



# Precision Medicine Approaches to Prevent Gastric Cancer

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## Article Info

Received July 22, 2019

Revised October 18, 2019

Accepted November 15, 2019

Published online January 6, 2020

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Gastric cancer remains one of the most common causes of cancer-related death worldwide, although the incidence is declining gradually. The primary risk factor for gastric cancer is *Helicobacter pylori* infection. The Kyoto global consensus report recommends eradication of *H. pylori* in all infected patients. However, because it is difficult to stratify the risk of carcinogenesis among patients with a history of *H. pylori* infection, annual endoscopic surveillance is performed for everyone after eradication. This review summarizes the current approaches used to screen for novel molecules that could assist in the diagnosis of gastric cancer and reduce mortality. Most well-studied molecules are tissue protein biomarkers expressed by the gastric epithelium and associated with metaplasia-dysplasia-carcinoma sequences. Other strategies focus on the origin of cancer stem cell-related markers, such as CD44, and immune reaction-related markers, such as matrix metalloproteinases. Noninvasive methods such as blood-based approaches are more attractive. Serum pepsinogen levels predict the severity of gastric mucosal atrophy before *H. pylori* eradication, whereas plasma ghrelin levels are associated with atrophy even after eradication. Cell-free DNAs and RNAs are attractive tools for the early detection of cancer. These ideas could lead to the development of more personalized strategies for cancer prevention based on cutting-edge technologies. ([Gut Liver 2021;15:3-12](#))

**Key Words:** Stomach neoplasms; Ghrelin; CD44v9; Microbiota; MicroRNA

## INTRODUCTION

Worldwide, gastric cancer is the fifth most common malignancy and the fourth leading cause of cancer-related death.<sup>1</sup> More than half of the world's total incident gastric cancers occur in East Asia. Although many factors contribute to the risk of gastric cancer, the single greatest risk factor for the development of non-cardia gastric cancer is infection with *Helicobacter pylori* (relative risk of 5.9).<sup>2</sup> Screening strategies for the detection of gastric cancer at an early and more curable stage have led to a significant reduction in mortality from the disease.<sup>3</sup> In addition, *H. pylori* eradication therapy has been shown to be effective at preventing gastric cancer in several well-conducted clinical trials.<sup>4-8</sup> Therefore, according to the Kyoto global consensus report, it is recommended that all infected patients be treated, irrespective of age or the severity of the gastric mucosal lesions, especially in regions with a high incidence

of gastric cancer.<sup>9-12</sup>

However, Uemura *et al.*<sup>13</sup> reported that the rate of gastric cancer in patients with *H. pylori* infection was only 2.9% over a mean follow-up period of 7.8 years. Furthermore, according to data from previous trials, 125 patients would need to be treated with *H. pylori* eradication therapy to prevent one case of gastric cancer.<sup>14</sup> However, *H. pylori* eradication cannot reduce the cancer risk to zero.<sup>15</sup> Thus, no strategy to prevent gastric cancer has been established. Our next challenge will be the proper selection of target populations for screening endoscopies. In this review, we summarize potentially useful epidemiological, biological, and molecular findings to further optimize strategies for the early detection and risk stratification of gastric cancer (Table 1, Fig. 1).



**Table 1.** Methodology for Risk Stratification and/or the Early Detection of Gastric Cancer

Methods	Strength	Limitation
Endoscopic findings	<ul style="list-style-type: none"> <li>Essential for the diagnosis of cancer location</li> <li>Macroscopic diagnosis of the extent of metaplasia (Kimura and Takemoto classification) is associated with the cancer risk</li> <li>Improvement of image-enhancing endoscopy</li> </ul>	<ul style="list-style-type: none"> <li>Sometimes painful/stressful for some individuals</li> <li>Well-trained clinicians are necessary</li> <li>Risk of adverse events (perforation, etc.)</li> </ul>
Histological findings	<ul style="list-style-type: none"> <li>Essential for the definitive diagnosis of dysplasia and cancer</li> <li>OLGA staging system is well associated with the cancer risk</li> </ul>	<ul style="list-style-type: none"> <li>Invasive</li> <li>Risk of adverse events (bleeding, etc.)</li> <li>Results of the analysis depend on the sampling point</li> </ul>
Tissue biomarkers	<ul style="list-style-type: none"> <li>Molecules related to the metaplasia-dysplasia-adenocarcinoma sequence assist the histological diagnosis (TFF2, CDX2, miR-124a-3p methylation, etc.)</li> <li>CD44v9 pathway and MMPs could predict the cancer risk independently from histological findings</li> </ul>	<ul style="list-style-type: none"> <li>Invasive</li> <li>Risk of adverse events (bleeding, etc.)</li> <li>Results of the analysis depend on the sampling point</li> </ul>
Gastric microbiota	<ul style="list-style-type: none"> <li>Causal relationship between cancer development and the presence of carcinogenic microbes is expected</li> <li>Potentially less-invasive diagnosis by sampling of gastric juice</li> </ul>	<ul style="list-style-type: none"> <li>Limited evidences in human</li> </ul>
Serum pepsinogen	<ul style="list-style-type: none"> <li>Good association with the severity of gastric mucosal atrophy in <i>Helicobacter pylori</i>-positive individuals</li> <li>Minimally invasive</li> </ul>	<ul style="list-style-type: none"> <li>No association with the severity of gastric mucosal atrophy after <i>H. pylori</i> eradication</li> <li>Confirmation of the present <i>H. pylori</i> infection is necessary</li> </ul>
Plasma ghrelin	<ul style="list-style-type: none"> <li>Expected association with the severity of gastric mucosal atrophy irrespective of the presence of <i>H. pylori</i></li> <li>Minimally invasive</li> </ul>	<ul style="list-style-type: none"> <li>Limited evidences in human</li> </ul>
Innovative blood-based biomarkers (cell-free DNA, cell-free RNA, etc.)	<ul style="list-style-type: none"> <li>Highly accurate early stage diagnosis is expected</li> <li>Potential organ specificity is expected for some biomarkers</li> <li>Minimally invasive</li> </ul>	<ul style="list-style-type: none"> <li>Unclear molecular mechanisms</li> <li>Further improvement of analytical methodology is necessary</li> <li>Potentially expensive</li> </ul>
CEA, CA19-9	<ul style="list-style-type: none"> <li>Low cost</li> <li>Minimally invasive</li> </ul>	<ul style="list-style-type: none"> <li>Low sensitivity and specificity for early stage cancer</li> </ul>

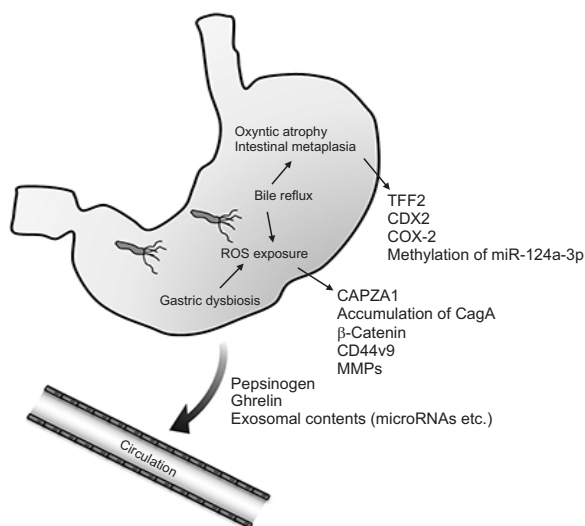
OLGA, Operative Link for Gastritis Assessment; TFF2, trefoil factor 2; CD44v9, CD44 variant 9; MMPs, matrix metalloproteinases; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

## RISK STRATIFICATION BASED ON HISTOLOGY AND MORPHOLOGY

Atrophic gastritis is the initial step in non-cardia gastric carcinogenesis. Gastric mucosal atrophy consists of both the absolute loss of resident glandular units and the transformation of native glands to intestinal metaplasia or spasmolytic polypeptide-expressing metaplasia (SPEM). Ruge *et al.*<sup>16,17</sup> have established a gastritis staging system (stage 0, I, II, III, and IV) based on histology called the Operative Link for Gastritis Assessment (OLGA) system. OLGA stage is determined by combining the atrophy scores in the corpus and antrum, and reflects the risk of progression to gastric cancer. The atrophy score is a four-tiered scale (0–3), based on the visual analogue scale of the updated Sydney system.<sup>18</sup> OLGA staging also requires a five-point biopsy: two from the antral mucosa, one from the mucosa of the angularis incisura, and two from the oxyntic area. In a large European study in which patients were followed for 5 years after *H. pylori* eradication, neoplastic lesions only occurred in patients that had stage III or IV gastritis at the

time of enrollment.<sup>19</sup>

In Japan, to avoid the risks of multiple gastric biopsies, the macroscopic extent of gastric mucosal atrophy is traditionally classified using the Kimura and Takemoto classification system,<sup>20</sup> which correlates with the results of histological evaluation.<sup>21</sup> Based on endoscopic findings, gastric mucosal atrophy is classified as the closed type (C-1 to C-3), which is characterized by borders of atrophy that do not exceed the cardia on the lesser curvature side of the gastric corpus, and the open-type (O-1 to O-3), which is characterized by borders of atrophy that exceed the cardia into the greater curvature side.<sup>20</sup> Open-type atrophy is a significant risk factor for gastric cancer, although follow-up strategies based on the Kimura and Takemoto classification have not been established.<sup>22,23</sup> Linked color imaging, a newly developed image-enhancing endoscopy technique, can detect map-like redness (a high risk feature) and regular arrangement of collecting venules (a low risk feature) more accurately than conventional white-color imaging.<sup>24</sup> Thus, histological and endoscopic approaches are useful for identifying high risk groups. However, carcinogenesis is



**Fig. 1.** Core etiologies of gastric carcinogenesis and potential biomarkers for risk stratification and early detection. Unhealthy intra-gastric conditions, such as *Helicobacter pylori* infection, dysbiosis, and bile reflux, induce the accumulation of reactive oxygen species (ROS) and gastric mucosal atrophy/intestinal metaplasia. Levels of several tissue biomarker molecules (as indicated) are altered during carcinogenesis. To achieve a minimally invasive means of evaluation, blood-based diagnostic strategies are needed.

TFF2, trefoil factor 2; COX, cyclooxygenase; CAPZA1, capping actin protein of muscle Z-line alpha subunit 1; CD44v9, CD44 variant 9; MMPs, matrix metalloproteinases.

observed frequently among histologically low risk patients. For more accurate risk stratification, a combination of histological evaluation and other markers is necessary.

### MOLECULAR DIAGNOSIS OF GASTRIC MUCOSAL ATROPHY AND INTESTINAL METAPLASIA

Immunostaining of several protein markers in non-cancer gastric tissues can assist diagnosis of gastric mucosal atrophy. For example, immunostaining for trefoil factor 2 (TFF2) assists histological assessment of SPEM.<sup>25,26</sup> LI-cadherin staining has good sensitivity and specificity for detecting intestinal metaplasia.<sup>27</sup>

In addition to *H. pylori* infection, the major etiological factor for the extension of atrophic mucosa is duodenogastric bile reflux.<sup>28</sup> Dixon *et al.*<sup>29</sup> reported that the independent risk factors for cardia intestinal metaplasia were increasing age, male sex, chronic inflammation, and significant bile reflux. Bile acids induce the production of reactive oxygen species (ROS), which cause DNA damage and activate nuclear factor- $\kappa$ B, resulting in production of inflammatory factors such as CDX2, epidermal growth factor receptor, interleukin (IL)-1 $\beta$ , IL-8, and cyclooxygen-

ase (COX)-2.<sup>30-33</sup>

Epigenetic alterations, represented by aberrant DNA methylation, are deeply involved in carcinogenesis. Aberrant methylation accumulates during gastric mucosal atrophy and can contribute to cancer development.<sup>34,35</sup> This suggests that the mucosal methylation status could be useful for the molecular diagnosis of the severity of gastric mucosal atrophy.<sup>36</sup> In fact, the methylation of miR-124a-3p, *EMX1*, and *NKX6-1* in biopsy samples taken from the lesser curvature 2 cm from the pyloric ring was significantly associated with the cumulative incidence of metachronous gastric cancers in patients after endoscopic resection of early gastric cancer.<sup>37</sup>

### STEM CELL MARKER CD44 VARIANT 9-RELATED MOLECULES

CD44, a major adhesion molecule and receptor for hyaluronic acid, has been implicated in a variety of physiological processes in addition to cancer cell growth, invasion, and metastasis.<sup>38</sup> Alternative splicing of CD44 mRNA produces a standard isoform of CD44 (CD44s) plus several variant isoforms. Among these, CD44 variant 9 (CD44v9) is the most unique phenotype that characterizes cancer stem cell-like properties.<sup>39</sup> In fact, reciprocal silencing of total CD44 results in reduced tumor-initiating potential of gastric cancer cells, which can be rescued by expression of CD44v9 but not by that of CD44s.<sup>40</sup> CD44v9 interacts with xCT, a glutamate–cystine transporter, and to contribute to ROS defense by promoting the synthesis of the primary intracellular antioxidant glutathione, thereby promoting tumor growth and chemoresistance.<sup>41,42</sup> CD44v9 expression is observed not only in cancer tissues, but also in gastric mucosal epithelium infected with *H. pylori*.<sup>43,44</sup> We reported that CD44v9-positive cells originate from capping actin protein of muscle Z-line alpha subunit 1 (CAPZA1)-overexpressing cells in the gastric epithelium.<sup>45</sup> CAPZA1 overexpression enhanced the expression of  $\beta$ -catenin, which is a transcription factor for CD44, and ESRP1 (epithelial splicing regulatory protein 1), which increases alternative splicing of CD44 to generate CD44v9.<sup>45</sup>

On the other hand, *H. pylori* produces the oncogenic protein CagA and delivers it into the gastric epithelial cell cytoplasm via a type IV transporter. CagA induces cell proliferation and division by interacting with target molecules such as the cytoplasmic Src homology 2 domain of SHP-2 (Src homology 2 phosphatase).<sup>46</sup> CagA is characterized by the presence of five repeating amino acid sequences (Glu-Pro-Ile-Tyr-Ala), designated EPIYA motifs. Four different EPIYA motifs (EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-

D) have been defined. Based on the EPIYA motifs, the CagA protein has been classified into Western and Eastern types. The Western type contains EPIYA-A and EPIYA-B, followed by up to five repeated sequences of EPIYA-C, whereas the East Asian strain contains EPIYA-A, EPIYA-B, and EPIYA-D.<sup>47</sup> The East Asian strain has been shown to be more virulent than the Western strain with respect to clinical outcomes.<sup>48</sup>

As a defense mechanism of host cells, CagA is degraded by autophagy irrespective of the type of EPIYA motif.<sup>49</sup> However, in CAPZA1-overexpressing cells, autolysosome formation is inhibited and CagA escapes autophagic degradation.<sup>50</sup> The accumulated CagA promotes CD44v9 expression by inducing the nuclear accumulation of  $\beta$ -catenin.<sup>45,49</sup> Furthermore, ROS triggers CAPZA1 expression by increasing histone H3 acetylation of the CAPZA1 promoter.<sup>45</sup> Furthermore, a CD44v9-xCT system-targeting intervention prevents development of preneoplastic lesions and cancer in mice.<sup>51</sup> Thus, the ROS/CAPZA1/CagA/ $\beta$ -catenin/CD44v9/xCT axis could be a key pathway in gastric cancer development. In fact, we showed that CD44v9 expression was a better predictor of gastric cancer recurrence after resection of early gastric cancer than the presence of open-type gastric mucosal atrophy.<sup>22</sup>

Although other variant isoforms are expressed in the gastric mucosa, the functions of each variant are unclear.<sup>52</sup> CD44v6 acts as a co-receptor for the function of c-Met in response to *H. pylori* infection and CagA-induced epithelial proliferation.<sup>53</sup>

## MATRIX METALLOPEPTIDASES

Studies of the interaction between gastric epithelium and immune responses reveal that matrix metallopeptidases (MMPs) play key roles. MMPs are a multimember family of zinc-containing endopeptidases with a wide substrate specificity; MMPs degrade extracellular matrix proteins during tissue morphogenesis and remodeling in wound healing. In addition, MMPs modify a multitude of non-matrix substrates such as cytokines, chemokines, growth factors, and growth factor receptors, as well as cell-surface adhesion receptors.<sup>54</sup>

*H. pylori* infection and translocation of CagA to gastric epithelial cells induces EPIYA phosphorylation-dependent up-regulation of MMP-1, MMP-3, MMP-7, MMP-9, and MMP-10, along with CD44.<sup>38,54-57</sup> MMP-10-associated inflammation is characterized by influx of CD8<sup>+</sup> T cells, whose migration is induced via the MMP-10-CXCL16 axis in gastric epithelial cells.<sup>58</sup> The MMP-9-1562 SNP results in gender-specific differences in the risk of intestinal metapla-

sia after *H. pylori* infection.<sup>59</sup> By contrast, MMP-7 inhibits *H. pylori*-induced gastric injury and development of pre-malignant lesions via Th1- and Th17-mediated pathways, and by suppressing M1 macrophage polarization.<sup>60,61</sup> Thus, altered expression of MMPs is associated with immune responses and carcinogenesis in the gastric epithelium, and it may serve as a tissue biomarker for cancer risk stratification and/or be a molecular target for cancer prevention.

## GASTRIC MICROBIOTA COMPOSITION

Until recently, the acidic environment of the stomach was thought to prevent bacterial colonization since indigenous bacteria from the stomach had not been successfully isolated in culture. However, recent technological advances such as DNA sequencing of ribosomal RNA genes and phylogenetic analysis confirmed the existence of a diverse gastric microbiota in humans. The gastric microbiota is strongly influenced by *H. pylori* infection.<sup>62-64</sup> Changes in the gastric environment such as changes in gastric acid secretion can drastically change the gastric microbiota. The overgrowth of pathobionts can promote inflammatory responses in the stomach, potentially leading to the development of gastric cancer.

Lofgren *et al.*<sup>65</sup> used human gastrin-overexpressing transgenic mice (INS-GAS mice), which develop gastric cancer upon *H. pylori* infection, to understand this effect. Under specific pathogen-free (SPF) conditions, *H. pylori* infection induced the onset of gastric cancer within 7 months of infection, whereas infection of germ-free INS-GAS mice with *H. pylori* did not cause tumorigenesis.<sup>65</sup> In addition, co-infection of germ-free INS-GAS mice with *H. pylori* and three other intestinal bacteria species (ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus*, and ASF519 *Bacteroides* species) led to gastric neoplasia formation, similar to *H. pylori* infection under SPF conditions.<sup>66</sup> These results suggest that gastric cancer onset is dependent on the presence of symbiotic bacteria that synergize with *H. pylori* infection.

According to analysis of human samples, the most common bacteria detected in individuals with *H. pylori*-associated gastritis are *Helicobacter*, *Streptococcus*, *Prevotella*, and *Neisseria*.<sup>67,68</sup> These symbiotic bacteria mainly colonize the oral cavity and esophagus. However, in individuals with gastric cancer, the numbers of *Helicobacter* falls significantly, whereas *Citrobacter*, *Clostridium*, *Lactobacillus*, *Achromobacter*, and *Rhodococcus* are more abundant.<sup>67</sup> These bacteria are mainly symbionts colonizing the lower gastrointestinal tract mucosa. Interestingly, abundantly detected bacteria in patients with gastric cancer have high

nitric acid reductase and nitrous acid reductase activity. These bacteria effectively regenerate nitrite, the precursor of *N*-nitroso compounds, from nitrate.<sup>67</sup> Conjugated bile acids are a major source of nitrosamides in humans. Bile acids are normally present as cholic acid amides of glycine or taurine, and these amides are *N*-nitrosated by gastric bacteria. *N*-nitrosoglycocholic acid and *N*-nitroso-taurocholic acid are mutagenic and can contribute to the development of gastric cancer.<sup>69,70</sup> Thus, the investigation of non-*H. pylori* components of the gastric microbiota will be of crucial importance for the risk stratification of gastric cancer.

### BLOOD-BASED TESTING FOR RISK STRATIFICATION

Altered levels of serum pepsinogens (PGs), which are mainly produced by the chief cells of the fundic glands of the stomach, reflect the atrophic status of the gastric mucosa.<sup>71</sup> While PG II is produced by both the antrum and the corpus mucosa, PG I is a marker of oxyntic mucosa status: any (metaplastic or non-metaplastic) loss of oxyntic glands results in lower PG I levels and a lower PG I/II ratio. The combination of the serum PG I level and the PG I/II ratio is a good predictor of gastric cancer occurrence in *H. pylori*-infected populations.<sup>72-74</sup> In fact, a 20-year prospective cohort study of a Japanese population revealed that the combination of serum *H. pylori* antibody and PG levels is a significant predictor of gastric cancer risk.<sup>75</sup> When participants were categorized into four groups (group A [*H. pylori*−, PG−], group B [*H. pylori*+, PG−], group C [*H. pylori*+, PG+], and group D [*H. pylori*−, PG+]), the risk of gastric cancer in group B had a hazard ratio of 4.08 (95% confidence interval [CI], 1.62 to 10.28), whereas that in groups C/D was 11.1 (95% CI, 4.45 to 27.46) when group A was used as the reference group.<sup>75</sup> However, PG I and II decrease, and the PG I/II ratio increases, after successful *H. pylori* eradication, irrespective of any improvement in mucosal atrophy.<sup>76,77</sup> Therefore, the serum PG level is a useful tool for gastric cancer risk stratification among individuals with *H. pylori* infection. For individuals after eradication, other noninvasive biomarkers of atrophic status are needed for risk stratification.

Ghrelin is a 28-amino-acid appetite-stimulating peptide hormone secreted by the A-like cells of the oxyntic glands of the stomach. Under conditions of gastric mucosal atrophy, as the number of A-like cells decreases, the plasma ghrelin concentration also decreases.<sup>78</sup> In fact, the plasma levels of total and active ghrelin correlate with the serum PG I level, as well as the serum PG I/II ratio, and decrease

as the extent of gastric mucosal atrophy increases.<sup>79,80</sup> More studies are needed on the reliability of plasma ghrelin levels for gastric cancer risk stratification.

TFF2 (produced during SPEM) and TFF3 (produced during intestinal metaplasia) can be detected in serum. Kuo *et al.*<sup>81</sup> investigated whether serum levels of TFF2 and TFF3 predict cancer-associated histology. Neither IM nor SPEM were associated with serum TFF2 or TFF3. Serum TFF2 levels were higher in participants with corpus gastritis who also had advanced SPEM. For participants without corpus gastritis, elevated serum TFF2 levels correlate with higher *H. pylori* density and more severe gastritis in the antrum. These results suggest that serum levels of TFF2 are associated with the extent of SPEM, as well as with the risk of gastric cancer.

### BLOOD-BASED TESTING FOR EARLY DETECTION

Conventional serum tumor biomarkers such as carcinoembryonic antigen and carbohydrate antigen (CA) 19-9 are unsuitable for early gastric cancer detection due to their insufficient specificity and sensitivity. Compared with these, serum TFF3 is a good biomarker for predicting the presence of gastric cancer.<sup>82</sup> The area under the receiver operating characteristics curve (AUROC) of TFF3 for predicting gastric cancer was 0.65 to 0.81.<sup>82-84</sup>

Furthermore, recent liquid biopsy technologies could provide us with more accurate diagnostic tools based on the analysis of cell-free DNA (cfDNA), microRNA (miRNA), or extracellular vesicles.<sup>85</sup> Circulating cfDNA is cell-free extracellular DNA originating from normal and cancerous cells in the blood. The fraction of cfDNA that derives from primary tumors, metastases, or circulating tumor cells is called circulating tumor DNA (ctDNA). A recent meta-analysis showed that detection of ctDNA resulted in increased gastric cancer diagnosis specificity over conventional protein biomarkers, but there was no increase in sensitivity.<sup>86</sup>

Dysregulated miRNAs can contribute to cancer initiation and development. miRNAs are short regulatory RNA molecules of 17 to 25 nucleotides in length. They modulate target gene expression at the post-translational level by guiding the RNA-induced silencing complex to miRNA target sites in the 3' untranslated region of mRNAs, leading to mRNA degradation or the inhibition of translation. Expression profiling has shown distinctive miRNA signatures in different cancers, including gastric cancer.<sup>87</sup> miRNAs are secreted by cells mainly via exosomes, and are detectable in the circulation.<sup>88,89</sup> Circulating miRNA profiles are associ-

ated with various disease conditions and can be good biomarkers.<sup>85</sup> Previous reports have shown that miRNA profiles can accurately discriminate between patients with and without gastric cancer. For example, Shin *et al.*<sup>90</sup> showed that the combination of plasma miR-627, miR-629, and miR-652 resulted in the highest AUROC with a sensitivity of 86.7% and a specificity of 85.5%. Liu *et al.*<sup>91</sup> reported that serum levels of miR-1, miR-20a, miR-27a, miR-34, and miR-423-5p achieved an AUROC of 0.879. Zhou *et al.*<sup>92</sup> reported that plasma levels of miR-185, miR-20a, miR-210, miR-25, and miR-92b resulted in an AUROC of 0.87. Hou *et al.*<sup>93</sup> reported that serum miRNA-206 not only served as a novel diagnostic biomarker (AUROC, 0.89), but also predicted cancer recurrence and patient prognosis. Zhu *et al.*<sup>94</sup> reported that the combination of plasma miR-16, miR-25, miR-92a, miR-451, and miR-486-5p acted as a potential biomarker for detecting non-cardia gastric cancer, with an AUROC of 0.812. A recent review summarizes other related reports.<sup>95</sup> Thus, patients with gastric cancer seem to have distinct circulating miRNA profiles. However, the specific miRNAs that were identified in these studies varied. The protocols that are used for RNA extraction and miRNA quantification can significantly affect the results.<sup>96</sup> Worldwide standardization of the methods for evaluating circulating miRNAs will be essential for the clinical application of the miRNA profile.

Other cell-free RNAs, such as LMX1A mRNA,<sup>97</sup> long non-coding RNAs (lncRNAs),<sup>98</sup> and circular RNAs (circRNAs),<sup>99</sup> are also potential biomarkers for cancer detection. Tumor-educated platelet RNA signatures could be useful for detecting early stage cancer.<sup>100</sup> Thus, knowledge of various innovative blood-based cancer detection methods is rapidly accumulating, which could lead to marked changes in future clinical practice.

## CONCLUSIONS

Given recent advances in DNA sequencing technology, many next generation biomarkers are being utilized. Traditionally, clinicians have tried to predict the risk of gastric carcinogenesis based on the severity of gastric mucosal atrophy. To achieve further risk stratification, further investigation of ROS-related alterations in the gastric mucosa and gastric pathobionts are warranted. Pathogenic gastric microbial species could be a good biomarker for the risk of carcinogenesis as well as a potential source of therapeutic targets. Establishment of less invasive blood-based examinations is expected and desirable. Ultimately, surveillance of gastric cancer should be performed via blood tests. To determine the appropriate interval for blood-based sur-

veillance, risk stratification based on blood tests should be performed. Only individuals with suspected gastric cancer will undergo endoscopy for definitive diagnosis and to determine the therapeutic strategy. Every promising biomarker should be verified strictly and prospectively, although it will take many years to obtain evidence that these markers reduce mortality. We must keep on addressing the issue and construct an ideal screening strategy, based on several biomarkers, for the next generation.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Scientific Research C (17K09471, to J.M.) from the Japan Society for the Promotion of Science (JSPS).

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