Journal of Clinical and Translational Pathology **2026** | Epub
DOI: 10.14218/JCTP.2026.00024

Review Article

DNA Methylation Biomarkers: From Precancerous Lesions to Adenocarcinoma in the Stomach and Esophagus

Maylynn Hu[1] and Zhongren Zhou[2*]

[1]*Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, USA;* [2]*Department of Pathology & Laboratory Medicine, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, USA*

Received: April 29, 2026 | **Revised:** July 17, 2026 | **Accepted:** August 25, 2026 | **Published online:** September 24, 2026

Abstract

Background and objectives: Esophageal adenocarcinoma (EAC) and gastric cancer (GC) remain major causes of cancer-related mortality worldwide, largely because of late-stage diagnosis. Barrett's esophagus (BE) is the established precursor setting for EAC, whereas gastric intestinal metaplasia is an important precursor lesion in the intestinal-type gastric carcinogenesis pathway. Aberrant DNA methylation arises early in both pathways and may offer greater sensitivity and specificity than histology or serology alone, while also illuminating the biology of neoplastic progression. This review evaluates the evidence and translational potential of DNA methylation biomarkers in upper gastrointestinal adenocarcinoma.

Methods: PubMed, Web of Science, and EMBASE were searched for studies published before May 2026 addressing DNA methylation biomarkers in BE, EAC, gastric intestinal metaplasia, and GC. Tissue-based, minimally invasive, and circulating biomarkers were reviewed, with emphasis on diagnostic performance, progression risk, biological significance, and clinical translation.

Results: Methylation alterations accumulate during progression from precancerous lesions to invasive cancer and distinguish disease states in tissue and minimally invasive specimens. Methylation-based assays show promising diagnostic performance in BE and EAC, including nonendoscopic cytology-based approaches and risk-stratification assays, and circulating methylated DNA shows potential for noninvasive detection of EAC and GC. However, heterogeneity in populations, specimens, assay platforms, and study designs, together with limited prospective validation, remains a barrier to implementation.

Conclusions: The field is moving rapidly from discovery toward practice: Cytosponge-TFF3 with biomarker panels has been evaluated in real-world UK surveillance pathways, the EsoCheck/EsoGuard methylation assay is clinically available, and Esopredict is the first epigenetic prognostic assay clinically validated to risk-stratify BE, while multicancer early detection assays undergo prospective evaluation. Multicenter studies of clinical utility and cost-effectiveness remain the decisive step before routine adoption.

Introduction

Upper gastrointestinal adenocarcinomas, comprising esophageal adenocarcinoma (EAC) and gastric cancer (GC), remain among the leading causes of cancer-related mortality worldwide despite substantial advances in endoscopic imaging, surgical techniques, and systemic therapies.[1] The poor prognosis associated with both malignancies is largely attributable to delayed diagnosis, as early-stage disease is frequently asymptomatic, and many patients present with locally advanced or metastatic cancer. Five-year survival ranges from 49% to 77% when EAC and GC are detected at an early stage but declines dramatically to 5–8% following regional or distant spread, highlighting the importance of effective strategies for early detection and risk stratification.[2,3]

Both cancers develop through well-characterized precursor lesions that provide a window of opportunity for intervention. EAC typically arises through the intestinal metaplasia–dysplasia–

Keywords: Barrett's esophagus; Gastric cancer; Adenocarcinoma; Epigenetics; DNA methylation; Predictive biomarkers.
***Correspondence to:** Zhongren Zhou, Department of Pathology & Laboratory Medicine, Robert Wood Johnson Medical School, Rutgers University, 1 Robert Wood Johnson Place, New Brunswick, NJ 08903, USA. ORCID: https://orcid.org/0000-0002-6977-8414. Tel: +1-732-667-0497, Fax: +1-732-235-8124, E-mail: zz442@rwjms.rutgers.edu
How to cite this article: Hu M, Zhou Z. DNA Methylation Biomarkers: From Precancerous Lesions to Adenocarcinoma in the Stomach and Esophagus. *J Clin Transl Pathol*. Published online: Sep 24, 2026. DOI: https://doi.org/10.14218/JCTP.2026.00024.

carcinoma sequence, whereas intestinal-type GC develops through the Correa cascade, progressing from chronic *Helicobacter pylori*–associated gastritis to atrophic gastritis, gastric intestinal metaplasia (GIM), dysplasia, and invasive adenocarcinoma.[4] Current surveillance strategies rely primarily on endoscopic evaluation with local biopsy and histopathologic assessment; however, these approaches are invasive, resource-intensive, susceptible to sampling error, and associated with considerable interobserver variability, limiting their effectiveness for population-based screening and longitudinal surveillance.[5-7] These limitations have stimulated intense interest in molecular biomarkers capable of identifying high-risk lesions before the development of invasive malignancy.

Among the molecular alterations implicated in upper gastrointestinal carcinogenesis, aberrant DNA methylation has emerged as one of the earliest and most reproducible epigenetic events. Promoter CpG island hypermethylation can silence tumor suppressor genes involved in cell-cycle regulation, apoptosis, DNA repair, epithelial differentiation, and Wnt signaling, thereby contributing to neoplastic progression while remaining detectable in tissue, cytology specimens, and circulating cell-free DNA (cfDNA).[8,9] Unlike many genetic mutations that occur later during tumor evolution, methylation changes frequently precede histopathologic metaplasia or dysplasia, making them attractive candidates for early detection, risk prediction, and minimally invasive surveillance. Advances in high-throughput methylation profiling, liquid biopsy technologies, and artificial intelligence have accelerated the discovery of increasingly accurate methylation signatures for both EAC and GC.[10,11]

Several recent reviews have comprehensively summarized DNA methylation biomarkers in GC and Barrett's-associated neoplasia.[12,13] However, these reviews primarily evaluate EAC and GC as separate disease entities and focus predominantly on cataloging candidate biomarkers. Comparatively little attention has been given to the shared epigenetic mechanisms that underlie upper gastrointestinal carcinogenesis, the overlap between methylation biomarkers identified across both organ systems, the comparative strengths and limitations of emerging methylation detection technologies, and the practical challenges that currently limit clinical implementation. Furthermore, rapid advances in minimally invasive sampling platforms, cfDNA methylation profiling, and multicancer early detection (MCED) assays have substantially expanded the translational landscape since many earlier reviews were published, warranting an updated synthesis of the field.

Accordingly, this review provides a comprehensive and comparative evaluation of DNA methylation biomarkers across Barrett's esophagus (BE), EAC, GIM, and gastric adenocarcinoma. Rather than considering these malignancies independently, we examine their shared and disease-specific epigenetic mechanisms, compare emerging tissue-based, nonendoscopic, and liquid biopsy methylation platforms, critically evaluate barriers to clinical translation—including assay standardization, regulatory approval, and implementation—and discuss future directions for integrating methylation biomarkers into precision screening and surveillance strategies for upper gastrointestinal adenocarcinomas.

EAC and BE

Definitions and clinical context

Esophageal cancer comprises two major histologic subtypes: EAC and esophageal squamous cell carcinoma. Since the 1980s, the incidence of EAC has increased approximately six-fold in North America, representing one of the most rapidly rising malignancies in Western countries.[14] More recent epidemiologic data suggest that although incidence continues to remain substantially elevated compared with historical levels, growth rates have begun to plateau in several Western populations, likely reflecting improvements in reflux management and changes in obesity trends. The 5-year survival rate for EAC is only 18–22% because most patients present at an advanced stage.[15] The single major risk factor for the development of EAC is BE, in which intestinal-like glandular epithelium replaces the normal squamous mucosa of the esophagus.[16,17] BE is a consequence of chronic gastroesophageal reflux disease (GERD).[17,18] Roughly 15–40% of adults in Western countries have GERD, while 8–20% of adults with GERD develop BE.[19,20] Patients with BE have a substantially increased risk of EAC compared with the general population; large population-based cohorts have reported annual EAC progression rates as low as approximately 0.12%, with risk estimates varying according to dysplasia status and cohort definition.[16-18,21] Current guidelines generally recommend endoscopic surveillance every 3–5 years for patients with BE in order to identify dysplasia or early cancer.[5,18] However, the limited cost-effectiveness and inconsistent patient compliance associated with these guidelines have resulted in a substantial number of EAC cases being diagnosed at advanced stages. Consequently, only a small fraction (10–15.9%) of EAC patients undergo regular BE surveillance.[22]

Current surveillance paradigm for BE

Currently, surveillance of individuals with BE relies on esophagogastroduodenoscopy (EGD) with systematic biopsy, which aims to detect dysplasia or early carcinoma before symptom onset. The American Society for Gastrointestinal Endoscopy guidelines recommend that patients with nondysplastic BE undergo surveillance EGD at regular intervals—typically every 3–5 years in the absence of dysplasia—using a systematic biopsy sampling protocol (Seattle protocol) with four-quadrant biopsies every 1–2 cm along the Barrett's segment.[5,23,24] More frequent surveillance is advised for those with confirmed low- or high-grade dysplasia (HGD), and targeted biopsies of any visible mucosal irregularities are also obtained during each procedure. Endoscopic assessment and histologic grading of dysplasia drive clinical decision-making, with treatment options such as endoscopic resection or radiofrequency ablation being effective in reducing progression for high-grade lesions.[24,25] However, this surveillance paradigm is limited by sampling error and interobserver variability, resulting in suboptimal risk stratification,[26] and many patients with a BE diagnosis follow divergent disease courses.[27] There is an urgent need for clinically reliable biomarkers that can accurately stratify BE patients by their risk of progression to EAC. Despite advances in endoscopic imaging, population-based screening for BE remains limited by the invasive nature, cost, and relatively low diagnostic yield of conventional upper endoscopy. As highlighted by Lao-Sirieix and Fitzgerald, these limitations have driven the development of minimally invasive screening strategies capable of identifying BE in larger at-risk populations while maintaining accept-

able diagnostic accuracy and patient acceptability.[28]

The Cytosponge is a nonendoscopic cell-collection device consisting of a swallowable capsule containing a compressed mesh sponge attached to a string; after the capsule dissolves in the stomach, the expanded sponge is withdrawn to collect esophageal cells for analysis.[29-33] When combined with trefoil factor 3 (TFF3) immunohistochemistry, the Cytosponge-TFF3 test demonstrates high sensitivity (91.5%) for detecting BE, with a landmark randomized controlled trial showing a 10-fold increase in Barrett's detection compared with usual care in primary care patients on acid-suppressant therapy.[5,34,35] The device exhibits excellent safety, tolerability, and patient acceptability. Clinical translation of nonendoscopic sampling devices accelerated following the BEST2 trial, in which Cytosponge coupled with TFF3 immunohistochemistry demonstrated high sensitivity and specificity for detecting BE in a multicenter case-control cohort. This landmark prospective study established the feasibility of combining minimally invasive cell collection with molecular biomarker assessment and has served as the foundation for subsequent incorporation of DNA methylation biomarkers into Cytosponge-based diagnostic platforms.[36] EsophaCap has been evaluated in clinical studies combining cytology and MUC2 immunohistochemistry, demonstrating reasonable sensitivity and high specificity for detecting intestinal metaplasia, dysplasia, and early EAC when compared with standard biopsies.[37] When combined with panels of methylated DNA biomarkers, EsophaCap has demonstrated the ability to collect sufficient cytologic material to distinguish BE and early neoplastic changes with high sensitivity and specificity in prospective validation studies.[38-40] Another class of devices, such as balloon-based esophageal samplers, similarly retrieve esophageal cells for analysis of methylated DNA markers like those used in commercially available assays, showing strong clinical utility and high rates of successful sample collection in real-world cohorts.[41] These technologies illustrate how cytology-based, nonendoscopic tools may augment traditional EGD surveillance, particularly in settings where endoscopy access is limited or for prescreening at the point of care.

Methylation markers in EAC as molecular signatures of malignancy

Epigenetic dysregulation, particularly aberrant DNA methylation, is now firmly established as a central molecular hallmark of EAC and a critical driver of Barrett's-associated neoplastic progression. CpG islands are CpG-rich genomic regions, typically located within gene promoters, whose methylation suppresses gene transcription and represents one of the principal mechanisms of epigenetic gene silencing. Early candidate-gene studies demonstrated progressive promoter hypermethylation of tumor suppressor genes such as *CDKN2A* and *APC*, establishing epigenetic silencing as a stable and early event in the transition from BE to invasive carcinoma (Table 1).[8,10,40,42-47] Subsequent genome-wide methylation profiling revealed widespread epigenetic remodeling across the Barrett's–dysplasia–EAC sequence, identifying distinct methylation signatures that differentiate normal squamous epithelium, nondysplastic BE, HGD, and EAC.[10,43-46] These studies demonstrated that global CpG methylation alterations accumulate progressively and define molecular subclasses of disease.

More recent large-scale discovery efforts have refined these observations by identifying restricted panels of CpG loci consistently hypermethylated in HGD and EAC, mapping to genes involved in transcriptional regulation, epithelial differentiation, and Wnt signaling pathways—including *ZNF682*, *ZNF132*, *CDO1*, and *SFRP1*.[10,40,48] These loci form the basis of multimarker methylation panels that demonstrate strong diagnostic accuracy for distinguishing advanced neoplasia from nondysplastic BE. Importantly, ongoing translational work has extended these findings beyond tissue-based diagnostics. Analytical validation studies and multicenter case-control trials have demonstrated that methylated DNA marker panels retain high sensitivity and specificity when applied to minimally invasive, nonendoscopic sampling platforms such as EsophaCap and EsoCheck.[38,49,50] In parallel, more studies continue to expand methylome-wide datasets, identifying additional biomarkers with potential utility for early detection and nonendoscopic screening of BE and EAC.[40,47]

Collectively, these advances position DNA methylation not only as a mechanistic hallmark of EAC progression but also as a clinically actionable biomarker platform capable of enabling nonendoscopic detection, risk stratification, and potentially surveillance optimization in BE. Whether aberrant DNA methylation functions primarily as a driver of carcinogenesis or as a biomarker of malignant transformation remains an area of active investigation. Functional studies demonstrate that promoter hypermethylation of tumor suppressor genes, including CDKN2A, APC, RUNX3, and secreted frizzled-related protein (SFRP) family members, can directly suppress transcription and alter cell-cycle regulation, apoptosis, and Wnt signaling, supporting a mechanistic contribution to neoplastic progression. However, many methylation alterations likely reflect broader epigenomic instability accompanying chronic inflammation and metaplastic evolution. Consequently, individual methylation events may serve both as biologically relevant contributors to carcinogenesis and as clinically useful biomarkers of disease progression.

Diagnostic methylation biomarkers for BE

Aberrant DNA methylation is an early and pervasive event in the BE–dysplasia–EAC sequence, making it an attractive platform for molecular diagnosis. Genome-wide profiling studies have demonstrated that nondysplastic BE already harbors extensive epigenetic reprogramming distinct from normal squamous epithelium, with additional methylation changes accumulating in dysplasia and invasive carcinoma (Table 2).[38,43,44,51-53] One study tested *CCNA1* DNA methylation as a BE biomarker in cytology brushings of the distal esophagus from 173 individuals with or without BE. *CCNA1* DNA methylation demonstrated an area under the curve (AUC) of 0.95 for discriminating BE-related metaplasia and neoplasia cases from normal individuals. When *VIM* and *CCNA1* were combined, the resulting two-biomarker panel was 95% sensitive and 91% specific. In balloon samples from 86 individuals, tests of *CCNA1* plus *VIM* DNA methylation detected BE metaplasia with 90.3% sensitivity and 91.7% specificity.[52] Wang *et al*.[38] also studied five methylation biomarkers (*p16*, *HPP1*, *NELL1*, *TAC1*, and *AKAP12*), comparing BE with controls. The AUC was 0.894, with a sensitivity of 94.4% and specificity of 62.2% in the training set and a sensitivity of 78.6% and specificity of 92.8% in the test set.[38] Another 5-marker methylated DNA panel, including *ZNF682, VAV3, NDRG4, BMP3, and ZNF568*,

Table 1. Genome-wide methylation biomarkers in EAC and precancerous lesions

Approach	Key findings	Clinical relevance	Notes	References
Genome-wide methylation profiling	Distinct signatures across normal → BE → dysplasia → EAC	Disease classification	Defines molecular subtypes	[43,45,46]
Integrated methylome studies	Epigenetic subclasses of BE/EAC	Risk stratification	Links to transcriptome/genome	[10]
CpG island methylation accumulation	Progressive methylation changes	Early carcinogenesis	Stepwise progression model	[8,44]
Expanded methylome datasets	Novel biomarker discovery	Early detection potential	Enables nonendoscopic testing	[40,47]

BE, Barrett's esophagus; CpG, cytosine–phosphate–guanine; EAC, esophageal adenocarcinoma

Table 2. Diagnostic methylation biomarkers for Barrett's esophagus

Biomarker / Panel	Sample type	Clinical use	Performance	Key notes	References
CCNA1	Cytology brushings	BE detection	AUC 0.95	Strong single biomarker	[52]
CCNA1 + VIM	Cytology / balloon	BE detection	Sens 90–95%, Spec ~91%	Validated in nonendoscopic samples	[52]
p16, HPP1, NELL1, TAC1, AKAP12	Cytology (EsophaCap)	BE diagnosis	AUC 0.894; Sens 78.6–94.4%, Spec 62.2–92.8%	Multimarker panel	[38]
ZNF682, VAV3, NDRG4, BMP3, ZNF568	Cytology	BE detection	Sens 93%, Spec 90–93%; AUC 0.96–0.97	Highly validated panel	[53]

AUC, area under the curve; BE, Barrett's esophagus; Sens, sensitivity; Spec, specificity.

was tested in 199 patients in the training group and 89 patients in the test group. The sensitivity of the 5-marker panel for BE diagnosis was 93% at 90% specificity in the training set and 93% at 93% specificity in the test set. AUCs were 0.96 and 0.97 in the training and test sets, respectively.[53] These methylation signatures retain performance when applied to minimally invasive, nonendoscopic platforms such as EsophaCap, EsoCheck, and capsule sponge devices, supporting their feasibility for large-scale screening and surveillance applications (Table 3).[31,32,37-39,41,49,52,53] Collectively, these studies establish methylation panels as robust molecular tools for diagnosing BE beyond the limits of histopathology alone.

Methylation biomarkers for predicting high-risk BE progression

Importantly, many of the methylation alterations observed in EAC emerge earlier along the BE–dysplasia–adenocarcinoma sequence, indicating that epigenetic reprogramming precedes overt histologic malignancy.[45] Recently, the role of epigenetic changes in the pathogenesis of BE and esophageal cancer was extensively studied (Table 4).[10,45,51,54-62] Multiple methylation biomarkers have been identified that can discriminate between high-risk and low-risk BE, including *APC/p16*,[59] *MGMT*,[55,58] *PKP1*,[51] *TIMP3/TERT*,[54] *RUNX3/HPP1*,[58] and OR3A4.[60] Targeted genetic and epigenetic profiling has further confirmed frequent APC, CDKN2A, MGMT, and TIMP3 promoter hypermethylation in both nondysplastic BE and EAC.[63] Dilworth *et al*.[60] identified 44 methylation markers that may be able to discriminate between nondysplastic BE that either progresses to adenocarcinoma or remains static. Hypomethylation of tumor suppressor *OR3A4* (probe cg09890332) was validated in a separate cohort of samples. For *OR3A4*, median methylation was 67.8% in progressors versus 96.7% in nonprogressors. Using receiver operating characteristic curve modeling, the AUC of this model was 0.70, and adjustment for a prevalence of 0.7% using a threshold of 58% demonstrated a sensitivity of 33.3% and specificity of 78.6%.[60] Another notable example of a translational effort is Previse's *Esopredict* test, a methylation-based prognostic assay developed to risk-stratify patients with BE according to their likelihood of progression to HGD or EAC within 5 years. *Esopredict* quantifies methylation at four loci—*p16* (*CDKN2A*), *RUNX3*, *HPP1* (*TMEFF2*), and *FBN1*—and incorporates patient age into a locked risk algorithm that generates a continuous risk score and categorical risk designation.[61,62] In a multicenter clinical validation study of 240 patients with BE, the average 5-year risk of progression to HGD or EAC was 21.5% among patients classified as high risk, compared with 1.85% among those classified as low risk. Importantly, the approximately four-fold figure reported in the study refers to the high-risk group's risk relative to the overall 5-year progression prevalence in the BE population, rather than to the low-risk group. Compared with patients in the low-risk category, those in the high-risk category were 11.6 times more likely to progress within 5 years.[62] These findings support the potential utility of methylation-based risk stratification for distinguishing patients with substantially different risks of neoplastic progression, although prospective studies are needed to determine whether incorporating *Esopredict* into

Table 3. Nonendoscopic sampling platforms for Barrett's esophagus

Platform	Biomarker(s)	Clinical use	Performance	Key notes	References
Cytosponge-TFF3*	TFF3 (IHC) ± methylation panels	BE detection	Sens ~91.5%	10-fold increase in detection vs. usual care	[31,32]
EsophaCap	Cytology + methylation markers	BE/EAC detection	High sensitivity & specificity	U.S.-developed capsule device	[37-39]
EsoCheck	EsoCheck + Methylated DNA panel	BE/EAC detection	High diagnostic accuracy	Balloon-based sampling	[41,49]

*TFF3 is an immunohistochemical biomarker rather than a DNA methylation marker. The Cytosponge platform is included because it provides a sampling device compatible with methylation assays. BE, Barrett's esophagus; DNA, deoxyribonucleic acid; EAC, esophageal adenocarcinoma; IHC, immunohistochemistry; Sens, sensitivity; TFF3, trefoil factor 3.

Table 4. Predictive methylation biomarkers for detecting BE patients at high risk of progression to EAC

Biomarker / Panel	Clinical use	Key findings	Performance	Notes	References
APC / p16	BE progression risk	Early tumor suppressor methylation	Associated with progression	Foundational markers	[42,59]
MGMT	BE progression	Promoter methylation	Risk association	DNA repair pathway	[55,58]
PKP1	BE progression	Aberrant methylation	—	Cell adhesion gene	[51]
TIMP3 / TERT	BE progression	Distinguishes high-risk BE	—	Telomere biology	[54]
RUNX3 / HPP1	BE progression	Risk stratification	—	Frequently replicated	[58]
OR3A4	BE progression	Hypomethylation in progressors	AUC 0.70; Sens 33%, Spec 79%	Limited standalone utility	[60]
Esopredict (p16, RUNX3, HPP1, FBN1 + age)	5-year progression risk	High vs. low risk: 21.5% vs. 1.85%	Strong risk stratification	Clinically validated prognostic assay	[61,62]

AUC, area under the curve; BE, Barrett's esophagus; DNA, deoxyribonucleic acid; EAC, esophageal adenocarcinoma; Sens, sensitivity; Spec, specificity.

surveillance strategies improves clinical outcomes.

Summary: Emerging genomic and epigenomic data support a model in which epigenetic instability—particularly aberrant DNA methylation—arises early in Barrett's metaplasia and persists throughout disease evolution, functioning as both a biomarker and a mechanistic driver of malignant transformation. While diagnostic methylation panels for detecting EAC and HGD compared with normal tissue or BE now demonstrate reproducibly high accuracy, biomarkers predicting progression from BE to EAC remain more challenging to develop, reflecting the biological heterogeneity of BE and the relatively low annual progression rate.

Gastric cancer biomarker review

Definitions and clinical context

Gastric adenocarcinoma is the fifth most common cancer worldwide, and demonstrates striking geographic variation in incidence and mortality.[64-66] The highest age-standardized incidence rates—often exceeding 30 per 100,000 persons—are observed in Eastern Asia, Eastern Europe, and parts of South America. The disease shows a clear male predominance and a median age at diagnosis of 68 years in the United States. Globally, nearly 85% of tumors arise in the distal stomach (noncardia), whereas in North America and Western Europe, 30–40% originate in the cardia or gastroesophageal junction. Significant racial and ethnic disparities persist, with Asian, Hispanic, Black, and American Indian populations experiencing substantially higher incidence rates of noncardia gastric cancer compared with non-Hispanic White individuals. These epidemiologic patterns underscore the combined influence of environmental exposures, infection prevalence, and host susceptibility in disease distribution.[64-66]

H. pylori infection remains the dominant etiologic factor, accounting for an estimated 75–89% of noncardia gastric cancers. Classified as a World Health Organization Group 1 carcinogen, *H. pylori* infects approximately 43.9% of adults globally (about 17.6% in the United States), yet only 1–3% of infected individuals ultimately develop malignancy.[67-69] Carcinogenic progression is driven by bacterial virulence factors—particularly CagA and VacA—host genetic susceptibility, and environmental influences. The Correa cascade describes the well-established sequence from chronic active gastritis to atrophic gastritis, intestinal metaplasia, dysplasia, and ultimately invasive adenocarcinoma.[67,68] Emerging data further highlight synergistic interactions between *H. pylori* infection and germline pathogenic variants in homologous recombination genes such as BRCA1, BRCA2, ATM, and PALB2; infected carriers may face a markedly elevated lifetime risk compared with uninfected carriers.[68] Although approximately

10% of cases demonstrate familial aggregation, recognized hereditary cancer syndromes account for only 1–3%, including hereditary diffuse gastric cancer due to CDH1 mutations and Lynch syndrome. Additional risk factors include pernicious anemia, prior gastric ulcer disease, advanced age, lower socioeconomic status, and immigration from high-incidence regions, collectively reflecting the multifactorial pathogenesis of gastric adenocarcinoma.[64-66]

Current screening and prevention

Standard clinical surveillance for GC relies primarily on endoscopic evaluation with systematic biopsy in high-risk populations, combined with serologic screening when appropriate. Guidelines for gastric cancer screening vary among countries and high-risk populations. The fundamental difference between US and Asian guidelines lies in their population-based screening strategies. Japan and South Korea implement universal screening programs for all adults starting at age 40–50 years with biennial endoscopy or radiography, with Japan starting screening at age 50 and South Korea at age 40, while the US employs a risk-stratified approach targeting only high-risk individuals.[3,70-75] The American Gastroenterological Association (AGA) Clinical Practice Update recommends high-quality endoscopy with systematic biopsies for individuals at elevated risk, such as those with extensive GIM, family history of GC, or first-generation immigrants from high-incidence regions, with intervals typically ranging from 3–5 years depending on histologic findings.[3]

H. pylori testing and eradication are critical components of both primary and secondary prevention strategies, given its role as the dominant etiologic factor for GC, particularly in East Asian populations, where prevalence rates remain high and *H. pylori* infection accounts for the majority of sporadic GC cases.[3,67-69,73,76,77] Epidemiologic studies indicate that, although eradication programs and declining infection rates have begun to reduce GC incidence in younger cohorts, the overall disease burden in Asian countries such as Japan, Korea, and China remains substantially higher than in Western populations.[68,73,77,78] Therefore, surveillance strategies in these regions emphasize endoscopic screening combined with risk-stratified *H. pylori* management to detect premalignant lesions early and prevent progression to carcinoma.[3]

Traditional biomarkers for screening

Traditional serologic biomarkers for gastric cancer screening primarily include serum pepsinogen I (PGI), pepsinogen II (PGII), the PGI/PGII ratio (PGR), carcinoembryonic antigen (CEA), CA19-9, CA72-4, gastrin-17 (G-17), and *H. pylori* IgG antibodies, which are used to detect atrophic gastritis and stratify gastric cancer risk, particularly in high-incidence Asian populations (Table 5).[69,70,73,76,79-89] Reduced PGI (≤70 μg/L) and PGR (≤3.0) reflect corpus atrophy and form the basis of pepsinogen screening; pooled Japanese data demonstrate sensitivities of 59–77% and specificities of 73–89% for gastric cancer detection, while meta-analyses report a summary sensitivity of approximately 0.69 and specificity of 0.73 (AUC ~0.76) for cancer and higher accuracy for atrophic gastritis (AUC ~0.85).[81,82] Pepsinogen testing performs best for noncardia gastric cancer and was associated with an 8–11-fold increased cancer risk in pepsinogen-positive individuals in a US PLCO-based study, suggesting potential value even in Western populations.[90,91] Gastrin-17 reflects antral function and shows a J-shaped association with cancer risk, with both low and high levels associated with increased risk; when combined with PGI and PGII, diagnostic performance improves substantially (AUC up to 0.93 in some studies).[76,80,83] *H. pylori* serology identifies current or past infection—the dominant etiologic factor for noncardia cancer—and is most effective when combined with pepsinogen testing in the ABC method, which stratifies individuals into stepwise risk groups and confers a 6–8-fold higher cancer risk among those with serologic atrophy.[68,69,73,78,84,85] Expanded multimarker "serological biopsy" panels incorporating PGI, PGII, PGR, G-17, and *H. pylori* antibodies further improve discrimination (C-statistic ~0.80) for precancerous lesions and future cancer risk.[86] However, these biomarkers have important limitations, including variability by ethnicity, tumor subtype, medication use, and infection status, and they are not routinely recommended for population screening in low-incidence regions such as the United States due to inconsistent validation and limited availability.[3,69] CEA, CA19-9, and CA72-4 have limited diagnostic utility for gastric cancer. When used individually at standard cutoff values, CEA demonstrates 20–28% sensitivity, CA19-9 shows 27–39% sensitivity, and CA72-4 achieves 28–43% sensitivity.[87-89] While combination testing improves sensitivity to 48–75%, this remains inadequate for screening purposes, and these markers are most frequently elevated in advanced, incurable disease rather than early-stage cancer. These markers have greater value for prognosis and monitoring recurrence than for initial diagnosis.[87-89]

DNA methylation biomarkers for gastric cancer

Although traditional models of gastric carcinogenesis emphasize the multistep Correa cascade—from chronic gastritis to atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma—recent genomic and epigenomic studies indicate that epigenetic alterations, particularly aberrant DNA methylation, arise early and persist throughout disease evolution.[12,13,92-95] Genome-wide and targeted methylation profiling demonstrates that premalignant gastric lesions, including intestinal metaplasia, already harbor extensive DNA methylation changes distinct from normal gastric mucosa, supporting a model in which epigenetic dysregulation is a foundational driver of malignant transformation rather than a late event.[96]

cfDNA released from tumor cells into the bloodstream provides a noninvasive source for detecting epigenetic alterations associated with cancer. DNA methylation changes occur early in gastric carcinogenesis and are often more frequent than somatic mutations, making them attractive biomarkers for early cancer detection. Recent studies have demonstrated that cfDNA methylation profiling can detect gastric cancer with reasonable accuracy (Table 6).[13,95-108]

In gastric cancer, plasma methylated SEPT9 has demonstrated sensitivities ranging from approximately 48–61% with specificities around 86–87%, depending on assay algorithms. However, SEPT9 methylation is not specific to gastric cancer, as it is also frequently hypermethylated in colorectal, pancreatic, and esophageal cancers, limiting its diagnostic specificity for gastric malignancy.[99,100,109] Methylation of SFRP2, RNF180, RPRM, APC, and PCDH10 has been detected in serum or plasma of gastric cancer patients and is associated with tumor presence and

Table 5. Traditional serologic biomarkers for gastric cancer

Biomarker	Biological basis	Clinical use	Performance	Key notes	References
Pepsinogen I (PGI)	Corpus gland function	Detect atrophic gastritis	Sens 59–77%, Spec 73–89%	Low in corpus atrophy	[81,82]
Pepsinogen II (PGII)	Gastric inflammation	Used with PGI	—	Elevated in inflammation	[81,82]
PGI/PGII Ratio (PGR)	Gastric atrophy index	GC risk stratification	AUC ~0.76 (GC), ~0.85 (atrophy)	≤3 suggests atrophy	[81,82]
Gastrin-17 (G-17)	Antral function	GC risk	AUC up to 0.93 (combined)	J-shaped association	[76,80,83]
H. pylori IgG	Infection status	Risk stratification	—	Used in ABC method	[69,73,84,85]
CEA	Tumor antigen	Prognosis/monitoring	Sens 20–28%	Poor early detection	[88,89]
CA19-9	Tumor marker	Prognosis	Sens 27–39%	Limited screening utility	[87,88]
CA72-4	Tumor marker	Prognosis	Sens 28–43%	Elevated in advanced disease	[88,89]
Multimarker panels	Combined markers	Risk prediction	C-stat ~0.80	Improved discrimination	[86]

AUC, area under the curve; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; CEA, carcinoembryonic antigen; C-stat, concordance statistic; G-17, gastrin-17; GC, gastric cancer; IgG, immunoglobulin G; PGI, pepsinogen I; PGII, pepsinogen II; PGR, pepsinogen I/II ratio; Sens, sensitivity; Spec, specificity.

Table 6. Circulating DNA methylation biomarkers for gastric cancer

Biomarker	Sample type	Clinical use	Performance	Key notes	References
SEPT9	Plasma cfDNA	GC detection	Sens 48–61%, Spec 86–87%	Not GC-specific	[99,100]
SFRP2	Plasma/serum	GC detection	Sens up to ~70%	Wnt signaling gene	[102,103]
SFRP2 + RPRM (Reprimo)	Plasma/serum	GC detection	Sens 57–79%, Spec >90% (combo)	Cell cycle regulator	[105]
RNF180	Plasma	GC detection	—	Tumor suppressor	[101,104]
PCDH10	Serum	GC detection	Detected in 12.9% of GC sera; 0% in controls	Low serum detection despite frequent tissue methylation	[105]
APC	Serum	GC detection	Detected in 6.25% of GC sera; 0% in controls	Low serum detection; limited standalone sensitivity	[105]
ELMO1 + ZNF569 + C13orf18	Plasma	GC detection	Sens 86% (95% CI 71–95%), Spec 95%	Three-marker plasma methylated DNA panel	[106]
GHR + GLRB ± GATM	Plasma (dPCR)	Early GC detection	Sens 83–87%, Spec ~90%	Strong early detection	[107]
FGFR2	Blood leukocyte DNA	GC vs. IM	Sens 85%, Spec 80%	Nontumor DNA source	[108]

cfDNA, cell-free DNA; CI, confidence interval; DNA, deoxyribonucleic acid; dPCR, digital polymerase chain reaction; GC, gastric cancer; IM, intestinal metaplasia; Sens, sensitivity; Spec, specificity.

progression.[101-105] A dual-gene panel of SFRP2 and RPRM demonstrated sensitivities up to 57–79% with specificities exceeding 90% in distinguishing gastric cancer from controls.[105] In a separate plasma pilot study, a three-marker methylated DNA panel comprising ELMO1, ZNF569, and C13orf18 detected 86% of gastric adenocarcinomas (95% confidence interval (CI), 71–95%) at 95% specificity.[106] More recently, a plasma-based digital polymerase chain reaction (PCR) assay targeting methylated GHR and GLRB achieved 83.3% sensitivity and 90% specificity, and adding GATM as a third marker increased sensitivity to 86.7% overall and 81.6% for stage I disease while maintaining high specificity.[107] FGFR2 methylation in blood leukocytes demonstrated 85% sensitivity and 80% specificity for distinguishing gastric cancer from intestinal metaplasia patients.[108]

Comprehensive genome-scale methylation analysis using methods such as methylated CpG tandem amplification and sequencing (MCTA-Seq) has identified extensive panels of methylation biomarkers (Table 7).[109-114] One study identified 153 cfDNA methylation biomarkers, including DOCK10, CABIN1, and KCNQ5, achieving sensitivities of 44%, 59%, 78%, and 100% for stages I, II, III, and IV, respectively, at 92% specificity.[110] Another genome-wide approach identified 21 differentially methylated regions, genomic regions demonstrating statistically significant methylation differences between normal and diseased tissues, that enabled gastric cancer detection with 93.9% sensitivity and 95.2% specificity in the discovery set, and 88.4% sensitivity and 94.2% specificity in validation.[111] MCED approaches based on cfDNA methylation sequencing have also demonstrated the ability to identify tumor-specific methylation signatures and classify cancer types with high accuracy.[11] A meta-analysis of 32 studies including 4,172 patients found that blood-based DNA methylation testing had an overall sensitivity of 57% and specificity of 97% for gastric cancer detection.[114] Plasma-based testing showed superior sensitivity (71%) compared with serum-based testing (50%), though with lower specificity (89% vs. 98%). Multigene panels achieved 76% sensitivity at 85% specificity, significantly outperforming single markers. For early-stage (TNM I+II) disease, methylation testing achieved 55% sensitivity at 96% specificity.[114] The GutSeer assay, combining DNA methylation and fragmentomics across 1,656 markers, demonstrated 65.3% sensitivity for gastric cancer specifically, with overall gastrointestinal cancer detection sensitivity of 82.8% at 95.8% specificity.[112]

Methylation DNA biomarkers for high-risk GIM

GIM is a key precancerous lesion in the Correa cascade leading to gastric adenocarcinoma. However, only a subset of intestinal metaplasia lesions progress to cancer, creating a critical need for molecular biomarkers that stratify high-risk patients. Recent advances in genomics, epigenomics, and transcriptomics have identified several candidate biomarkers reflecting early malignant transformation.

CpG island hypermethylation and genomic DNA hypomethylation are found not only in gastric cancers but also in associated premalignant lesions. Aberrant DNA methylation represents one of the earliest molecular alterations in gastric carcinogenesis, occurring even before histologically detectable neoplastic changes. *H. pylori* infection induces aberrant CpG island hypermethylation in gastric mucosa. The timing of methylation varies by gene. THBS1 and TIMP3 show marked increases in hypermethylation frequency from chronic gastritis to intestinal metaplasia, while *MLH1* and p16 methylation increases more prominently from metaplasia to cancer (Table 8).[13,96,112,115-117] This temporal pattern suggests that different methylation events contribute to distinct stages of the carcinogenic cascade.

Recent genomic profiling reveals that incomplete (colonic-type) intestinal metaplasia exhibits distinct methylation patterns compared with complete intestinal metaplasia. Incomplete intestinal metaplasia demonstrates extensive intergenic hypermethylation resembling native antral mucosa, while complete intestinal metaplasia displays promoter hypermethylation of tumor suppressor genes with a more fully intestinalized epigenetic profile. Incomplete intestinal metaplasia represents a phenotypically unstable, epigenetically deregulated state with molecular resemblance to gastric cancer, establishing it as a true precursor lesion.[116]

RIMS1 has emerged as one of the most robustly validated single-gene methylation markers for gastric cancer risk stratification (Table 9).[117-121] A prospective multicenter study of 1,624 patients after *H. pylori* eradication demonstrated that those in the highest quartile of RIMS1 methylation had a gastric cancer incidence rate of 972.8 per 100,000 person-years compared with 127.1 in the lowest quartile (adjusted hazard ratio (HR) 5.7, 95% CI 1.3–25.5). A methylation cutoff of 25.7% identified a "super-high-risk" population requiring more frequent screening than currently recommended.[118]

A prospective cohort study of 782 patients after endoscopic resection of early gastric cancer demonstrated that the highest quartile of miR-124a-3 methylation had a significant multivariate-adjusted HR of 2.30 (95% CI 1.03–5.10) for developing metachronous gastric cancers. Similar trends were observed for EMX1 and NKX6-1.[119] In a Caucasian population, MIR124-3 hypermethylation was significantly associated with development of metachronous gastric lesions (adjusted HR 2.31, 95% CI 1.03–5.17), particularly in females and *H. pylori*-negative patients. NKX6-1 was found to be hypermethylated in patients with synchronous lesions. A molecular-based methylation model incorporating both genes was associated with a threefold increased risk for metachronous gastric lesion development (adjusted HR 3.10, 95% CI 1.07–8.95).[120] In a 16-year Colombian cohort study, methylation levels of AMPH, PCDH10, RSPO2, SORCS3, and ZNF610 predicted progression of gastric lesions independent of *H. pylori* infection duration, baseline diagnosis, or inflammatory scores.[122] Although these biomarkers demonstrated statistically significant associations with progression in the original longitudinal cohort, independent external validation remains limited, and their clinical utility has yet to be established. Blood-based markers also show potential. Hypermethylated *IGF2* and N33 in blood leukocyte DNA were elevated at least 5 years before clinical gastric cancer diagnosis, with markedly increased frequency in intestinal metaplasia patients who progressed to cancer (odds ratio 12.52 for *IGF2* in intestinal metaplasia progressors).[121] Comprehensive epigenomic profiling of 138 intestinal metaplasia cases from a 10-year prospective study revealed that intestinal metaplasia patients with shortened telomeres and chromosomal alterations combined with hypermethylation signatures were associated with subsequent dysplasia or gastric cancer, while those with normal-like epigenomic patterns showed association with regression.[123]

Longitudinal studies confirm that intestinal metaplasia partially recapitulates patterns of aberrant methylation of intestinal-type gastric cancer, independently of *H. pylori* status. Genes such as RASSF1A and APC consistently showed increased methylation in intestinal metaplasia with respect to earlier precursor lesions. Importantly, *H. pylori*-dependent methylation in intestinal metaplasia suggests that eradication in late stages of precursor lesions may not prevent epigenome reprogramming toward a cancer signature.[117]

Clinical implementation considerations

Current AGA guidelines identify high-risk intestinal metaplasia patients based on incomplete (vs. complete) histology (3.3-fold relative risk), extensive (corpus-extended) vs. limited gastric intestinal metaplasia (2.1-fold relative risk), and family history of

Table 7. Genome-wide methylation panels for gastric cancer

Panel / Approach	Markers	Sample	Clinical use	Performance	Key notes	References
MCTA-Seq–derived 153-marker cfDNA panel	DOCK10, CABIN1, KCNQ5	Plasma	GC detection by stage	Sens 44% (I), 59% (II), 78% (III), 100% (IV); Spec 92%	Genome-scale cfDNA methylation analysis	[110]
21 DMR panel	Differential regions	Plasma	GC detection	Sens 88–94%, Spec 94–95%	High accuracy	[111]
GutSeer assay	1,656 markers	Plasma	Multicancer detection	GC Sens 65.3%	Includes fragmentomics	[112]
Meta-analysis panels	Multigene	Blood	GC detection	Sens 76%, Spec 85%	Superior to single markers	[114]

cfDNA, cell-free DNA; DMR, differentially methylated region; GC, gastric cancer; MCTA-Seq, methylated CpG tandem amplification and sequencing; Sens, sensitivity; Spec, specificity.

Table 8. Methylation biomarkers for gastric carcinogenesis

Stage	Biomarkers	Methylation pattern	Key insight	References
Chronic gastritis → IM	THBS1, TIMP3	Increasing hypermethylation	Early carcinogenic event	[115]
IM → Dysplasia/GC	*MLH1*, p16	Increased methylation later	Late-stage progression	[115]
Intestinal metaplasia (IM)	RASSF1A, APC	Elevated methylation	IM resembles GC epigenetically	[117]
Incomplete IM subtype	Intergenic hypermethylation	Cancer-like profile	High-risk precursor	[116]
H. pylori-associated mucosa	Multiple CpG loci	Infection-driven methylation	Early driver of carcinogenesis	[96,115]
Advanced GC	Global CpG alterations	Widespread dysregulation	Defines tumor biology	[13,112]

CpG, cytosine–phosphate–guanine; GC, gastric cancer; IM, intestinal metaplasia.

Table 9. Prognostic and risk-associated methylation markers for gastric cancer

Biomarker	Clinical context	Key findings	Performance	Notes	References
RIMS1	Post–*H. pylori* eradication	High methylation → high GC risk	HR 5.7	Strong prospective evidence	[118]
miR-124a-3 methylation	Metachronous GC risk	Increased recurrence risk	HR 2.30	Validated cohort	[119]
EMX1	GC progression	Risk association	—	Often combined	[119]
NKX6-1	GC progression	Combined risk model	HR ~3.1	Multimarker model	[120]
RPRM	IM → GC progression	Progressive hypermethylation	—	Early transformation marker	[117]
ZNF793	IM progression	Hypermethylation	—	Early epigenetic shift	[117]
IGF2 (blood DNA)	Preclinical detection	Elevated ≥5 years prior	OR 12.5	Strong early signal	[121]
N33 (blood DNA)	Early detection	Elevated in progressors	—	Noninvasive biomarker	[121]

GC, gastric cancer; HR, hazard ratio; IM, intestinal metaplasia; miR, microRNA; OR, odds ratio.

gastric cancer (4.5-fold relative risk).[3] Methylation biomarkers provide an additional molecular layer for risk stratification that may complement these histologic and clinical factors. Several challenges remain for clinical implementation. Most reported methylation markers are in early discovery stages and have been evaluated primarily in tissue samples. Noninvasive blood-based assays lack standardized sampling protocols, preanalytical procedures, and multicenter validation. Many markers exhibit insufficient sensitivity for early-stage gastric cancer detection, and most are pan-cancer markers rather than gastric cancer-specific.[13]

Emerging prediction markers

Beyond DNA methylation, emerging work also supports combining methylation profiles with other biomarkers (e.g.,

microbiome indices) to enhance predictive performance, especially in *H. pylori*-negative GC cases, underscoring the complexity and heterogeneity of epigenomic influences in gastric tumorigenesis.[124] Other nucleic acid-based biomarkers are under investigation for early GC detection. Blood microRNA signatures, including miR-124-3p and miR-125a-3p, have demonstrated high predictive performance for early detection and prognosis.[94] Epigenetic alterations also intersect with other layers of gene regulation, including histone modifications and noncoding RNA dysregulation, which collectively shape transcriptional networks that drive proliferation, invasion, immune evasion, and resistance to therapy.[92] Integrative epigenomic profiling continues to refine molecular taxonomy of GC, revealing subtypes with distinct prognostic and therapeutic implications and creating opportunities for precision risk stratification beyond traditional histopathology.[13,92] When comparing performance, targeted methylation panels generally provide higher specificity, whereas microRNA and transcriptomic signatures may enhance sensitivity and enable multilayered risk stratification when combined with methylation markers.[94,106,125]

Shared epigenetic mechanisms across upper gastrointestinal adenocarcinomas

Although EAC and GC arise from distinct epithelial lineages and exhibit important differences in epidemiology, environmental risk factors, and clinical management, both malignancies develop through remarkably similar epigenetic mechanisms. Chronic inflammation induced by GERD in BE and *H. pylori* infection in the stomach initiates progressive epigenetic remodeling that accompanies the transition from metaplasia to dysplasia and ultimately invasive adenocarcinoma. These observations suggest that aberrant DNA methylation represents a common molecular hallmark of upper gastrointestinal carcinogenesis, while organ-specific methylation signatures reflect differences in tissue lineage, inflammatory microenvironment, and selective pressures during tumor evolution.

A defining feature shared by both diseases is the early establishment of widespread promoter CpG island hypermethylation. Genome-wide methylation profiling has demonstrated that extensive methylation abnormalities are already present in nondysplastic BE and GIM, well before the development of invasive carcinoma.[8,10,13,43,117] These findings support a model in which epigenetic dysregulation is not merely a late consequence of malignant transformation but rather an early event that creates a permissive environment for neoplastic progression.

Several methylation targets appear consistently across both EAC and GC, suggesting that common biological pathways are disrupted despite differences in tissue origin. Promoter hypermethylation of CDKN2A (p16), APC, RUNX3, and members of the SFRP family has been repeatedly identified in Barrett's-associated neoplasia as well as gastric carcinogenesis.[62,92-95,112,126-130] These genes regulate fundamental cellular processes, including cell-cycle control, apoptosis, epithelial differentiation, and Wnt/β-catenin signaling, indicating that convergent epigenetic silencing of tumor suppressor pathways represents a shared mechanism underlying upper gastrointestinal adenocarcinoma development. Similarly, methylation of RASSF1A, TIMP3, and additional genes involved in extracellular matrix remodeling and genomic stability has been reported across multiple stages of both disease pathways, further supporting common mechanisms of inflammation-associated epigenetic reprogramming.[131]

Among these shared pathways, dysregulation of canonical Wnt signaling deserves particular attention. Aberrant methylation of APC, SFRP1, and SFRP2 results in loss of negative regulation of Wnt signaling, promoting epithelial proliferation, stem-cell expansion, and resistance to apoptosis. Because activation of the Wnt pathway is observed in both Barrett's-associated carcinogenesis and gastric carcinogenesis, these methylation events likely represent fundamental drivers of upper gastrointestinal tumor initiation rather than organ-specific phenomena.[130] Likewise, epigenetic silencing of RUNX3, an important mediator of transforming growth factor-β signaling and immune regulation, has been implicated in both diseases, suggesting that impaired differentiation and altered immune surveillance may represent another common mechanism of malignant transformation.[36,58,105,127,132]

Despite these similarities, important lineage-specific methylation patterns distinguish EAC from GC and likely reflect differences in epithelial origin and disease biology. Barrett's-associated neoplasia demonstrates recurrent methylation of genes including ZNF682, ZNF132, VAV3, NDRG4, BMP3, PKP1, and OR3A4, many of which have shown promising diagnostic or prognostic performance in nonendoscopic sampling platforms such as Cytosponge, EsophaCap, and EsoCheck.[133,134] In contrast, gastric adenocarcinoma exhibits frequent methylation of RNF180, RPRM, RIMS1, GHR, GLRB, FGFR2, and PCDH10, several of which have demonstrated encouraging performance in plasma cell-free DNA assays for early cancer detection.[104,135-137] These organ-specific methylation signatures likely reflect differences in developmental lineage, exposure to distinct inflammatory stimuli, and divergent selective pressures imposed by reflux-associated versus *H. pylori*-associated carcinogenesis.

An additional shared feature of both malignancies is the phenomenon of epigenetic field cancerization. Chronic inflammatory injury induces widespread methylation alterations throughout histologically normal-appearing mucosa, creating an epigenetically altered field from which dysplastic lesions subsequently emerge. In BE, methylation abnormalities extend beyond areas of visible dysplasia and are detectable throughout the Barrett's segment, supporting the concept of diffuse molecular field defects. Similarly, *H. pylori* infection produces persistent methylation changes throughout the gastric mucosa that frequently remain even after bacterial eradication, potentially explaining the continued risk of gastric cancer in patients with established intestinal metaplasia. This persistence of epigenetic alterations provides a biological rationale for long-term surveillance despite apparent histologic regression.

These common mechanisms have important implications for biomarker development. Rather than viewing BE and GIM as entirely separate diseases, their shared epigenetic architecture suggests that similar biomarker discovery strategies may be applicable across upper gastrointestinal adenocarcinomas. Genome-wide methylation profiling, machine learning-based biomarker selection, and minimally invasive sampling approaches have already demonstrated success in both disease settings. Furthermore, emerging MCED platforms, including those

developed through the Circulating Cell-free Genome Atlas (CCGA) program and evaluated prospectively in the PATHFINDER study, leverage methylation signatures across multiple tumor types to simultaneously detect cancer and predict tissue of origin.[11] Although current MCED assays demonstrate lower sensitivity for localized upper gastrointestinal cancers than for some other malignancies, continued refinement of organ-specific methylation classifiers may substantially improve their performance for EAC and GC.

Nevertheless, important challenges remain before a unified methylation-based approach to upper gastrointestinal cancer detection can be realized. Significant heterogeneity exists across published studies with respect to patient selection, specimen type (tissue, cytology, plasma, serum, or cell-free DNA), laboratory platform, methylation assay, reference standards, and statistical modeling. Consequently, direct comparison of reported sensitivities, specificities, and AUC values across studies should be interpreted cautiously, as differences often reflect methodological variation rather than true biomarker performance. Standardization of assay platforms, multicenter prospective validation, and integration of methylation biomarkers with established clinical risk factors and histopathologic staging systems will be essential before these assays can be incorporated into routine clinical practice.

Collectively, these observations support a conceptual framework in which DNA methylation represents a shared biological hallmark of upper gastrointestinal adenocarcinoma while preserving sufficient tissue-specific information to distinguish organ of origin and disease stage. Future biomarker development should therefore emphasize both the common molecular pathways linking reflux-associated and *H. pylori*-associated carcinogenesis and the lineage-specific methylation signatures that enable accurate diagnosis, risk stratification, and precision surveillance.

Clinical translation of DNA methylation biomarkers: Barriers to implementation and the path toward clinical adoption

The development of DNA methylation biomarkers for BE, EAC, and GC has progressed rapidly over the past decade, with several assays demonstrating excellent diagnostic accuracy in retrospective and prospective validation studies. Nevertheless, despite encouraging analytical performance, no methylation-based assay has achieved widespread adoption as the standard of care for screening, surveillance, or risk stratification in upper gastrointestinal malignancies. This disconnect highlights a fundamental challenge in translational biomarker research: demonstrating diagnostic accuracy alone is insufficient for clinical implementation. Successful translation requires robust evidence of analytical validity, clinical validity, clinical utility, cost-effectiveness, regulatory approval, reimbursement, and integration into existing healthcare systems.[138-143]

Esopredict represents one of the potential methylation assays developed for BE surveillance, incorporating multiple methylation biomarkers to identify patients at increased risk of dysplasia or progression to EAC. Early validation studies have reported encouraging discrimination between low-risk and high-risk Barrett's lesions, suggesting that molecular risk stratification may complement conventional histopathologic assessment. The clinical validation study demonstrated substantial separation of progression risk across assay categories, but prospective evidence that assay-guided management improves outcomes remains limited.[62] The current evidence remains insufficient to support replacement of established surveillance strategies based on high-definition endoscopy with systematic biopsy protocols. Most validation cohorts remain relatively small, enriched for high-risk populations, and originate from specialized tertiary referral centers, limiting generalizability to community practice. Large prospective multicenter studies demonstrating improved patient outcomes compared with current surveillance strategies are still lacking.

The major obstacle is the lack of prospective evidence demonstrating clinical utility.[139,140] Most published studies focus on diagnostic performance metrics, including sensitivity, specificity, and AUC, rather than patient-centered outcomes. Regulatory agencies, clinical guideline committees, and healthcare payers increasingly require evidence that introducing a new biomarker changes clinical management, reduces cancer incidence or mortality, decreases unnecessary endoscopic procedures, or improves cost-effectiveness.[141] Demonstrating statistical superiority over existing biomarkers is therefore insufficient if the assay does not clearly improve clinical decision-making. Consequently, randomized or pragmatic prospective studies comparing methylation-guided surveillance with current guideline-directed management represent an essential next step for clinical translation.[142,143]

Cost-effectiveness also remains a critical consideration. Current surveillance for BE relies on periodic upper endoscopy with systematic biopsy following the Seattle protocol, an approach that is invasive, resource-intensive, and associated with substantial interobserver variability in histopathologic interpretation.[7,144] Although methylation assays have the potential to reduce unnecessary endoscopic procedures by identifying patients at genuinely elevated risk of progression, widespread implementation will depend on demonstrating that molecular testing provides meaningful economic value relative to existing surveillance strategies.[25] Formal health economic analyses evaluating laboratory costs, downstream diagnostic procedures, treatment expenditures, and quality-adjusted life years remain limited. Without clear evidence of cost-effectiveness, reimbursement by public and private insurers is likely to remain inconsistent, substantially slowing adoption into routine clinical practice.

An equally important limitation is the absence of standardized analytical methodology across published methylation studies. Differences in specimen acquisition, DNA extraction protocols, bisulfite conversion efficiency, methylation detection platforms, bioinformatic processing, and statistical modeling contribute substantially to variability in reported diagnostic performance. Identical biomarkers frequently demonstrate different sensitivities and specificities depending on whether they are measured using methylation-specific PCR (MSP), pyrosequencing, droplet digital PCR (ddPCR), targeted bisulfite sequencing, or next-generation sequencing (NGS). Without standardized laboratory protocols and external quality assurance programs, reproducibility between institutions remains uncertain, limiting confidence in routine clinical implementation.[9]

Regulatory approval represents an additional hurdle. Before a methylation assay can be incorporated into evidence-based clinical

guidelines, it must satisfy regulatory standards for analytical validity, reproducibility, and clinical performance. Incorporation of the biomarkers into professional society guidelines, including those issued by U.S. organizations such as the American College of Gastroenterology, AGA, American Society for Gastrointestinal Endoscopy, and European organizations such as European Society of Gastrointestinal Endoscopy, requires consistent evidence from multiple independent prospective studies demonstrating reproducible benefit across diverse patient populations. At present, methylation biomarkers are recognized as promising investigational tools but are not recommended as replacements for standard endoscopic surveillance.[5,23,145]

Implementation within routine pathology and gastroenterology practice presents further practical challenges. Adoption of molecular diagnostics requires laboratory infrastructure capable of performing standardized methylation analyses, specialized personnel trained in molecular pathology and bioinformatics, validated quality assurance protocols, and efficient workflows for integrating molecular results into electronic health records and clinical decision-making. These requirements may be readily achievable in academic medical centers but remain substantially more difficult in community hospitals and lower-resource healthcare systems, where most BE surveillance is performed. Ensuring equitable access to molecular diagnostics therefore represents an important consideration as these technologies mature.[145]

The emergence of MCED assays further illustrates both the promise and complexity of clinical translation. Large prospective studies, including the CCGA program and the PATHFINDER trial, have demonstrated that genome-wide methylation signatures can detect multiple malignancies simultaneously while accurately predicting tissue of origin.[11,146] However, the sensitivity of current MCED platforms for early-stage EAC and GC remains lower than for several other solid tumors, underscoring the need for disease-specific methylation classifiers and continued refinement of upper gastrointestinal-focused biomarker panels. Rather than replacing organ-specific surveillance, these technologies may ultimately function as complementary screening tools that identify individuals requiring targeted endoscopic evaluation.

Future implementation will likely require integration of methylation biomarkers into multivariable clinical prediction models rather than reliance on molecular markers alone. Combining methylation profiles with established clinical risk factors—including age, sex, obesity, GERD duration, smoking history, Barrett's segment length, histopathologic findings, and established risk stratification systems such as the Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM)—may improve predictive performance while facilitating individualized surveillance intervals. Such integrated models are more likely to gain acceptance within evidence-based clinical guidelines because they complement, rather than replace, existing standards of care.

Ultimately, the greatest challenge facing methylation-based assays is not whether they demonstrate promising diagnostic accuracy, but whether they can consistently improve patient outcomes within real-world healthcare systems. Future research should therefore prioritize multicenter prospective validation, standardized laboratory methodologies, cost-effectiveness analyses, regulatory evaluation, and implementation science alongside continued biomarker discovery. Addressing these barriers will determine whether methylation-based diagnostics evolve from promising investigational tools to routinely incorporated components of precision surveillance for upper gastrointestinal adenocarcinomas.

Technical platforms for DNA methylation analysis: Advantages, limitations, and clinical translation

The rapid expansion of DNA methylation biomarkers for EAC, BE, gastric adenocarcinoma, and GIM has been accompanied by significant advances in methylation detection technologies. However, differences in assay platforms represent a major source of heterogeneity across published studies and remain a significant barrier to clinical implementation. Variations in analytical sensitivity, quantitative accuracy, genomic coverage, DNA input requirements, cost, and laboratory standardization complicate direct comparisons between studies and contribute to the limited reproducibility of many candidate biomarkers. Consequently, understanding the strengths and limitations of individual methylation platforms is essential when interpreting published performance metrics and evaluating their suitability for clinical translation.[9]

Bisulfite-based DNA methylation analysis

Sodium bisulfite conversion remains the cornerstone of most DNA methylation assays. Bisulfite treatment selectively converts unmethylated cytosine residues to uracil while preserving methylated cytosines, allowing subsequent discrimination between methylated and unmethylated CpG sites by PCR amplification or sequencing.[147] Many conventional targeted methylation assays rely on bisulfite-converted DNA. Therefore, the efficiency of this conversion step critically influences assay accuracy and reproducibility. Incomplete conversion may lead to false-positive methylation calls, whereas DNA degradation during treatment can reduce assay sensitivity, particularly when analyzing low-abundance cfDNA samples.[9]

Pyrosequencing

Pyrosequencing is one of the most widely used quantitative methods for validating candidate methylation biomarkers. Following bisulfite conversion and PCR amplification, pyrosequencing quantitatively measures methylation at individual CpG sites through sequencing-by-synthesis, providing highly reproducible estimates of methylation percentages.[148] Compared with MSP, pyrosequencing offers superior quantitative accuracy and is particularly useful for validating methylation gradients across the metaplasia–dysplasia–carcinoma sequence.

Numerous studies investigating CDKN2A, RUNX3, APC, and SFRP family methylation in BE and gastric carcinogenesis have employed pyrosequencing because of its high analytical precision and relatively straightforward implementation.[43,122] However, pyrosequencing is limited to predefined genomic regions, requires moderate DNA input, and is less suitable for large-scale genome-wide discovery studies.[148]

MSP and quantitative MethyLight assays

MSP and its quantitative derivative, MethyLight PCR, remain among the most commonly used techniques in translational

methylation studies because of their simplicity, low cost, and excellent analytical sensitivity.[8,149] These assays use methylation-specific primers following bisulfite conversion to selectively amplify methylated DNA, making them particularly useful for detecting low-frequency methylated alleles in plasma, cytology specimens, or nonendoscopic sampling devices.

Many early studies evaluating VIM, CCNA1, VAV3, ZNF682, RNF180, and RPRM employed MSP- or MethyLight-based approaches because of their robust performance using limited DNA input.[52,150] Nevertheless, these assays are generally limited to a small number of candidate loci and may exhibit interlaboratory variability due to differences in primer design, amplification conditions, and threshold determination. Consequently, although MSP remains valuable for targeted clinical assays, its limited multiplexing capacity restricts its utility for comprehensive biomarker discovery.[9]

ddPCR

ddPCR has emerged as one of the most promising technologies for clinical methylation testing, particularly for liquid biopsy applications. In ddPCR, DNA samples are partitioned into thousands of nanoliter-sized droplets, allowing absolute quantification of methylated DNA molecules without requiring standard calibration curves.[151] This partitioning substantially improves analytical sensitivity and enables reliable detection of rare methylated alleles present in circulating tumor DNA.

Because tumor-derived methylated DNA may be present at very low abundance within circulating cfDNA in patients with early-stage gastrointestinal cancers, ddPCR offers important advantages over conventional quantitative PCR.[152] Recent investigations evaluating plasma methylation biomarkers for EAC and GC have demonstrated that digital PCR platforms improve analytical sensitivity and quantitative reproducibility while requiring only minimal DNA input.[107] Its relatively rapid turnaround time and compatibility with Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories further support its potential for future clinical implementation. However, ddPCR remains restricted to targeted genomic regions and is therefore better suited for biomarker validation than initial discovery.

Targeted next-generation bisulfite sequencing

Targeted NGS combines bisulfite conversion with massively parallel sequencing, allowing simultaneous quantitative assessment of hundreds to thousands of CpG loci within predefined genomic panels. Compared with single-gene assays, targeted NGS provides substantially greater multiplexing capacity while maintaining high analytical sensitivity.

Recent methylation panels developed for BE and gastric cancer increasingly utilize targeted NGS to evaluate multiple biomarkers simultaneously, thereby improving diagnostic accuracy through machine learning-based classification algorithms rather than reliance on individual genes.[10,52] Targeted sequencing also permits assessment of methylation density, regional methylation patterns, and differentially methylated regions, all of which may outperform single CpG measurements for cancer detection. Nevertheless, higher costs, increased bioinformatic complexity, and the need for specialized laboratory infrastructure currently limit widespread clinical adoption.

Genome-wide DNA methylation arrays

Genome-wide methylation arrays, including the Illumina HumanMethylation450 BeadChip and the more recent Infinium MethylationEPIC array, have transformed biomarker discovery by enabling interrogation of more than 450,000 and 850,000 CpG sites, respectively.[153] These platforms provide comprehensive epigenomic profiling across promoters, enhancers, CpG islands, shores, shelves, and open sea regions, facilitating unbiased identification of novel methylation biomarkers.

Numerous genome-wide studies have used these arrays to identify candidate biomarkers associated with BE progression, GIM, and early gastric cancer.[10,43,117] These approaches have substantially expanded understanding of upper gastrointestinal epigenetic evolution and enabled the development of multimarker diagnostic panels. However, methylation arrays remain research tools rather than routine clinical assays because they require relatively high-quality DNA, sophisticated bioinformatic analysis, and extensive external validation before translation into clinical diagnostics.[9]

Cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq)

cfMeDIP-seq represents an emerging technology that enriches methylated cfDNA fragments using antibodies against 5-methylcytosine prior to NGS. Unlike bisulfite-based methods, cfMeDIP-seq preserves DNA integrity and requires substantially lower DNA input, making it particularly attractive for liquid biopsy applications where circulating tumor DNA concentrations are extremely limited.[154]

Recent studies have demonstrated that cfMeDIP-seq can identify highly cancer-specific methylation signatures while preserving sufficient tissue-specific information for tumor localization.[154] These characteristics have generated considerable interest in integrating cfMeDIP-seq into MCED assays, where methylation patterns can simultaneously detect malignancy and predict tissue of origin.[11] Although early results are encouraging, prospective validation in upper gastrointestinal cancers remains limited, and standardization of laboratory protocols will be necessary before routine clinical implementation.

Whole-genome bisulfite sequencing (WGBS)

WGBS provides single-base resolution of DNA methylation across the entire genome and is considered the gold standard for comprehensive methylome characterization.[155] WGBS enables identification of novel differentially methylated regions, enhancer methylation, and non-CpG methylation patterns that may not be captured by targeted panels or methylation arrays.

Despite its unparalleled genomic coverage, WGBS remains prohibitively expensive for routine clinical use because of high sequencing costs, extensive computational requirements, and large DNA input needs. Consequently, WGBS currently serves primarily as a discovery platform for identifying candidate biomarkers that are subsequently validated using more clinically practical targeted assays.[155]

Comparative considerations for clinical translation

Although remarkable progress has been made in methylation assay development, no single platform is optimal for every clinical application. Genome-wide technologies, including EPIC arrays

and WGBS, excel in biomarker discovery but are currently impractical for routine diagnostics because of their cost and analytical complexity. In contrast, pyrosequencing, ddPCR, and targeted bisulfite sequencing provide highly reproducible quantitative assessment of predefined biomarkers and are better suited for clinical validation and eventual implementation.[9]

Importantly, differences in assay methodology represent one of the principal reasons reported diagnostic performance varies considerably across published studies. Factors including DNA source (fresh tissue, formalin-fixed paraffin-embedded tissue, cytology specimens, plasma, serum, or cfDNA), bisulfite conversion efficiency, sequencing depth, normalization strategies, and statistical classification algorithms all influence measured sensitivity, specificity, and AUC. Therefore, direct comparison of biomarker performance across studies should be interpreted cautiously, particularly in the absence of standardized laboratory protocols or prospective multicenter validation.[139,140]

Future clinical implementation will likely rely on targeted, multiplexed methylation assays capable of simultaneously evaluating multiple biomarkers from minimally invasive specimens such as plasma, Cytosponge, EsoCheck, or gastric brushing specimens. Integration of these assays with machine learning algorithms, clinicopathologic risk factors, and established surveillance strategies may substantially improve early detection and personalized risk stratification for patients with BE, GIM, and upper gastrointestinal adenocarcinomas.[11] Continued standardization of analytical methods, external validation across diverse populations, and adherence to reporting guidelines such as Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) will be essential for translating promising methylation biomarkers from discovery into routine clinical practice.[139]

Limitations

Several limitations should be considered when interpreting the findings presented in this review. First, the conclusions drawn from the existing literature are inherently limited by substantial heterogeneity among published studies. Considerable variability exists in study design, patient demographics, disease stage, specimen type (tissue, cytology, plasma, or cfDNA), methylation detection platforms, gene panels, and statistical methodologies. Consequently, reported measures of diagnostic performance, including sensitivity, specificity, and AUC, should not be compared directly across studies, as differences in patient selection, reference standards, and laboratory methods preclude meaningful quantitative comparisons without formal meta-analytic approaches.[9,140]

Second, although numerous methylation biomarkers have demonstrated promising diagnostic and prognostic performance, relatively few have undergone external validation in large, prospective, multicenter cohorts. Most available studies remain retrospective or case-control in design and evaluate biomarker performance under controlled research conditions rather than real-world clinical settings. Furthermore, many investigations report analytical performance without assessing whether methylation-guided testing improves patient-centered outcomes, such as earlier cancer detection, reduced mortality, optimized surveillance intervals, or decreased healthcare costs. As a result, the clinical utility of many candidate biomarkers remains to be established despite encouraging preliminary findings.[139,142,143]

Third, while this review emphasizes shared epigenetic mechanisms across Barrett's-associated EAC and gastric carcinogenesis, important biological differences exist between these malignancies with respect to their etiologic factors, tissue microenvironments, molecular evolution, and clinical management. The comparative framework presented herein is intended to highlight common principles of DNA methylation biology and opportunities for translational biomarker development rather than imply that these diseases are biologically identical. Similarly, although several methylation markers are shared across both disease pathways, their functional significance, temporal acquisition, and diagnostic performance may differ substantially depending on tissue context and disease stage.[10,13]

Finally, as a narrative review, this manuscript does not employ a formal systematic review methodology or quantitative meta-analysis. Although every effort was made to include landmark studies, recent advances, and clinically relevant investigations, publication bias and selective reporting within the existing literature remain unavoidable limitations. In addition, the rapid evolution of methylation profiling technologies, liquid biopsy platforms, and MCED assays means that new biomarkers and validation studies are likely to emerge following publication. Consequently, the conclusions presented should be interpreted as a contemporary synthesis of a rapidly evolving field rather than definitive evidence supporting immediate clinical implementation.

Future directions and clinical translation of DNA methylation biomarkers in upper gastrointestinal adenocarcinoma

DNA methylation has emerged as one of the most promising molecular approaches for improving the early detection, risk stratification, and surveillance of Barrett's-associated EAC and gastric adenocarcinoma. However, despite the growing number of candidate biomarkers with excellent reported diagnostic performance, relatively few have progressed beyond the discovery or early validation stages. Future research should therefore prioritize rigorous clinical validation rather than continued identification of novel methylation targets. A major challenge remains the substantial methodological heterogeneity among published studies, including differences in specimen types, methylation detection platforms, laboratory protocols, and bioinformatic analyses, which complicate direct comparison of biomarker performance and limit reproducibility.[9] Standardization of analytical methods and adherence to reporting frameworks such as REMARK will be essential for facilitating multicenter validation and eventual clinical implementation.[139]

Future biomarker development is likely to shift from single-gene assays toward multiplexed methylation signatures integrated with machine learning algorithms and complementary molecular datasets. Rather than relying on individual methylation events, emerging diagnostic models increasingly combine panels of biomarkers to improve diagnostic accuracy and tissue-of-origin prediction. Shared methylation events involving CDKN2A (p16), APC, RUNX3, and members of the SFRP family have been consistently implicated in both Barrett's-associated neoplasia and gastric carcinogenesis, reflecting common epigenetic mechanisms associated with chronic inflammation, Wnt signaling dysregula-

tion, cell-cycle disruption, and progressive metaplasia.[8,10,13,43] In contrast, biomarkers such as VIM, CCNA1, VAV3, and ZNF682 appear to exhibit greater specificity for BE and EAC, whereas RNF180, RPRM, BARHL2, PCDH10, SOX17, and ZIC1 have demonstrated particular promise in GIM and gastric adenocarcinoma.[13,52,106,122] Integrating these shared and lineage-specific methylation signatures with established clinicopathologic risk factors—including Barrett's segment length, dysplasia grade, *H. pylori* infection status, and histologic staging systems such as OLGIM—may improve personalized risk prediction models capable of more accurately identifying patients who would benefit from intensified surveillance.[6,10,156] Continued investigation is also needed to determine whether these epigenetic alterations function primarily as causal drivers of neoplastic progression or as molecular biomarkers reflecting chronic inflammatory injury and metaplastic evolution, as accumulating evidence suggests that both mechanisms likely contribute to upper gastrointestinal carcinogenesis.[8,10,13,43]

Minimally invasive sampling strategies and liquid biopsy technologies offer significant opportunities to expand molecular screening beyond conventional endoscopy. Devices such as Cytosponge, EsoCheck, and EsophaCap, together with plasma-derived cfDNA, have demonstrated the feasibility of combining nonendoscopic sampling with methylation-based diagnostics.[36,49,52,157,158] More recently, genome-wide cfDNA methylation profiling has enabled the development of MCED assays capable of identifying multiple malignancies while predicting tissue of origin, as demonstrated by the CCGA and PATHFINDER studies.[11,146] Although current performance for early-stage upper gastrointestinal cancers remains suboptimal, continued refinement of tissue-specific methylation classifiers and targeted biomarker panels may substantially enhance the role of liquid biopsy in early detection, surveillance, and treatment monitoring.

Successful clinical translation, however, will require considerably more than high diagnostic accuracy. Before methylation biomarkers can be incorporated into routine practice, prospective multicenter studies must demonstrate analytical validity, clinical validity, clinical utility, and cost-effectiveness while establishing meaningful improvements over existing surveillance strategies.[8,139,143] Integration into evidence-based clinical guidelines will additionally require standardized laboratory workflows, regulatory approval, reimbursement, and implementation across diverse healthcare settings. Rather than replacing established endoscopic and histopathologic evaluation, methylation biomarkers will most likely serve as complementary tools that improve patient selection for screening, personalize surveillance intervals, and reduce unnecessary procedures through more precise molecular risk stratification.[5,6]

Conclusions

Barrett's-associated EAC and GC represent biologically distinct malignancies that nevertheless share fundamental epigenetic mechanisms characterized by progressive DNA methylation during chronic inflammation and metaplastic transformation. Advances in high-throughput methylation profiling, liquid biopsy technologies, and computational risk modeling have accelerated the field toward precision prevention and early detection. Several of these tools have already crossed into clinical or near-clinical use: Cytosponge-TFF3 coupled with biomarker panels has been prospectively evaluated in real-world UK surveillance pathways, the EsoCheck/EsoGuard methylated VIM and CCNA1 assay is available as a CLIA laboratory-developed test, and Esopredict has become the first epigenetic prognostic assay clinically validated to risk-stratify patients with BE, while blood-based MCED assays incorporating cfDNA methylation are undergoing prospective evaluation. Continued collaboration among molecular biologists, gastroenterologists, pathologists, bioinformaticians, and implementation scientists will be critical to standardize assays, validate biomarkers across diverse populations, and integrate molecular diagnostics into clinical care. Addressing these translational challenges will ultimately determine whether DNA methylation biomarkers evolve from promising investigational tools to evidence-based components of routine management for patients at risk of upper gastrointestinal adenocarcinomas.

Acknowledgments

None.

Funding

No specific funding was received for the preparation of this review.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization (MH, ZZ), literature search (MH), data curation (MH), investigation (MH), original draft preparation (MH), review and editing (MH, ZZ), supervision (ZZ), validation (ZZ), and project administration (ZZ). Both authors have approved the final version and publication of the manuscript.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71(3):209–249. DOI: 10.3322/caac.21660, PMID: 33538338.

[2] Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. Lancet 2020;396(10251):635–648. DOI: 10.1016/S0140-6736(20)31288-5, PMID: 32861308.

[3] Shah SC, Wang AY, Wallace MB, Hwang JH. AGA Clinical Practice Update on Screening and Surveillance in Individuals at Increased Risk for Gastric Cancer in the United States: Expert Review. Gastroenterology 2025;168(2):405–416.e1. DOI: 10.1053/j.gastro.2024.11.001, PMID: 39718517.

[4] Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52(24):6735–6740. PMID: 1458460.

[5] Shaheen NJ, Falk GW, Iyer PG, Souza RF, Wani S. Guideline to

Practice: Diagnosis and Management of Barrett's Esophagus: An Updated ACG Guideline. Am J Gastroenterol 2022;117(8): 1177–1180. DOI: 10.14309/ajg.0000000000001788, PMID: 35467555.

[6] Banks M, Graham D, Jansen M, Gotoda T, Coda S, di Pietro M, *et al*. British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma. Gut 2019;68(9):1545–1575. DOI: 10.1136/gutjnl-2018-318126, PMID: 31278206.

[7] Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, *et al*. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. Am J Gastroenterol 2010;105(7):1523–1530. DOI: 10.1038/ajg.2010.171, PMID: 20461069.

[8] Eads CA, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, *et al*. Epigenetic patterns in the progression of esophageal adenocarcinoma. Cancer Res 2001;61(8):3410–3418. PMID: 11309301.

[9] Laird PW. Principles and challenges of genomewide DNA methylation analysis. Nat Rev Genet 2010;11(3):191–203. DOI: 10.1038/nrg2732, PMID: 20125086.

[10] Jammula S, Katz-Summercorn AC, Li X, Linossi C, Smyth E, Killcoyne S, *et al*. Identification of Subtypes of Barrett's Esophagus and Esophageal Adenocarcinoma Based on DNA Methylation Profiles and Integration of Transcriptome and Genome Data. Gastroenterology 2020;158(6):1682–1697.e1. DOI: 10.1053/j.gastro.2020.01.044, PMID: 32032585.

[11] Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV; CCGA Consortium. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol 2020;31(6):745–759. DOI: 10.1016/j.annonc.2020.02.011, PMID: 33506766.

[12] Zeng Y, Rong H, Xu J, Cao R, Li S, Gao Y, *et al*. DNA Methylation: An Important Biomarker and Therapeutic Target for Gastric Cancer. Front Genet 2022;13:823905. DOI: 10.3389/fgene.2022.823905, PMID: 35309131.

[13] Xue Y, Huang C, Pei B, Wang Z, Dai Y. An overview of DNA methylation markers for early detection of gastric cancer: current status, challenges, and prospects. Front Genet 2023;14:1234645. DOI: 10.3389/fgene.2023.1234645, PMID: 37560387.

[14] Pohl H, Sirovich B, Welch HG. Esophageal adenocarcinoma incidence: are we reaching the peak? Cancer Epidemiol Biomarkers Prev 2010;19(6):1468–1470. DOI: 10.1158/1055-9965.EPI-10-0012, PMID: 20501776.

[15] Njei B, McCarty TR, Birk JW. Trends in esophageal cancer survival in United States adults from 1973 to 2009: A SEER database analysis. J Gastroenterol Hepatol 2016;31(6):1141–1146. DOI: 10.1111/jgh.13289, PMID: 26749521.

[16] Thiruvengadam SK, Tieu AH, Luber B, Wang H, Meltzer SJ. Risk Factors for Progression of Barrett's Esophagus to High Grade Dysplasia and Esophageal Adenocarcinoma. Sci Rep 2020;10(1): 4899. DOI: 10.1038/s41598-020-61874-7, PMID: 32184470.

[17] Roumans CAM, van der Bogt RD, Steyerberg EW, Rizopoulos D, Lansdorp-Vogelaar I, Sharma P, *et al*. Adherence to recommendations of Barrett's esophagus surveillance guidelines: a systematic review and meta-analysis. Endoscopy 2020;52(1):17–28. DOI: 10.1055/a-0995-0134, PMID: 31529444.

[18] Sharma P, Shaheen NJ, Katzka D, Bergman JJGHM. AGA Clinical Practice Update on Endoscopic Treatment of Barrett's Esophagus With Dysplasia and/or Early Cancer: Expert Review. Gastroenterology 2020;158(3):760–769. DOI: 10.1053/j.gastro.2019.09.051, PMID: 31730766.

[19] Modiano N, Gerson LB. Barrett's esophagus: Incidence, etiology, pathophysiology, prevention and treatment. Ther Clin Risk Manag 2007;3(6):1035–1045. PMID: 18516262.

[20] Karimian M, Salamati M, Azami M. The relationship between metabolic syndrome and increased risk of Barrett's esophagus: an updated systematic review and meta-analysis. BMC Gastroenterol 2020;20(1):138. DOI: 10.1186/s12876-020-01267-2, PMID: 32375671.

[21] Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, *et al*. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103(13):1049–1057. DOI: 10.1093/jnci/djr203, PMID: 21680910.

[22] Gibbens Y, Iyer PG. What is the optimal surveillance strategy for non-dysplastic Barrett's esophagus? Curr Treat Options Gastroenterol 2020;18(3):369–383. DOI: 10.1007/s11938-020-00297-9, PMID: 33737800.

[23] Qumseya B, Sultan S, Bain P, Jamil L, Jacobson B, Anandasabapathy S, *et al*. ASGE guideline on screening and surveillance of Barrett's esophagus. Gastrointest Endosc 2019;90(3):335–359.e2. DOI: 10.1016/j.gie.2019.05.012, PMID: 31439127.

[24] Rubenstein JH, Sawas T, Wani S, Eluri S, Singh S, Chandar AK, *et al*. AGA Clinical Practice Guideline on Endoscopic Eradication Therapy of Barrett's Esophagus and Related Neoplasia. Gastroenterology 2024;166(6):1020–1055. DOI: 10.1053/j.gastro.2024.03.019, PMID: 38763697.

[25] Shaheen NJ, Falk GW, Iyer PG, Gerson LB, American College of Gastroenterology. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. Am J Gastroenterol 2016; 111(1):30–50; quiz 51. DOI: 10.1038/ajg.2015.322, PMID: 26526079.

[26] Coco DP, Goldblum JR, Hornick JL, Lauwers GY, Montgomery E, Srivastava A, *et al*. Interobserver variability in the diagnosis of crypt dysplasia in Barrett esophagus. Am J Surg Pathol 2011;35(1):45–54. DOI: 10.1097/PAS.0b013e3181ffdd14, PMID: 21164286.

[27] Kota L, Tyberg A. Risk of Barrett's esophagus progression to esophageal adenocarcinoma. Clin Endosc 2024;57(6):763–764. DOI: 10.5946/ce.2024.273, PMID: 39623931.

[28] Lao-Sirieix P, Fitzgerald RC. Screening for oesophageal cancer. Nat Rev Clin Oncol 2012;9(5):278–287. DOI: 10.1038/nrclinonc.2012.35, PMID: 22430857.

[29] Benaglia T, Sharples LD, Fitzgerald RC, Lyratzopoulos G. Health benefits and cost effectiveness of endoscopic and nonendoscopic cytosponge screening for Barrett's esophagus. Gastroenterology 2013;144(1):62–73.e6. DOI: 10.1053/j.gastro.2012.09.060, PMID: 23041329.

[30] di Pietro M, Modolell I, O'Donovan M, Price C, Pilonis ND, Debiram-Beecham I, *et al*. Use of Cytosponge as a triaging tool to upper gastrointestinal endoscopy during the COVID-19 pandemic. Lancet Gastroenterol Hepatol 2020;5(9):805–806. DOI: 10.1016/S2468-1253(20)30242-9, PMID: 32738939.

[31] Fitzgerald RC, di Pietro M, O'Donovan M, Maroni R, Muldrew B, Debiram-Beecham I, *et al*. Cytosponge-trefoil factor 3 versus usual care to identify Barrett's oesophagus in a primary care setting: a multicentre, pragmatic, randomised controlled trial. Lancet 2020; 396(10247):333–344. DOI: 10.1016/S0140-6736(20)31099-0, PMID: 32738955.

[32] Paterson AL, Gehrung M, Fitzgerald RC, O'Donovan M. Role of TFF3 as an adjunct in the diagnosis of Barrett's esophagus using a minimally invasive esophageal sampling device-The Cytosponge(TM). Diagn Cytopathol 2020;48(3):253–264. DOI: 10.1002/dc.24354, PMID: 31814330.

[33] Ghimire B, Landy R, Maroni R, Smith SG, Debiram-Beecham I, Sasieni PD, *et al*. Predictors of the experience of a Cytosponge test: analysis of patient survey data from the BEST3 trial. BMC Gastroenterol 2023;23(1):7. DOI: 10.1186/s12876-022-02630-1, PMID: 36627580.

[34] Spechler SJ, Katzka DA, Fitzgerald RC. New Screening Techniques in Barrett's Esophagus: Great Ideas or Great Practice? Gastroenterology 2018;154(6):1594–1601. DOI: 10.1053/j.gastro.2018.03.031, PMID: 29577931.

[35] Spechler SJ. Screening for Barrett's oesophagus: are we looking for the right thing? Gut 2021;70(8):1426–1427. DOI: 10.1136/gutjnl-2020-323378, PMID: 33239343.

[36] Ross-Innes CS, Chettouh H, Achilleos A, Galeano-Dalmau N, Debiram-Beecham I, MacRae S, *et al*. Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: a cohort study. Lancet Gastroenterol Hepatol 2017;2(1):23–31. DOI: 10.1016/S2468-1253(16)30118-2, PMID: 28404010.

[37] Zhou Z, Kalatskaya I, Russell D, Marcon N, Cirocco M, Krzyzanowski PM, *et al*. Combined EsophaCap cytology and MUC2 immunohistochemistry for screening of intestinal metaplasia, dysplasia and carcinoma. Clin Exp Gastroenterol 2019;12:219–229. DOI: 10.2147/CEG.S186958, PMID: 31190949.

[38] Wang Z, Kambhampati Thiruvengadam S, Cheng Y, Ma K, Simsek C, Tieu AH, *et al*. Methylation Biomarker Panel Performance in EsophaCap Cytology Samples for Diagnosing Barrett's Esophagus: A Prospective Validation Study. Clin Cancer Res 2019;25(7):2127–2135. DOI: 10.1158/1078-0432.CCR-18-3696, PMID: 30670490.

[39] Iyer PG, Taylor WR, Johnson ML, Lansing RL, Maixner KA, Hemminger LL, *et al*. Accurate Nonendoscopic Detection of Barrett's Esophagus by Methylated DNA Markers: A Multisite Case Control Study. Am J Gastroenterol 2020;115(8):1201–1209. DOI: 10.14309/ajg.0000000000000656, PMID: 32558685.

[40] Kalra A, Ma K, Cheng Y, Tsai HL, Wang H, Cope L, *et al*. Discovery of Methylated DNA Biomarkers for Potential Nonendoscopic Detection of Barrett's Esophagus and Esophageal Adenocarcinoma. Am J Gastroenterol 2025;120(10):2268–2279. DOI: 10.14309/ajg.0000000000003323, PMID: 39819761.

[41] Greer KB, Blum AE, Faulx AL, Deming EM, Hricik LL, Siddiqui H, *et al*. Nonendoscopic Screening for Barrett's Esophagus and Esophageal Adenocarcinoma in At-Risk Veterans. Am J Gastroenterol 2025;120(3):545–553. DOI: 10.14309/ajg.0000000000002962, PMID: 38989889.

[42] Wang JS, Guo M, Montgomery EA, Thompson RE, Cosby H, Hicks L, *et al*. DNA promoter hypermethylation of p16 and APC predicts neoplastic progression in Barrett's esophagus. Am J Gastroenterol 2009;104(9):2153–2160. DOI: 10.1038/ajg.2009.300, PMID: 19584833.

[43] Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, *et al*. DNA methylation profiling in Barrett's esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics 2011;6(12):1403–1412. DOI: 10.4161/epi.6.12.18199, PMID: 22139570.

[44] Kaz AM, Grady WM. Epigenetic biomarkers in esophageal cancer. Cancer Lett 2014;342(2):193–199. DOI: 10.1016/j.canlet.2012.02.036, PMID: 22406828.

[45] Kaz AM, Wong CJ, Varadan V, Willis JE, Chak A, Grady WM. Global DNA methylation patterns in Barrett's esophagus, dysplastic Barrett's, and esophageal adenocarcinoma are associated with BMI, gender, and tobacco use. Clin Epigenetics 2016;8:111. DOI: 10.1186/s13148-016-0273-7, PMID: 27795744.

[46] Luebeck EG, Curtius K, Hazelton WD, Maden S, Yu M, Thota PN, *et al*. Identification of a key role of widespread epigenetic drift in Barrett's esophagus and esophageal adenocarcinoma. Clin Epigenetics 2017;9:113. DOI: 10.1186/s13148-017-0409-4, PMID: 29046735.

[47] Xu Y, Wang Z, Pei B, Wang J, Xue Y, Zhao G. DNA methylation markers in esophageal cancer. Front Genet 2024;15:1354195. DOI: 10.3389/fgene.2024.1354195, PMID: 38774285.

[48] Yu M, Moinova HR, Willbanks A, Cannon VK, Wang T, Carter K, *et al*. Novel DNA Methylation Biomarker Panel for Detection of Esophageal Adenocarcinoma and High-Grade Dysplasia. Clin Cancer Res 2022;28(17):3761–3769. DOI: 10.1158/1078-0432.CCR-22-0445, PMID: 35705525.

[49] Ghosal A, Verma S, Le IT, Lee VT, deGuzman BJ, Aklog L. Analytical Validation of a DNA Methylation Biomarker Test for the Diagnosis of Barrett's Esophagus and Esophageal Adenocarcinoma from Samples Collected Using EsoCheck(®), a Non-Endoscopic Esophageal Cell Collection Device. Diagnostics (Basel) 2024;14(16):1784. DOI: 10.3390/diagnostics14161784, PMID: 39202271.

[50] Tan WK, Ross-Innes CS, Somerset T, Markert G, Markowetz F, O'Donovan M, *et al*. Biomarker risk stratification with capsule sponge in the surveillance of Barrett's oesophagus: prospective evaluation of UK real-world implementation. Lancet 2025;406(10500):271–282. DOI: 10.1016/S0140-6736(25)01021-9, PMID: 40570865.

[51] Kaz AM, Luo Y, Dzieciatkowski S, Chak A, Willis JE, Upton MP, *et al*. Aberrantly methylated PKP1 in the progression of Barrett's esophagus to esophageal adenocarcinoma. Genes Chromosomes Cancer 2012;51(4):384–393. DOI: 10.1002/gcc.21923, PMID: 22170739.

[52] Moinova HR, LaFramboise T, Lutterbaugh JD, Chandar AK, Dumot J, Faulx A, *et al*. Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. Sci Transl Med 2018;10(424):eaao5848. DOI: 10.1126/scitranslmed.aao5848, PMID: 29343623.

[53] Iyer PG, Taylor WR, Slettedahl SW, Lansing RL, Hemminger LL, Cayer FK, *et al*. Validation of a methylated DNA marker panel for the nonendoscopic detection of Barrett's esophagus in a multisite case-control study. Gastrointest Endosc 2021;94(3):498–505. DOI: 10.1016/j.gie.2021.03.937, PMID: 33857451.

[54] Clément G, Braunschweig R, Pasquier N, Bosman FT, Benhattar J. Methylation of APC, TIMP3, and TERT: a new predictive marker to distinguish Barrett's oesophagus patients at risk for malignant transformation. J Pathol 2006;208(1):100–107. DOI: 10.1002/path.1884, PMID: 16278815.

[55] Kuester D, El-Rifai W, Peng D, Ruemmele P, Kroeckel I, Peters B, *et al*. Silencing of MGMT expression by promoter hypermethylation in the metaplasia-dysplasia-carcinoma sequence of Barrett's esophagus. Cancer Lett 2009;275(1):117–126. DOI: 10.1016/j.canlet.2008.10.009, PMID: 19027227.

[56] Lü L, Liu T, Gao J, Zeng H, Chen J, Gu X, *et al*. Aberrant methylation

of microRNA-193b in human Barrett's esophagus and esophageal adenocarcinoma. Mol Med Rep 2016;14(1):283–288. DOI: 10.3892/mmr.2016.5225, PMID: 27176876.

[57] Cotton CC, Wolf WA, Overholt BF, Li N, Lightdale CJ, Wolfsen HC, *et al*. Late Recurrence of Barrett's Esophagus After Complete Eradication of Intestinal Metaplasia is Rare: Final Report From Ablation in Intestinal Metaplasia Containing Dysplasia Trial. Gastroenterology 2017;153(3):681–688.e2. DOI: 10.1053/j.gastro.2017.05.044, PMID: 28579538.

[58] Saikia S, Rehman AU, Barooah P, Sarmah P, Bhattacharyya M, Deka M, *et al*. Alteration in the expression of MGMT and RUNX3 due to non-CpG promoter methylation and their correlation with different risk factors in esophageal cancer patients. Tumour Biol 2017;39(5):1010428317701630. DOI: 10.1177/1010428317701630, PMID: 28468586.

[59] Wang H, DeFina SM, Bajpai M, Yan Q, Yang L, Zhou Z. DNA methylation markers in esophageal cancer: an emerging tool for cancer surveillance and treatment. Am J Cancer Res 2021;11(11):5644–5658. PMID: 34873485.

[60] Dilworth MP, Nieto T, Stockton JD, Whalley CM, Tee L, James JD, *et al*. Whole Genome Methylation Analysis of Nondysplastic Barrett Esophagus that Progresses to Invasive Cancer. Ann Surg 2019;269(3):479–485. DOI: 10.1097/SLA.0000000000002658, PMID: 29384778.

[61] Laun S, Pierre F, Kim S, Lunz D, Maddala T, Braun JV, *et al*. Analytical Validation of Esopredict, an Epigenetic Prognostic Assay for Patients with Barrett's Esophagus. Diagnostics (Basel) 2024;14(18):2003. DOI: 10.3390/diagnostics14182003, PMID: 39335682.

[62] Laun SE, Kann L, Braun J, Gilbert S, Lunz D, Pierre F, *et al*. Validation of an Epigenetic Prognostic Assay to Accurately Risk-Stratify Patients With Barrett Esophagus. Am J Gastroenterol 2024;120(6):1296–1306. DOI: 10.14309/ajg.0000000000003030, PMID: 39140473.

[63] Pinto R, Hauge T, Jeanmougin M, Pharo HD, Kresse SH, Honne H, *et al*. Targeted genetic and epigenetic profiling of esophageal adenocarcinomas and non-dysplastic Barrett's esophagus. Clin Epigenetics 2022;14(1):77. DOI: 10.1186/s13148-022-01287-7, PMID: 35701814.

[64] Wong MCS, Huang J, Chan PSF, Choi P, Lao XQ, Chan SM, *et al*. Global Incidence and Mortality of Gastric Cancer, 1980-2018. JAMA Netw Open 2021;4(7):e2118457. DOI: 10.1001/jamanetworkopen.2021.18457, PMID: 34309666.

[65] Sundar R, Nakayama I, Markar SR, Shitara K, van Laarhoven HWM, Janjigian YY, *et al*. Gastric cancer. Lancet 2025;405(10494):2087–2102. DOI: 10.1016/S0140-6736(25)00052-2, PMID: 40319897.

[66] Patel AK, Sethi NS, Park H. Gastric Cancer: A Review. JAMA 2026;335(5):439–450. DOI: 10.1001/jama.2025.20034, PMID: 41499132.

[67] Fox JG, Wang TC. Helicobacter pylori--not a good bug after all. N Engl J Med 2001;345(11):829–832. DOI: 10.1056/NEJM200109133451111, PMID: 11556306.

[68] Usui Y, Taniyama Y, Endo M, Koyanagi YN, Kasugai Y, Oze I, *et al*. Helicobacter pylori, Homologous-Recombination Genes, and Gastric Cancer. N Engl J Med 2023;388(13):1181–1190. DOI: 10.1056/NEJMoa2211807, PMID: 36988593.

[69] Chey WD, Howden CW, Moss SF, Morgan DR, Greer KB, Grover S, *et al*. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. Am J Gastroenterol 2024;119(9):1730–1753. DOI: 10.14309/ajg.0000000000002968, PMID: 39626064.

[70] Hamashima C; Systematic Review Group and Guideline Development Group for Gastric Cancer Screening Guidelines. Update version of the Japanese Guidelines for Gastric Cancer Screening. Jpn J Clin Oncol 2018;48(7):673–683. DOI: 10.1093/jjco/hyy077, PMID: 29889263.

[71] Yashima K, Shabana M, Kurumi H, Kawaguchi K, Isomoto H. Gastric Cancer Screening in Japan: A Narrative Review. J Clin Med 2022;11(15):4337. DOI: 10.3390/jcm11154337, PMID: 35893424.

[72] He S, Zhang Z, Song G, Wang Z, Li H, Cao M, *et al*. Personalized starting age of gastric cancer screening based on individuals' risk profiles: a population-based, prospective study. J Natl Cancer Inst 2024;116(11):1775–1783. DOI: 10.1093/jnci/djae162, PMID: 38976632.

[73] Li P, Li Z, Linghu E, Ji J; Society of Digestive Endoscopy of the Chinese Medical Association; Colorectal Surgery Group of the Chinese Medical Association, *et al*. Chinese national clinical practice guidelines on the prevention, diagnosis, and treatment of early gastric cancer. Chin Med J (Engl) 2024;137(8):887–908. DOI: 10.1097/CM9.0000000000003101, PMID: 38515297.

[74] Shitara K, Fleitas T, Kawakami H, Curigliano G, Narita Y, Wang F, *et al*. Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with gastric cancer. ESMO Open 2024;9(2):102226. DOI: 10.1016/j.esmoop.2023.102226, PMID: 38458658.

[75] Kim IH, Kang SJ, Choi W, Seo AN, Eom BW, Kang B, *et al*. Korean Practice Guidelines for Gastric Cancer 2024: An Evidence-based, Multidisciplinary Approach (Update of 2022 Guideline). J Gastric Cancer 2025;25(1):5–114. DOI: 10.5230/jgc.2025.25.e11, PMID: 39822170.

[76] Xu Y, Miremadi A, Link A, Malfertheiner P, Fitzgerald RC, Bornschein J. Feasibility of combined screening for upper gastrointestinal adenocarcinoma risk by serology and Cytosponge testing: the SUGAR study. J Clin Pathol 2019;72(12):825–829. DOI: 10.1136/jclinpath-2019-205700, PMID: 31235543.

[77] Ichita C, Kusano C, Gotoda T, Hagiwara Y, Kemmoto Y, Li R, *et al*. Prevalence and Incidence of Gastric Cancer Among Helicobacter pylori-Naïve Individuals in Japan: A Systematic Review and Meta-Analysis. J Gastroenterol Hepatol 2026;41(2):454–464. DOI: 10.1111/jgh.70217, PMID: 41462573.

[78] Lee YC, Chiang TH, Chiu HM, Su WW, Chou KC, Chen SL, *et al*. Screening for Helicobacter pylori to Prevent Gastric Cancer: A Pragmatic Randomized Clinical Trial. JAMA 2024;332(19):1642–1651. DOI: 10.1001/jama.2024.14887, PMID: 39348147.

[79] Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. Gut 1999;44(5):693–697. DOI: 10.1136/gut.44.5.693, PMID: 10205207.

[80] Leung WK, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, *et al*. Screening for gastric cancer in Asia: current evidence and practice. Lancet Oncol 2008;9(3):279–287. DOI: 10.1016/S1470-2045(08)70072-X, PMID: 18308253.

[81] Huang YK, Yu JC, Kang WM, Ma ZQ, Ye X, Tian SB, *et al*. Significance of Serum Pepsinogens as a Biomarker for Gastric Cancer and Atrophic Gastritis Screening: A Systematic Review and Meta-Analysis. PLoS One 2015;10(11):e0142080. DOI: 10.1371/journal.pone.0142080, PMID: 26556485.

[82] Bang CS, Lee JJ, Baik GH. Prediction of Chronic Atrophic Gastritis and Gastric Neoplasms by Serum Pepsinogen Assay: A Systematic Review and Meta-Analysis of Diagnostic Test Accuracy. J Clin Med

2019;8(5):657. DOI: 10.3390/jcm8050657, PMID: 31083485.

[83] Chen H, Xu H. Effect of Gastrin G-17 Combined with Pepsinogen PGI and PGII on the Early Screening of Gastric Cancer in the Department of Gastroenterology. Altern Ther Health Med 2024; 30(9):141–145. PMID: 39110041.

[84] Murphy JD, Olshan AF, Lin FC, Troester MA, Nichols HB, Butt J, *et al*. A Predictive Model of Noncardia Gastric Adenocarcinoma Risk Using Antibody Response to Helicobacter pylori Proteins and Pepsinogen. Cancer Epidemiol Biomarkers Prev 2022;31(4): 811–820. DOI: 10.1158/1055-9965.EPI-21-0869, PMID: 35131882.

[85] Hirai R, Hirai M, Otsuka M, Mitsuhashi T, Shimodate Y, Mouri H, *et al*. Endoscopic evaluation by the Kyoto classification of gastritis combined with serum anti-Helicobacter pylori antibody testing reliably risk-stratifies subjects in a population-based gastric cancer screening program. J Gastroenterol 2023;58(9):848–855. DOI: 10.1007/s00535-023-02010-w, PMID: 37340218.

[86] Tu H, Sun L, Dong X, Gong Y, Xu Q, Jing J, *et al*. A Serological Biopsy Using Five Stomach-Specific Circulating Biomarkers for Gastric Cancer Risk Assessment: A Multi-Phase Study. Am J Gastroenterol 2017;112(5):704–715. DOI: 10.1038/ajg.2017.55, PMID: 28323271.

[87] Yang AP, Liu J, Lei HY, Zhang QW, Zhao L, Yang GH. CA72-4 combined with CEA, CA125 and CAl9-9 improves the sensitivity for the early diagnosis of gastric cancer. Clin Chim Acta 2014;437: 183–186. DOI: 10.1016/j.cca.2014.07.034, PMID: 25086284.

[88] Liang Y, Wang W, Fang C, Raj SS, Hu WM, Li QW, *et al*. Clinical significance and diagnostic value of serum CEA, CA19-9 and CA72-4 in patients with gastric cancer. Oncotarget 2016;7(31): 49565–49573. DOI: 10.18632/oncotarget.10391, PMID: 27385101.

[89] Xu Y, Zhang P, Zhang K, Huang C. The application of CA72-4 in the diagnosis, prognosis, and treatment of gastric cancer. Biochim Biophys Acta Rev Cancer 2021;1876(2):188634. DOI: 10.1016/j.bbcan.2021.188634, PMID: 34656687.

[90] In H, Sarkar S, Ward J, Friedmann P, Parides M, Yang J, *et al*. Serum Pepsinogen as a Biomarker for Gastric Cancer in the United States: A Nested Case-Control Study Using the PLCO Cancer Screening Trial Data. Cancer Epidemiol Biomarkers Prev 2022;31(7): 1426–1432. DOI: 10.1158/1055-9965.EPI-21-1328, PMID: 35534235.

[91] Zhou MJ, Huang RJ. Catching Up with the World: Pepsinogen Screening for Gastric Cancer in the United States. Cancer Epidemiol Biomarkers Prev 2022;31(7):1257–1258. DOI: 10.1158/1055-9965.EPI-22-0372, PMID: 35775231.

[92] Christodoulidis G, Koumarelas KE, Kouliou MN, Thodou E, Samara M. Gastric Cancer in the Era of Epigenetics. Int J Mol Sci 2024; 25(6):3381. DOI: 10.3390/ijms25063381, PMID: 38542354.

[93] Lupan I, Bintintan V, Deleanu D, Samasca G. Epigenetic Regulation of DNA Methylation and RNA Interference in Gastric Cancer: A 2024 Update. Biomedicines 2024;12(9):2001. DOI: 10.3390/biomedicines12092001, PMID: 39335515.

[94] Wang X, Dong Y, Zhang H, Zhao Y, Miao T, Mohseni G, *et al*. DNA methylation drives a new path in gastric cancer early detection: Current impact and prospects. Genes Dis 2024;11(2):847–860. DOI: 10.1016/j.gendis.2023.02.038, PMID: 37692483.

[95] Huang HY, Lan J, Zhuang W. Role of circulating tumor DNA methylation in gastric cancer initiation and progression: A comprehensive review. World J Gastrointest Oncol 2025;17(8):107412. DOI: 10.4251/wjgo.v17.i8.107412, PMID: 40837757.

[96] Tahara S, Tahara T, Yamazaki J, Shijimaya T, Horiguchi N, Funasaka K, *et al*. Helicobacter pylori infection associated DNA methylation in primary gastric cancer significantly correlates with specific molecular and clinicopathological features. Mol Carcinog 2024; 63(2):266–274. DOI: 10.1002/mc.23650, PMID: 37846801.

[97] Vahidi S, Mirzajani E, Norollahi SE, Aziminezhad M, Samadani AA. Performance of DNA Methylation on the Molecular Pathogenesis of Helicobacter pylori in Gastric Cancer; targeted therapy approach. J Pharmacopuncture 2022;25(2):88–100. DOI: 10.3831/KPI.2022.25.2.88, PMID: 35837145.

[98] De Marco K, Sanese P, Simone C, Grossi V. Histone and DNA Methylation as Epigenetic Regulators of DNA Damage Repair in Gastric Cancer and Emerging Therapeutic Opportunities. Cancers (Basel) 2023;15(20):4976. DOI: 10.3390/cancers15204976, PMID: 37894343.

[99] Zhao L, Li M, Zhang S, Liu Y. Plasma-Methylated SEPT9 for the Noninvasive Diagnosis of Gastric Cancer. J Clin Med 2022;11(21): 6399. DOI: 10.3390/jcm11216399, PMID: 36362627.

[100] Huang Y, Yang S, Cun Y, He J, Xu G, Shi P, *et al*. Diagnostic value of methylated SEPT9 and RNF180 for gastric cancer in high-risk population: a multi-centre retrospective study in Yunnan, Southwest China. Arch Med Sci 2024;20(6):2077–2081. DOI: 10.5114/aoms/196807, PMID: 39967944.

[101] Zhang X, Zhang X, Sun B, Lu H, Wang D, Yuan X, *et al*. Detection of aberrant promoter methylation of RNF180, DAPK1 and SFRP2 in plasma DNA of patients with gastric cancer. Oncol Lett 2014;8(4): 1745–1750. DOI: 10.3892/ol.2014.2410, PMID: 25202403.

[102] Miao J, Liu Y, Zhao G, Liu X, Ma Y, Li H, *et al*. Feasibility of Plasma-Methylated SFRP2 for Early Detection of Gastric Cancer. Cancer Control 2020;27(2):1073274820922559. DOI: 10.1177/1073274820922559, PMID: 32379490.

[103] Yan H, Chen W, Ge K, Mao X, Li X, Liu W, *et al*. Value of Plasma Methylated SFRP2 in Prognosis of Gastric Cancer. Dig Dis Sci 2021; 66(11):3854–3861. DOI: 10.1007/s10620-020-06710-8, PMID: 33216241.

[104] Dai Z, Jiang J, Chen Q, Bai M, Sun Q, Feng Y, *et al*. Combining methylated RNF180 and SFRP2 plasma biomarkers for noninvasive diagnosis of gastric cancer. Transl Oncol 2025;51:102190. DOI: 10.1016/j.tranon.2024.102190, PMID: 39541711.

[105] Dede S, Şakalar Ç, Yılmaz B, Çubukcı G, Acar M, Yavuz A, *et al*. SFRP2 and RPRM as methylation based serum biomarkers for the detection of gastric cancer. Discov Oncol 2025;16(1):1606. DOI: 10.1007/s12672-025-03472-5, PMID: 40849852.

[106] Anderson BW, Suh YS, Choi B, Lee HJ, Yab TC, Taylor WR, *et al*. Detection of Gastric Cancer with Novel Methylated DNA Markers: Discovery, Tissue Validation, and Pilot Testing in Plasma. Clin Cancer Res 2018;24(22):5724–5734. DOI: 10.1158/1078-0432.CCR-17-3364, PMID: 29844130.

[107] Lee YY, An J, Han J, Moon Y, Lee SI. Plasma-based digital PCR assay for early detection of gastric cancer using multiple methylation biomarkers. Sci Rep 2026;16(1):1727. DOI: 10.1038/s41598-025-31314-5, PMID: 41381655.

[108] Aleyasin SA, Moradi A, Abolhasani N, Abdollahi M. Investigating FGFR2 gene as a blood-based epigenetic biomarker in gastric cancer. Mol Biol Rep 2024;51(1):253. DOI: 10.1007/s11033-023-09082-0, PMID: 38302798.

[109] Nagano S, Kurokawa Y, Hagi T, Yoshioka R, Takahashi T, Saito T, *et al*. Extensive methylation analysis of circulating tumor DNA in plasma of patients with gastric cancer. Sci Rep 2024;14(1):30739. DOI: 10.1038/s41598-024-79252-y, PMID: 39730450.

[110] Ren J, Lu P, Zhou X, Liao Y, Liu X, Li J, *et al*. Genome-Scale

Methylation Analysis of Circulating Cell-Free DNA in Gastric Cancer Patients. Clin Chem 2022;68(2):354–364. DOI: 10.1093/clinchem/hvab204, PMID: 34791072.

[111] Qi J, Hong B, Wang S, Wang J, Fang J, Sun R, *et al*. Plasma cell-free DNA methylome-based liquid biopsy for accurate gastric cancer detection. Cancer Sci 2024;115(10):3426–3438. DOI: 10.1111/cas.16284, PMID: 39038922.

[112] Huang A, Guo DZ, Su ZX, Zhong YS, Liu L, Xiong ZG, *et al*. GUIDE: a prospective cohort study for blood-based early detection of gastrointestinal cancers using targeted DNA methylation and fragmentomics sequencing. Mol Cancer 2025;24(1):163. DOI: 10.1186/s12943-025-02367-x, PMID: 40468355.

[113] Wang Y, Jin L, He W, Peng J, Yin K, Liu X, *et al*. Plasma cell-free DNA methylation markers for detection and prognosis of gastric cancer: A case-control study. Chin J Cancer Res 2025;37(5):851–864. DOI: 10.21147/j.issn.1000-9604.2025.05.14, PMID: 41229986.

[114] Hu W, Zheng W, Liu Q, Chu H, Chen S, Kim JJ, *et al*. Diagnostic accuracy of DNA methylation in detection of gastric cancer: a meta-analysis. Oncotarget 2017;8(68):113142–113152. DOI: 10.18632/oncotarget.22613, PMID: 29348893.

[115] Kang GH, Shim YH, Jung HY, Kim WH, Ro JY, Rhyu MG. CpG island methylation in premalignant stages of gastric carcinoma. Cancer Res 2001;61(7):2847–2851. PMID: 11306456.

[116] Kim H, Kim J, Jeong IH, Park E, Yoo M, Yoon S, *et al*. Hybrid identity and distinct methylation profiles of incomplete intestinal metaplasia in the stomach. Gut 2026;75(1):10–23. DOI: 10.1136/gutjnl-2025-335793, PMID: 40691053.

[117] Gómez A, Pato ML, Bujanda L, Sala N, Companioni O, Cosme Á, *et al*. Follow-Up Study Confirms the Presence of Gastric Cancer DNA Methylation Hallmarks in High-Risk Precursor Lesions. Cancers (Basel) 2021;13(11):2760. DOI: 10.3390/cancers13112760, PMID: 34199386.

[118] Yamada H, Abe S, Charvat H, Ando T, Maeda M, Murakami K, *et al*. Precision risk stratification of primary gastric cancer after eradication of H. pylori by a DNA methylation marker: a multicentre prospective study. Gut 2025;74(9):1410–1418. DOI: 10.1136/gutjnl-2025-335039, PMID: 40240063.

[119] Asada K, Nakajima T, Shimazu T, Yamamichi N, Maekita T, Yokoi C, *et al*. Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. Gut 2015;64(3):388–396. DOI: 10.1136/gutjnl-2014-307094, PMID: 25379950.

[120] Lopes C, Almeida TC, Macedo-Silva C, Costa J, Paulino S, Jerónimo C, *et al*. MIR124-3 and NKX6-1 hypermethylation profiles accurately predict metachronous gastric lesions in a Caucasian population. Clin Epigenetics 2024;16(1):113. DOI: 10.1186/s13148-024-01712-z, PMID: 39169394.

[121] Zhang Y, Su HJ, Pan KF, Zhang L, Ma JL, Shen L, *et al*. Methylation status of blood leukocyte DNA and risk of gastric cancer in a high-risk Chinese population. Cancer Epidemiol Biomarkers Prev 2014;23(10):2019–2026. DOI: 10.1158/1055-9965.EPI-13-0994, PMID: 25086101.

[122] Schneider BG, Mera R, Piazuelo MB, Bravo JC, Zabaleta J, Delgado AG, *et al*. DNA Methylation Predicts Progression of Human Gastric Lesions. Cancer Epidemiol Biomarkers Prev 2015;24(10):1607–1613. DOI: 10.1158/1055-9965.EPI-15-0388, PMID: 26269563.

[123] Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, *et al*. Genomic and Epigenomic Profiling of High-Risk Intestinal Metaplasia Reveals Molecular Determinants of Progression to Gastric Cancer. Cancer Cell 2018;33(1):137–150.e5. DOI: 10.1016/j.ccell.2017.11.018, PMID: 29290541.

[124] Kim MJ, Kim HN, Jacobs JP, Yang HJ. Combined DNA Methylation and Gastric Microbiome Marker Predicts Helicobacter pylori-Negative Gastric Cancer. Gut Liver 2024;18(4):611–620. DOI: 10.5009/gnl230348, PMID: 38509701.

[125] Wang J, Zhao G, Zhao Y, Zhao Z, Yang S, Zhou A, *et al*. N(6)-methylation in the development, diagnosis, and treatment of gastric cancer. J Transl Int Med 2024;12(1):5–21. DOI: 10.2478/jtim-2023-0103, PMID: 38525439.

[126] Wang Y, Liu H, Zhang M, Xu J, Zheng L, Liu P, *et al*. Epigenetic reprogramming in gastrointestinal cancer: biology and translational perspectives. MedComm (2020) 2024;5(9):e670. DOI: 10.1002/mco2.670, PMID: 39184862.

[127] Smith E, De Young NJ, Pavey SJ, Hayward NK, Nancarrow DJ, Whiteman DC, *et al*. Similarity of aberrant DNA methylation in Barrett's esophagus and esophageal adenocarcinoma. Mol Cancer 2008;7:75. DOI: 10.1186/1476-4598-7-75, PMID: 18831746.

[128] Bansal A, Fitzgerald RC. Biomarkers in Barrett's Esophagus: Role in Diagnosis, Risk Stratification, and Prediction of Response to Therapy. Gastroenterol Clin North Am 2015;44(2):373–390. DOI: 10.1016/j.gtc.2015.02.008, PMID: 26021200.

[129] Riahi Dehkordi N, Kruusmaa K, Krishnadath KK, Bertossi A. Associations Between Non-Genetic Risk Factors and DNA Methylation Alterations in Barrett's Esophagus and Its Progression to Esophageal Adenocarcinoma. Int J Mol Sci 2025;26(23):11704. DOI: 10.3390/ijms262311704, PMID: 41373857.

[130] Kalra A, Meltzer SJ. The Role of DNA Methylation in Gastrointestinal Disease: An Expanded Review of Malignant and Nonmalignant Gastrointestinal Diseases. Gastroenterology 2025;168(2):245–266. DOI: 10.1053/j.gastro.2024.07.001, PMID: 38971197.

[131] Selaru FM, David S, Meltzer SJ, Hamilton JP. Epigenetic events in gastrointestinal cancer. Am J Gastroenterol 2009;104(8):1910–1912. DOI: 10.1038/ajg.2008.145, PMID: 19661933.

[132] Wang Y, Qin X, Wu J, Qi B, Tao Y, Wang W, *et al*. Association of promoter methylation of RUNX3 gene with the development of esophageal cancer: a meta analysis. PLoS One 2014;9(9):e107598. DOI: 10.1371/journal.pone.0107598, PMID: 25229459.

[133] Matchett CL, Slettedahl SW, Taylor WR, Berger CK, Anderson CE, Passe MA, *et al*. Discovery and Validation of Molecular Biomarkers for Differentiation of Nondysplastic Barrett's Esophagus from High-grade Dysplasia and Esophageal Adenocarcinoma. Cancer Prev Res (Phila) 2026;19(1):49–60. DOI: 10.1158/1940-6207.CAPR-25-0215, PMID: 41059926.

[134] Kosmidou C, Jammula S, Fitzgerald RC. DNA Methylation Biomarkers for the Diagnosis of Barrett's Oesophagus. Am J Gastroenterol 2018;113(11):1722. DOI: 10.1038/s41395-018-0226-1, PMID: 30333547.

[135] Zhao L, Liu Y, Zhang S, Li M. Plasma Methylated RNF180 for Noninvasive Diagnosis of Gastric Cancer. Biomed Res Int 2022;2022:6548945. DOI: 10.1155/2022/6548945, PMID: 36246966.

[136] Dai Y, Li H, Wu Q, Wang J, Wang K, Fei S, *et al*. A sensitive and robust plasma-based DNA methylation panel for early detection of target gastrointestinal cancers. Neoplasia 2023;46:100941. DOI: 10.1016/j.neo.2023.100941, PMID: 37918207.

[137] Nie Y, Gao X, Cai X, Wu Z, Liang Q, Xu G, *et al*. Combining methylated SEPTIN9 and RNF180 plasma markers for diagnosis and early detection of gastric cancer. Cancer Commun (Lond) 2023;

43(11):1275–1279. DOI: 10.1002/cac2.12478, PMID: 37584087.

[138] FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD): Food and Drug Administration (US); 2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326791/.

[139] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 2005;23(36):9067–9072. DOI: 10.1200/JCO.2004.01.0454, PMID: 16172462.

[140] Bossuyt PM, Parvin T. Evaluating Biomarkers for Guiding Treatment Decisions. EJIFCC 2015;26(1):63–70. PMID: 27683482.

[141] Fryback DG, Thornbury JR. The efficacy of diagnostic imaging. Med Decis Making 1991;11(2):88–94. DOI: 10.1177/0272989X9101100203, PMID: 1907710.

[142] Lord SJ, Irwig L, Simes RJ. When is measuring sensitivity and specificity sufficient to evaluate a diagnostic test, and when do we need randomized trials? Ann Intern Med 2006;144(11):850–855. DOI: 10.7326/0003-4819-144-11-200606060-00011, PMID: 16754927.

[143] Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, *et al*. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 2001;93(14):1054–1061. DOI: 10.1093/jnci/93.14.1054, PMID: 11459866.

[144] Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ; American Gastroenterological Association. American Gastroenterological Association technical review on the management of Barrett's esophagus. Gastroenterology 2011;140(3):e18–52; quiz e13. DOI: 10.1053/j.gastro.2011.01.031, PMID: 21376939.

[145] Weusten BLAM, Bisschops R, Dinis-Ribeiro M, di Pietro M, Pech O, Spaander MCW, *et al*. Diagnosis and management of Barrett esophagus: European Society of Gastrointestinal *Endoscopy* (ESGE) Guideline. Endoscopy 2023;55(12):1124–1146. DOI: 10.1055/a-2176-2440, PMID: 37813356.

[146] Schrag D, Beer TM, McDonnell CH 3rd, Nadauld L, Dilaveri CA, Reid R, *et al*. Blood-based tests for multicancer early detection (PATHFINDER): a prospective cohort study. Lancet 2023;402(10409):1251–1260. DOI: 10.1016/S0140-6736(23)01700-2, PMID: 37805216.

[147] Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, *et al*. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc Natl Acad Sci U S A 1992;89(5):1827–1831. DOI: 10.1073/pnas.89.5.1827, PMID: 1542678.

[148] Tost J, Gut IG. DNA methylation analysis by pyrosequencing. Nat Protoc 2007;2(9):2265–2275. DOI: 10.1038/nprot.2007.314, PMID: 17853883.

[149] Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 1996;93(18):9821–9826. DOI: 10.1073/pnas.93.18.9821, PMID: 8790415.

[150] Qin Y, Wu CW, Taylor WR, Sawas T, Burger KN, Mahoney DW, *et al*. Discovery, Validation, and Application of Novel Methylated DNA Markers for Detection of Esophageal Cancer in Plasma. Clin Cancer Res 2019;25(24):7396–7404. DOI: 10.1158/1078-0432.CCR-19-0740, PMID: 31527170.

[151] Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, *et al*. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 2011;83(22):8604–8610. DOI: 10.1021/ac202028g, PMID: 22035192.

[152] Whale AS, Huggett JF, Cowen S, Speirs V, Shaw J, Ellison S, *et al*. Comparison of microfluidic digital PCR and conventional quantitative PCR for measuring copy number variation. Nucleic Acids Res 2012;40(11):e82. DOI: 10.1093/nar/gks203, PMID: 22373922.

[153] Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, *et al*. High density DNA methylation array with single CpG site resolution. Genomics 2011;98(4):288–295. DOI: 10.1016/j.ygeno.2011.07.007, PMID: 21839163.

[154] Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, *et al*. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature 2018;563(7732):579–583. DOI: 10.1038/s41586-018-0703-0, PMID: 30429608.

[155] Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, *et al*. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 2009;462(7271):315–322. DOI: 10.1038/nature08514, PMID: 19829295.

[156] Capelle LG, de Vries AC, Haringsma J, Ter Borg F, de Vries RA, Bruno MJ, *et al*. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. Gastrointest Endosc 2010;71(7):1150–1158. DOI: 10.1016/j.gie.2009.12.029, PMID: 20381801.

[157] Iyer PG, Taylor WR, Johnson ML, Lansing RL, Maixner KA, Yab TC, *et al*. Highly Discriminant Methylated DNA Markers for the Non-endoscopic Detection of Barrett's Esophagus. Am J Gastroenterol 2018;113(8):1156–1166. DOI: 10.1038/s41395-018-0107-7, PMID: 29891853.

[158] Chettouh H, Mowforth O, Galeano-Dalmau N, Bezawada N, Ross-Innes C, MacRae S, *et al*. Methylation panel is a diagnostic biomarker for Barrett's oesophagus in endoscopic biopsies and non-endoscopic cytology specimens. Gut 2018;67(11):1942–1949. DOI: 10.1136/gutjnl-2017-314026, PMID: 29084829.