Surgical and Molecular Pathology of Barrett Esophagus
Sherma Zibadi, MD, PhD, and Domenico Coppola, MD

Background: Patients with Barrett esophagus (BE) are predisposed to developing dysplasia and cancer. Adenocarcinoma, which is associated with BE, is the most common type of esophageal tumor and, typically, it has an aggressive clinical course and a high rate of mortality.

Methods: The English-language literature relating to tumor epidemiology, etiology, and the pathogenesis of BE was reviewed and summarized.

Results: The role of pathologists in the diagnosis and pitfalls associated with grading Barrett dysplasia is addressed. Current molecular testing for Barrett neoplasia, as well as testing methods currently in development, is discussed, focusing on relevant tests for diagnosing tumor types, determining prognosis, and assessing therapeutic response.

Conclusions: Grading is essential for developing appropriate treatment plans, follow-up visits, and therapeutic interventions for each patient. Familiarity with current molecular testing methods will help physicians correctly diagnose the disease and select the most appropriate therapy for each of their patients.

Introduction
Barrett mucosa refers to a metaplastic process induced by the acid-peptic content of the stomach that then erodes the esophageal squamous mucosa. During endoscopy, changes of any length recognized as columnar-type mucosa in the distal esophageal epithelium that, on biopsy, are confirmed to have intestinal metaplasia are also defined as Barrett mucosa.1

Barrett esophagus (BE) is more common in men and has a male:female ratio of 3:1 to 7:1.2 Although BE can present at any age, its peak incidence occurs in the sixth decade of life and it occurs more frequently in whites (80% of patients) than patients of other races.3 Patients with BE are predisposed to esophageal adenocarcinoma (EAC; 5-year survival rate of 14%-22%), which has increased in incidence during the last 30 years.4,5 Guidelines for diagnosis and surveillance intervals for patients diagnosed with BE have been established and developed by the American College of Gastroenterology (Table 1).1

Etiology and Pathogenesis
Typically, patients acquire BE as a result of gastroesophageal reflux.6,7 The factors putting patients at risk for BE are several and include duodenogastric
reflux, which is a delay in the clearance of acid juice from the esophagus, a resting pressure in the sphincter of the lower esophagus less than the optimal rate, and the presence of hiatal hernia.5-11

Other etiological factors include postgastrectomy bile reflux12 and esophageal injury (eg, lye ingestion, tobacco use).13,14 Alcohol intake does not promote BE15 and Helicobacter pylori infection has a protective effect, particularly if Helicobacter possesses the CagA gene.16 In infants, BE may arise from congenital rests of gastric columnar epithelium reacting to gastroesophageal reflux.5,17

The way in which normal squamous epithelium transitions into specialized columnar epithelium is not yet clear.18 However, based on the theory of restitution and replication proposed by Jankowski et al,19 after the acid-peptic content of the stomach erodes the parabasal and superficial layers of the squamous mucosa (Fig 1A), the basal progenitor cells move into denuded areas.5 In the setting of persistent gastroesophageal reflux, these multipotential stem cells become resistant to acid and bile, selectively differentiating into columnar epithelium that secretes mucin (Fig 1B).5,19 By contrast, the upward movement of the columnar epithelium from the stomach could cause metaplasia due to wound healing of the ulcerated mucosa.5

Cancer Risk
BE is a premalignant condition associated with EAC and dysplasia.20,21 During the last several decades, the incidence of EAC has risen.21-23 Approximately 10% to 20% of patients with symptomatic gastroesophageal reflux who undergo endoscopy have BE.24 However, many patients with BE are asymptomatic.25 The prevalence of dysplasia in the setting of BE varies between 14% and 40%,26 and 7% to 15% of patients will have EAC at the time of initial diagnosis of BE.27

Pathology
Grossly, the esophagogastric junction in BE is displaced by areas of velvety, gastric-type mucosa that macroscopically appear salmon in color.11 These areas of gastric-type mucosa proximally extend from their typical location. The area of BE may be ulcerated or contain islands of residual, whitish squamous mucosa surrounded by pink-colored, gastric-type mucosa (Fig 2). It is possible that the health care professional may be unable to distinguish the appearance of simple BE — grossly and endoscopically — from BE with early cancer and dysplasia.28 With regard to its location, BE characteristically involves the lower one-third of the esophagus, but it may involve the middle and upper esophagus as it progresses.11

Previously, BE was histologically indicated by the presence of intestinal metaplasia or the gastric, cardia, or oxyntic type of columnar mucosa in the esophagus.29,30 However, in the modern era of endoscopy, the American Gastroenterological Association requires the presence of unequivocal intestinal metaplasia for there to be a diagnosis of BE.31,32

The nonintestinalized, gastric-type mucosa was previously thought to have no potential for malignant transformation; however, in recent years, small (presumably early) EACs arising in the absence of intestinal metaplasia have been reported.33-35 Thus, these findings suggest that the identification of goblet cells as a requirement for diagnosing BE should be challenged.26-28 For example, the requirement no longer exists in Japan, which was the first country to propose the existence of malignant potential for nonintestinalized BE.35,36 In recent years, this view has been supported by study findings indicating that goblet cell–containing epithelium has similar DNA abnormalities as metaplastic esophageal columnar epithelium without goblet cells.39,40

Although goblet cell metaplasia is not always an obligate precursor of malignancy, it is important to point out that the recognition of goblet cell metaplasia remains the most important criterion in clinical practice for the routine histological recognition and diagnosis of BE.

In BE, the intestinalized mucosa rarely has a villiform appearance and Paneth cells, absorptive cells, and intestinal-type endocrine cells are typically absent because of the incomplete form of metaplasia present. Uncommonly, the complete form of metaplasia presents in conjunction with the usual incomplete form of metaplasia.

Table 1. — ACG Guidelines for BE

<table>
<thead>
<tr>
<th>Dysplasia</th>
<th>Diagnosis</th>
<th>Follow-Up</th>
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<tbody>
<tr>
<td>None present</td>
<td>2 EGDs with biopsy in 1 y</td>
<td>EGD every 3 y</td>
</tr>
<tr>
<td>Low grade</td>
<td>Highest grade on repeat EGD</td>
<td>1-y interval until no dysplasia (× 2)</td>
</tr>
<tr>
<td></td>
<td>with biopsies within 6 mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expert pathology confirmation</td>
<td></td>
</tr>
<tr>
<td>High grade</td>
<td>Mucosal irregularity</td>
<td>Continued 3-mo surveillance or intervention based on results and outcomes</td>
</tr>
<tr>
<td></td>
<td>Repeat EGD with biopsies to rule out esophageal carcinoma within 3 mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expert pathology confirmation</td>
<td></td>
</tr>
<tr>
<td>Invasive</td>
<td>Expert pathology confirmation</td>
<td>Esophagectomy</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td></td>
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</tbody>
</table>

ACG = American College of Gastroenterology, BE = Barrett esophagus, EGD = esophagogastroduodenoscopy.

Fig 1A–C. — (A) Basal progenitor cells move into denuded areas after the gastric acid-peptic content from the stomach has eroded the squamous mucosa. (B, C) Persistent gastroesophageal reflux causes multipotential stem cells to differentiate into columnar, mucin-secreting epithelium resistant to acid and bile. Reprinted from *Am J Pathol*, 154(4), Jankowski JA, Wright NA, Meltzer SJ, et al, Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus, 965-973, Copyright 1999, with permission from Elsevier.
cytoplasm filled with acidic mucin and can be identified using hematoxylin and eosin stain, and, if necessary, also highlighted with either Periodic acid–Schiff or Alcian blue (pH 2.5) stain.

**Role of Pathologists**

The role of pathologists when dealing with esophagogastric biopsy or resection is twofold:

1. Identification of BE: The pathologist must differentiate between nonmetaplastic columnar epithelium (eg, hiatal hernia, ectopic gastric mucosa), intestinal metaplasia of the cardia, and true BE.
2. Identification and grading of dysplasia: This task will affect patient treatment.

When dealing with endoscopic mucosal resection specimens, pathologists are responsible for assessing the resection margins. If invasive cancer is present, then pathologists are also required to report the distance of the invasive carcinoma from the deep margin, the presence of angiolymphatic invasion, and, if possible, the level of invasion within the muscularis mucosa, submucosa, or both.

**Dysplasia**

The presence of the neoplastic epithelium confined within the basement membrane of the gland from which it arises and within the superficial layer of epithelium is the defining characteristic of dysplasia. Criteria have been established for grading dysplasia in BE that consider a combination of features, such as gland architecture, the presence of inflammation, ulceration, or erosion, cytological features, and the level of maturation of the surface epithelium.

**Negative**

When cases of BE are negative for dysplasia, the underlying glands will not be as mature as the surface epithelium. The nuclear:cytoplasmic ratio is lower than that of the deeper glands. Lamina propria is abundantly present between the glands. Cytologically, nuclear polarity is retained, the nuclear membrane is smooth, and the nucleoli are inconspicuous. In the event of prominent inflammation, reactive changes will be present but surface maturation is maintained.

**Indefinite**

A diagnosis of indefinite for dysplasia is used for cases with worrisome changes but not diagnostic for dysplasia, typically because of the presence of marked inflammation. Normal glandular architecture may be observed but crowding will be present, and, cytologically, nuclear hyperchromasia, nuclear membrane irregularities, and increased mitoses at the base of the glands (but with surface maturation) may be present. Nuclear polarity is not lost in cases of indefinite for dysplasia. Such a diagnosis may also be used in the presence of tangential embedding that does not allow for the assessment of the superficial portion of the glands.

**Low Grade**

In cases of BE with low-grade dysplasia (LGD), the mucosal surface is similar to the underlying glands, which reveal nuclear stratification and nuclear polarity (Fig 3). Glandular crowding can be seen, but the lamina propria is still present between the glands. The nuclei are elongated, hyperchromatic, and have irregular contours and sustain a moderate increase in mitotic activity. The nuclear:cytoplasmic ratio will be mildly increased. In the setting of LGD, minimal inflammation may be present and nucleoli are not prominent.

**High Grade**

In the setting of BE with high-grade dysplasia (HGD), surface maturation is lacking; glandular distortion and glandular crowding are both present with a minimal amount of lamina propria between the glands. The glands may become dilated with luminal necrotic debris. The nuclei display marked enlargement, anisocytosis, pleomorphism, hyperchromasia, membrane irregularity, and prominent nucleoli. The mitotic figures are numerous and the nuclear:cytoplasmic
The ratio will be large. Nuclear polarity is lost (Fig 4). Inflammation will be minimal. In the setting of HGD, the presence of superficial ulceration, cytologically high-grade nuclei, solid nests of dysplastic cells with multiple secondary lumina, and/or dilated dysplastic tubules containing granular eosinophilic and nuclear debris may indicate an unsampled invasive carcinoma. Additional biopsies should be performed when observing these findings.\textsuperscript{48,49}

**Clinical Significance**

One study found that HGD was a marker of metachronous or synchronous adenocarcinoma in approximately 40% of cases.\textsuperscript{50} In another study, Weston et al\textsuperscript{51} found that HGD progressed to adenocarcinoma or multifocal HGD in 53% of cases.

**Pitfalls Associated With Grading**

The grading of dysplasia in BE is subjective due to sampling errors as well as interobserver and intraobserver variations. In addition, the biological behavior of the lesion and its degree of dysplasia may not correlate.\textsuperscript{34,52} However, the task of grading dysplasia in BE is important and can sometimes have crucial therapeutic implications (see Table 1).\textsuperscript{3} For this reason, biopsy findings in cases of BE, particularly in the setting of HGD and carcinoma, should be reviewed by a second pathologist with expertise in gastrointestinal pathology.\textsuperscript{53}

Morphological and molecular data suggest that dysplasia in BE begins at the base of the crypts.\textsuperscript{54} Crypt dysplasia is thought to progress into the remaining portion of the crypt and surface epithelium.\textsuperscript{55} Histologically, crypt dysplasia may exhibit the same cytological changes characteristic of LGD or, rarely, have high-grade cytological features with very large nuclei, a large nuclear:cytoplasmic ratio, irregular nuclear membranes, nuclear polarity loss, and eosinophilic cytoplasm. One study had good interobserver agreement when diagnosing crypt dysplasia.\textsuperscript{55}

**Intramucosal Carcinoma**

In intramucosal adenocarcinoma, the neoplastic cells are limited to the mucosa (Fig 5); however, these cells will make their way to the basement membrane and
into the muscularis mucosae or the lamina propria, but they do not go beyond this point. Histologically, an effacement of the architecture of the lamina propria can be seen, as well as back-to-back microglands, small clusters, or infiltrating single cells. A syncytial growth pattern may also be observed. Typically, desmoplasia is absent or not completely developed, perhaps accounting for the poor intraobserver reproducibility in the diagnosis of this entity.

**Invasive Adenocarcinoma**

Invasive adenocarcinoma has a variable gross appearance and size (≤ 10 cm) and typically involves the distal one-third of the esophagus. Tumor cells infiltrate beyond the muscularis mucosa in the setting of invasive adenocarcinoma and oftentimes evoke a desmoplastic reaction. The microscopic pattern is mostly of the intestinal form, however diffuse, and signet ring cell or mixed types can be observed. The tumor cells may produce mucin, and the tumor itself will generally be well to moderately differentiated.

**Intestinal Metaplasia of the Cardia**

Intestinal metaplasia of the cardia has a prevalence of 6% to 25%. Generally in this setting, the cardia contains areas of intestinal metaplasia with goblet cells and a normal Z line. *H. pylori* infection, gastroesophageal reflux, and carditis can all be causes for intestinal metaplasia of the cardia. Patients with intestinal metaplasia of the cardia are at increased risk for gastric cardia cancer; however, this type of intestinal metaplasia may go into regression if its cause is treated; furthermore, research suggests that this condition is not typically associated with dysplasia.

**Endoscopic Mucosal Resection**

Gastroesophageal endoscopic mucosal resection is a procedure to remove dysplastic or carcinomatous mucosa from the stomach, esophagus, or both. It is performed on low-risk adenocarcinomas using either an injection- or cap-assisted endoscopic mucosal resection. Low-risk adenocarcinoma is defined as a stage 1A tumor no larger than 20 mm in size without vascular or lymphatic invasion and a histological grade of G1 or G2.

Following endoscopic mucosal resection, a complete tumor response was reported in 99% of patients after a follow-up time of 36.7 months. Recurrent or metachronous lesions were present in 11% of patients and the 5-year survival rate was 98%.

**Molecular Alterations**

Jankowski et al highlight restitution, cell cycle and apoptosis, adhesion, DNA instability, and invasion as potential factors in the molecular pathways associated with the progression of BE to adenocarcinoma. For example, following mucosal injury, the mucosa rapidly heals by restitution. During this phase, metaplastic clones with specialized intestinal metaplasia propagate in the presence of persistent gastroesophageal reflux, followed by the loss of cell-cycle checkpoints. In addition, because of increasing proliferation, genomic instability is thought to be associated with slow clonal expansion. The inhibition of apoptosis occurs late and only in cases of HGD. Altering cell adhesion can precede invasive cancer, and the accumulation of genetic errors that follows may be associated with the generation of multiple, transformed cell clones, thus expanding the number of altered cells with metastatic or angiogenic potential.

**Aberrant p53**

Several studies have observed alterations in p53 during the progression of BE. Typically, aberrant p53 is accumulated in the nucleus of the neoplastic cells, particularly in cases of HGD. When they were detected by polymerase chain reaction single-strand conformation polymorphism, the p53 molecular alterations coincided with the level of p53 protein overexpression detected by immunohistochemistry in Barrett adenocarcinoma and BE-HGD but were discordant in BE-LGD.

Currently, the only accepted predictor for progression is a histological diagnosis of LGD; its predictive value is low. However, the immunohistochemistry status of p53 has diagnostic value and can help predict neoplastic progression in patients with BE. Using more than 12,000 biopsy samples from 635 patients, including those with BE who developed HGD, those with invasive adenocarcinoma, and those without neoplastic progression, Kastelein et al studied the protein expression of p53 via immunohistochemistry. The researchers found that 49 patients (8%) developed HGD or invasive adenocarcinoma during the follow-up period. The results of the study indicated that patients with a diagnosis of LGD had a
positive predictive value for neoplastic progression of 15%. Conversely, patients with LGD and concurrent altered p53 expression had a positive predictive value of 33%. Thus, these data show that p53 alterations are a better predictor of tumor progression than BE with LGD.

**ERBB2 Amplification and Overexpression**

Using a tissue microarray, Hu et al looked at 34 cases of BE, 18 cases of LGD, 15 cases of HGD, and 116 cases of EAC to study ERBB2 (formerly known as HER2 or HER2/neu) amplification using chromogenic in situ hybridization. They found ERBB2 amplification in 21 of the cases of EAC (18.1%), ERBB2 overexpression in another 14 cases of EAC (12.1%), and ERBB2 protein overexpression in 1 case of HGD (6.7%). All of the cases of LGD and BE had negative results for fluorescence in situ hybridization (FISH) and immunohistochemistry.

A recent study looked at the immunohistochemical expression of ERBB2 in BE, BE with dysplasia, and adenocarcinoma. The overexpression of ERBB2 in a significant number of HGD cases was an unexpected finding that may have clinical implications. The low expression level of ERBB2 in LGD was a novel finding and may indicate the role of ERBB2 in the early stages of Barrett carcinogenesis.

**Molecular Testing**

Molecular testing has been proposed to accurately detect and grade dysplasia and adenocarcinoma in patients with BE. Among these tests, FISH can be performed on endoscopic esophageal brushings, which can be used to sample a large diseased area of the esophagus. In a study by Brankley et al, various probe combinations were used to select a combination of FISH probes specific for identifying dysplasia and carcinoma in the setting of BE (Table 2). A total of 170 brushing specimens from 138 patients with BE were examined. Biopsy results were used as the gold standard. The authors found that probes to 8q24, 9p21, 17q11.2, and 20q13.2 detected LGD with sensitivity and specificity rates of 70% and 89%, respectively. The same probes were also able to identify HGD with sensitivity and specificity rates of 84% and 93%, respectively, and identify adenocarcinoma with sensitivity and specificity rates of 94% and 93%, respectively. Thus, these probes have been proposed for testing in prospective clinical trials.

Selaru et al used global gene expression profiling to study BE and esophageal cancer using DNA microarrays. They examined 13 esophageal resections or biopsies, including 7 cases of BE and 6 cases of EAC, using a microarray platform containing 8,000 complementary DNA (cDNA) clones. Gene profiles were similar in all cases of BE, which clustered together and were different from the EAC cases (also clustered together). The researchers also differentiated adenocarcinoma from squamous cell carcinoma and signet ring cell carcinoma.

In another study, Helm et al analyzed microarrays so that they could obtain expression profiles from individual genes based on biopsy samples (9 cases of EAC/BE from which 3 cancers had arisen). To use as a reference point, the researchers pooled samples of BE from 6 patients with BE but without cancer or dysplasia. Their findings showed that increasing changes in gene expression were related to the progression from BE to EAC. The early loss of gene function that governs differentiation began prior to such histological change, whereas a gain in gene function was associated with the progression of invasiveness and remodeling.

Using DNA microarrays, Hao et al focused on the contributions of the stroma to the neoplastic process, studying the gene expression profiles of EAC or BE specimens obtained via biopsy and the associated normal duodenum and esophagus among 17 volunteers. The researchers found a unique expression profile for each tissue. Both EAC and BE had a unique set of 37 stromal genes distinct from those found in normal tissue. Collagen 5A2 and periostin were validated by in situ hybridization. No differences in gene expression were seen between long- and short-segment-type BE. The authors concluded that stromal gene changes precede transformation.

**Ultrasensitive Complementary DNA Microarray Chip**

In 2007, Ito et al introduced an ultrasensitive cDNA microarray chip for gene-expression profiling to be used in preoperative esophageal cancer biopsies that requires 0.1 to 0.01 µg of total ribonucleic acid (RNA). However, no RNA amplification is needed.

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Spectrum Red</th>
<th>Spectrum Green</th>
<th>Spectrum Aqua</th>
<th>Spectrum Gold</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LSI 9p21</td>
<td>LSI 5p15</td>
<td>CEP 9</td>
<td>LSI 5q21–22</td>
</tr>
<tr>
<td>II</td>
<td>CEP Y</td>
<td>LSI 17q11.2–12</td>
<td>CEP 17</td>
<td>LSI 17p13.1</td>
</tr>
<tr>
<td>II</td>
<td>CEP Y</td>
<td>LSI 17q11.2–12</td>
<td>CEP 17</td>
<td>LSI 17p13.1</td>
</tr>
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Formerly known as HER2/neu. CEP = centromere enumeration, FISH = fluorescence in situ hybridization, LSI = locus-specific indicator.

This method is highly sensitive. It might be suitable for clinical diagnosis because it discriminates noncancerous tissue from cancerous tissue and has accuracy, sensitivity, and specificity rates of 95.2%, 95.7%, and 94.7%, respectively.

**MicroRNA**

The expression of short, noncoding RNA influences genes by blocking the translation of target messenger RNAs or by quickening their degradation. RNA = ribonucleic acid. Reprinted from Curr Opin Pharmacol, 9(6), Kan T, Melzter SJ. MicroRNAs in Barrett's esophagus and esophageal adenocarcinoma, 727-732, Copyright 2009, with permission from Elsevier.

![Diagram of MicroRNA expression](Image)

Fig 7. — The expression of short, noncoding RNA influences genes by blocking the translation of target messenger RNAs or by quickening their degradation (Fig 7). Yang et al examined microRNA expression in 5 cases of BE with LGD, 5 cases of BE with HGD, and 6 cases of EAC using a human microRNA microarray kit and then validated by real-time reverse transcription–polymerase chain reaction. Unsupervised hierarchical clustering and class comparison analyses showed different microRNA expression profiles between either EAC or BE with HGD and their corresponding normal tissues. No differences in microRNA expression profiles were observed between BE with LGD and their normal counterparts (refer to Table 4 in Yang et al).

In addition, microRNA signatures specific to each stage of Barrett carcinogenesis have been identified; each correlated with their target gene expression.

**Conclusions**

We presented a review of the clinical and pathological characteristics of Barrett esophagus, underscoring the role of pathologists in recognizing and grading this disease. Potential pitfalls during diagnosis, detection, and grading of Barrett esophagus, including associated dysplasia, were also topics addressed. Finally, we discussed select molecular techniques that may soon be introduced in the clinical setting for the personalized management of Barrett esophagus. Pathologists are essential for planning the most appropriate follow-up period needed for each patient. The advent of molecular testing for Barrett esophagus also provides new tools for selecting the most appropriate therapy for each patient.

**References**


