Barrett’s Esophagus

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Barrett’s esophagus is an acquired condition in which the stratified squamous epithelium normally lining the esophagus is replaced by metaplastic, intestinal-type columnar epithelium. It develops as a result of chronic gastroesophageal reflux disease, and predisposes to the development of esophageal adenocarcinoma. This review is focused on histologic diagnosis and differential diagnosis of Barrett’s esophagus.

Key Words: Barrett’s esophagus; Esophageal adenocarcinoma; Histology

INTRODUCTION

Barrett’s esophagus is an acquired condition in which the stratified squamous epithelium normally lining the esophagus is replaced by metaplastic, intestinal-type columnar epithelium, defined by the presence of goblet cells. Barrett’s esophagus occurs in both adults and children, in whom it has a similar pathogenesis. It develops as a result of chronic gastroesophageal reflux disease (GERD), and predisposes to the development of esophageal adenocarcinoma.

Our understanding of Barrett’s esophagus has undergone considerable change since the influential British surgeon, Dr. Norman Barrett, first described this condition over half a century ago. Advances have occurred in the definition of Barrett’s esophagus, the pathologic and clinical diagnostic criteria, and its management.

The main difficulties in the pathology of Barrett’s esophagus continue to revolve around: 1) the over-diagnosis of Barrett’s esophagus itself, and 2) the over-diagnosis of high-grade dysplasia in Barrett’s esophagus. These are serious matters that result not only in inappropriate and lifelong cancer surveillance, but also unwarranted invasive therapy, including even esophagectomy. The critical elements for avoiding these problems in Barrett’s esophagus are presented.

Management options for Barrett’s esophagus with high-grade dysplasia have rapidly expanded over the past decade from surgery alone. Alternatives now include endoscopic ablative therapy, endoscopic mucosal resection (EMR), and expanded use of continued biopsy surveillance. Prospective natural history data from adequately sampled patients have significantly informed this last option. Whereas the diagnostic threshold to pursue esophagectomy formerly rested upon the pathologist’s ability to reliably diagnose high-grade dysplasia, the newer non-surgical options have largely moved the esophagectomy diagnostic threshold to the level of adenocarcinoma invading into the submucosa or deeper. This of course relies on accurate separation of high-grade dysplasia from carcinoma on mucosal biopsies. Unfortunately, such distinctions are unreliable on forceps biopsies, even by expert gastrointestinal (GI) pathologists in high volume practices. This serious shortfall of forceps biopsy sampling is obvious to pathologists as they struggle to apply unreliable histologic criteria to endoscopic biopsy specimens, but this problem remains relatively unrecognized by gastroenterologists and surgeons who actually make the treatment decisions. Fortunately EMR has become much more widely available and because they provide far greater tissue they are the optimal pre-surgical sample for pathologists to determine when high-grade dysplasia ends and adenocarcinoma begins. These aspects
of Barrett’s neoplasia among others are considered in detail toward the goal of achieving reliable and accurate diagnoses upon which rational management decisions can be made.

**DIAGNOSIS OF BARRETT’S ESOPHAGUS**

1. **Definition of Barrett’s esophagus**

Careful definition of Barrett’s esophagus is essential, as this cancer predisposing condition confers untold patient anxiety, insurance rate elevations, and commitment of the patient to lifelong endoscopic cancer surveillance. As advocated by the American College of Gastroenterology,6 and the American Gastroenterological Association,7,8 and the recent reversal of opinion by the British Society of Gastroenterology,10 Barrett’s esophagus is defined by two components, one endoscopic and one histologic (Fig. 1). The diagnosis should not be established unless both are present as will be discussed.

The endoscopic component requires proximal extension of columnar mucosa into the tubular esophagus in continuity with the stomach. Columnar mucosa is recognized endoscopically by virtue of its salmon-pink color relative to the more tan-colored appearance of the squamous esophageal mucosa (Fig. 1A). This endoscopic criterion was added to the definition of Barrett’s esophagus based on the discovery that up to a third of reflux patients will have a few glands of intestinal metaplasia at an otherwise endoscopically normal gastroesophageal junction (GEJ).11-13 The extremely high prevalence of this finding relative to the uncommon occurrence of Barrett’s tumorigenesis, renders it obvious that a few intestinalized junctional glands in reflux disease cannot confer the same increased cancer risk as endoscopically visible Barrett’s esophagus involving the tubular esophagus. This important knowledge mandates that there be an endoscopic diagnostic component of Barrett’s esophagus.

The defining histologic component for Barrett’s esophagus requires intestinalized columnar epithelium with goblet cells (Fig. 1B) within the endoscopically identified glandular mucosa in the distal tubular esophagus. Endoscopists cannot discern whether columnar-lined, salmon-pink mucosa in the esophagus is gastric mucosa or intestinalized mucosa with goblet cells. This distinction can only be achieved histologically by the pathologist. In the early decades of Barrett’s esophagus, it was held that there were three types of Barrett’s mucosa, gastric cardiac, gastric fundic and intestinalized with goblet cells (also known as metaplastic or specialized columnar epithelium). This thinking evolved over time to include only intestinalized mucosa due to overwhelming evidence that it conferred the cancer risk in the columnar-lined esophagus. To confuse matters, some authors have recently returned to the idea that the goblet cell histologic requirement is not necessary to diagnose Barrett’s and that any columnar epithelium (i.e., gastric as well as intestinal epithelia) in the distal esophagus constitutes Barrett’s esophagus.7,8,10,14 As discussed further below, the evidence that gastric cardiac or fundic columnar mucosa also confers a significant cancer risk is almost entirely flawed by sampling error. Furthermore, and unfortunately, the concept that Barrett’s does not require intestinalized mucosa tremendously dilutes the patient population at highest risk.

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Fig. 1. Barrett’s esophagus is defined by both endoscopic (A) and histologic (B) components. Endoscopically there must be visible pink columnar epithelium within the tubular esophagus that on biopsy has intestinalized metaplastic columnar epithelium defined by the presence of true goblet cells (H&E, ×200). Barrett’s esophagus should not be diagnosed without both components.
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2. anatomic and mucosal landmarks of
barrett’s esophagus

the literature concerning barrett’s esophagus is difficult to interpret because of variable pathologic criteria and imprecise endoscopic definitions of important anatomic and mucosal landmarks. the following definitions of the various landmarks within the lower esophageal region have helped to refine the endoscopic identification of barrett’s esophagus. if abnormalities are identified, endoscopists should, at a minimum, separately identify and biopsy the following three landmarks.10 the gej is the anatomic junction at which the tubular esophagus joins the saccular stomach. it is generally agreed upon that it occurs where the perpendicularly radiating superior most gastric folds end.16 the squamocolumnar junction (scj), also known as the “z-line”, is a mucosal junction, which may not necessarily line up with the gastroesophageal anatomic junction. in fact, the squamocolumnar mucosal junction may be irregular and in as many as half of normal individuals, it may be proximally displaced from the gej to lie anywhere within the distal 2–3 cm of the tubular esophagus in patients without barrett’s esophagus. this distal most 2–3 cm region of the tubular esophagus is the increased pressure zone that is also termed the lower esophageal sphincter (les) region. thus, if a proximally displaced scj relative to the gej is found, an accurate diagnosis of barrett’s esophagus requires separate biopsies specifying: 1) the gej, 2) the scj or z-line, and 3) the intervening columnar (salmon-pink colored) mucosa of potential barrett’s metaplastic epithelium to document goblet cells, whether the intervening mucosa is a tongue or a circumferential segment, long or short.

single biopsies designated as “rule out barrett’s” or as “gej” alone are clearly insufficient for a definitive diagnosis. intestinal epithelium in this area could also derive from intestinalized gastritis due to either helicobacter pylori or autoimmune gastritis. further and as already mentioned, the diagnosis of barrett’s esophagus also requires endoscopically visible columnar epithelium within the esophagus to avoid over-diagnosing the highly prevalent finding in gerd of only rare intestinalized glands at an otherwise normal gej. these factors cannot be inferred
from biopsies labeled as “rule out Barrett’s” or “GEJ” alone. If the intervening salmon-pink mucosa between the GEJ and SCJ and within the tubular esophagus has intestinal metaplasia, this is true Barrett’s esophagus, whether it is a long or short segment. If this intervening mucosa is only gastric, or if there is no endoscopically visible columnar mucosa in the tubular esophagus, the findings are insufficient to establish a definitive diagnosis of Barrett’s esophagus.

Despite these definitions, the endoscopic diagnosis of Barrett’s esophagus may be difficult. The minimal criteria for establishing any medical condition are fraught with difficulty, and Barrett’s esophagus is not exempt from this problem. The anatomy of the distal esophagus moves up and down with respiration during esophagoscopy. The endoscopic diagnosis can also be particularly challenging in patients with large hiatal hernias, especially if they are combined with patulous or widely open LESs, making it difficult to identify the end of the radiating gastric folds. In such patients, the GEJ usually becomes apparent if air is insufflated to better demarcate the saccular hiatal hernia and its proximal-most gastric folds radiating up to the tubular esophagus. The GEJ is located within one cm or so of the proximal margins of the gastric folds, even when a hiatal hernia is present.17

3. LES region in normal individuals and GERD patients

1) Pancreatic acinar metaplasia

Biopsy specimens obtained from the region of the GEJ, particularly in the setting of GERD, not infrequently have foci of pancreatic acinar metaplasia.18,19 Pancreatic acinar cells are readily identified histologically by their unique supranuclear eosinophilic cytoplasm and subnuclear basophilic cytoplasm, growing in small acini or glands. This is also an incidental finding, like gastric mucosa, that has no known clinical importance.18,19

2) Gastric cardiac and fundic mucosa

Normal individuals and reflux patients without Barrett’s esophagus may have endoscopically visible columnar or salmon pink-colored epithelium within the tubular esophagus that is composed of gastric cardiac or fundic mucosa (Fig. 2). These two gastric types of columnar mucosa are endoscopically identical in appearance to Barrett’s metaplastic mucosa with goblet cells. Importantly, gastric cardiac or fundic-type mucosa within the distal 2∼3 cm (LES zone) of the esophagus is not Barrett’s esophagus.1,6-8,20 This is why the histologic component for the diagnosis of Barrett’s esophagus becomes critical. In fact, gastric mucosa occurs in as many as half of biopsies from the distal esophagus.1,20 The mucosal SCJ or Z-line may be irregular and project into the distal 2∼3 cm of the tubular esophagus as tongues, or it may lie entirely within

Fig. 2. Gastric mucosa of cardiac (A) and fundic (B) types in biopsies from endoscopically visible pink columnar mucosa within the lower esophageal sphincter (LES) region (H&E, ×200). Note the absence of goblet cells, the foveolar surface of columnar mucinous cells and the gastric-type mucinous or oxyntic glands. Gastric epithelium within the LES region occurs in approximately half of normal patients and virtually all gastroesophageal reflux disease patients, is not Barrett’s esophagus, and does not confer an increased neoplastic risk.
the distal tubular esophagus in normal individuals.16,21 None of these endoscopic mimics are Barrett’s esophagus. Biopsy histology is mandatory to distinguish whether the endoscopic columnar mucosa is normal or inflamed gastric mucosa or true Barrett’s metaplasia with goblet cells.

Debate exists over whether gastric cardiac or fundic or admixed cardiofundic mucosa in this region are truly “normal” (present congenitally) or alternatively represent an acquired alteration from reflux disease.18,19 Regardless of this congenital versus acquired debate, neither cardiac nor fundic epithelia in this region have been proven to confer an increased cancer risk in well-designed studies with sufficient biopsy sampling (4-quadrant biopsies at 1 to 2 cm intervals, with the most proximal biopsy straddling the SCJ).22 Concern, however, has been raised that nonintestinalized gastric mucosa may also confer risk and that this in fact should change the entire definition of Barrett’s esophagus to one that no longer requires goblet cells.7,8,22 Careful scrutiny of many of the studies purported to document cancer risk in pure gastric mucosa that supposedly lack goblet cells reveals inadequate or even unstated sampling of the esophageal mucosa to establish whether there truly was pure gastric mucosa as advertised, or whether these patients actually had unsampled intestinalized Barrett’s esophagus. A recent study from the renowned esophageal center at the University of Southern California provides highly enlightening data and literature review on this point. Chandrasoma et al.20 selected 214 patients with a visible columnar-lined esophagus who underwent systematic biopsies and 109 patients without systematic biopsy. The systematic protocol included 4-quadrant biopsies at 1 to 2 cm intervals, with the most proximal biopsy straddling the SCJ. In the well-sampled group, 187 patients (87.4%) had intestinal metaplasia, and 27 (12.6%) had only cardiac epithelium. Dysplasia or adenocarcinoma was present in 55 patients, all with intestinal metaplasia; none of the cardiac only patients had dysplasia or adenocarcinoma (P=0.01). In the second limited sampling group, 49 had only tumor tissue in the biopsy. Of 60 poorly sampled patients with non-tumor epithelium, only 34 (56.7%) had intestinal metaplasia. These data document that “inadequate sampling is a powerful reason why the near absolute association between intestinal metaplasia and adenocarcinoma is not seen in some studies”.22 Thus, so-called cardiac “only” cases with neoplasia are likely unsampled Barrett’s patients with intestinal metaplasia, especially if biopsy sampling is poor. Metaplastic goblet cells in Barrett’s esophagus range from diffuse and numerous to more patchy, requiring more than 1 or 2 limited biopsies but not unreasonable sampling for detection.6-8,22

4. Summary on the definition of Barrett’s esophagus

The above definitional information on Barrett’s esophagus, from both the endoscopic and histologic perspectives, represents a very definite evolution of understanding over the past many decades. Earlier, three types of Barrett’s epithelia were recognized, namely gastric cardiac-type, gastric fundic-type, and intestinal-type, but it is now reasonably certain that only intestinal-type mucosa with goblet cells clearly confers a sufficiently increased neoplastic risk to warrant surveillance for early detection and prevention. It is also clear that salmon-pink glandular mucosa within the distal esophagus is required endoscopically to assign a diagnosis of Barrett’s, due to the well-established rare intestinalized glands that occur frequently at the normal GEJ aligning with the SCJ. Both the histologic and endoscopic components are mandatory. It cannot be emphasized strongly enough that the histologic component of Barrett’s esophagus is limited to intestinal metaplasia with true goblet cells.1,7,8,16 Without this criterion, over half of the American population would meet a non-goblet definition of Barrett’s esophagus, as gastric mucosa occurs in the LES region of this segment of the US population, be they normal or GERD patients. Lack of emphasis on this critical point is the major factor generating incorrect diagnoses of Barrett’s esophagus, with the serious and unnecessary consequences this poses for patients. The most recent American Gastroenterological Association definition is very clear: “Presently, intestinal metaplasia is required for the diagnosis of Barrett’s esophagus because intestinal metaplasia is the only type of esophageal columnar epithelium that clearly predisposes to malignancy”.

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5. Histology of Barrett’s epithelium with true goblet cells

Barrett’s metaplastic epithelium, also known as specialized columnar epithelium, is histologically identical to gastric intestinal metaplasia of the incomplete type or less commonly the complete type. Subtyping of intestinal metaplasia, however, has no practical clinical significance, as neoplasia may develop in either type. The epithelium of Barrett’s esophagus has three major cell types—goblet cells and two different types of intervening columnar cells that either resemble intestinal-type absorptive cells with poorly formed brush borders, or gastric foveolar-type mucinous cells (Fig. 3). Paneth cells may also occasionally be seen within the complete-type of intestinal metaplasia. True goblet cells not only have a rounded goblet shape, but also contain acid mucin that stains intensely blue on an alcian blue stain at pH 2.5. Histochemical analyses show that this acid mucin most often contains a mixture of sialomucins and sulfomucins, but the sialomucins generally predominate. Demonstration of the acid mucin subtype, similar to the subtyping of intestinal metaplasia, also has no clinical or prognostic significance. Neither aspect need be analyzed or reported for practical diagnostic purposes.

Routine alcian blue staining at pH 2.5 is costly and not necessary for the great majority of cases, as most often specialized columnar metaplasia can be readily recognized on H&E alone. This is achieved by noting the singly dispersed distribution of true goblet cells among the remaining epithelial cells. This is especially true if the hematoxylin being used in the H&E stain is optimized to stain the goblet cell mucin slightly blue. Multiple types of hematoxylin preparations are available with varying abilities to stain goblet cell acid mucins blue. Alcian blue staining or the lack thereof can, however, help in the setting of gastric cardiac mucosa with goblet-shaped cells (so-called pseudo-goblet cells), as discussed further below.

The columnar cells between the goblet cells may resemble gastric foveolar cells or intestinal-type absorptive cells, but they do not have all of the typical features of either. Microvillus brush borders, if present, are only partially developed, in contrast to the thick and refractile brush border of the mature intestinal absorptive cell. The mucinous columnar cells differ from normal gastric foveolar surface cells because they frequently contain acid mucin (rather than normal gastric neutral mucin) in variable quantities (resulting in their nickname of “tall columnar blue cells”).

6. Long segments of gastric only mucosa

 Virtually all of the columnar epithelium in adults extending proximal to the LES (i.e., more than 2–3 cm above the GEJ) is composed of specialized columnar epithelium with admixed goblet cells, intestinal-type absorptive cells and gastric mucinous-type cells. While an individual biopsy specimen may contain only cardiac or fundic-type mucosa in a patient who has specialized columnar metaplasia in other specimens, it is only an extremely rare well-sampled patient with only cardiac-type mucosa without goblet cells extending well above the LES region. Due to the rarity of this finding, its clinical significance or neoplastic risk remains unknown. Terminology that has been suggested for this condition is “columnar-lined esophagus of the non-Barrett’s type” in order to distinguish it from Barrett’s esophagus and its established cancer risk.
7. Minimum histologic requirements for Barrett’s esophagus or “how many goblet cells are enough?”

While there is a major emphasis on goblet cells to diagnose Barrett’s, this requirement is eliminated in the presence of glandular dysplasia of the tubular esophagus. Mucin (goblet) loss is common once dysplasia becomes established. This is not unexpected as cytoplasmic mucin as a feature of cellular differentiation, which is commonly lost in neoplasia. Thus goblets are not required to diagnose Barrett’s, if the mucosa is already dysplastic. When either of these findings (goblet cells or dysplasia) is identified in a biopsy specimen taken from endoscopically visible columnar epithelium within the tubular esophagus (above the GEJ) it is abnormal, regardless of whether it occupies a 1 or a 10 cm segment. This is true Barrett’s esophagus.

“Short segment” Barrett’s esophagus is defined arbitrarily by 3 cm or less of metaplastic epithelium within the esophagus. However, the minimum amount of goblet cell containing epithelium required to confer an increased cancer risk remains unknown. A few metaplastic glands from an endoscopically normal LES region, in which the SCJ and the GEJ align, are quite common, and in fact have been found in up to a third of patients undergoing upper endoscopy for reflux symptoms (Fig. 4). Thus, the very high prevalence of rare intestinalized glands in the distal esophagus versus the low prevalence of Barrett’s cancers, means these common minute patches of intestinalized glands cannot possibly confer the same neoplastic risk endoscopically visible segments of Barrett’s epithelium. Thus, it seems ill-advised to diagnose Barrett’s esophagus based upon predominantly gastric-type mucosa containing only rare metaplastic glands with goblet cells. As a practical matter but admittedly one of personal opinion, this author defines “rare metaplastic glands” as five intestinalized glands or less, although this is entirely and admittedly arbitrary. There are no data that such minute foci of metaplastic epithelium confer an increased cancer risk, nor are such data likely to ever become available. The high prevalence of this finding renders the requisite study sample size, the numbers of biopsies and the follow-up period to large. Nonetheless, based on current knowledge that a few intestinalized glands in GERD patients are extremely prevalent, it seems unwise to give such patients a diagnosis of Barrett’s esophagus with its attendant lifelong endoscopic surveillance. In such cases, a diagnosis of focal intestinal metaplasia that is negative for dysplasia, rather than Barrett’s esophagus seems a better option, along with an explanatory comment on the high prevalence of rare metaplastic glands in GERD and the mandatory requirement of endoscopically visible columnar mucosa in the tubular esophagus to establish a definitive diagnosis of Barrett’s esophagus. Additional biopsies from well-characterized endoscopic landmarks as discussed above may be helpful. Finally, the clinical practice of taking biopsies from an otherwise endoscopically normal GEJ aligning with the SCJ should be discouraged, as there is no known clinical significance to histologic findings in the setting of a normal GEJ, and the practice leads to the over diagnosis of Barrett’s esophagus. While many endoscopists place patients with focal intestinal metaplasia into surveillance, this practice is not evidence based.

8. Histologic mimics of Barrett’s mucosa

1) Pseudogoblet cells

Not uncommonly, cardiac-type gastric mucosa may contain foveolar cells with barrel-shaped or distended cytoplasmic mucin-containing vacuoles resembling goblet cells at the gastroesophageal junction are insufficient to establish a diagnosis of Barrett’s esophagus. Alcian blue at pH 2.5 (×200).
cells. These distended gastric foveolar cells are also called “pseudogoblet” cells and can be a large source of error in the incorrect diagnosis of Barrett’s esophagus. They are recognizable on H&E staining in most cases as pseudogoblet cells because they are not singly dispersed among columnar absorptive and gastric foveolar-type cells as with true goblet cells. Rather, pseudogoblet cells are characteristically arranged in linear contiguous stretches along the surface or they completely fill glands without intervening columnar cells. Alcian blue staining at pH 2.5 generally stains pseudogoblet cells pale blue if at all (as opposed to the intense blue of true goblet cells). This differential staining by Alcian blue is the only utility of this stain (Fig. 5). Fortunately, the H&E morphologic features of pseudogoblet cells mentioned above are fully diagnostic without additional staining is almost all cases.

The columnar cells of gastric cardiac-type mucosa, including the barrel-shaped “pseudogoblet” cells and the columnar foveolar-type cells, may show positive Alcian blue staining at pH 2.5. This is usually weakly blue in color but occasionally may be intense and also should not be mistaken for Barrett’s metaplastic epithelium. In the majority of cases, the pattern and types of cells present will permit this distinction, as discussed above. Thus, as with pseudogoblet cells, alcian blue positive columnar cells are usually not singly dispersed, but rather occur in linear, contiguous stretches, and are not diagnostic of Barrett’s esophagus. Alcian blue positivity is common in reactive gastric foveolar mucosa in reflux disease alone without Barrett’s metaplasia. It is also noteworthy that the submucosal esophageal glands and the ducts that drain them onto the esophageal surface are intensely alcian blue positive at pH 2.5; this is not Barrett’s either. These false positive pitfalls with alcian blue staining at pH 2.5 are important to know for proper interpretation in the rare case this stain is needed.

2) Inflamed and reactive gastric mucosa resembling Barrett’s dysplasia

Distinguishing reactive, inflamed gastric cardiac-type mucosa (Fig. 6) from dysplastic Barrett’s epithelium is another difficulty, which may be considerable. In both of these epithelia, there is a strong tendency for loss of cytoplasmic mucin, whether it is a reactive loss of foveolar gastric mucin due to inflammatory injury (Fig. 6A, B), or loss of cytoplasmic differentiation in Barrett’s dysplasia (Fig. 7∼13). Both epithelia also show cytologic atypia that can be marked. These combined features render these epithelia remarkably similar looking, but of course they have entirely different clinical significances. Differential diagnostic considerations between these epithelia are further discussed below in the section on problems in the diagnosis of dysplasia.

3) Inlet patches

Barrett’s esophagus should not be confused with congenital islands of ectopic gastric or intestinalized mucosa in the proximal esophagus. These so-called “inlet patches” are found in up to 10% of individuals undergoing endoscopy, occur principally in the cervical esophagus, and are separated from the stomach by a large zone of intact squamous epithelium. Inlet patches may have gastric or even intestinal metaplasia, but this should not to be confused with Barrett’s esophagus, which always begins distally in the LES region and arises in continuity with the stomach. While extremely rare cases of cancer arising in inlet patches have been reported, surveillance is not justified even if they contain intestinal metaplasia, due to the extremely low neoplastic risk of this highly preva-
Fig. 6. Similarities between reactive gastric cardiac mucosa (A∼C) and dysplastic Barrett’s mucosa may lead not only to the over diagnosis of Barrett’s esophagus itself, but of dysplastic Barrett’s as well. The similarities include mucin loss and nuclear atypia, as seen in A (at higher magnification) and B. The differences include the often more bland gastric mucinous deeper glands (B and C) relative to the more “top heavy” atypical surface (A and B) in comparison to the opposite “bottom heavy” atypia pattern in Barrett’s (Fig. 7∼9, 14). Mitotic figures may also be helpful (A, white arrowhead), as the mitotic or regenerative zone of gastric mucosa resides in the central or neck region of the gastric crypt, leading to the “top-heavy” appearance of the atypia, whereas in Barrett’s and in any intestinal-type epithelium, the regenerative zone emanates from the deepest parts of the crypts leading to the “bottom-heavy” atypia. Finally, reactive gastric foveolar cells commonly retain a linear array of small apical foveolar mucin caps along the mucosal surface (A, black arrow), which are not usually well developed in dysplastic Barrett’s epithelium (H&E; A: ×300, B: ×200, C: ×400).

9. Barrett’s esophagus in children

Children with reflux disease who do not initially have metaplastic epithelium may eventually develop it. Of children with reflux disease with metaplastic epithelium containing goblet cells, the youngest was 5 years of age.

10. Intestinal metaplasia of the cardia: reflux disease or Helicobacter gastritis?

An ongoing debate exists over whether intestinal metaplasia in biopsies taken from the GI tract represents reflux disease-induced Barrett’s esophagus or alternatively represents intestinalized pangastritis caused by either Helicobacter pylori or autoimmune gastritis. Goldblum and colleagues demonstrated differences in the cytokeratin 7 and 20 staining patterns for these different etiologies, but subsequent studies have rendered keratin profiling less useful. The clinical significance of these distinctions is also unknown as long-term prospective follow-up data are lacking in relation to keratin profiles. Distinction between GERD versus Helicobacter pylori or autoimmune
Fig. 7. Surface extension of dysplasia from the base of the mucosa (black arrow) onto the surface (black arrowhead) in this example of low-grade dysplasia. Surface extension is the single most important criterion for the diagnosis of dysplasia. The enlarged, stratified nuclei of low-grade dysplasia, in this example, also reveal maintenance of nuclear polarity whereby the long axes of the nuclei remain perpendicular to the basement membrane. The asterisk (*) denotes a non-dysplastic gland for comparison with small normal nuclei that mature even further as they extend onto the biopsy surface (H&E, ×300).

Fig. 8. Indefinite for dysplasia in Barrett’s esophagus with cytologic atypia that partially but incompletely matures onto the biopsy surface. The changes do not yet cross the author’s threshold for unequivocal low-grade dysplasia. Diagnostic thresholds are impossible to precisely define because they based on innumerable variables. Threshold standardization can be refined; however, through high volume and continual practice of Barrett’s pathology (H&E, ×200).

Fig. 9. High-grade dysplasia in Barrett’s esophagus demonstrating crowded irregular gland architecture and marked cytologic atypia that includes loss of nuclear polarity, wherein the nuclei are no longer oriented perpendicularly to the basal lamina and are disorderly and maloriented one to another (inset: ×400) (H&E, ×300).

Fig. 10. Numerous dilated glands with luminal necrotic or apoptotic debris in high-grade dysplasia. This feature constitutes marked distortion of glandular architecture and a warning is appropriate that invasive adenocarcinoma cannot be excluded (H&E, ×200).

11. Summary on establishing a diagnosis of Barrett’s esophagus

The consequences of a diagnosis of Barrett’s esophagus are high, with its attendant cancer predisposition, lifelong surveillance, major insurance repercussions and the con-

intestinalized gastritides as the etiologies of junctional intestinal metaplasia will continue to require identification of either Barrett’s esophagus or evidence of gastritis based on antral and body biopsies, serologies or pernicious anemia.
Fig. 11. High-grade dysplasia with marked distortion of glandular architecture such that invasive adenocarcinoma cannot be excluded, due to the severe crowding of glands in a “back-to-back” pattern without intervening lamina propria (H&E, ×300).

Fig. 12. Intramucosal adenocarcinoma in Barrett’s esophagus, showing (A) numerous single malignant cells invading the lamina propria (black arrowheads), (B) sheets of invasive malignant cells, (C) abortive and angulated glands invading the lamina propria, and (D) never-ending gland pattern where the lumen of the glands appears continuous (H&E; A: ×400, B: ×200, C: ×200, D: ×400).

Considerable psychological burden for the patient. Accordingly, the diagnosis should not be established when the data on an individual patient are ill-defined and without proven significance. Endoscopic landmarks, including 1) the anatomic GEJ, 2) the proximally displaced mucosal SCJ (Z-line), and 3) the intervening salmon-pink colored columnar mucosa suspected of being Barrett’s esophagus, should each be separately biopsied and identified for the pathologist. The intervening mucosa must contain specialized columnar or metaplastic intestinalized epithelium with true goblet cells. Attention should be paid to potential mimics of Barrett’s mucosa, particularly pseudogoblet cells and inflamed gastric cardiac mucosa. The interpretation of alcian blue staining at pH 2.5 is complicated by the fact that several cell types within the esophagus may be alcian blue positive at pH 2.5 that are not Barrett’s epithelium. Finally, caution should be exercised...
regarding diagnosing rare intestinalized glands from an endoscopically normal appearing LES region. This finding is very common in reflux patients and due to its prevalence cannot confer the high cancer risk of true Barrett’s esophagus.

**BARRETT’S NEOPLASIA**

Barrett’s esophagus predisposes to the development of esophageal adenocarcinoma, but the frequency with which it does so is somewhat controversial. Part of the difficulty in defining the cancer risk in Barrett’s is that the prevalence of Barrett’s esophagus itself, and thus the denominator in the equation, is not clearly established. Barrett’s esophagus is present in about 10% to 12% of patients with symptomatic GERD who undergo endoscopy. The reported prevalence of adenocarcinoma in Barrett’s esophagus averages about 10%, i.e., at the time the initial diagnosis of Barrett’s esophagus is made, about 10% of patients will have adenocarcinoma. In a systematic review of 47 studies from 1950 to 2006, the estimated incidence of Barrett’s adenocarcinoma was 5.3 per 1,000 person-years (0.5% per year). Adenocarcinoma of the esophagus appears to be essentially limited to patients who have metaplastic epithelium, other than the extremely rare salivary-type adenocarcinomas that presumably develop from esophageal submucosal glands. The length of the endoscopically visible columnar-lined segment does not have a significant influence on cancer risk, as patients with even very short segments develop cancer at a similar rate. Cancer appears to arise in Barrett’s esophagus through a multi-step sequence of events that is initiated by chronic gastroesophageal reflux, leading to metaplasia, then dysplasia, and finally adenocarcinoma. Progression is not inexorable, and in fact, increasingly smaller subsets of patients progress from each step, presumably related to the genetic and cellular complexity of neoplastic progression.
1. Diagnosis of dysplasia and early carcinoma

Dysplasia is defined as neoplastic epithelium that remains confined within the basement membrane of the epithelium it arises within.34 When dysplastic epithelium proliferates to form a visible lesion, the term adenoma may be applied, but this is uncommon in Barrett’s esophagus35 and of no significance. Dysplasia in Barrett’s esophagus is recognized histologically by a combination of architectural and cytologic abnormalities. Dysplastic glands may retain their normal configuration, but more often have irregular, crowded or even markedly distorted architecture.

The cytologic changes in dysplasia vary depending on whether the dysplasia is of the intestinal-type or the recently recognized gastric foveolar-type. In the far more common intestinal-type of dysplasia, the glands are usually lined by cells with enlarged, irregular, hyperchromatic, crowded and stratified nuclei. However, because Barrett’s metaplasia is comprised of several cell types, including goblet, pseudogoblet, pseudoabsorptive and gastric foveolar cell types, it should not be surprising that not all dysplasias in Barrett’s esophagus are of the classic intestinal or adenomatous-type that simulate intestinal adenomas. The gastric foveolar-type of dysplasia is quite different exhibits large and hyperchromatic nuclei that often contain macronucleoli but lack the crowding and stratification seen in intestinal-type dysplasia. Rather, the nuclei in the gastric foveolar-type of dysplasia maintain a basal and largely monolayer arrangement within the cells as the major distinguishing feature from intestinal type dysplasia. Both types of dysplasia are further detailed below.

In the case of discrepancy between cytology and architecture, cytology generally takes precedence in the grading of dysplasia. The one exception to this is when architecture is extremely abnormal as detailed in the criteria below for high-grade dysplasia.

In virtually all cases of dysplasia the cytologic features extend from the glands onto the epithelial surface (Fig. 7, 9~11, 13). This surface extension is perhaps the single most important criterion for the diagnosis of dysplasia in gastrointestinal epithelium at any location and in large part permits distinction between dysplasia and reactive inflammatory change.

In the presence of severe inflammation, with or without erosion/ulceration; however, assignment of unequivocal dysplasia is done only in rare circumstances. In general, all of the cytologic alterations of neoplasia may be completely mimicked by inflammatory change, whereas architectural changes of dysplasia have somewhat greater fidelity for neoplastic change. Thus, extensively crowded glands with necrosis or cribriform architecture, sheets of cells and markedly angulated glands are generally not seen in inflammatory change and strongly indicate neoplasia. Nonetheless, the overlap is pronounced and overwhelming majority of ulcerated/eroded biopsies with cytoarchitectural alterations suggesting neoplasia should be classified as indefinite for dysplasia. Strong cautionary comments in such situations are warranted to advise the endoscopists that neoplastic change is very possible but obscuring inflammation precludes a definitive diagnosis. In such situations, the patient should be re-biopsied after aggressive anti-reflux therapy aimed at inducing inflammatory remission.

Further criteria applying to all grades of dysplasia are that biopsies that stand out as significantly different from others, such as those with relative mucin loss or hypermucinous change, dystrophic goblet cells, or varying architecture or cytology, are clues for dysplasia.

Slightly more cytoarchitectural atypia occurs normally precisely at the SCJ, so that more caution should be exercised when diagnosing dysplasia immediately at the junction.

For purposes of clinical utility, unequivocal dysplasia in Barrett’s esophagus has been divided into low and high-grade categories, in a manner analogous to dysplasia in idiopathic inflammatory bowel disease.134,35 It is important to note that “moderate dysplasia” is not a diagnostic option, due to the strong tendency to over select the middle category in any three-tiered system, saving the far ends of the spectrum for the low and high options. Thus if moderate dysplasia were an option, it would become the majority diagnosis rendering clinical management uncertain. The present two-tiered system fosters either conservative (for low-grade dysplasia) or aggressive
action (for high-grade dysplasia), providing clearer options to clinicians. Although the changes of Barrett’s neoplasia certainly form a continuum and there is undoubtedly a middle zone, pathologists diagnoses of unequivocal dysplasia should comply with the 2-tier only system of either low or high-grade to promote clinical utility.

2. Criteria for grading intestinal type Barrett’s epithelium

1) Negative for dysplasia

The glandular architecture and cellular morphology are free of neoplastic alterations, but may contain reactive or regenerative change from inflammatory injury in the negative for dysplasia category. The glandular architecture is orderly and not crowded, with abundant lamina propria surrounding most glands. The basal-most intestinalized glands, which are closest to the muscularis mucosae, make up the regenerative compartment of Barrett’s metaplastic mucosa. These deeper glands are characteristically atypical in intestinalized metaplasia, and typically exhibit nuclear enlargement, hyperchromasia, pleomorphism, and nuclear membrane irregularity. These findings simulate dysplasia except for the critically important fact they are limited to the basal glands and there is normal surface maturation as the epithelium extends onto the biopsy surface (Fig. 14). This is the baseline deep glandular atypia that is quite characteristic of intestinalized metaplastic epithelium without dysplasia. The deep glandular atypia may be particularly striking in comparison to frequently admixed and directly adjacent mucinous or oxyntic gastric-type glands, which are usually quite bland and also occur within the basal portions of Barrett’s biopsies (Fig. 14). The basal regenerative intestinalized glands should never be compared to the bland basal gastric glands to assess for dysplasia; rather, the comparison should be to the surface epithelium. Thus, if the basal atypia of the intestinal metaplasia matures to the surface, it is virtually always negative for dysplasia.

Care must also be taken at the surface of biopsies not to over interpret tangential sectioning artifact, which creates the false appearance of nuclear stratification simulating dysplasia. Stratification of nuclei is one criterion of dysplasia, but as a solitary feature, it is almost never sufficient to diagnose true dysplasia. In tangential sectioning artifact, the uniform and bland appearance of the surface nuclei along with the simultaneous elongation of the cytoplasm as well as the nuclei help to exclude dysplasia.

Outside of the deepest regenerative crypt zone where the nuclei are usually enlarged, normal epithelial cell nu-

Fig. 14. Baseline deep glandular atypia in Barrett’s metaplasia that still falls within the spectrum that is negative for dysplasia. This baseline glandular atypia with nuclear enlargement, stratification and hyperchromasia occurs commonly in the basal regenerative compartment of metaplastic Barrett’s epithelium. It can be quite marked, as in this example, especially when viewed in comparison to often adjacent non-metaplastic and entirely bland gastric cardiac glands (A, black arrow). The crucial criterion to differentiate this deep baseline glandular atypia simulating dysplasia is its maturation onto the surface of the mucosa, where the atypical nuclear features are lost and the nuclei mature to a small, bland and non-stratified appearance (B, black arrowhead) (H&E; A: ×300, B: ×200).
clear size should be no more than 1∼2 times the size of normal lamina propria cell nuclei, such as fibroblasts, endothelial cells or inflammatory cells. Dysplastic nuclei are characteristically greater than twice these internal size markers. This relative measurement takes into account the vagaries of tissue fixation, processing, sectioning and staining, by assessing epithelial nuclear size in relation to an internally normalized control cell population.

Reactive cytologic alterations in the presence of active inflammation (defined as intraepithelial granulocytic inflammation), with or without granulation tissue, are also part of the spectrum of negative for dysplasia, if the cytologic changes mature to the surface of the biopsy and the glandular architecture remains intact. Surface maturation is critical to distinguishing inflammatory reaction from dysplasia. Reactive inflammatory change often also produces a more “open” (less hyperchromatic) nuclear chromatin structure along with cytoplasmic mucin depletion. Mucin depletion is commonly observed in dysplasia as well, so that care must be taken not to over interpret mucin loss.

Regenerative cytoarchitectural alterations in relation to erosion or ulceration may also be classified as negative for dysplasia. Regenerative change consists of a surface monolayer or near monolayer of cells overlying eroded/ulcerated mucosa that is either devoid of deeper glands or shows prominent gland loss with replacement by granulation tissue. The surface regenerative cells may have variably atypical and even bizarre cytologic abnormalities, but in general they maintain a characteristic monolayer growth pattern and usually have abundant cytoplasm as well as enlarged nuclei. At times, the monolayer will contain multinucleated cells or will appear to lack cell borders and form a syncytium along the surface. Despite the sometimes extreme cytologic abnormalities, regenerative change is so stereotypical by growing as a monolayer over stroma devoid of glands, that it can still be readily diagnosed as negative for dysplasia if it fulfills the indicated criteria.

2) Indefinite for dysplasia

The glandular architecture of epithelium that is indefinite for dysplasia is intact or may exhibit mild crowding or mild loss of orderly architecture. The cytologic changes usually show partial but incomplete maturation onto the mucosal surface (Fig. 8). Goblet or columnar cell mucin is often diminished and may be absent. So-called “dystrophic” goblet cells may be seen, in which the goblet mucin vacuoles are disorganized or jumbled or the goblet mucin vacuoles fail to communicate with the luminal surface. In the presence of pronounced inflammation or erosion/ulceration, the cells may be of markedly atypical and lack surface maturation altogether. Numerous mitotic figures may be present.

Controversy exists over the correct classification of marked crypt atypia accompanied by a normally maturing overlying epithelial surface. The concept of “crypt dysplasia” has been proposed for this finding and it is noteworthy that despite its existence in the literature for at least ten years, it remains almost entirely the contribution of a single group of investigators. Serious caution is recommended in applying this concept relative to the characteristic atypia of virtually all metaplastic crypts that remain negative for dysplasia is Barrett’s esophagus. Metaplastic mucosa is not normal intestinal-type epithelium. It is metaplastic and as such is an abnormal mucosa at its baseline. Direct comparison of normal small bowel or colonic intestinal mucosa (non-metaplastic) to Barrett’s metaplastic mucosa (or gastric intestinal metaplasia for that matter) of even the most bland variety, will disclose the considerable atypia of intestinal metaplasia that remains negative for dysplasia. The concept of “crypt dysplasia”; however, is based on the contention that dysplasia must logically involve stem cells in order to persist within the mucosa and as such could be limited to the crypt region at a highly transitory phase. This concept does not incorporate the rapid turnover of gastrointestinal mucosa, which occurs continually every few days normally and is markedly elevated in Barrett’s mucosa. The rapid turnover combined with the complexity and rarity of neoplastic progression, make it improbable that “crypt dysplasia” will be observed with significant frequency.

Of far greater importance than these dynamic mucosal turnover considerations is that the concept of “crypt dysplasia” contradicts the most important criterion available to pathologists to distinguish dysplasia from the far more commonplace baseline crypt atypia of Barrett’s metaplasia
that is negative for dysplasia, or the inflammatory/reparative change so common in Barrett’s esophagus. In other words, “crypt dysplasia” fully contradicts the criterion of surface maturation to exclude dysplasia. At its core, Barrett’s esophagus is an injurious and inflammatory disease of the esophagus, with great propensity to mimic dysplasia. “Crypt dysplasia” therefore poses a serious diagnostic problem by detracting from the importance of surface maturation for the exclusion of dysplasia. The authors of this concept do not provide criteria to help with this distinction, nor are observer variability data provided in comparison to baseline or inflammatory atypia that remains negative for dysplasia. While true “crypt dysplasia” undoubtedly exists, it cannot be overly stressed that it is a very minor exception to the rule that surface maturation excludes dysplasia in the great majority of biopsies. As a practical matter, dysplasia limited to crypts is only diagnosed once or twice annually by this author among more than 5,000 Barrett’s biopsies reviewed. Thus, the great majority of atypical crypts with surface maturation should be diagnosed as negative for dysplasia (Fig. 14), less commonly as indefinite for dysplasia, and only extremely rarely as “crypt dysplasia” with surface maturation.

When there is doubt as to the significance of the epithelial abnormalities in a biopsy, the diagnosis of “indefinite for dysplasia” should be made. The wide range of appearances of mucosa in the indefinite category is belied by this simple and solitary name, creating the false impression that this is only a single type of epithelium. In reality there may be hundreds or even thousands of variations on the cytoarchitectural changes in the indefinite for dysplasia category. Pathologists strive to classify the vast array of alterations in this category into the single and utterly limited designation of indefinite for dysplasia. Understandably, therefore, this leads to marked inter- and intra-observer diagnostic variability, which is not surprisingly highest in the indefinite category among the entire grading scheme of Barrett’s esophagus. Much of this problem could be avoided if endoscopists would refrain from biopsying obviously inflamed mucosa and instead would get patients into inflammatory remission through aggressive anti-reflux treatment prior to surveillance biopsies.

It is useful to consider four general categories to help organize the possible alterations of changes that are indefinite for dysplasia. Reactive inflammatory change, especially in biopsies taken from the edges of ulcers may be indistinguishable from dysplasia. In cases with marked inflammation or ulceration, the atypia may be so severe that not only is dysplasia in contention, but even carcinoma may be suspected. Surface maturation is usually absent in this form of indefinite for dysplasia, which is the most concerning and difficult general category in the indefinite group (Fig. 15). Cautionary language should be provided to clinicians for this type of indefinite biopsy.

**Fig. 15.** Indefinite for dysplasia in Barrett’s esophagus with severe cytologic changes (A) in the setting of active ulceration (B). The cytologic changes here are concerning not only for dysplasia but even adenocarcinoma (A). The severe active inflammation with ulceration that accompanies the atypia here on lower magnification (B) are notorious for producing changes that mimic dysplasia and are strong justification for a diagnosis of indefinite for dysplasia. Commentary are appropriate that the findings are very concerning for possible advanced neoplasia, and that aggressive anti-reflux therapy followed by multiple additional biopsies may be helpful (H&E; A: ×400, B: ×200).
that the findings are very concerning for dysplasia or even carcinoma and that repeat biopsies should be obtained after intensive anti-reflux therapy. Followup biopsies will often show resolution of the abnormalities, affirming the use of caution in the setting of marked inflammation.

Cases with more mild inflammatory change and atypia, which are likely negative for dysplasia, form the second major general type of indefinite for dysplasia. These biopsies are far less worrisome. The inflammation offers a probable explanation for mild changes but the cytologic abnormalities do not entirely mature onto the surface so that the diagnosis of indefinite for dysplasia is appropriate here as well.

The third category of general change that may be classified as indefinite involves non-inflamed Barrett’s epithelium that is not negative for dysplasia, but yet has insufficient alterations for a diagnosis of unequivocal low-grade dysplasia. A common issue again is that the cytologic alterations mature partially but incompletely as the cells extend onto the surface of the biopsy and/or there is only mild architectural concerns (Fig. 8). These alterations are presumably on the pathway of neoplastic progression, but have not yet crossed the threshold for low-grade dysplasia.

Mechanical issues comprise a fourth general type of change that may be classified as indefinite for dysplasia, such as when the biopsy surface is denuded or the biopsy is maloriented and the surface is otherwise unavailable for evaluation. Crush and cautery or other mechanical artifacts as well as poor histologic preparations or tiny biopsy size can also be placed into this category.

Fortunately, the distinction between indefinite and low-grade dysplasia has no practical clinical significance, for both categories are essentially managed in the same manner clinically, namely by conservative continued periodic surveillance with diligent anti-reflux therapy to eliminate as much causative and obscuring inflammation as possible. On the other hand, while the distinction between indefinite for dysplasia and low-grade dysplasia is not clinically important, the distinction between indefinite changes and those that remain negative for dysplasia is essential. Specifically, less surveillance with longer intervals between endoscopies apply to the negative category. Furthermore, the great majority, estimated at 90% or greater of Barrett’s patients should fall within the negative category, varying only by the diligence and effectiveness of anti-reflux therapy. As such, even a minimal to modest over diagnosis of the indefinite for dysplasia category will greatly inflate unnecessary surveillance rates and patient anxiety. Thus, endoscopists are strongly advised to achieve inflammatory remission prior to surveillance biopsies and pathologists are strongly advised to focus on distinguishing the negative for dysplasia category from the indefinite category rather than the less important distinction between indefinite change and low-grade dysplasia.

3) Low-grade dysplasia (intestinal type)

The crypt architecture in low-grade dysplasia tends to be preserved, and distortion, if present, is mild to moderate. It should be noted that the crypts in low-grade dysplastic epithelium may still be relatively more abnormal cytologically than the surface, but unequivocal dysplastic change will extend fully onto the surface as well. Involvement of the surface as well as the crypts in low-grade dysplasia is the major criterion for distinguishing low-grade dysplasia from the negative and indefinite categories (Fig. 7).

The nuclear cytology of low-grade dysplasia (Fig. 7) usually consists of stratified, elongated or pencillate shaped nuclei that are typically enlarged, measuring 2-fold or more greater than internal control lamina propria nuclei, such as small mature lymphocyte nuclei. Low-grade dysplastic nuclei are also usually hyperchromatic, crowded, and arranged in an overlapping and stratified configuration. Importantly for the distinction from high-grade dysplasia, the nuclei of low-grade dysplasia maintain nuclear polarity. This is defined by the maintenance of a perpendicular orientation of the long axes of the stratified nuclei to the basal lamina. The nuclei in low-grade dysplasia are also orderly and parallel to each other within the epithelial layer as the remaining aspect of maintenance of nuclear polarity.

Abnormal mitotic figures may be present but are a soft criterion for diagnosing dysplasia. Mitotic activity itself is unhelpful, even if the mitoses extend out of the basal re-
generative zone to occur near or at the surface. This is because Barrett’s metaplasia has an elevated proliferative rate at its baseline and is even more mitotically active in the setting of active inflammation. Atypical mitoses may be a bit more helpful, but even these are not specific to dysplasia.

Goblet or columnar cell mucin is often diminished and may be absent in any grade of dysplasia, including low-grade dysplasia (Fig. 7). Hypermucinous change may, however, accompany a minority of Barrett’s dysplasias. So-called “dystrophic” goblet cells with mucin vacuoles that fail to connect to glandular lumen, may be present in low-grade dysplasia as well. These cytoplasmic mucin-related features are never diagnostic of dysplasia on their own, as dysplasia is based on nuclear cytology and architectural change. However, the mentioned cytoplasmic findings can be helpful.

4) High-grade dysplasia (intestinal type)

Distortion of crypt architecture usually occurs and is frequently marked (Fig. 9), including branching and lateral budding of crypts, asymmetrical gland shapes, marked glandular crowding with little to no intervening lamina propria, villiform configuration at the mucosal surface, and/or intraglandular bridging of epithelium to form cribriform patterns with multiple lumens confined to a single gland. Dilated glands containing necrotic or apoptotic luminal debris are a markedly concerning architectural change observed in high-grade dysplasia (Fig. 10). If this feature is seen in multiple glands per biopsy fragment, a warning that intramucosal carcinoma cannot be excluded is appropriate. While nuclear features usually take precedence over architectural change in determining the grade of dysplasia, dilated glands with luminal debris or extremely crowded back to back glands with high-grade dysplasia without intervening lamina propria (Fig. 11) are notable exceptions to this general principle that cytology is the most important factor. Such severe architectural changes typically indicate a diagnosis of high-grade dysplasia, despite more bland cytology.

Extremely crowded high-grade dysplasia without significant intervening stroma and can also border on intramucosal invasion (Fig. 11). Where the continuum of high-grade dysplastic alterations end and intramucosal carcinoma begins is difficult to assess, if not impossible. Combined with abnormal cytology, such extremely crowded architectural changes and/or numerous dilated glands with necrotic luminal debris make it difficult to be certain that intramucosal carcinoma is absent. In such cases, the terminology coined by the late Dr. Roger C. Haggitt is an honest and appropriate appraisal: namely, “high-grade dysplasia with marked distortion of glandular architecture such that intramucosal adenocarcinoma cannot be excluded”.¹ The major difficulties in differentiating high-grade dysplasia from early carcinoma are considered further below.

Cytologically, high-grade dysplasia has greater nuclear enlargement, more irregularity of nuclear membranes, more pleomorphism and more hyperchromasia than low-grade dysplasia. These are all continuous variables, so that unfortunately, precise diagnostic cutoffs cannot be defined. Pathologists must therefore acquire their own thresholds through high volume and continual experience. Most importantly, high-grade dysplasia will usually exhibit loss of nuclear polarity, such that the long axes of the nuclei are no longer oriented perpendicularly to the basement membrane (as in low-grade dysplasia), and the orderly parallel orientation of one nucleus to the next is lost (Fig. 9). With loss of polarity, the nuclei often also assume a more rounded and less pencillate shape in comparison to low-grade dysplasia (Fig. 9). Loss of nuclear polarity is the most objective criterion for distinguishing low and high-grade dysplasia because it is a more dichotomous, yes or no, criterion relative to all of the other continuous variables discussed. As such, heavy emphasis on the criterion of loss of nuclear polarity is appropriate for high-grade dysplasia.

As a rule with virtually no exceptions, nuclear abnormalities extend from the base of the crypts onto the surface epithelium in high-grade dysplasia. It should be noted that the crypts may still be slightly more atypical than the surface even in high-grade dysplasia, but if there is a significant degree of maturation with dysplasia extending onto the surface, then low-grade dysplasia is probably more appropriate than high-grade dysplasia.

As in the indefinite and low-grade dysplastic categories, goblet cell and columnar cell mucin are usually dimin-
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5) Intramucosal adenocarcinoma

Well-defined criteria are not widely accepted for this category of neoplastic progression. However, because the esophageal lamina propria contains a rich lymphatic network, intramucosal carcinoma has a defined, albeit very low rate of lymph node metastasis of approximately 1–2%.[38-41] It is therefore a biologically malignant diagnostic category and attempts to separate it from premalignant dysplasia are important. Unfortunately, it can be extremely difficult to distinguish this earliest invasive category from high-grade dysplasia on the one hand and more deeply invasive adenocarcinoma on the other, particularly using endoscopic forceps biopsies. Suggested criteria for the diagnosis of intramucosal carcinoma include: 1) numerous individual invasive cells invading into the lamina propria (numerous refers to at least three to five such single cells, which helps to exclude sectioning artifact in which only the uppermost cell of a gland is sectioned so that it falsely appears to represent a single cell invading the lamina propria); and/or 2) sheets of malignant cells without gland formation invading the lamina propria; and/or 3) angulated, infiltrative and/or abortive glands invading the lamina propria; and/or 4) the so-called “never-ending-gland” pattern of complex anastomosing glands infiltrating the lamina propria (Fig. 12).[41]

6) Adenocarcinoma with at least submucosal invasion

If a well-defined desmoplastic stroma with infiltrating malignant glands can be identified separately from inflammatory stromal changes of scarring and granulation tissue, the diagnosis of at least submucosal invasive adenocarcinoma can be made (Fig. 16). These distinctions can be very difficult, especially on the basis of endoscopic forceps biopsies.[41] This category is difficult and at times impossible to distinguish from high-grade dysplasia and intramucosal adenocarcinoma, a problem that is considered further below.

3. Diagnostic criteria for grading gastric foveolar-type Barrett’s dysplasia

Because Barrett’s epithelium is an admixture of goblet cells, pseudoabsorptive cells and gastric foveolar cells, it should come as no surprise that dysplasia arising in Barrett’s may develop from any one of these cell types and have varying appearances and criteria because of this. In fact, precancerous dysplasia in Barrett’s esophagus indeed does have two distinct histologic subtypes, parallel ing what also occurs in gastric epithelial dysplasia. The far more common “adenomatous” or intestinal-type dysplasia in Barrett’s esophagus is widely recognized and has been defined above. However, the second category of Barrett’s dysplasia, namely, gastric foveolar-type dysplasia, has gone virtually unrecognized in the Barrett’s literature. It is poorly characterized with regards to its prevalence, histologic features, and natural history. Diagnostic criteria were first described by Mahajan et al.[41]. Due to its dis-

Fig. 16. Invasive adenocarcinoma with at least submucosal invasion in Barrett’s esophagus, showing infiltrative, angulated glands within a well-developed desmoplasia showing the characteristic myofi-broblastic spindled stroma and lack of vessels (H&E, ×300).
tinctly different morphology, gastric foveolar-type dysplasia does not readily conform to standard intestinal-type dysplasia diagnostic or grading criteria. As such, and despite being a minority, it can pose serious difficulties for the diagnostic pathologist.

The prevalence, diagnostic criteria, and natural history of gastric-type Barrett’s dysplasia were systematically evaluated in 1,854 endoscopic biopsies from a cohort of 200 consecutive Barrett’s dysplasia patients. In this study, the prevalence of Barrett’s gastric-type dysplasia was 15% at the patient level (30 of 200 patients) and 19.5% at the biopsy level (166 of 852 dysplastic biopsies). As the major distinguishing diagnostic criterion, gastric-type dysplasia uniformly showed non-stratified, basally oriented nuclei (Fig. 13), in comparison to the highly stratified nuclei of intestinal-type Barrett’s dysplasia. As such, loss of nuclear polarity, as the most objective criterion to distinguish intestinal-type low and high-grade dysplasia, cannot be applied to gastric-type dysplasia. Rather, discriminatory features between low and high-grade gastric-type dysplasia include increased nuclear size (Fig. 13) with a high-grade dysplasia cutoff by ROC analysis approximating 3–4 times the size of a mature lymphocyte (Fig. 13B), providing an optimal sensitivity, specificity, and area under the curve of 0.78, 0.90, and 0.90 (95% CI 0.87, 0.93), respectively. Crowded, irregular glandular architecture (P<0.001) was more common in high-grade lesions (P<0.001), as was eosinophilic and oncocytic cytoplasm relative to mucinosus cytoplasm (P<0.001), prominent nucleoli (P<0.001), mild nuclear pleomorphism (P<0.001) and villiform architecture (P<0.001; Fig. 13). During follow-up, 64% of patients (7 of 11) with pure gastric and 26.3% (5 of 19) with mixed gastric and intestinal dysplasia underwent neoplastic progression, supporting that gastric-type dysplasia is a real precursor of Barrett’s neoplasia. The recognition of Barrett’s gastric-type dysplasia and use of the proposed grading criteria in this initial study should promote better diagnostic classification of the Barrett’s neoplastic spectrum. The summary diagnostic criteria for gastric-type dysplasia in Barrett’s esophagus are included in Table 1.

Of major importance, goblet cells were present in all 200 consecutive Barrett’s dysplasia patients in this series (100%), affirming this defining feature of Barrett’s esophagus. This also documents that gastric-type dysplasia relates only to the recognition and grading of Barrett’s dysplasia and not to the definition of Barrett’s esophagus itself. Gastric-type foveolar cells are present in the distal most esophageal epithelium in essentially all patients with GERD and in Barrett’s esophageal mucosa. This of course

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<th>Gastric-type dysplasia grade</th>
<th>Criteria</th>
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<tr>
<td>Negative for dysplasia</td>
<td>- Uncrowded glands within abundant lamina propria</td>
<td>- Regular nonstratified bland epithelial cell nuclei that are &lt; 1–1.5 times the size of a small mature lymphocyte</td>
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<td>Indefinite/low-grade dysplasia (Fig. 13A)</td>
<td>- Uncrowded to minimally crowded glands without cribri-forming or glandular dilatation containing luminal necrotic debris - Obscuring biopsy artifacts (small size, inflammatory change, crush, inadequate histology) preventing an unequivocal diagnosis of dysplasia</td>
<td>- Regular nonstratified epithelial cell nuclei that are 2–3 times the size of a small mature lymphocyte - Patchy but mild nuclear pleomorphism, if present at all - Patchy nuclei</td>
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<td>High grade dysplasia (Fig. 13B)</td>
<td>- Crowded glandular architecture with little to no intervening lamina propria - Cribriform glands and dilated glands containing necrotic luminal debris may be present</td>
<td>- Full thickness mucosal involvement - Regular nonstratified epithelial cell nuclei that are 3–4 times or greater than the size of a small mature lymphocyte - Mild to moderate nuclear pleomorphism (note: marked nuclear pleomorphism is not typical of any type of gastric-type dysplasia) - Regular nuclei - Full thickness mucosal involvement</td>
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renders gastric-type mucosa itself completely nonspecific for defining Barrett’s separately from GERD. It might be tempting to misconstrue the concept of gastric foveolar-type dysplasia in Barrett’s as evidence that Barrett’s esophagus should also be redefined to include any type of glandular epithelium in the esophagus, including gastric-type without goblet cells. This would be a serious error, based on the non-specificity of gastric mucosa for distinguishing GERD and Barrett’s and the extremely high prevalence of GERD without a significant cancer risk. Barrett’s most definitely and always contains gastric-type cells, which in turn generate the ~15% per patient prevalence of gastric-type Barrett’s dysplasia, but gastric-type epithelium cannot be used to define Barrett’s esophagus. To do so would dilute the risk of esophageal adenocarcinoma from its already very limited level in Barrett’s esophagus, histologically defined by goblet cells, to a level far below that necessary for effective cancer screening if all of GERD were to be included in the definition of Barrett’s. Improved cancer screening in Barrett’s mandates increased specificity to include only the highest risk patients, and not the greatly diminished specificity that would result from redefining Barrett’s esophagus to include gastric mucosa without goblet cells. Thus, the definition of Barrett’s esophagus and the requirement for goblet cells must remain separate from the diagnosis Barrett’s gastric-type dysplasia.

4. Distinction between foveolar Barrett’s dysplasia and reactive cardia in GERD

The distinction between Barrett’s gastric-type dysplasia and reactive gastric cardiac mucosa in GERD has been systematically studied, and several diagnostic criteria are very useful (Table 2). A total of 3,698 endoscopic biopsies from a cohort of 461 Barrett’s patients were reviewed to identify 43 patients (80 biopsies) with gastric-type Barrett’s dysplasia (13 had low-grade gastric-type dysplasia and 30 had high-grade gastric-type dysplasia). These were compared histologically to biopsies from 60 GERD patients with markedly inflamed and reactive gastric cardiac mucosa. The findings are shown in the table below. Overall, nuclear stratification and surface predominant or “top-heavy” atypia accompanied by non-crowded, villiform architecture are features that most reliably distinguish marked reactive cardiac atypia in GERD from gastric-type Barrett’s dysplasia with its full-thickness, non-stratified, crowded glands (Fig. 6, 13).

5. General problems in the diagnosis of dysplasia in Barrett’s esophagus

1) Sampling error

Mapping studies of esophagectomy specimens have shown that dysplasia can involve a highly variable extents of esophageal mucosa with or without endoscopically visible lesions or cancer. Neoplastic mucosa is frequently flat and invisible to the endoscopist, although evolving endoscopic imaging techniques show promise for improved endoscopic targeting. At present, the endoscopist must still thoroughly sample the mucosa to avoid missing small areas of dysplasia or carcinoma. Four-quadrant, well-oriented, expertly prepared and interpreted jumbo biopsies taken at 2 cm intervals or less throughout the length of the Barrett’s segment are recommended, combined with additional biopsies of any endoscopic lesions. Shortening the biopsy interval to every 1 cm, based on a computerized modeling approach, has been recommended for patients with high-grade dysplasia who are maintained in endoscopic surveillance. Adherence to this or similar protocols in a research setting can produce excellent correlation between the pre-operative endoscopic diagnosis and the final diagnosis in the resected specimen, but the difficulty of transferring these research results to routine practice has also been emphasized.
2) Histologic spectrum of dysplasia with imprecise cutoffs

As the epithelial abnormalities in dysplasia form a continuous spectrum, from relatively mild atypism to overt dysplasia, the boundaries between the grades cannot be sharply defined. Thus, observer variation exists in the diagnosis and grading of dysplasia, particularly at the indefinite/low-grade interface. For this reason, the categories of indefinite and low-grade are combined in most endoscopic protocols for practical clinical management purposes. Fortunately, at the high end of the spectrum of abnormalities, namely separating high-grade dysplasia from the lower grades, there is excellent agreement by GI pathologists within and between observers. Similarly, there is good reliability for the diagnosis of negative for dysplasia as well. Distinction between gastric and intestinal type Barrett’s dysplasia may further improve diagnostic accuracy, especially considering the very different criteria for these two categories.

3) High-grade dysplasia versus carcinoma

When high-grade dysplasia develops, architectural distortion may reach a point at which the diagnosis of carcinoma is impossible to exclude with certainty on the basis of a superficial biopsy. This occurs when glands grow in a cribriform or dense “back-to-back” pattern, when they are closely packed together, or when dilated glands with luminal necrotic debris are present. High-grade dysplasia in the setting of an ulcer is another very serious risk factor for carcinoma. While high-grade dysplasia and intramucosal adenocarcinoma can be defined by criteria indicated earlier, application of these criteria can be quite challenging. Now that ablative therapies are entering the therapeutic armamentarium for Barrett’s high-grade dysplasia, and natural history data on dysplasia in Barrett’s esophagus reveal that continued endoscopic surveillance can be a safe option even for high-grade dysplasia, especially incident high-grade dysplasia, the precise distinction between high-grade dysplasia and carcinoma is becoming ever more important. This is increasingly the diagnostic threshold for esophagectomy.

Despite the increasing importance of distinguishing high-grade dysplasia from adenocarcinoma on biopsy specimens, only limited inter- and intra-observer variability data exist on this distinction. Pathologists are acutely aware that this interface in the morphologic spectrum of neoplastic progression in Barrett’s esophagus is the most challenging of all on biopsy samples. Data from a high volume Barrett’s practice at the Cleveland Clinic (Cleveland, OH, USA) reveal that the histologic distinction between high-grade dysplasia and more advanced neoplasia on biopsy samples is quite unreliable. This study assessed observer variability in 168 preoperative biopsies with at least high-grade dysplasia. Four diagnostic categories were defined, including high-grade dysplasia, high-grade dysplasia with marked distortion of glandular architecture not excluding carcinoma, intramucosal adenocarcinoma, and adenocarcinoma with at least submucosal invasion. Seven GI pathology subspecialists who all practiced in the high volume Barrett’s setting at the Cleveland Clinic made up the participants. Overall diagnostic agreement was poor (Kappa=0.30). Agreement for high-grade dysplasia versus more advanced neoplasia was only moderate (Kappa=0.46), while agreement for the other three defined categories was poor (Kappa=0.21, 0.31 and 0.14, respectively). These data call into serious question clinical management practices based on pathologists’ ability to differentiate high-grade dysplasia from carcinoma on forceps biopsy specimens. Fortunately, and as discussed further below in the section on preoperative staging of early Barrett’s adenocarcinoma, EMRs provide improved sensitivity and specificity over forceps biopsies, so the situation is not hopeless.

4) Squamous overgrowth

Successful medical or surgical anti-reflux therapy may be associated with some downward migration of the SCJ and the development of squamous “islands” within the Barrett’s mucosa; however, complete regression of all Barrett’s epithelium rarely occurs. Prior biopsy sites or mucosal resection sites, proton pump inhibitor therapy and ablative therapies have also been associated with the development of squamous islands and unfortunately, Barrett’s metaplastic epithelium may persist beneath the surface squamous mucosa. A major concern is that this could lead to an underestimation of the endoscopic extent of the Barrett’s mucosa lying beneath areas of squamous regrowth. It has become clear that patients...
undergoing non-surgical ablative or endoscopic resection therapies and/or continued surveillance must be biopsied over the entire original length of the Barrett’s segment, including areas of squamous regrowth. This is necessary to detect subsquamous neoplastic Barrett’s.44-49

Squamous overgrowth after ablative therapy has received considerable scrutiny.45,46,49 A prospective 5-year randomized trial of photodynamic therapy (PDT) for Barrett’s high-grade dysplasia controlled for all factors known to cause squamous overgrowth, most importantly proton pump inhibitor therapy and healing biopsy sites.49 The only variable between the two treatment arms in this study was PDT itself. Over 33,000 biopsies were analyzed for squamous overgrowth and no differences were found with the non-ablation control arm with continued biopsy surveillance and proton pump inhibitor therapy. The lack of difference was true on both a per patient (31% vs. 33% with squamous overgrowth) and per biopsy basis (1.2% vs. 2.2% with squamous overgrowth). Importantly, no cases with subsquamous Barrett’s neoplasia were missed through the practice of biopsying the original length of the Barrett’s segment in this study.49

Other studies suggesting that ablative therapies increase squamous overgrowth have not controlled for biopsy numbers or proton-pump inhibitor therapy, both of which are known to increase squamous overgrowth.45,48 Instead, the other reported ablation studies compared patients’ pre-ablation biopsies to their post-ablation biopsies,45,48 which does not control for these likely causative variables. An uncontrolled trial of 52 patients reported completely buried neoplasms at a rate of 17.3%, but it must be noted that almost 2/3’s of the patients in this trial had intramucosal and deeper adenocarcinoma at the start of the trial.45 Thus, this study is not directly comparable to the controlled trial of high-grade dysplasia only patients without visible lesions.49 Thus, the issue of squamous overgrowth is real, but as long as the original length of the Barrett’s segment remains in endoscopic surveillance, this phenomenon is clinically manageable.

In addition to the squamous overgrowth, most patients taking proton pump inhibitors develop parietal cell protrusions, or peculiar tongue-like projections or blebs of gastric parietal cell cytoplasm into the lumens of fundic glands, and many will also develop fundic gland polyps.50,51

6. Over diagnosis of high-grade dysplasia in Barrett’s esophagus

Barrett’s esophagus with high-grade dysplasia is a serious condition prone to over diagnosis by pathologists. As already mentioned the accuracy of this important diagnosis, with all of its attendant and major clinical consequences, is highly dependent upon the pathologist’s experience and continuous exposure to a high volume of Barrett’s material. Documentation of the magnitude of the problem of the over diagnosing high-grade dysplasia is provided by a multi-institutional international trial of ablation therapy in 208 patients with Barrett’s high-grade dysplasia.52 Prior to study enrollment, all potential patients had received a biopsy diagnosis from their local hospital of Barrett’s esophagus with high-grade dysplasia. In order to enroll 200 patients with actual Barrett’s with high-grade dysplasia, at total of 485 patients had to be screened (Table 3). Thus, there were 237 or 49% of patients who were thought to have high-grade dysplasia but ultimately did not. This was uncovered through a rigorous endoscopic and pathologic screening protocol, including review of the original material thought to have high-grade dysplasia, combined with a new protocol endoscopy using 4-quadrant jumbo biopsies every 2 cm. The 237 patients (49%) who did not qualify for the trial had a variety of pathologies (Table 3).

As shown in Table 3, 18 patients who failed to qualify for the ablation trial had only inflamed gastric mucosa or GERD. These patients did not even have Barrett’s esophagus, much less Barrett’s esophagus with high-grade dysplasia and all were facing esophagectomy. These and the

### Table 3. Reinterpretation of High-Grade Dysplasia in Barrett’s Esophagus in 237 of 485 Patients

<table>
<thead>
<tr>
<th>Reinterpreted diagnoses</th>
<th>Patient No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric only</td>
<td>18 (4)</td>
</tr>
<tr>
<td>Other, not Barrett’s</td>
<td>4 (&lt;1)</td>
</tr>
<tr>
<td>Barrett’s negative</td>
<td>35 (7)</td>
</tr>
<tr>
<td>Barrett’s indefinite for dysplasia</td>
<td>61 (12)</td>
</tr>
<tr>
<td>Barrett’s low-grade dysplasia</td>
<td>79 (16)</td>
</tr>
<tr>
<td>Barrett’s carcinoma</td>
<td>45 (9)</td>
</tr>
<tr>
<td>Total</td>
<td>237 (49)</td>
</tr>
</tbody>
</table>
rest of a total of 194 patients who had lesser diagnoses than Barrett’s with high-grade dysplasia were all being considered for esophagectomy. This underscores the problem of the over diagnosis not only of Barrett’s esophagus itself, as reviewed extensively earlier, but also of Barrett’s with high-grade dysplasia.

The over diagnosis of Barrett’s esophagus with high-grade dysplasia can be reduced by attention to several diagnostic issues. First, the baseline atypia of the regenerative glands in Barrett’s mucosa needs to be recognized as part of metaplastic epithelium that in negative for dysplasia. Second, high-grade dysplasia should rarely if ever be diagnosed if there is surface maturation of the overlying epithelium. Markedly inflamed and particularly eroded and ulcerated Barrett’s mucosa will readily simulate high-grade dysplasia cytologically. Especially if architectural changes of high-grade dysplasia are lacking, great caution should be exercised in such markedly inflamed biopsies, by diagnosing them as indefinite for dysplasia with cautionary comments about obscuring inflammation.

Third, the best and most objective criterion to separate the low and high-grade dysplasia is loss of nuclear polarity in high-grade dysplasia.

The nuclear polarity criterion is not 100% sensitive, as there are examples of high-grade dysplasia without loss of polarity, although these are uncommon exceptions. Reparative inflammatory change can also result in loss of nuclear polarity, making this criterion less than 100% specific as well, but in such cases the presence of erosion or ulceration and the lack of high-grade glandular architectural alterations are often very helpful in distinguishing these possibilities.

As high-grade dysplasia is rare in unselected patients with Barrett’s esophagus, and because most pathologists do not therefore have the opportunity to study many examples of it, pathologists who do not review a high volume of Barrett’s biopsies would be wise to seek a second opinion regarding the diagnosis of high-grade dysplasia before aggressive management is undertaken.

1) Reactive gastric mucosa

One of the more frequent mimics of Barrett’s dysplasia is gastric cardiac mucosa with reactive change. Such reactive change frequently develops from GERD and gas-tritic involvement of gastric mucosa in the lower esophageal region and hiatal hernias. This is a particularly unfortunate mimic, as it not only renders a false positive diagnosis of Barrett’s, with all of its attendant problems, but it may also cause the patient to undergo an unnecessary esophagectomy for a false positive diagnosis of high-grade dysplasia. The reasons for this mimic are at least 2-fold. First, damaged gastric cardiac mucosa commonly develops reactive mucin depletion. Mucin loss is also common in dysplastic Barrett’s epithelium. Thus, both of these epithelia tend to lack goblet cells or any other mucin production in the remaining columnar epithelial cells, so that this differentiating cytoplasmic feature is commonly absent. Second, reactive gastric mucosa may have marked cytologic atypia. The atypia of benign reactive gastric mucosa may in fact be worse than the atypia of dysplasia or even of cancer. Due to these issues, it is of no surprise that reactive gastric cardiac mucosa may be difficult to differentiate from Barrett’s dysplasia.

A useful criterion to distinguish reactive gastric cardiac mucosa from Barrett’s dysplasia involves the regenerative compartments of these differing epithelia (Fig. 6). The basal glands of gastric mucosa are characteristically not mitotically active. This is because the regenerative zone of gastric mucosa resides in the foveolar neck region, generally located in the upper mid-region of gastric mucosa, rather than within the deep glandular compartment as is typical of intestinal epithelium. Another diagnostic gastric feature is the presence of parietal cells. The deeper glands in reactive gastric mucosa also tend to retain most and commonly all of their mucin (Fig. 6B, C). Further, the mucinous cells within the deep glands (and the surface for that matter) involve the entire gland or crypt in a linear continuous fashion, rather than as scattered mucinous goblet cells typical of metaplastic mucosa. Reactive gastric mucosa may also retain some surface mucin in the form of markedly shortened but back-to-back foveolar mucin caps covering the surface of the biopsy (Fig. 6A). These features in combination with bland mitotically inactive mucinous glands are the best criteria for recognizing reactive gastric mucosa rather than Barrett’s or Barrett’s with dysplasia.

Dysplastic Barrett’s epithelium typically also shows loss
of goblet cell mucin and other features of cytoplasmic
differentiation, but the glandular compartment is charac-
teristically more atypical than the surface. This is in sharp
contrast to the opposite pattern in reactive gastric muco-
sa, where the deepest glands tend to be very bland and
the surface markedly atypical, the “top-heavy” pattern of
gastric atypia (Fig. 6A, B). Mitotic activity is also highest
in the regenerative deepest glandular compartment of
Barrett’s metaplastic epithelium, akin to the regenerative
compartment of intestinal epithelium elsewhere in the GI
tract, the “bottom-heavy” pattern of metaplastic in-
testinalized mucosa.

As in all areas of diagnostic pathology, it is not always
possible to differentiate reactive gastric mucosa from
Barrett’s with dysplasia or even high-grade dysplasia. In
these cases, a diagnosis of “atypical glandular epithelium
of uncertain origin (gastric versus Barrett’s) with alter-
ations indefinite for dysplasia” can be made, along with
requests for additional biopsies following aggressive an-
ti-reflux management to attempt to eliminate the obscuring
reactive inflammatory change.

7. Additional biomarkers in Barrett’s esophagus

Although many genetic abnormalities have been docu-
mented in Barrett’s esophagus, these tend to correlate
with advancing histologic grades of neoplastic progres-
sion. To date, long-term, prospective studies to de-
termine the potential clinical utility of these single gene
or gene panel markers have not proven their additional
utility beyond histology.

It has also been suggested that p53 in particular and
other markers could be useful to differentiate reactive change from true dysplasia. This question generally re-
volves around biopsies in the indefinite to low-grade dys-
plasia range of the neoplastic spectrum, as high-grade
dysplasia is obvious histologically. Aggressive clinical
management in these lower grade dysplasia patients is not
in question; indefinite to low-grade dysplasia changes are
managed conservatively by continued surveillance. There-
fore, the additional cost of p53 analysis in this setting is
difficult to justify. It is undoubtedly true that no patients
have ever been referred for esophagectomy or any other
high-risk therapy for findings other than a histologic di-
agnosis of dysplasia or cancer in Barrett’s esophagus. For
all of these reasons, neither p53 nor any other reported
 genomic marker can be recommended for diagnostic pur-
poses at this juncture. This may and hopefully will
change in the future, but the current standard of care
continues to rely upon histologic diagnosis.7,8

8. Significance and management of dysplasia
in Barrett’s esophagus

Management of patients with dysplasia complicating
Barrett’s esophagus presents a difficult task.7,8 The options
for high-grade dysplasia are the most controversial and
have broadened from esophagectomy alone to also in-
clude ablative therapies, EMR and continued intensive bi-
opsy surveillance. Published clinical management guide-
lines exist,5-8 which are partially data driven but still
largely empiric.

1) High-grade dysplasia as a marker for unsampled
carcinoma

When high-grade dysplasia is detected for the first time
in a patient with Barrett’s esophagus, early re-endoscopy
with extensive biopsies should be done to rule out a co-
existing early carcinoma.53-55 This concept has been
termed “the hunt”53 and is of critical significance if
non-surgical options are to be considered. Extensive sam-
pling of the mucosa is essential, as early carcinomas may
not be recognizable to the endoscopist and can be very
focal. Due to these sampling error issues, it has been sug-
gested that additional biopsies should be increased to 4
biopsies taken every 1 cm (as apposed to every 2 cm) in
the Barrett’s segment in patients with high-grade dyspla-
sia, and performed on repeated and shortened surveil-
ance intervals.40,53 The logic behind this increased sur-
veilance if non-surgical options are considered is that in
patients with a reasonable estimated operative risk, re-
section of early carcinomas provides an opportunity for
cure, including cure of early metastatic disease to esoph-
ageal lymph nodes,55-57 which non-surgical approaches do
not address.

Patients with an endoscopic biopsy diagnosis of
high-grade dysplasia, but not carcinoma, who have un-
dergone esophagectomy, have been reported to have a
relatively high prevalence of endoscopically unrecognized
carcinoma in resection specimens. This has lead to the conclusion that high-grade dysplasia is a “marker” for co-existing adenocarcinoma, but this conclusion has in part been based on single endoscopies or very small numbers of patients with limited biopsies, or patients with advanced symptomatic disease or endoscopic findings suggestive of carcinoma. When thorough endoscopic biopsy sampling is carried out by experienced gastroenterologists and pathologists according to the “hunt” protocol, biopsies can accurately determine whether or not a clinically unsuspected carcinoma accompanies dysplasia.

2) Natural history of high-grade dysplasia and continued surveillance

The natural history of neoplastic progression requires large numbers of patients followed for long enough periods using adequate numbers of biopsies to achieve a high degree of diagnostic confidence. Pertinent questions with direct impact on patient management include whether all dysplasia inevitably progresses to cancer, and over what time period. To begin to answer these critical questions, multiple large cohorts of patients with Barrett’s esophagus fulfilling these criteria have been published, including that of the Hines VA Hospital in Chicago and the University of Washington in Seattle. Based on these important data, we have begun to understand the natural history of neoplastic progression in Barrett’s esophagus, upon which to base data-driven management decisions.

At the Hines VA program, a total of 1,099 patients diagnosed with Barrett’s esophagus over a 20-year period were studied. A total of 79 patients (7.2%) in the cohort had high-grade dysplasia, of which 34 had prevalent high-grade dysplasia (present at the first endoscopy) and 45 had incident high-grade dysplasia (detected during surveillance and therefore probably earlier in their natural histories). Of the 75 who remained without detectable cancer during the first year of intensive biopsy surveillance, termed “the hunt”, only 12 patients (16%) developed cancer over a mean of 7.3 years of surveillance. Further, 11 of the 12 who were compliant with the surveillance protocol were considered cured of their early cancers by surgical or ablative therapy.

In the 15-year prospective longitudinal study at the University of Washington, a total of 327 patients were evaluated by rigorous surveillance endoscopy for progression from their baseline alterations. Median surveillance intervals were 24.4 months for baseline negative histology, 18.2 months for indefinite histology, 15.7 months for low-grade dysplasia, and 4.6 months for high-grade dysplasia. Mean and medium follow-up periods were 3.9 and 2.4 years, respectively. Overall, a total of 42 patients developed cancer and 35 of these developed it within 5 years of their first endoscopy. No patient with negative, indefinite or low-grade dysplasia with normal flow cytometric studies developed cancer within 5 years; this patient subset made up two-thirds of the entire cohort. No biomarkers were more powerful or diagnostically significant than the histologic detection of neoplastic progression and high-grade dysplasia in particular, which had a 5-year cancer incidence of 59%.

3) Incident versus prevalent high-grade dysplasia

The data from the Seattle and Hines VA cohorts, show that lesions with less than high-grade dysplasia (metaplasia, indefinite, and low-grade dysplasia) have a very low rate of progression over even greater than 10 years of follow-up. These data further document that high-grade dysplasia itself does not inexorably progress to cancer. In fact, these data demonstrate that one can increase the margin of safety if continued surveillance is performed for incident rather than prevalent high-grade dysplasia. Specifically, incident high-grade dysplasia is discovered after the patient has been under surveillance with adequate biopsy sampling for some period of time. The Seattle group showed that this type of high-grade dysplasia has only an ~20% progression rate to cancer after 6 years of follow-up. An even lower rate was observed in the Hines VA trial. This is undoubtedly because incident high-grade dysplasia is diagnosed closer to the time at which it actually begins, and is therefore much more likely to be early in its natural history, which probably takes many years to decades to progress to cancer if it does so at all. Prevalent high-grade dysplasia, on the other hand, is diagnosed at the patient’s first endoscopy, when detection is heralded by symptoms. As such, prevalent high-grade dysplasia has been present for an unknown and likely much longer period of time than incident high-grade dysplasia. Prevalent high-grade dysplasia would thus be
expected to have a higher rate of progression to cancer, as it is further along in its neoplastic progression than incident disease. Such is indeed the case in the longitudinal data of both the Seattle and Hines VA cohorts.53,54

Based on these natural history data, rational, evidence-based patient management can be formulated. Especially for poor surgical candidates, or in those who have incident high-grade dysplasia, continued intensive surveillance is an option. The reproducibility of the discussed Seattle and Chicago data, and the non-trivial issue of the transportability of this type of intensive surveillance from the research setting into private practice both remain serious and important caveats.

4) Focal versus extensive dysplasia

Several studies with significant patient numbers and length of follow-up have investigated the significance of focal versus extensive high-grade dysplasia, hypothesizing that focal disease may have a lower risk of progression to carcinoma and thus might be managed more conservatively. Focal high-grade dysplasia is arbitrarily defined but in essence consists of a limited extent, such as 5 or fewer glands of high-grade dysplasia in one biopsy from adequately sampled Barrett’s segments using the Seattle protocol. The results and conclusions are conflicting. The Mayo Clinic group found that diffuse high-grade dysplasia had a 3.7-fold increased cancer risk in comparison to focal high-grade dysplasia (P=0.02). On the other hand, the Cleveland Clinic group, who followed all of these patients to esophagectomy as an added strength of that study, found no difference in cancer risk using the same definition of focal high-grade dysplasia. In the Cleveland Clinic series, adenocarcinoma was present in 5 of 7 patients (72%) with focal disease compared with 19 of 34 (54%) with diffuse disease (P=0.68). The presence of incident or prevalent disease was not addressed by these studies and may explain some of the discrepancies.

5) Endoscopic therapy of Barrett’s neoplasia

Non-surgical endoscopic methods for the treatment of Barrett’s neoplasia show promising results and are becoming the standard of care for patient subsets. These include a multitude of ablative therapies and EMR.6-8,61-77

PDT received FDA approval after completion of 2 and 5-year phase III prospective randomized placebo-controlled trials of 208 Barrett’s patients with high-grade dysplasia. At 5 years, PDT was significantly more effective than omeprazole alone in eliminating high-grade dysplasia (77% [106/138] vs. 39% [27/70], P<0.0001). Progression to cancer also showed a significant difference (P=0.027) with about half the likelihood of cancer occurring in PDT arm (21/138 [15%]) compared to the omeprazole alone control arm (20/70 [29%]), with a significantly longer time to progression to cancer with PDT (P=0.004). A high stricture rate was the most significant complication of PDT ablation therapy, which has limited its use. This work, however, undoubtedly paved the way for the many advances in ablative therapy that followed.

Radiofrequency ablation is a popular option in ablative techniques. In a large multicenter, randomized placebo controlled trial with 2-year follow-up data, the intention-to-treat analyses among patients with low-grade dysplasia showed complete eradication of dysplasia in 90.5% of the ablation group, as compared with 22.7% of the control group (P<0.001). Among patients with high-grade dysplasia, complete eradication occurred in 81.0% of the ablation group and only 19.0% of the control group (P<0.001). Overall, 77.4% of patients in the ablation group had complete eradication of intestinal metaplasia, as compared with 2.3% of the control group (P<0.001). Patients in the ablation group had less disease progression (3.6% vs. 16.3%, P=0.03) and fewer cancers (1.2% vs. 9.3%, P=0.045). The esophageal stricture rate was much reduced in comparison to PDT. Radiofrequency ablation has also been shown to be effective for Barrett’s intramucosal adenocarcinoma in a small trial of 36 patients (31 also had pre-ablation EMR) followed for a mean of 24±19 months, with 81% complete durable eradication of dysplasia and carcinoma and a 19% stricture rate.75

While no randomized trials of endoscopic therapy versus esophagectomy for the treatment of Barrett’s high-grade dysplasia exist, nor are they likely to be performed, a retrospective cohort study from the Mayo Clinic in Rochester, MN compared 129 patients receiving PDT plus EMR versus 70 treated with esophagectomy. The overall mortality in the ablation group was 9% over a median of 57.4 months and 8.5% over 63.4 months in the
esophagectomy group. Remarkably, there were no deaths from Barrett’s adenocarcinoma in either group. Extensive biopsy sampling was performed prior to surgery in all patients, using a 4 quadrant, jumbo forceps protocol every 1 cm throughout the length of Barrett’s esophagus, which is important prior to selecting an endoscopic management approach. Nonetheless, this comparison of ablative therapy to esophagectomy shows similar survival after nearly 5 years of follow-up and supports that ablative therapy is an important option for the management of high-grade dysplasia in Barrett’s esophagus.

EMR is the other advance in the non-surgical management of Barrett’s neoplasia. Innovations in endoscopic technology and technique have made it possible to resect sizeable, visible lesions endoluminally, despite the relatively challenging and narrow diameter of the esophagus relative to other gastrointestinal organs. The EMR procedure is now a mainstream clinical practice and a major step forward in the management of Barrett’s neoplasia. EMR is used for either visible Barrett’s dysplastic lesions or early adenocarcinomas, as defined by endoscopic ultrasound and biopsy. The endoscopist injects saline into the submucosa deep to the lesion to lift it into the lumen and enhance the likelihood of clear deep resection margins. If lesions fail to lift with saline injection, this is a poor prognostic sign that may preclude adequate deep margins and suggests more advanced invasion. Unlike forceps biopsies, EMR specimens need to be processed pathologically in an identical fashion to surgical specimens, with careful attention to designation and inking of lateral mucosal and deep submucosal margins. Endoscopists must strive to resect the specimen as a single piece with visibly free lateral and deep margins, rather than in a piecemeal resection fashion that obscures margin analysis. However, if the lesion fractures during resection or is removed in multiple pieces, the endoscopist can often orient the specimen in the fresh state by reassembling the pieces and inking the true margins with diagrams to instruct the pathologists. These are neoplastic resections rather than biopsies and endoscopists must be prepared to treat them like oncologic resections with careful orientation and designations of margins. Close endoscopic-pathologic correlation renders the best diagnostic outcome for the patient.

9. Staging and treatment of early esophageal adenocarcinoma

Staging and endoscopic treatment of early Barrett’s cancers have become ever more important as ablative and mucosal resection methods continue to advance. Important issues include the diagnostic yield from forceps biopsies versus EMRs, the staging significance imparted by the split muscularis mucosae in Barrett’s, and the major difference in the metastatic risk posed by T1a (intramucosal) versus T1b (submucosal) Barrett’s cancers.

As already indicated, Barrett’s adenocarcinoma is difficult to diagnose by endoscopic forceps biopsies. Even expert Barrett’s pathologists at the same institution sharing diagnostic criteria cannot achieve high levels of agreement using forceps biopsies. The critical question in the neoplastic spectrum is where high-grade dysplasia ends and intramucosal versus submucosal adenocarcinoma begin. The reason for the poor agreement histologically is obvious to surgical pathologists, as forceps biopsies are small and have proportionately higher degrees of crush and orientation artifact, making definitive diagnosis difficult to impossible. These diagnostic distinctions are nonetheless critical biologically. The risk of metastasis from intramucosal Barrett’s adenocarcinoma (TMN stage T1a) is only 1–2%, whereas the risk from submucosal Barrett’s adenocarcinoma (TMN stage T1b) is markedly higher at 30%. Fortunately, all is not lost, as EMRs provide much larger samples with proportionately less artifact and improved diagnoses. Multiple studies have shown that EMRs either upstage or downstage biopsy diagnoses from 15–48% of the time. Interobserver agreement in diagnosis by nine pathologists reviewing 25 patients’ materials was significantly improved using EMRs in comparison to biopsies (P=0.015). Thus, it appears clear that endoscopic pathologic staging should be based on mucosal resection specimens rather than forceps biopsies.

The issue of how the split muscularis mucosae in Barrett’s esophagus impacts staging and metastatic risk has been of great interest. The muscularis mucosae in the esophagus is normally the thickest of all regions of the entire gastrointestinal tract—this fact of normal histology
is often overlooked. Further, in Barrett’s esophagus, with its repeated inflammatory and ulcerative injuries, the muscularis mucosae is not only further thickened, it also frequently splits into two separate layers of smooth muscle with intervening fibroadipose, neural, and vascular tissue that appears similar to submucosal tissue. These two factors conspire to trick pathologists into thinking that the muscularis present in EMRs could even be muscularis propria (it can be that thick) or that the stroma deep to the superficial layer of a split muscularis mucosae is submucosal tissue. If Barrett’s cancers are falsely assigned to involve these structures, they will be over-staged as T2 or T1b cancers, when in fact they are still intramucosal T1a cancers with very low 1~2% risks of metastasis. One study revealed 60% over-staging of intramucosal cancers in relation to this split muscularis mucosae issue. As a matter of practical clinical significance, EMRs essentially never extend deep enough to obtain muscularis propria, so T2 staging simply is not viewable on EMRs. The most important research studies in this arena have been performed by the group at MD Anderson Cancer Center. They were the first to systematically report the phenomenon of the split muscularis mucosae in Barrett’s esophagus and later answered the critical question of whether invasion into the submucosa-like stroma intervening between a split muscularis mucosae would behave like intramucosal T1a cancer or have the far greater metastatic risk of submucosal T1b cancer. In their seminal study of 99 early esophageal cancers, if the depth of invasion extended into the true submucosa, nodal metastases developed in 10 of 30 patients (33.3%), whereas if it was limited to the mucosa or anywhere within a split muscularis mucosae, nodal metastasis developed in only 1 of 69 such patients (1.4%), with mean follow up of well over 10 years. These data strongly document that even with a split muscularis mucosae, cancers invading to this depth remain intramucosal cancers biologically (T1a) with very low rates of metastasis. This finding has major impact on the utility of endoscopic resections for early esophageal cancers.

CONCLUSION

Barrett’s esophagus is a chronic inflammatory disease of the lower esophagus that predisposes to glandular dysplasia and adenocarcinoma. The two most important challenges posed by this disorder are the over diagnosis of Barrett’s itself, and the over diagnosis of high-grade dysplasia in Barrett’s esophagus. Additional challenges have evolved over the past decade to include issues with intestinal versus gastric-type dysplasias, ablation therapy, EMRs, and the split muscularis mucosae. This review details the pitfalls that have been discovered and advances that have been made to achieve better diagnoses and improved patient care in Barrett’s esophagus.

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REFERENCES

enterological Association technical review on the management of Barrett’s esophagus. Gastroenterology 2011;140:e18-e52.


42. Patil DT, Bennett AE, Mahajan D, Allende D, Fraser C, Bronner MP. Can pathologists distinguish Barrett’s gastric-type dysplasia from reactive gastric cardiac mucosa in GERD? Mod Pathol 2010;23(Suppl 1):S161A.


67. Lightdale CJ. Ablation therapy for Barrett’s esophagus: is it time to choose our weapons? Gastrointest Endosc 1999;49:122–125.


70. Vieith M, Ell C, Gossner L, May A, Stolte M. Histological analysis of endoscopic resection specimens from 326 patients with Barrett’s esophagus and early neoplasia. Endoscopy 2004;36:
776-781.