting was conducted but failed to show superiority, and authors concluded that The results of this trial do not provide any evidence for the use of bevacizumab in combination with peri-operative epiribicin, cisplatin, and capecitabine chemotherapy for patients with resectable gastric, oesophagogastric junction, or lower oesophageal adenocarcinoma [19]. ToGA trial [11] was conducted to confirm the efficacy of trastuzumab (Tmab) plus capecitabine and cisplatin therapy or 5-FU + CDDP (FP) in patients with gastric cancer whose immunohistochemistry (IHC) was strong positive (3+) or FISH (fluorescence in situ hybridization) positive. In the OS which is the primary endpoint, superiority of Tmab combination group was shown to standard treatment (MST, 13.8 months vs. 11.1 months; hazard ratio, 0.74; $P = 0.0046$).

Table 12.2 Results of current key clinical trials for targeted agents

Line	Target	Agent	Trial		Result
0 line	VEGF-A	Bevacizumab	ST03	Cunningham et al. [19]	Negative
1st line	HER2	Trastuzumab	ToGA	Bang et al. [11]	Positive
		Lapatinib	LOGiC	Hecht et al. [20]	Negative
		Pertuzumab	JACOB	NCT01774786	Negative
	VEGF-A	Bevacizumab	AVAGAST	Ohtsu et al. [10]	Negative
			AVATAR	Shen et al. [21]	Negative
	EGFR	Panitumumab	REAL 3	Waddell et al. [22]	Negative
		Cetuximab	EXPAND	Lordick et al. [23]	Negative
	HGF	Rilotumumab	RILOMET-1	Catenacci et al. [24]	Negative
	MET	Onartuzumab	MET Gastric	Shah et al. [25]	Negative
2nd, 3rd line	HER2	Lapatinib	TyTAN	Satoh et al. [26]	Negative
		T-DM1	GATSBY	Kang et al. [34]	Negative
	mTOR	Everolimus	GRANITE-1	Ohtsu et al. [27]	Negative
	VEGFR-2	Ramucirumab	RAINBOW	Wilke et al. [28]	Positive
			REGARD	Fuchs et al. [29]	Positive
	PARP	Olaparib	GOLD	Bang et al. [30]	Negative
	STAT3	BBI608	BRIGHTER	Press release 2017	Negative

In subgroup analysis, survival prolonging effect in IHCO/1 + and FISH-positive HER2 low-expression group was not observed by Tmab combination. The effect is more prominent (MST, 16.0 months vs. 11.8; 95% confidence interval, 0.678–0.962; $P = 0.017$; hazard ratio, 0.65) in IHC3, IHC2 +, and FISH-positive high HER2 high-expression group. Approximately 15–20% of unresectable/recurrent gastric cancer patients are human epidermal growth factor receptor 2 (HER 2)-positive [31, 32]. Since it can be expected that the combination effect of anti-HER2 antibody trastuzumab (Tmab) can be expected in Her2-positive patients, it is recommended that a therapeutic strategy be divided into HER2-negative stomach cancer and HER2-positive one.

On the other hand, Phase III trial (LOGiC trial) using lapatinib, a HER 1/HER 2 inhibitor, showed no prolongation of survival when used in combination with capecitabine plus oxaliplatin (CapeOX) therapy (hazard ratio, 0.91; 95% confidence interval, 0.73–1.12; $P = 0.3492$) [20]. In addition, pertuzumab which is an anti-HER2 antibody that binds to a domain different from Tmab among HER2 receptors, acting complementarily to Tmab and with HER2-positive advanced breast cancer, was reported to show superiority over Tmab plus capecitabine/CDDP in JACOB trial [26].

Treatment development based on the expression of HER2 protein was also attempted in the second-line treatment as with the first-line treatment.

In the TyTan study conducted in Asia including Japan, the significance of using lapatinib, a HER1/HER2 inhibitor, for WPTX therapy was tested for gastric cancer patients who were found to have amplified HER2 gene by FISH test. Lapatinib +WPTX failed to show superiority in OS over wPTX (MST, 11 months vs. 8.9 months; hazard ratio, 0.84; 95% confidence interval, 0.64–1.11; $P = 0.1044$). In this study, 35% of cases with IHC 0 or 1+ were registered (23% in the ToGA test), and no additional effect of lapatinib was observed in this subgroup (hazard ratio, 1.07). However, in the IHC 3 + subgroup, the hazard ratio was 0.59, suggesting the possibility that the combined use of lapatinib may be effective [33].

In the GATSBY study, patients with gastric cancer with IHC 2 + and FISH-positive or IHC 3 + in HER 2 test were treated with Tmab, and emtansine as a microtubule polymerization inhibitor (DM1) antibody drug complex T-DM1 was compared with the physician's choice taxane (wPTX or DTX). The T-DM1 group failed to show superiority in the OS to the taxane group (MST, 7.9 months vs. 8.6 months; hazard ratio, 1.15; 95% confidence interval, 0.87–1.51; $P = 0.86$) [28]. Currently, a randomized Phase II study (WJOG 7112 G test: UMINOOOO 9297) comparing wPTX + Tmab therapy with wPTX therapy in secondary treatment is in progress for HER2-positive gastric cancer with Tmab in combination with paclitaxel.

AVAGAST did not reach its primary objective OS, although adding bevacizumab to chemotherapy was associated with significant increases in progression-free survival and overall response rate in the first-line treatment of AGC [10]. AVATAR trial revealed that addition of bevacizumab to capecitabine-cisplatin in Chinese patients with advanced gastric cancer did not improve outcomes since there was no difference in OS between the two arms and PFS was similar in both arms [21]. Two international cooperative Phase III trials were conducted using RAM, an antibody drug that inhibits vascular endothelial growth factor receptor 2 (VEGFR2). First, in the RAINBOW trial which also participated in Japan, it was a trial to verify the significance of using RAM together with wPTX. As a result, in the primary end-

point OS, the wPTX + RAM group showed superiority to the wPTX + placebo (MST, 9.6 months vs. 7.4 months; hazard ratio, 0.807; 95% confidence interval, 0.678 ~ 0.962; $P = 0.017$), and the survival benefit of RAM on wPTX was demonstrated [28].

Addition of either panitumumab or cetuximab to Standard of care in first line unselected population did not increase overall survival in REAL3 and EXPAND for AGC [22, 23].

Rilotumumab is a fully human monoclonal antibody that selectively targets the ligand of the MET receptor, hepatocyte growth factor (HGF). However rilotumumab combined with epirubicin, cisplatin, and capecitabine, is not effective in improving clinical outcomes in patients with MET-positive gastric or gastro-oesophageal adenocarcinoma [24]. Similar results were reported from MET Gastric trial in which addition of MET inhibitor onartuzumab to first-line mFOLFOX6 did not significantly improve clinical benefits in the ITT or MET 2+/3+ populations [25].

The REGARD trial was a comparison study between RAM monotherapy and placebo (PBO). RAM monotherapy showed clear survival benefit on PBO group (MST, 5.2 months vs 3.8 months; hazard ratio, 0.776; 95% confidence interval, 0.603–0.998; $P = 0.047$) [29].

An international cooperative Phase III study comparing BSC with monotherapy of nivolumab (NIVO), an anti-programmed death-1 (PD-1) antibody, was conducted in Japan/Korea/Taiwan. It was reported that nivolumab's significant survival extension effect was demonstrated at the primary endpoint of OS (MST, 5.32 months vs, 4.14 months; hazard ratio, 0.63; 95% confidence interval, 0.50–0.78; $P < 0.0001$) [34].

In GRANITE-1 trial it is reported that everolimus, the oral mammalian target of rapamycin inhibitor, did not significantly improve overall survival for advanced gastric cancer that progressed after one or two lines of previous systemic chemotherapy [27]. GOLD trial was reported that it did not meet its primary objective of showing a significant improvement in overall survival with olaparib in the overall or ATM-negative population of Asian patients with AGC [30].

In LoGic trial, addition of lapatinib to CapeOx did not increase OS in patients with HER2-amplified gastroesophageal adenocarcinoma [20].

12.3 Future Prospects

Ongoing key clinical trials of testing new agents or concepts are summarized in Table 12.3.

In order to examine the significance of conducting a combination therapy of pushed pyrimidine plus platinum for the elderly, a randomized Phase II study comparing S-1 monotherapy and SOX therapy for elderly people aged 70 years or older (WJOG 8315 G test/UMINOOOO 20864) is in progress.

Patients with massive ascites or peritoneal metastasis or patients who cannot be ingested orally are out of the scope of major clinical trials so far, and standard treatment including first line is not yet defined. Randomized first-line Phase I/Phase III trial (JCOG 1108/WJOG 7312 G/UMINOOOO 10949) of FULTAX, which is a com-

Table 12.3 Ongoing key clinical trials of new agents

Trial	Target	Sample size	Reference	Experimental	ID
SOLAR	1st	686	SP	TAS118+l-OHP	NCT02322593
JCOG1013	1st	740	SP	DCS	UMIN000007652
RAINFALL	1st	616	XP	XP+RAM	NCT02314117
BRIGHTER	2nd	700	wPTX	WPTX+BBI608	NCT02178956
RINDBeRG	3rd	400	IRI	IRI+RAM	UMIN000023065
ANGEL	3rd, 4th	459	PBO	Apatinib	NCT030462611
ATTRACTION4	1st	680	SOX/ CapOX	SOX/CapOX+NIVO	NCT02746796
Checkmate649	1st	1266	CapOX/ FOLFOX	NIVO+CapOX/ FOLFOX, NIVO+IPI	NCT028272116
KEYNOTE-062	1st	750	FP	Pembrolozumab, Pembrolizumab+FP	NCT02494583
JAVELIN-100	1st	666	CapOX/ FOLFOX	Avelumab	NCT02625610
KEYNOTE-061	2nd	720	wPTX	Pembrolizumab	NCT02370498
JAVELIN-300	3rd	330	wPTX/IRI	Avelumab	NCT02325623

SP S-1+cisplatin, *XP* capecitabine +ciplatin, *wPTX* weekly Paclitaxel, *IRI* irinotecan, *PBO* placebo, *SOX* S-1+oxaliplatin, *CapeOX* capecitabine +oxaliplatin, *FOLFOX* 5-fluorouracil +leucovorine+oxaliplatin, *FP* 5-fluorouracil cisplatin, *DCS* docetaxel+cisplatin+S-1, *RAM* ramcirumab, *NIVO* nivolumab

bination therapy of PIX and 5-FU comparing 5-FU/LV, is currently under way for this subject. The SOLAR trial (NCT02322593) comparing SP with a combination therapy of TAS 118 which is a combination of S-1 + leucovorin and l-OHP has been performed. In randomized Phase II study [35], S-1 + leucovorin + l-OHP therapy shows a very promising result that OS hazard ratio to SP therapy was 0.59 (95% confidence interval 0.37–0.93). JCOG 1013 test (UMIN00007652) comparing SP therapy with DTX + CDDP + S-1 therapy (DCS), a triple drug combination regimen, is ongoing. Phase III trial using RAM, the RAINFALL trial (NCTO 2314117), to verify the additional effect of RAM on XP therapy in first-line treatment is under way but reported it was negative. The RINDBeRG trial (UMINOOO 23065), which examines the significance of continued administration of RAM in combination with IRI of third-line therapy, was started as an intergroup trial for patients where RAM was incompatible with prior treatment. Therapeutic development of immune checkpoint inhibitors is advancing at a rapid pace, and phase III trials are being conducted in each treatment line.

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Part V
Prevention

Chapter 13
Gastric Cancer Prevention Using *Helicobacter pylori* Eradication in Japan

Masahiro Asaka

Abstract The annual number of deaths from gastric cancer is approximately 50,000, and there has been no change over the last 50 years in Japan. All efforts have been directed toward improving the detection of early gastric cancer by barium X-ray and endoscopy, since early cancer has a good prognosis, resulting in Japan having the best diagnostic capability for early gastric cancer worldwide.

H. pylori eradication therapy for chronic gastritis achieved the world's first coverage by the Japanese national health insurance scheme in 2013, making a dramatic decrease of gastric cancer-related deaths more realistic. Combining *H. pylori* eradication therapy with endoscopic surveillance can prevent the development of gastric cancer. Even if gastric cancer develops, most patients are likely to be diagnosed while it is at an early stage, possibly resulting in a large decrease of gastric cancer deaths.

Approximately 1.6 million prescriptions for *H. pylori* eradication therapy were written annually. Gastric cancer deaths fell each year: 48,427 in 2013, 47,903 in 2014, 46,659 in 2015, and 45,509 in 2016, showing a significant decrease after expansion of insurance coverage for *H. pylori* eradication therapy ($P < 0.0001$). Prescriptions for *H. pylori* eradication therapy increased markedly after approval of the gastritis indication by the national health insurance scheme and were associated with a significant decrease in gastric cancer deaths.

Keywords Gastric cancer prevention · *Helicobacter pylori (H. pylori)* · *H. pylori*-associated gastritis · Elimination of gastric cancer

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13.1 Introduction

Gastric cancer is the second most common cancer death worldwide [1]. Until the early twentieth century, Europe and the United States (USA) suffered a high incidence of gastric cancer. The twentieth century also saw that incidence rapidly decreases coincidence with changes in lifestyle, sanitation, and the widespread adoption of refrigeration for food preservation. Currently, three East Asian countries, Japan, China, and Korea, account for about 60% of new gastric cancers [1]. Early studies of the possible cause of gastric cancer emphasized dietary factors such as excessive intake of salt or nitrates and hereditary factors. The culture of *Helicobacter pylori* (*H. pylori*) in 1983 [2] resulted in research focused on proving the causal relationship between *H. pylori* infection and gastritis and gastric cancer. *Helicobacter pylori* causes chronic gastric mucosal inflammation which underlies various disorders of the stomach [3, 4], including atrophic gastritis from which intestinal type gastric cancer can develop. It has also been reported that *H. pylori* gastritis is etiologically associated with gastroduodenal ulcers, gastric mucosa-associated lymphoid tissue lymphoma, functional dyspepsia, hyperplastic gastric polyps, idiopathic thrombocytopenic purpura, and undifferentiated gastric cancer [5, 6]. As a result, in 1994, *H. pylori* was classified as a definite carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) [7]. A multicenter randomized study performed by the Japan Gastric Study Group showed that *H. pylori* eradication therapy decreased the incidence of secondary gastric cancer by approximately two-thirds in patients undergoing endoscopic mucosal resection of early gastric cancer [8], demonstrating a preventive effect of *H. pylori* eradication therapy against gastric cancer. The study also showed that eradication could not completely prevent gastric cancer such that periodic follow-up for gastric cancer would be required even after eradicating *H. pylori* in high-risk patients. It has been suggested that *H. pylori* infection causes more than 95% of all gastric cancers in Japan and Korea [9, 10].

Japan has long placed emphasis on secondary prevention of gastric cancer with use of barium studies for early detection [11]. However, the number of gastric cancer deaths has remained stable at approximately 50,000 per year over the past 40 years in Japan, showing almost no change after the start of screening with barium studies [12]. *H. pylori* eradication therapy for chronic gastritis (*H. pylori* gastritis) was approved for coverage by the Japanese national health insurance (NHI) scheme in February 2013. According to the Ministry of Health, Labour, and Welfare (MHLW) notification, eradication therapy is only covered by NHI when a patient has endoscopically diagnosed chronic gastritis and is positive for *H. pylori*. After this change, prescription of *H. pylori* eradication therapy increased markedly, and approximately six million patients have been treated in the 4 years since approval [13]. This raises the possibility that deaths from gastric cancer may have begun to decrease in Japan.

13.1.1 Previous Preventative Measures for Gastric Cancer in Japan

In Japan, the prevention of cancer, including gastric cancer, has primarily focused on secondary measures for early detection of cancer, rather than on primary prevention aimed at elimination of the causes. Indirect barium contrast imaging has been employed as the screening method for gastric cancer, but despite the long interest and emphasis, the screening rate was only 9.6% in 2010 [11]. Screening for gastric cancer based on barium contrast imaging also does not have a high sensitivity for detecting early cancer [11] and is associated with considerable exposure to radiation. Moreover, *H. pylori*-negative patients with minimal or no atrophy of the gastric mucosa are very unlikely to develop gastric cancer [14, 15] such that these patients are unlikely to benefit from annual barium contrast screening and are still exposed to the adverse effects of radiation. Overall, as currently employed, conventional screening for gastric cancer based on barium contrast studies has proved to be impractical as a method to reduce the incidence of gastric cancer.

The most serious disadvantage with Japan's attempts to prevent gastric cancer was the inability to implement primary prevention which is understandable as the cause of gastric cancer had not been identified in the 1970s when programs of screening for this cancer were begun. As a general rule for cancers caused by infections, such as liver cell cancer and cervical carcinoma, primary prevention based on preventing the infection or early eradication before significant damage is done and is preferred over screening (i.e., primary prevention is superior to secondary prevention). Due to the aging of the population (i.e., more people at risk), the number of patients dying from gastric cancer has remained unchanged remaining around 50,000 per year. The lack of a reduction in overall mortality provided important evidence to the Japanese Government that current programs were not effective in the prevention of gastric cancer deaths.

13.1.2 Prevention of Gastric Cancer by Eradication of **H. pylori**

As it has become clear that *H. pylori* infection is an important risk factor for gastric cancer, the issue of whether *H. pylori* eradication therapy can decrease the incidence of gastric cancer has attracted increasing attention. Intervention studies to assess the preventative effect of *H. pylori* eradication on gastric cancer have been conducted in healthy individuals worldwide. However, the incidence of gastric cancer is very low in the United States and Europe, and the study populations were not large enough to detect a significant effect of eradication therapy, resulting in the discontinuation of most studies [16].

Assessment of the design of a new prospective study on the basis of previous studies indicated that a clinical trial with a small sample size and short follow-up period should enroll patients with early gastric cancer who have undergone EMR, since they represent the population most likely to develop advanced gastric cancer. The annual incidence of gastric cancer has been reported to be only 0.1–0.4% in *H. pylori*-positive patients with atrophic gastritis [15, 17], while the annual incidence of metachronous recurrence is far higher (3–5%) in patients who have undergone endoscopic surgery for early gastric cancer [18, 19]. We investigated the metachronous recurrence of gastric cancer in 544 patients who had undergone endoscopic treatment for early gastric cancer. They were randomly allocated to *H. pylori* eradication or non-eradication groups and were followed up by annual endoscopic examination for 3 years. As a result, metachronous recurrence was detected in 9 and 24 subjects from the eradication group and the non-eradication group, respectively, and the former had a significantly lower relapse rate ($p < 0.01$ according to intention-to-treat analysis) [3]. This prospective study had an adequate sample size to provide a definitive answer to the long controversial issue of whether gastric cancer could be prevented through *H. pylori* eradication. It demonstrated that *H. pylori* eradication therapy reduced the incidence of intestinal-type gastric cancer by at least two-thirds and this effect was noted irrespective of whether patients had atrophic gastritis, intestinal metaplasia, or early gastric cancer. Thus, it was confirmed that most gastric cancer is associated with *H. pylori* infection and that the disease can be effectively prevented by eradication of this microorganism. Maehata et al. investigated the long-term clinical outcome following *H. pylori* eradication therapy and whether it prevented metachronous gastric cancer. They reported that eradication therapy inhibited the development of metachronous gastric cancer for 5 years, but there was no significant difference after longer follow-up [20]. However, the mean observation period of this study was only 3 years, and the 10-year prognosis was assessed in very few patients, leading to lack of reliability. That is, the findings about the short-term prognosis may well be accurate, but no conclusion can be drawn regarding the long-term outcome.

After the JGSG study was completed and data obtained at 8–10 years were analyzed, it was found that there was still a difference in the incidence of metachronous gastric cancer between the *H. pylori* eradication and non-eradication groups [21]. This indicates that the preventive effect of eradication therapy on gastric cancer persists for a long time.

13.1.3 Health Insurance Coverage for H. pylori *Eradication Therapy in Japan*

Cancers are classified into two broad categories, which are lifestyle-related and infection-related cancers. In the United States and Europe, cancers related to infection account for a low percentage (10% or less) of all cancers [22, 23]. In Japan, however, it has become clear that infection-related cancers account for

approximately 25%, including liver cancer caused by hepatitis viruses, cervical cancer due to papillomavirus, and gastric cancer related to *H. pylori*. Although cervical cancer is uncommon and accounts for a low percentage (1.3%) of all cancers, gastric cancer and liver cancer account for about 17% and 6.5%, respectively, and the total for these three cancers is nearly 25% [24]. Since it has become clear that most gastric cancer is due to *H. pylori* infection rather than lifestyle factors, it is time for major revision of the preventative strategies for gastric cancer. When it is suspected that a cancer is caused by infection, proactive preventative measures are likely to lead to a dramatic decrease in the incidence of that cancer, resulting in a significant decrease of cancer mortality. The annual number of deaths from gastric cancer has remained at around 50,000 for the last few decades [24], suggesting that the current preventative measures are inadequate. Thus, the fundamental measures for preventing gastric cancer should be shifted from conventional secondary prevention based on barium X-ray screening to primary prevention focused on *H. pylori* eradication therapy.

The Japanese Society for Helicobacter Research published a guideline in which it is recommended that all *H. pylori*-infected people receive bacterial eradication therapy in 2009 [25]. In response to this, the MHLW approved the extension of national health insurance coverage to *H. pylori* eradication therapy for three indications (i.e., patients with gastric mucosa-associated lymphoid tissue [MALT] lymphoma, patients who have undergone endoscopic surgery for early gastric cancer, and patients with idiopathic thrombocytopenic purpura [ITP]), in addition to patients with gastroduodenal ulcer. This was the first time in the world that insurance cover has been provided for *H. pylori* eradication therapy for indications other than gastroduodenal ulcer and represents an innovative approach. Regarding the potential expansion of health insurance coverage for eradication therapy to include patients with chronic gastritis, the Japanese Society of Gastroenterology, the Japan Gastroenterological Endoscopy Society, and the Japanese Society for Helicobacter Research submitted a joint petition to the Minister of the MHLW. This public knowledge-based application led to the inclusion of *H. pylori* eradication therapy for patients with chronic gastritis on February 21, 2013. The MHLW notification states that eradication therapy is covered by the national health insurance scheme when a patient with endoscopically diagnosed chronic gastritis is positive for *H. pylori*.

Gastritis with neutrophil and lymphocyte infiltration develops in almost 100% of patients who have *H. pylori* infection within a few months of being infected. Such gastritis is called chronic active gastritis and is said to be specific to *H. pylori* infection [26]. Persistent inflammation gradually increases the fragility of the gastric mucosa, and *H. pylori*-associated gastritis progresses to atrophic gastritis over time. It has been demonstrated that progression takes 10–20 years in about 80% of Japanese patients [4], and some cases of atrophic gastritis then progress to intestinal type gastric cancer. The effects of gastric acid and stress on a background of *H. pylori*-associated gastritis can lead to the development of peptic ulcer. In contrast, gastritis that is not associated with *H. pylori* usually does not progress to ulceration even when stress occurs. It has become obvious that *H. pylori*-associated gastritis is

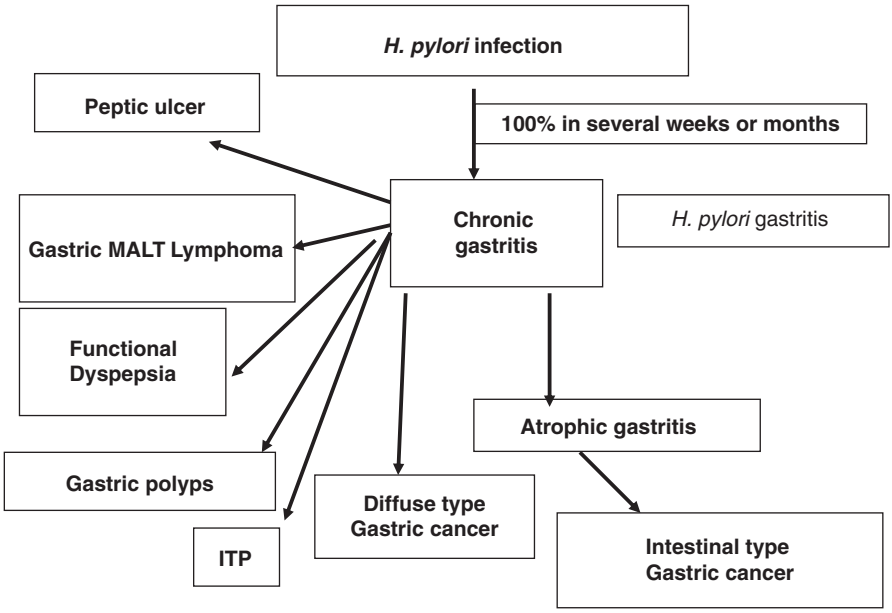

Fig. 13.1 Progress of *H. pylori* infection [27]

also closely associated with gastric MALT lymphoma, functional dyspepsia (FD), hyperplastic gastric polyps, idiopathic thrombocytopenic purpura (ITP), and diffuse-type gastric cancer (Fig. 13.1) [5, 6]. Thus, *H. pylori*-associated gastritis is the underlying cause of almost all gastric diseases; hence treatment of this gastritis through bacterial eradication therapy is likely to prevent most gastric conditions, including gastric cancer.

13.1.4 Effect on Strategy for the Elimination of Gastric Cancer in Japan

Prescription of *H. pylori* eradication therapy increased markedly in the 4-year period after Japanese national health insurance (NHI) coverage for *H. pylori* eradication therapy was expanded to include chronic gastritis, and the number of deaths from gastric cancer decreased significantly during the same period. The increase in the prescription of eradication therapy is attributable to the fact that treatment became available for all of millions. After eradication therapy for *H. pylori* gastritis was approved, patients with *H. pylori* gastritis could receive eradication therapy if the diagnosis of *H. pylori* gastritis was confirmed by endoscopy. In other words, the fact that patients diagnosed with *H. pylori* gastritis required gastroscopy to receive eradication therapy resulted in a rapid increase in gastroscopy procedures along with the prescription of *H. pylori* eradication therapy. Approximately 1.6 million

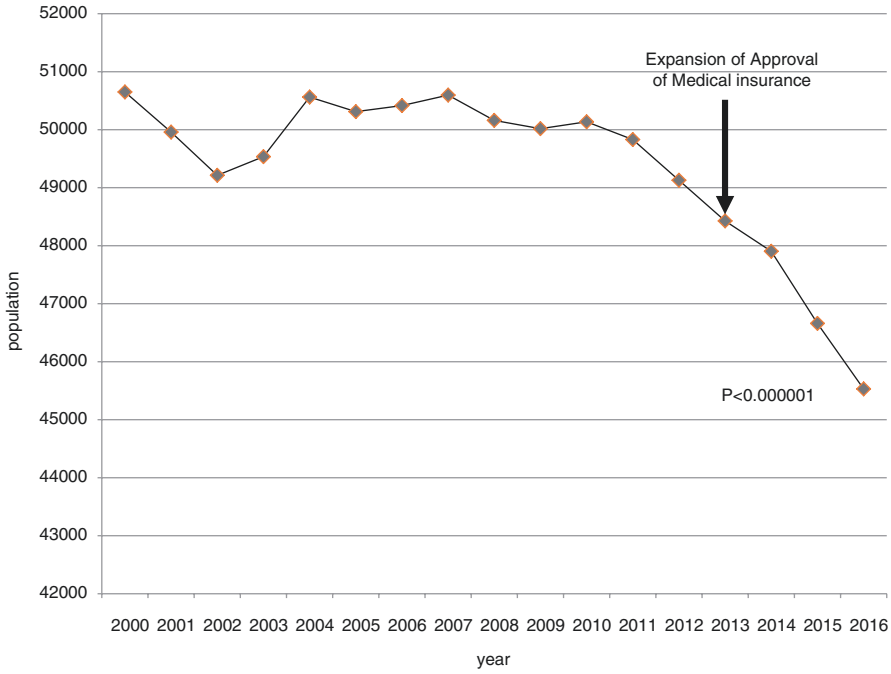

Fig. 13.2 Changes of gastric cancer deaths in Japan

patients received eradication therapy each year after the indications for *H. pylori* eradication therapy were expanded to include chronic gastritis, with approximately six million patients being treated by eradication therapy over 4 years [13]. *H. pylori* eradication therapy for gastroduodenal ulcer was approved for coverage by the Japanese NHI scheme in 2000. Subsequently, the incidence of gastroduodenal ulcer decreased dramatically by approximately 60% over 10 years [27], and medical costs for treatment of gastroduodenal ulcer were also reduced by 47% during this period.

The number of deaths from gastric cancer was 48,632 in 2013, 47,903 in 2014, 46,659 in 2015, and 45,509 in 2016 showing a decreasing trend after widening of the NHI indications for *H. pylori* eradication therapy, according to cancer mortality data (1958–2014) from the Vital Statistics in the Cancer Registry and Statistics. The decrease in the number of gastric cancer deaths to 45,509 in 2016 represented a fall of 9.2% in the 4 years after the indications for *H. pylori* eradication therapy were expanded (Fig. 13.2) [13]. If the incidence of *H. pylori* gastritis can be reduced by eradication therapy, the incidence of atrophic gastritis (a premalignant condition for gastric cancer) will also decrease. Although it is unclear whether the outcome for improvement of gastric cancer will be comparable to that for gastroduodenal ulcer, if the incidence of atrophic gastritis decreases, a reduction in the incidence of intestinal type gastric carcinoma that develops from atrophic gastritis would also be expected. Our study showed that the number of deaths from gastric cancer, which has remained stable over past 40 years, decreased during the last 4 years along with

an increase in the prescription of *H. pylori* eradication therapy. According to NHI criteria, the diagnosis of gastritis must be established by endoscopy before eradication therapy is performed, and this increased requirement for endoscopy could lead to detection of gastric cancer in many patients.

It was predicted that the number of deaths from gastric cancers would be 60,000 in 2020 if no new measures were taken [27]. However, our research revealed that the annual number of deaths from gastric cancer (which remained at about 50,000 over the period of 40 years before the expansion of health insurance coverage) showed a significant decrease of about 9.2% over 4 years after expansion of the indications for *H. pylori* eradication. It is estimated that if patients with *H. pylori* infection continue to receive eradication therapy, the number of deaths from gastric cancer will be reduced by 40% to about 30,000 per year in 2020, along with a protective effect against future development of this cancer.

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Chapter 14
Prevention Strategy for Gastric Cancer

Osamu Handa and Yuji Naito

Abstract In Japan, the major cause of gastric cancer is *Helicobacter pylori* infection. Therefore, targeting *H. pylori* eradication is considered an effective strategy to prevent gastric carcinogenesis. However, the prevention strategy should be decided based on the risk of individual patients. The risk of gastric cancer has been reported to depend on the severity of atrophic mucosa caused by *Helicobacter pylori*, and the earlier *H. pylori* eradication has been thought to be more effective in the prevention of future gastric carcinogenesis. Consequently, there are several "screen-and-treat" projects at the prefectural level in Japan for junior high and high school students. On the contrary to the high-risk group, the severity of gastric mucosal atrophy is much severer than the younger generation, and follow-up surveillance for gastric cancer is more important in addition to *H. pylori* eradication.

Keywords Helicobacter pylori · Younger generation · Student · Screen and treat · Gastric cancer

14.1 Introduction

In general, a positive correlation has been found between the incidence of gastric cancer and *Helicobacter pylori* infection rate as shown in Fig. 14.1 [1]. However, in Eastern Asian countries, a higher incidence of gastric cancer is reported than in Europe and North America [2]. In Japan, gastric cancer is a major cause of cancer deaths, and most gastric cancers are caused by *H. pylori* infection. The incidence of gastric cancer among patients who have never been infected by *H. pylori* is extremely low; the incidence of *H. pylori*-negative gastric cancer has been reported to be very low in Japan, ranging from 0.42 to 0.66% [3, 4]. Therefore, targeting *H. pylori* eradication is considered an effective strategy to prevent gastric carcinogenesis.

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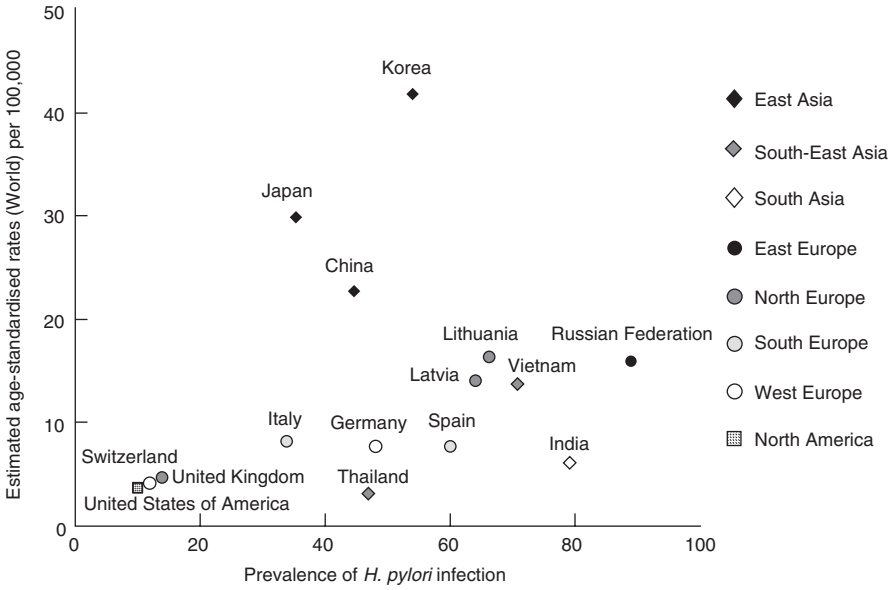

Fig. 14.1 The geographic distribution of the prevalence of *H. pylori* infection and gastric cancer incidence (Suzuki H, Mori H. J Gastroenerol 2017)

However, the prevention strategy should be decided based on the prevalence of *H. pylori* in each region and the risk of individual patients. In this chapter, we describe a prevention strategy for high-risk groups and the younger generation.

14.2 Prevention Strategy for the Younger Generation

In Japan, the main route of infection is from a mother to her infants [5–7]. It has been reported that in most cases *H. pylori* infects infants during early childhood and that infection after childhood is rare [4, 8, 9]. Without eradication, *H. pylori* continues to infect the stomach and causes chronic atrophic gastritis, which is a known precancerous lesion for gastric cancer [10, 11]. Consequently, the risk for gastric cancer in *H. pylori*-positive patients has been reported to be 15 times higher than those without *H. pylori* [12]. Also, the risk of gastric cancer has been reported to depend on the severity of atrophic mucosa [13]. Therefore, earlier *H. pylori* eradication has been thought to be more effective in the prevention of future gastric carcinogenesis. In humans, no direct evidence has been reported regarding the effect of *H. pylori* eradication on gastric cancer chemoprevention in the younger generation compared to the older generation; however, an apparent effect has been reported using an animal model of gastric carcinogenesis (Fig. 14.2). In this experiment, to

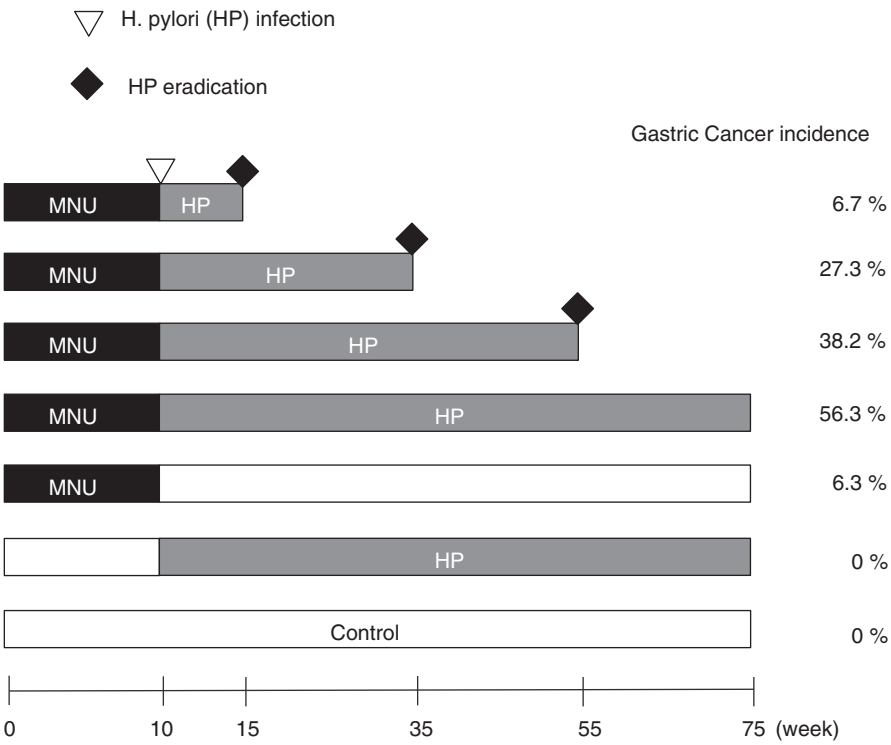

Fig. 14.2 Effect of early eradication on *H. pylori*-related gastric carcinogenesis in Mongolian gerbils (Nozaki K et al. Cancer Sci. 2003)

evaluate the effect of eradication on gastric carcinogenesis, an animal model with eradication in the early, middle, or late period was studied using *H. pylori*-infected and *N*-methyl-*N*-nitrosourea (MNU)-treated Mongolian gerbils. MNU is a well-known chemical carcinogen with a potent tumor initiation effect. In the MNU-treated and *H. pylori*-infected group, the incidence of stomach cancer was 56.3% at 75 weeks. However, eradication therapy significantly decreased the cancer incidence, depending on the period of eradication after *H. pylori* infection, suggesting that the tumor incidence was related to the duration of inflammation induced by *H. pylori* infection and eradication for the younger generation seems a promising strategy for gastric cancer prevention in humans.

In addition, the population infected with *H. pylori* is another important issue. Due to the improvement in environmental hygiene, the infection rate of *H. pylori* is decreasing in Japan [14–16]. It was reportedly more than 40% among individuals born before 1950, 20% in the 1970s, and 12% in the 1980s (Fig. 14.3) [16]. Therefore, targeting the younger generation with *H. pylori* infection for preventing gastric cancer might be cost-effective.

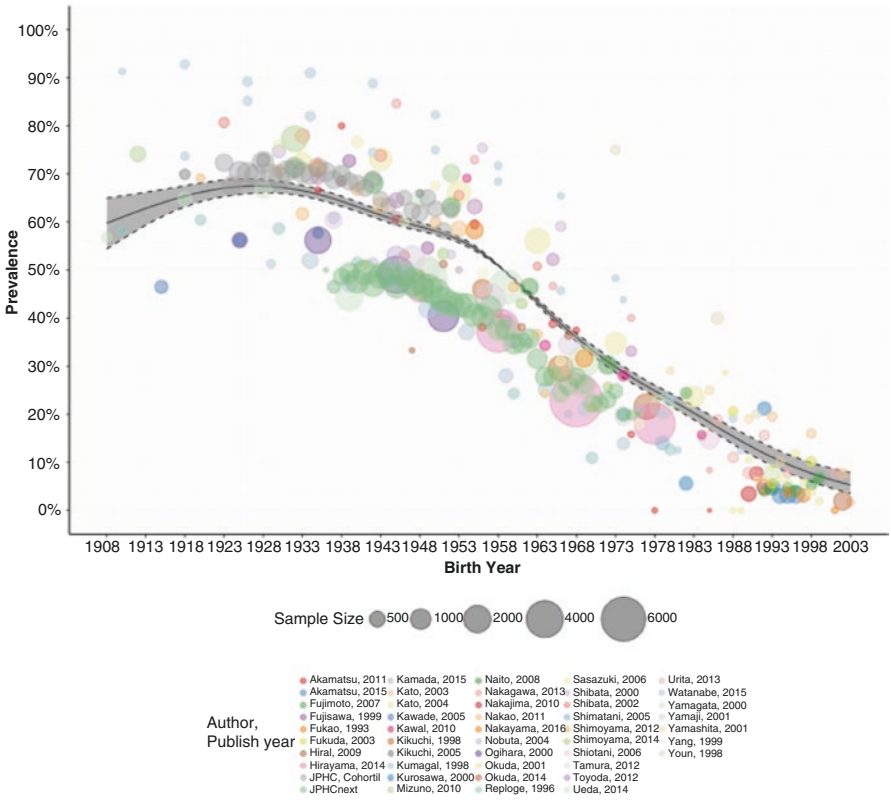

Fig. 14.3 Multivariable-adjusted prevalence of *H. pylori* infection in Japan by birth year from 1908 to 2003 (Wang, C. et al. Sci Rep 2017)

14.3 "Screen-and-Treat" Strategy for High School Students in Kyoto

Consequently, there are several "screen-and-treat" projects at the prefectural level in Japan for junior high and high school students. We have also started a multicenter prospective "screen-and-treat" project for high school students in the first grade from April 2015 as a part of the gastric cancer elimination project in Kyoto Prefecture.

The procedure of our project is as follows**:**

1. At first, collaborating with the Kyoto Prefecture Health Measures Division, we requested the cooperation of several high schools out of 104 high schools (approved by the ethics committee of Kyoto Prefectural University of Medicine). The number of high schools that agreed to partake in this project increased over time: 3 in 2015, 6 in 2016, and 15 high schools in 2017.

2. We explained the procedure of this project to teachers, parents, and students of each high school, and written informed consent was obtained from the parents and students.
3. For the students who agreed, urine-based enzyme-linked immunosorbent assay for anti-*H. pylori* immunoglobulin G antibody (urine-HpELISA) was performed.
4. Those students whose urine-HpELISA tests yielded positive results were re-examined using the *H. pylori* stool antigen test (HpSA).
5. Those students who tested positive for HpSA received, if they wished, *H. pylori* eradication therapy for free in collaborating hospitals near their high schools. The *H. pylori* eradication regimen was as follows: proton pump inhibitor (PPI) (rabeprazole 20 mg/day) + amoxicillin (AMPC) 1500 mg/day + metronidazole (MNZ) 500 mg/day (approved by the ethics committee of each hospital). Since the resistance rate for MNZ is very low in Japan compared to other Asian countries, we selected MNZ instead of clarithromycin (CAM) to which *H. pylori* has increased resistance.
6. A negative HpSA test at 2 months after the eradication therapy was considered to be successful eradication.

The results in 2015 and 2016 were as follows:

1. The number of students who enrolled in this project increased from 734 to 883.
2. The proportion of students who submitted their urine for examination increased from 83.9 to 88.1% of all students.
3. There was no significant difference between positivity rates of urine-HpELISA in 2015 (8.3%) and 2016 (8.4%).
4. There was no significant difference between positivity rates of HpSA in 2015 (4.7%) and 2016 (3.2%).
5. The successful eradication rate in 2015 was 84.2%, with no major side effects. The proportion of students who underwent eradication therapy in 2016 was 85.7%, and there were no significant adverse effects.

We are aiming to scale up this project across the entire prefecture by 2020. By continuing this project, we will be able to eliminate *H. pylori* and terminate the incidence of gastric cancer.

14.4 "Screen-and-Treat" Strategy for Junior High School Students

In other prefectures in Japan, there are several "screen-and-treat" projects for junior high school students at prefectural levels in Japan. The basis for targeting junior high school students is as follows: it has been reported that in children, (1) the reinfection rate of *H. pylori* after successful eradication, especially among those younger than 5 years old [17, 18], is higher than that in adults [19], (2) the sensitivity of the

antibody test against *H. pylori* is not high under 10 years old and that after 10 years old is the same as that of adults [20], and (3) high examination rate can be expected, since junior high school is compulsory in Japan. However, some pediatricians do not agree to the blind "screen-and-treat" strategy for asymptomatic junior high school students since the eradication therapy is not covered by insurance and the risk of long-term effect of eradication therapy on the younger generation has been speculated. In any case, informed consent is necessary before performing "screen and treat."

14.5 Screening Method and Eradication Therapy for the Younger Generation

For the screening of *H. pylori* infection in the younger generation, urine antibody or stool antigen test would be recommended. Since urine examination is easy, noninvasive, and inexpensive, it is preferable for mass screening. However, the false-positive rate of the urine antibody test has been reported to be around 30–40% [21] [22] because of proteinuria [23] in the younger generation. Therefore, another method is required for the confirmation of *H. pylori* infection. The stool antigen test is thought to be suitable for the screening of *H. pylori* infection in the younger generation, since it is easy to perform and is noninvasive. However, the cost of this test is higher than that of the urine test, the transportation of stool specimen at higher temperatures has been reported to increase the rate of false-negatives [24], and the students tend to be ashamed to submit samples of their stool.

Indeed, the urea breath test (UBT) is favorable for confirming *H. pylori* infection because of its high precision; however, it is expensive to perform and requires about half an hour per examination. Besides, testing the sensitivity of *H. pylori* to antibiotics is also recommended because of the high resistance (more than 50%) of *H. pylori* to CAM [25]. However, to assess the resistance of *H. pylori* to CAM, endoscopy needs to be performed; this is therefore not suitable for mass screening of the younger generation. Thus, the urine antibody test is widely used for mass screening of the younger generation, and the result is confirmed by stool antigen test or UBT. For symptomatic cases, endoscopic examination should be considered.

In Japan, under the health insurance system, PPI + AMPC + CAM (PAC therapy) has been used as the first-line therapy. In accordance with the increase in *H. pylori* resistance to CAM, the eradication rate of the PAC therapy has decreased (around 70%) year by year [26], and PPI + AMPC + MNZ (PAM therapy) has been used as the second-line therapy. For the younger generation below 15 years old, who are not covered by the health insurance, the components of the eradication therapy remain controversial. Recently, vonoprazan (VPZ), a potassium-channel-competitive acid blocker that strongly inhibits gastric acid secretion, has been reported to increase the eradication rate even with AMPC + CAM (VAC therapy) [27]. It has recently been reported that VAC therapy is also useful and safe for the younger generation [28]. More evidence needs to be accumulated to confirm this issue.

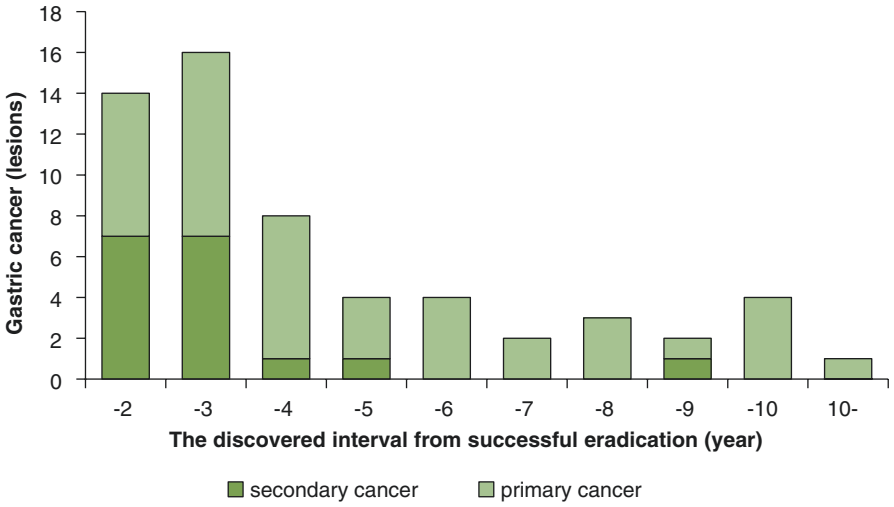

Fig. 14.4 The gastric cancer detection interval from successful eradication (Majima A, Handa O. et al. Digestion 2017)

14.6 Prevention Strategy for High-Risk Groups

As described above, *H. pylori* infection is acquired during childhood and persists for a lifetime if not eradicated. The long infection period is also correlated with the severity of gastric mucosal atrophy, a well-known precancerous lesion. Therefore, the "screen-and-treat" strategy for the younger generation is thought to be effective for the prevention of gastric cancer, especially in countries with high incidence of gastric cancer, such as Japan and Korea.

Although the gastric cancer preventive effect of *H. pylori* eradication has been reported to be expected even in the older generation [29, 30], it has been thought to be less effective since the severity of gastric mucosal atrophy tends to be higher than in the younger generation [13]. Therefore, for high-risk groups, especially those with severe atrophy of the gastric mucosa, screening for gastric cancer by endoscopy becomes more important than eradication therapy itself. In addition, since the risk of gastric cancer persists long after successful *H. pylori* eradication (Fig. 14.4) [31], follow-up surveillance for gastric cancer is also required for more than 10 years after eradication.

14.7 Conclusion

For gastric cancer prevention, *H. pylori* eradication is inevitable. The strategy for its prevention depends on the severity of gastric mucosal atrophy; for the generation with mild gastric mucosal atrophy (younger generation), the "screen-and-treat"

strategy might be effective, while for high-risk groups, follow-up surveillance is important in addition to *H. pylori* eradication.

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