The incidence of esophageal adenocarcinoma continues to increase in the United States, albeit at a slower rate than that recorded in the mid-1990’s.[1] The estimated number of new cases in the United States for 2015 is 16,980, with an estimated 15,590 deaths, making esophageal adenocarcinoma the 7th leading cause of death in men in 2015.[Cancer Facts & Figures 2015, http://www.cancer.org] Since Barrett esophagus (BE) is a known risk factor for the development of esophageal adenocarcinoma, various screening and surveillance programs for BE are currently utilized in an attempt to identify individuals at risk for progression to cancer. Pathologists play a critical role in these screening and surveillance protocols, as they
confirm the diagnosis of BE and assess for the presence and grade of dysplasia. Currently, morphology is the gold standard for the diagnosis of BE and BE-associated dysplasia. However, these diagnoses are not always straightforward. Numerous studies have attempted to identify ancillary stains that may help pathologists confirm the presence of BE and/or dysplasia, and to predict the progression to adenocarcinoma. In this review, representatives of the Rodger C. Haggitt Gastrointestinal Pathology Society evaluated the published literature and provide recommendations regarding the use of ancillary stains in the diagnosis of BE and BE-associated dysplasia.

**Background**

**Definition of BE**

In 1950, Norman Barrett, a British surgeon, published a learned discourse on peptic ulcer of the esophagus, in which he described the presence of a columnar-lined lower esophagus; he ascribed this phenomenon to the stomach being pulled up into the chest as the result of a congenital short esophagus.[2] Intestinal metaplasia (IM) of the distal esophagus was first described in 1953 by Allison and Johnstone who published seven cases of clinical reflux esophagitis with a columnar-lined lower esophagus, two of which had goblet cells.[3] In this study, the authors were the first to speculate that this columnar lining was an acquired metaplasia secondary to reflux of acidic gastric contents.

Beginning in the mid-1970s, it gradually became apparent that BE was a precursor, and thus a risk factor, for adenocarcinoma.[4-6] It was noted that the prevalence of BE and adenocarcinoma of the gastroesophageal junction (GEJ) rapidly increased in some parts of the
world, including the United States, from the 1960s through the 2000s. At the same time, squamous cell carcinoma, the most common type of esophageal carcinoma worldwide, gradually decreased in prevalence in Western societies, including the United States, and was eventually replaced by adenocarcinoma as the most prevalent esophageal carcinoma.

Several guidelines for the diagnosis and management of BE have been published over time and in different countries (Table 1). In 1998, the American College of Gastroenterology (ACG) created the first practice guidelines for the diagnosis, surveillance, and therapy of BE.[7] These guidelines required IM to be present for a diagnosis of BE. In 2006, the British Society of Gastroenterology (BSG) issued guidelines for the diagnosis and management of BE.[8] BE was defined as an endoscopically apparent area above the GE junction suggestive of BE that was supported by the finding of columnar lined esophagus on histology. Under these guidelines, IM was not a requirement for diagnosis due to the presumption that inadequate sampling may result in missed IM.

In 2011, the American Gastroenterological Association (AGA) Institute Medical Position Panel issued guidelines on the definition of BE.[9] This group defined BE as “the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium.” This was the first definition to specify the association of BE with cancer. As with the ACG definition, the AGA definition requires the presence of IM due to the assertion that the presence of goblet cells is the risk factor that predisposes to malignancy. This definition did leave an open question as to the potential risk of columnar mucosa devoid of IM. However, this definition mentioned that the absence of IM did not necessitate ongoing surveillance.
In 2014, the BSG published modified guidelines that required an endoscopically abnormal segment of esophagus that is greater than or equal to 1 cm in length. [10] As in the 2006 guideline, this BSG definition did not require the presence of IM, but did note that the lack of IM in a segment of columnar-lined esophagus did show a lower risk of malignant transformation compared to those that contained IM. As in the 2011 AGA Guideline, the BSG noted that patients without IM may not require ongoing cancer surveillance.

Finally, in January 2016, the ACG issued a modified definition of BE: “BE should be diagnosed when there is extension of salmon-colored mucosa into the tubular esophagus extending ≥1 cm proximal to the GEJ with biopsy confirmation of intestinal metaplasia”. The main difference between this definition and the previous ACG definition from 2008[11] is that there is now a minimum required length of columnar mucosa identical to that in the BSG guidelines.[12]

In summary, all authorities agree that BE is a metaplastic phenomenon that is a risk factor for the development of adenocarcinoma of the GEJ or distal esophagus. All major professional organizations require an endoscopically abnormal segment of distal esophagus that currently must exceed 1 cm in length and contains columnar epithelium on biopsy. The necessity of IM for the diagnosis of BE varies; IM is required in the United States and parts of Europe, whereas IM is not necessary in The United Kingdom and Japan.

- **Definition of BE:** Endoscopically apparent abnormal mucosa extending ≥1 cm proximal to the GEJ, and, in the United States, with biopsy confirmation of intestinal metaplasia.
Challenges in the diagnosis of BE

As discussed above, the diagnosis of BE is determined by two factors: (1) endoscopic identification of columnar metaplasia in the esophagus; (2) histologic confirmation of columnar epithelium and/or IM.[13] In the absence of inflammation, the salmon pink and velvety appearance of columnar mucosa is readily differentiated from whitish, pearlescent squamous mucosa, but mucosal injury in the esophagus due to reflux may obscure this distinction. In order to identify columnar metaplasia in the distal esophagus, the endoscopist must be able to identify the GEJ. The proximal extent of the gastric folds[14] and the distal extent of the longitudinal palisade vessels of the distal esophagus[15] are two landmarks that have been proposed. The former landmark is preferred by most gastroenterologists in North America and Europe, while Japanese gastroenterologists favor the latter landmark.[13] The AGA and BSG guidelines for the diagnosis of BE recommend the proximal extent of the gastric folds due to moderately superior reproducibility[16] and historical use of this landmark in most Western studies.[9, 10]

To measure the BE segment length, the endoscopist must localize these landmarks with respect to the squamocolumnar junction, but localization may be compromised by esophagitis, peristalsis, patient respiration, degree of insufflation and anatomic variation of the vasculature of the distal esophagus.[9, 10] In patients with a hiatal hernia, the endoscopist must also identify the diaphragmatic hiatus in relation to the GEJ in order estimate the extent of BE.[17] The Prague C&M criteria were proposed to standardize the endoscopic diagnosis and grading of columnar metaplasia in the distal esophagus in an effort to improve diagnostic reproducibility.[18] This study specified the anatomic landmarks and criteria for measuring the
circumferential (C) and maximal (M) extent of columnar metaplasia in the esophagus. Reported kappa scores in the external validation study were excellent for BE≥1 cm in length (0.72), but substantially worse for BE<1cm in length (0.21).[18] This may be the reason that the recent BSG and ACG definitions both require a minimum length of one centimeter for a diagnosis of BE.

- **Challenge in the diagnosis of BE:** The GEJ can be difficult to identify in some patients.

  Esophageal columnar metaplasia is an amalgam of several types of glandular mucosa (including cardiac-type, fundic-type and hybrid cardio-fundic glands).[19] In addition, cells showing multiple lines of differentiation have been identified in the metaplastic glandular epithelium using electron microscopy and histochemical stains, including small intestinal and colonic-type goblet cells, intestinal surface epithelial cells, gastric-type foveolar cells, intermediate cell types, and Paneth cells.[20, 21] Pancreatic acinar metaplasia/heterotopia is frequently seen in biopsies from the GEJ, but has no correlation with the presence of BE.[22] In spite of this cellular and glandular heterogeneity, goblet cells are identifiable on routine hematoxylin and eosin stained sections in the vast majority of cases (**Figure 1A**). Goblet cells can be differentiated from distended, goblet-shaped surface mucous cells (‘pseudogoblet cells’) based on their distinctive (grey-blue) staining characteristics of the mucin vacuole and their distribution in the epithelium: pseudogoblet cells are often clustered while true goblet cells are usually single and interspersed among foveolar cells (**Figure 1B**). The utility of ancillary stains to detect true goblet cells is discussed later.

- **Challenge in the diagnosis of BE:** Distinguishing true goblet cells from pseudogoblet cells.
Prior to the current ACG and BSG definitions of BE as requiring ≥1 cm of columnar-lined esophagus proximal to the GEJ, one major interpretive challenge for clinicians and pathologists was the distinction of ultrashort segment BE (BE <1 cm in length) from IM of the proximal stomach immediately distal to the anatomic GEJ in biopsies taken near the GEJ. IM is a common finding in biopsies from the GEJ, reported in up to 18% of patients without endoscopically evident BE.[23] However, because BE is now defined by both the presence and extent of columnar metaplasia/IM (≥1 cm proximal to the GEJ), pathologists should be descriptive by including only the term “IM” in their diagnoses of biopsies obtained from the GEJ or unknown site (ie, “distal esophagus”) if more proximal biopsies were not taken or do not show IM, rather than assign a definitive diagnosis of BE. If pathologists want to comment on the presence or absence of BE in the biopsy, a comment such as the following can be added: “If the biopsy was taken from the tubular esophagus and there is endoscopic evidence of esophageal columnar metaplasia extending at least 1 cm proximal to the gastroesophageal junction, then the presence of intestinal metaplasia confirms the diagnosis of Barrett Esophagus.”

In a subset of cases, there are histologic clues to the esophageal origin of biopsies taken near the GEJ, including multilayered epithelium, “buried” IM (squamous epithelium overlying glandular epithelium with IM), and esophageal glands/ducts.[21, 24, 25] In the study by Srivastava et al., these findings were almost exclusively seen in biopsies from patients with endoscopic BE compared to controls.[24] Multilayered epithelium (MLE) is a distinctive epithelial type comprised of squamoid basal (reserve) cells with surface columnar cells usually at the mucosal surface that can mimic true goblet cell metaplasia.[26] Prospective studies involving targeted biopsies taken from the GEJ[25, 27] and squamocolumnar junction[28] found
MLE in 10-36% of cases in a background of cardia-type or cardiofundic-type mucosa.[25] It was strongly associated with the presence of BE [27, 28] and endoscopic evidence of gastroesophageal reflux.[27] Various authors have suggested that this finding is indicative of esophageal origin of the epithelium based on its phenotypic similarity and association with esophageal gland ducts [25-27] and suggest that it may represent an early transitional form of columnar metaplasia in the esophagus.[27, 28]

- **Challenge in the diagnosis of BE:** *Intestinal metaplasia at the GEJ is no longer considered ultrashort BE. Hence, pathologists should not make a diagnosis of BE when intestinal metaplasia is present in biopsies taken from the GEJ, as the current definition requires the Barrett segment to be ≥1 cm in length.*

There are no convincing data that cardia IM is associated with an increased risk of cancer. Cardia and GEJ IM is more frequently associated with *Helicobacter pylori* gastritis[29], while BE is more often associated with gastroesophageal reflux.[29, 30] In studies that compare these two groups of patients, there are low rates of progression to high grade dysplasia (HGD) or adenocarcinoma in patients diagnosed with BE, but progression was not seen in patients with IM of the cardia who undergo surveillance endoscopy with no HGD at baseline.[30, 31] Consequently, the BSG and ACG recommend against endoscopic surveillance of cardia IM and also recommend against taking biopsies of a normal appearing GEJ.[10] This recommendation was affirmed by an international consensus of BE experts in a report sponsored by the AGA.[17]
Dysplasia in BE

BE-associated dysplasia has been classified into four categories: (1) Negative for dysplasia (ND), (2) indefinite for dysplasia (IND), (3) low grade dysplasia (LGD), and (4) HGD. While an international consensus definition of each of these categories has yet to be created, the following are histologic criteria that are generally agreed upon by GI pathologists:[32]

- **ND (Figure 1B):** This epithelium shows a simple columnar epithelium with small nuclei and abundant apical cytoplasm. The nuclei are round and usually occupy <25% of the cell surface area; nuclear membranes are smooth. Nuclei are uniformly present at the basal aspect of the cell and show extreme monotony from cell to cell. Nuclear to cytoplasmic ratio is generally higher at the base of the pits and shows a progressive decrease towards the luminal surface. The architecture of non-dysplastic epithelium shows a well-organized, uniform distribution of pits.

- **LGD (Figure 2A):** Cytologically, nuclear changes include increased nuclear to cytoplasmic ratio, hyperchromasia, nuclear membrane irregularities and variation in normal chromatin distribution with intact nuclear polarity; significant nuclear anisocytosis is absent. These cytologic changes should extend to the luminal surface. Architecturally, only minimal gland crowding should be present.

- **HGD (Figure 2B):** Nuclear changes are more profoundly atypical than LGD with loss of nuclear polarity, marked hyperchromasia, atypical mitotic figures, and significant nuclear anisocytosis that extend to the luminal surface. Architectural features of HGD include significant gland crowding, cribriform architecture, and dilated glands containing necrotic debris. Single cell invasion of the lamina propria, solid sheets and/or cords of
neoplastic cells (i.e. the ‘never-ending gland’ pattern) should not be seen in HGD and suggest intramucosal adenocarcinoma.

- **IND**: This category should be utilized when there are histologic features that are suspicious, but not diagnostic of dysplasia. This is most often a result of some amount of neutrophilic inflammation within or adjacent to the abnormal epithelium. Cytologic atypia that is confined to the crypts/glands without extension to the surface epithelium is also generally regarded as IND. Some studies have shown that patients with cytologic features of dysplasia limited to the crypts (crypt dysplasia) may behave equivalently to patients with conventional dysplasia;[33] however, this has not been validated. Moreover, the majority of patients with crypt dysplasia were reported to have conventional dysplasia elsewhere at the same endoscopy,[33] limiting the practical utility of this morphological finding.

The diagnosis of BE-associated dysplasia is a marker of increased risk of progression to adenocarcinoma. Progression rates of LGD range from 2-28% annually, while progression of HGD ranges from 6-60% per year.[34-40] The aggregate rate of annual progression to either HGD or adenocarcinoma in groups of patients with IND is 0.86% to 1.4%.[41-44] The large range of rates of progression are very like due to the fact that the diagnosis of both LGD and IND are plagued by significant interobserver variability with Cohen’s Kappa statistics of approximately 0.4 (moderate agreement); interobserver agreement tends to better at the ends of the interpretive spectrum (i.e. negative for dysplasia and HGD/intramucosal adenocarcinoma) and rather poor for LGD/IND.[32, 45] Nevertheless, dysplasia remains the gold standard for predicting risk of neoplastic progression in BE. The current standard of care prescribes that
patients with LGD receive increased surveillance; patients diagnosed with HGD require endoscopic interventions, such as radiofrequency ablation, endoscopic mucosal resection of visible lesions, and, in selected cases, esophagogastrectomy. Because of the important management implications for a diagnosis of dysplasia coupled with the interobserver variability in the interpretation of dysplasia, it is recommended that any new diagnosis of dysplasia be confirmed by two pathologists, including at least one with expertise in gastrointestinal pathology.[10, 12]

- **Dysplasia in BE:** *BE-associated dysplasia is a marker of increased risk of progression to adenocarcinoma. Any new diagnosis of dysplasia should be confirmed by two pathologists, including at least one with expertise in gastrointestinal pathology.*

**Methods for Developing the Recommendations**

In 2014, the Rodger C. Haggitt Gastrointestinal Pathology Society (GIPS) asked the membership via a survey what practice topics members would like to see addressed by the GIPS. The GIPS executive team chose this topic from among the top three responses that would best lend itself to the format of a GIPS recommendation. A senior author was identified, who then created a team of pathologists with interest and expertise in BE and who were willing to co-author the manuscript. The authors reviewed the literature and referenced papers that addressed the two chosen questions: (1) Are there scientific data to support the statement that special stains are necessary to diagnose BE, and (2) Are there scientific data to support the statement that special stains are necessary to diagnose dysplasia in BE? After analyzing the literature, the authors summarized the data to formulate the recommendations. The
recommendations were vetted by members of the Executive Committee of GIPS and then reviewed by a separate group of pathologists with interest and expertise in the field, none of whom were authors of the manuscript or members of the Executive Committee. After final acceptance by the GIPS Executive Committee, the recommendations were made available to the GIPS membership through the society website (http://usgips.com) for a one-month comment period. Submitted comments were analyzed for validity and incorporated into the recommendations prior to submission for publication.

**Are ancillary stains needed to diagnose BE?**

As noted above, in the United States and parts of Europe, the identification of goblet cells by the pathologist is necessary for a diagnosis of BE. In the vast majority of cases, true goblet cells can be easily and accurately recognized by their characteristic morphology in routine H&E sections.[46] However, a common mimic of goblet cells, named “pseudogoblet” cells, occurs frequently in inflamed cardia-type epithelium. These consist of scattered mucinous cells with distended cytoplasmic mucin droplets that can be confused with goblet cells.[47] With experience, these so-called “pseudogoblet” cells can be separated from true goblet cells in H&E sections. If there is any doubt, an Alcian blue (at pH 2.5)/PAS stain can be utilized to confirm the H&E impression: The columnar mucinous cells of the foveolar epithelium contain neutral mucins that stain red or magenta, while the acidic mucin in goblet cells stain bright blue (Figure 3A).[48, 49]

A pitfall of using an Alcian blue stain alone is the detection of so-called “columnar blue cells.” Not infrequently, columnar cells without the goblet shaped mucin droplet of true goblet
cells will stain blue with the Alcian blue pH 2.5 stain (Figure 3B). Some investigators have suggested that these columnar blue cells represent an earlier phase of IM and should be regarded as BE, or at least a precursor of BE.[48, 50-52] However, subsequent studies have demonstrated that Alcian blue positive columnar cells in cardiac-type mucosa are sometimes present in patients without BE.[47] One small prospective study that addressed the utility of the Alcian blue stain in BE determined that patients with Alcian blue positive pseudogoblet cells (but no true goblet cells) experienced no increased risk of future dysplasia.[53] There is one study that demonstrates that columnar blue cells containing sulfomucin (brown staining in the high iron diamine/Alcian blue pH 2.5 stain) were more commonly present in the metaplastic esophageal mucosa that contained goblet cells than those that contained only sialomucins (blue staining with the Alcian blue pH 2.5 stain).[50] The authors suggest that this type of columnar blue cell may in fact represent an incomplete form of IM, but there are no prospective studies to address the clinical significance of this finding. Hence, the utility of the Alcian blue pH 2.5 stain alone is limited by these columnar blue cells that must not be confused with true goblet cell metaplasia.

Since the detection of goblet cells plays a central role in the diagnosis of BE, there has been some interest in goblet cell distribution and number. One well-designed study indicated that at least eight biopsies from the endoscopically defined Barrett segment were necessary to be confident that goblet cells were in fact present by H&E examination.[46] In this study, Alcian blue/PAS stains were also performed to identify goblet cells missed in the initial H&E sections. Obtaining the Alcian blue/PAS stain led to only an additional 5.6% of patients being diagnosed as having BE. However, this study did not address whether simply performing additional H&E
sections would have led to a similar increase in the detection of goblet cells. Another study that
did look at the effect of obtaining deeper H&E levels found that goblet cells were detected in
4.7% of cases that had no goblet cells in the original 3 levels of serial sections.[54] Obviously,
identification of columnar mucosa devoid of goblet cells can be accomplished by H&E stain
alone, and reflexive use of AB-PAS staining of squamous epithelium in order to "exclude
Barrett's epithelium" is unjustifiable.

- **Recommendations for the use of Alcian blue (at pH 2.5) and/or PAS stains to diagnose
  BE:**
  
  - *Should not be used reflexively on all esophageal biopsies because goblet cells
    are almost always identifiable on routine H&E stained sections.*
  
  - *Alcian blue/PAS stains can be utilized to distinguish pseudogoblet cells from
    true goblet cells if there is a doubt on the H&E section.*

A number of studies have been performed analyzing mucin glycoprotein subtypes by
immunohistochemistry in Barrett epithelium. MUC2 is regarded as a reliable marker of an
intestinal epithelial phenotype, while MUC5AC is expressed in normal gastric foveolar epithelial
cells. Goblet cells in BE are consistently reactive with the MUC2 antibody and non-goblet
columnar cells are reactive with the MUC5AC antibody.[55] Interestingly, MUC2 is also
expressed in a proportion of the non-goblet cell columnar epithelium in BE, particularly in the
epithelium adjacent to goblet cell containing Barrett mucosa.[56] It has been suggested that
MUC2+ non-goblet columnar cells indicates an earlier stage in intestinal differentiation and may
be used as a surrogate marker of goblet cells in Barrett mucosa when goblet cells are not
identified in endoscopic biopsies as a result of sampling issues.[56] However, no study has been performed to determine whether MUC2 immunostains could be used to predict the future development of goblet cells. In fact, none of the published studies have advocated the use of any of the mucin glycoprotein immunostains as diagnostic or prognostic markers.[57-62]

- **Recommendation for the use of mucin glycoprotein immunostains to diagnose BE: Not indicated.**

Markers of intestinal phenotype have also been studied in immunohistologic studies, including CDX2, Das-1, villin, and HepPar-1. CDX2 is an important nuclear transcription factor that promotes epithelial intestinal differentiation during normal development.[63] The Das-1 antibody recognizes an antigen specific to a colonic epithelial cell protein, and is regarded as a sensitive and specific marker of IM.[64] Likewise, villin and HepPar-1 proteins are highly expressed in intestinal epithelia and are regarded as markers of intestinal differentiation in metaplastic epithelia.[65, 66] Many studies have demonstrated reactivity with these markers in non-goblet cell columnar epithelium in patients with BE.[67-72] In most studies, these markers are more often present in non-goblet cell columnar epithelial component in patients who also have goblet cell containing epithelium, suggesting that perhaps they are markers of an earlier phase of intestinal differentiation. However, none of these studies explored their use as predictors of the future development of goblet cell containing metaplastic epithelium in the esophagus. There is one study that reported that immunoreactivity with either CDX2 or SOX9 (a transcription factor downstream of the sonic hedgehog signaling pathway) in patients with only non-goblet cell metaplastic epithelium in the esophagus was predictive of the future detection
of goblet cell containing BE with a sensitivity of 85% and specificity of 85%.[73] Because the vast majority of studies that have evaluated the risk of neoplastic progression in BE are based primarily on histologic identification of IM,[74] the clinically accepted definition of IM is based on the identification of goblet cells on routine histology rather than immunophenotypic evidence of IM.

- **Recommendation for the use of markers of intestinal phenotype (CDX2, Das-1, villin, and HepPar-1) to diagnose BE:** These stains may be markers of an earlier phase of intestinal differentiation, but none have shown to be predictors of the future development of goblet cell containing BE. Thus, the use of these stains is not currently indicated.

- **Recommendation for the use of CDX2 and SOX9 immunostains to diagnose BE:** These stains show promise, but too few studies have been performed to date. Thus, a recommendation cannot be made at this time.

Because there are no entirely reliable endoscopic landmarks to precisely identify the GEJ, even with the new definition of BE, there may continue to be some degree of uncertainty regarding the distinction between BE and cardia IM. As discussed above, mucin histochemical stains reveal overlapping patterns of mucin glycoprotein expression in gastric and esophageal IM. However, there are some differences in the expression of cytokeratin subtypes between the two conditions. Barrett epithelium most often exhibits strong cytoplasmic CK7 reactivity of both the surface epithelium and glandular compartment, with weak reactivity for CK20 confined to the surface epithelium. In contrast, gastric IM less frequently demonstrates this
pattern of reactivity.[75-77] However, this characteristic pattern of expression in BE is not specific enough to utilize as a diagnostic marker, and is difficult to identify in suboptimally oriented endoscopic biopsy specimens.[68, 78, 79]

- **Recommendation for the use of CK7 and CK20 immunostains to distinguish IM of the gastric cardia from BE:** These stains are not specific enough to be utilized as diagnostic markers. Also, in suboptimally oriented biopsies, these stains can be difficult to interpret. Thus, their use is not indicated.

**Are ancillary stains needed for dysplasia diagnosis or risk stratification in patients with BE?**

It is well established that the vast majority of patients with known BE do not develop esophageal adenocarcinoma on follow up, and that most patients diagnosed with esophageal adenocarcinoma do not have a prior diagnosis of BE.[80] However, the current standard of care for patients diagnosed with BE is periodic surveillance for early detection of neoplasia. As noted above, a morphological diagnosis of dysplasia remains the gold standard for identification of patients at increased risk for the development of cancer. The surveillance biopsy diagnosis guides the recommended surveillance intervals in these patients. The recent ACG guidelines recommend a 3-5 year surveillance interval for patients with non-dysplastic BE, a 12 month surveillance for an IND diagnosis, and either definitive therapy or a 12 month surveillance for patients with LGD. Surveillance alone is not recommended for HGD unless significant comorbidities preclude definitive endoscopic therapy. [12]

In order for an ancillary stain to be helpful in the clinical care of patients with BE, the stain should ideally either: (1) Aid in the diagnosis of dysplasia, and/or (2) Stratify risk in
patients with BE. The morphological diagnosis of dysplasia lacks interobserver reproducibility, particularly for the IND and LGD categories. Thus, any special stains that improve interobserver reproducibility and thereby standardize dysplasia diagnosis would be immensely beneficial to patient care. Regarding risk stratification, there is wide variation in the reported rates of progression to adenocarcinoma for BE patients with LGD and HGD. Part of this variation is obviously related to the problem of interobserver reproducibility mentioned above. Another reason for this discrepancy may be the presence of heterogeneous biological subsets within each morphological dysplasia diagnosis category. An ancillary test that can help identify high risk subsets of patients within each group could be useful in guiding surveillance recommendations. Finally, given the lack of any progression in the vast majority of BE patients, an ancillary test that can identify the truly low risk subset of patients whose surveillance can be extended beyond the usual 3-5 years for non-dysplastic BE would have beneficial consequences to cost of care for these patients.

Studies analyzing the utility of ancillary immunostains in BE and BE-associated dysplasia fall into three broad categories. 1) The first and largest group includes those that have used morphology as the gold standard to classify biopsies and then determined the prevalence of the marker in question in each morphologically defined category. Progressively increasing prevalence of positivity in the HGD and carcinoma categories has been then taken as evidence for the utility of the stain being a marker of high risk in BE. The obvious limitation of such studies is that they cannot improve upon morphology because they use it as the gold standard for determining biomarker positivity in the non-dysplastic and dysplastic groups. Moreover, most of these studies do not directly assess the association of biomarker positivity with
subsequent disease progression. 2) A case control design overcomes some of these limitations as it can be used to assess the odds of biomarker positivity in BE patients who progress to HGD or cancer on follow up (“cases”) in comparison to those who remain free of neoplasia during follow up (“controls”). HGD is considered a relevant end point in some studies because patients usually exit surveillance and enter a phase of definitive treatment when they are diagnosed with HGD. However, the use of HGD as an end point is problematic because the distinction between low and HGD is not always straightforward. What is persistent LGD for some pathologists may be progression to HGD for others. Another issue with case control studies is the number of index and surveillance biopsies that need to be stained since both progressors and non-progressors are likely to have had multiple surveillance biopsies during follow up. Staining all available biopsies may be feasible in a research study but is not practical in a routine clinical setting unless very strong performance characteristics can be shown for a given biomarker that clearly separates the truly high risk patients from the low risk ones. 3) The last category of study design includes those that have evaluated biomarker status at index examination and then determined the likelihood of disease progression on follow up. The studies on ancillary stains described below include all three study designs.

Ancillary stain: p53

*TP53* is a tumor suppressor gene that prevents genomic mutations. In normal cells, the level of p53 protein is low, but when DNA damage or other stress occurs, p53 is upregulated, which results in growth arrest, DNA repair and apoptosis. *TP53* gene mutations are highly prevalent in esophageal adenocarcinoma as well as HGD, but are uncommon in non-dysplastic BE. Mutations often lead to increased nuclear accumulation of a faulty protein, but in the case
of truncating mutations, p53 can be completely lost. Thus, faint scattered p53 positivity within nuclei correlates best with a wild-type gene status whereas either intense nuclear positivity or complete absence ("null pattern") of staining correlate best with TP53 mutations (Figure 4). The latter null pattern of staining has been recognized only in recent studies and was largely ignored in earlier immunohistochemical studies on p53 expression in BE.[81, 82] Abnormal immunohistochemical expression of p53 can be detected across the entire morphological spectrum of BE but the prevalence is lower in non-dysplastic BE. [83]

Use in the Diagnosis of Dysplasia. It has been proposed that p53 immunohistochemistry may aid in improving diagnostic reproducibility in the diagnosis of dysplasia and in risk stratification in BE. A summary of studies on p53 and the diagnosis of dysplasia in BE is included in Table 2. One of the recommendations put forth by the 2014 BSG guidelines on the diagnosis and management of BE states: “the addition of a p53 immunostain to the histopathological assessment may improve the diagnostic reproducibility of a diagnosis of dysplasia in BE and should be considered as an adjunct to routine clinical diagnosis” (grade B recommendation).[10] This recommendation is largely based on studies that focused on the progression to HGD or adenocarcinoma (further discussed below) rather than pure histologic evaluation. One study that supported this recommendation examined the utility of p53 immunostaining in improving observer reproducibility in the diagnosis of BE-associated dysplasia; in this study, biopsies from 143 patients with BE dysplasia and 35 negative for dysplasia were stained with p53.[84] The conclusions from this study were that use of p53 improves observer reproducibility and disease progression was better predicted by incorporating p53 immunohistochemical findings into the morphological diagnosis. Pathologists
integrated the p53 findings into their morphologic diagnosis but no details were provided in the study regarding how exactly this evaluation was performed. Scoring p53 immunohistochemistry may itself be subject to interobserver variability. In fact, in the study cited above, strongly positive abnormal glands were considered p53 positive while p53 negative cases were either “weakly positive or completely negative.”[84] As mentioned earlier, this latter pattern of staining correlates with truncating mutations and should be considered an aberrant pattern of staining. A recent study of 72 patients by the same group evaluated reproducibility of p53 interpretation by 10 pathologists from four institutions.[82] P53 immunostain was interpreted as “significant” in the presence of strong or absent staining and as “not significant” when neither of the two patterns were present or findings were equivocal. The authors reported better interobserver reproducibility for p53 interpretation (average kappa value=0.6) when compared to morphological diagnosis of dysplasia using the four categories in the Vienna classification (average kappa value=0.3). Since the p53 staining was evaluated in two categories and the morphological diagnosis split across four, the higher kappa value for p53 is not surprising. In fact, when the morphological diagnosis was grouped into two categories of definite dysplasia versus no dysplasia, the average kappa value was 0.55, which is not much different from the p53 interpretation. Based on the results of this study, the authors suggested that including p53 can be a useful aid in dysplasia diagnosis but cannot be used to separate LGD from HGD. Abnormal p53 was not present in any of the 14 non-dysplastic BE cases included in the study though others have reported a prevalence of about 10% in this category (see more data below). Moreover, 21/22 cases of LGD showed abnormal p53 staining, even though molecular studies indicate TP53 mutations generally to be a late event and to be present in only
about 30% of BE-associated LGD.[85] Despite the current widespread use of p53 IHC, we believe that additional studies are needed to develop and validate precise criteria before p53 staining can be fully endorsed and incorporated into the morphological dysplasia diagnosis algorithm.

Use in Predicting Disease Progression. Immunoreactivity for p53 is the most widely studied and most promising marker among the many ancillary immunostains that have been evaluated as markers of high risk for disease progression in BE. A summary of studies on p53 and disease progression in BE is included in Table 2. Some of the earlier studies reported p53 positivity in biopsies IND or with LGD to be a highly sensitive and specific marker of progression to HGD or adenocarcinoma. In a study of 61 patients, 25 (41%) were diagnosed either as IND or as LGD and five (20%) of these developed HGD or adenocarcinoma on follow up. Five of nine patients with p53 positive indefinite or LGD progressed compared to none of the 16 with p53 negative IND or LGD.[86] Others have reported a 88% sensitivity and 75% specificity for p53 positivity in LGD as a marker for progression to HGD or carcinoma; however, this was based on a very small series of 16 cases in which agreement for a diagnosis of LGD among three pathologists was present in only 4/16 cases included in the study and 50% patients had no evidence of dysplasia on follow up. [39] Weston et al studied 48 patients with LGD (26 prevalent, 22 incident) who were followed up for a mean duration of 41.2 months and five of these progressed to either multifocal HGD (4 patients) or cancer (1 patient).[87] In this study, p53 positivity was present at index biopsy in the low grade dysplastic focus in 4/31 patients that regressed to no dysplasia, 3/12 that showed persistent LGD and 3/5 that progressed to HGD or carcinoma.[87] The limitations of this study include the use of quantitative image analysis,
which may have scored more than just the strong staining nuclei, and the inherent issue in many studies, the interobserver variability in grading dysplasia (some of the illustrations of p53 positive LGD show round nuclei with loss of polarity suggestive of HGD). A nested case control study from the Northern Ireland Barrett’s Esophagus Register (NIBR) included 29 cases with incident adenocarcinoma and 6 with incident HGD that were matched to at least three control patients with no progression.[88] With p53 positivity scored semi-quantitatively as 0, 1 (<10%; focal), 2 (10-50%; diffuse) and 3 (≥50%; intense), 32.4% of cases and 11.7% of controls showed p53 positivity. No increase in risk was observed with focal (<10%) staining, but patients with diffuse or intense p53 staining were nearly ten times more likely to progress to carcinoma or HGD compared to those with focal or no staining (OR=9.28; 95% CI, 1.78-48.3), underscoring the importance of establishing an appropriate definition of “abnormal” p53 expression. In this study, a third of all patients with incident adenocarcinoma had initial biopsies with diffuse or intense p53 positivity, suggesting that p53 is not sufficiently sensitive to predict progression to cancer in all patients at index biopsy as a single marker; however p53 did outperform the initial histologic assessment by a substantial margin (of note, on the initial histologic assessment, none of the cases had LGD and one had HGD).[88] A similar study of 27 patients with incident HGD or adenocarcinoma matched to an equal number of controls with no progression found moderate (15-40% positive nuclei) but not strong (>40%) p53 overexpression to be an independent predictor of progression to HGD or adenocarcinoma, when adjusted for age, gender and a diagnosis of LGD [89]. It is difficult to come up with a plausible biological explanation as to why moderate but not strong p53 positivity should be a better predictor of the risk of neoplastic progression in BE. Although it is less common than the “overexpression”
pattern, the null pattern of p53 staining that correlates with truncating TP53 mutations was not evaluated in the studies mentioned above. Also, the use of semi-quantitative percentage thresholds for determining p53 positivity is likely to be problematic in routine clinical practice because strong expression in a small focus of morphologically atypical glands may not be scored as positive if it represents only a small percentage of the cells on the slide (Figure 5).

A more recent study from the NIBR included 89 BE patients who progressed to adenocarcinoma or HGD of the esophagus or gastric cardia six months after the initial BE diagnosis; these patients were matched to 291 controls with no progression to HGD or adenocarcinoma.[90] P53 positivity, defined as “dark staining adjacent to background with no staining”, was present in 41% of cases and 28% of controls. P53 positivity was associated with a significantly increased risk of progression to adenocarcinoma (OR, 1.95; 95% CI, 1.04-3.67) but was not a significant predictor of combined HGD and adenocarcinoma outcomes (OR, 1.60; 95% CI, 0.91-2.82). Moreover, in this study, the combination of a morphological diagnosis of LGD, abnormal DNA ploidy and positivity for Aspergillus oryzae lectin most accurately discriminated between cases that progressed to HGD or adenocarcinoma and controls that did not.[90] In the largest study to date, aberrant p53 staining (defined as either overexpression or complete loss of staining in at least one crypt or gland), was evaluated in 635 patients, 8% of whom developed HGD (n=35; 6%) or carcinoma (n=14; 2%) on follow up.[91] P53 overexpression was present in 18% and complete loss was seen in 2% of all biopsies. Aberrant p53 staining was seen in 11% of biopsies with non-dysplastic BE, 38% of LGD, 83% of HGD and 100% of adenocarcinomas. Overall, aberrant p53 staining was seen in 49% of cases that progressed to HGD or carcinoma compared to 14% of controls with no progression on follow up. Importantly,
abnormal p53 expression was shown to be an independent risk factor (when controlled for
dysplasia diagnosis). The positive predictive value for neoplastic progression increased from
15% for a diagnosis of LGD to 33% for a diagnosis of LGD with aberrant p53 staining.
Immunohistochemistry for p53 was performed on tissue from all surveillance endoscopies in
patients with any form of dysplasia while in those with no dysplasia staining was performed in
biopsies from only one randomly chosen surveillance endoscopy. This makes it more likely to
detect aberrant p53 staining in the former compared to the latter group. A surprisingly high
number (223/635; 35%) of patients were diagnosed with LGD at index or follow up endoscopy
in this study and 85% of them showed no progression raising some concern about
morphological thresholds used for a diagnosis of LGD.

To summarize, most studies suggest that abnormal p53 immunohistochemical staining is
a risk factor for progression to HGD or cancer. However, there is a significant body of literature
on longitudinal outcomes for each dysplasia category based on morphology alone. The studies
discussed above do not precisely define the prospective risk of adverse outcomes based on
abnormal p53 immunohistochemistry, which limits the ability of abnormal p53 findings to alter
the recommended surveillance intervals based on morphology alone. Therefore,
implementation of p53 immunostaining as a routine diagnostic adjunct is hindered by several
factors: (1) there is no widely accepted definition of abnormal p53 staining, particularly
regarding minimum extent of staining; (2) there are no guidelines on how many biopsies need
to be tested and whether it should be performed at each surveillance endoscopy; (3) there are
no recommendations for integrating the histologic diagnosis and p53 immunohistochemical
result and its impact on recommended surveillance interval; and (4) it is unknown if patients
will benefit from the addition of p53 testing. There are no data to indicate that patients with non-dysplastic p53 positive biopsies would benefit from altering the surveillance interval from the usual 3-5 year interval to 12 months. False positive results in this setting may result in a substantial burden of unnecessary surveillance. With the exception of the study by Kaye et al, all of the studies mentioned above treated p53 immunohistochemistry independent of the morphologic diagnosis. Hence, there is no evidence at this time that a p53 result should modify the morphologic diagnosis. Additional studies are needed to determine the best definition of “abnormal” p53 staining and to show how integrating p53 testing into routine practice could improve patient care.

Other ancillary stains

A number of other biomarkers assessed by immunohistochemistry have been reported in small numbers of studies. Immunohistochemistry for cyclin D1 was evaluated in a prospective study of 307 patients, 12 of whom developed adenocarcinoma during follow up. Index biopsies positive for cyclin D1, defined as any positive nuclei irrespective of extent or intensity of staining, were significantly associated with progression to adenocarcinoma (OR=6.85; 95% CI=1.57-29.91). [92] In the same study, p53 positivity was not predictive of progression to adenocarcinoma [93] (OR=2.99; 95% CI=0.57-15.76). Others have reported that cyclin D1 is almost universally expressed across the entire BE morphological spectrum but high expression (>50% cells positive) is seen more often in HGD and adenocarcinoma. Cyclin D1 was not found to be predictive of progression to HGD or adenocarcinoma in a retrospective nested case control study by Murray et al (OR=0.81; 95%CI=0.14-4.5).[88] Surface expression of cyclin A has also been reported to correlate with degree of dysplasia in BE and proposed as a high risk
marker for progression to adenocarcinoma (OR=7.5; 95% CI=1.8-30.7) but this finding remains to be validated. [94]

AMACR (α-Methylacyl-CoA racemase) immunohistochemistry has been reported in initial studies to be completely negative in non-dysplastic BE and in BE with reactive atypia but positive in 11-38% of LGD and 64-81% of HGD cases, raising hopes that this could be a useful marker of dysplasia.[95] It was also suggested in some studies that AMACR positivity in cases diagnosed as IND may indicate a need for closer surveillance.[96] However, more recent longitudinal data from a larger series of patients showed weak AMACR positivity in 46% and strong expression in 3% of biopsies without dysplasia in BE. Strong but not weak AMACR expression was predictive of progression to adenocarcinoma in this study. [97] It appears, therefore, that variable AMACR positivity is not entirely specific for dysplasia and may also be present in non-dysplastic BE.

IMP3 is an oncofetal protein that has been reported to be expressed in >90% of BE-associated HGD and esophageal adenocarcinomas. [98] However, it is also expressed in 14% of LGD and 7% of non-dysplastic BE, limiting its utility in the diagnosis of BE-related HGD. Loss of SOX2 expression was recently reported to be associated with an increased risk of progression to adenocarcinoma. Complete absence of SOX2 expression was found in 2% of biopsies with no dysplasia, 28% of biopsies with LGD and 63% of biopsies with HGD.[99] Finally, MUC stains, MUC5AC and MUC2, CD10 and CDX2 have been used to diagnose foveolar-type dysplasia in BE[100], a form of dysplasia that has also been reported as gastric or non-adenomatous-type dysplasia in the literature. There is no consensus at present on the morphological and immunophenotypic definition of foveolar-type dysplasia and while these stains may be useful in
categorizing dysplasia as adenomatous or foveolar-type, they do not help in the distinction of BE with and without dysplasia.

- **Recommendation for the use of special stains to diagnose dysplasia and for risk stratification in BE:**
  - A diagnosis of dysplasia remains a morphological diagnosis; ancillary stains are not recommended for diagnosing dysplasia in BE at this time.
  - Whether p53 immunostaining can be used as a marker for identifying high risk BE patients requires additional studies before it can be recommended for routine use.

**Summary**

After a thorough and critical review of the literature, the Rodger C. Haggitt Gastrointestinal Pathology Society recommends that morphology should remain the gold standard for diagnosing BE and BE-associated dysplasia at this time. The Alcian blue stain at pH 2.5 combined with a periodic-acid Schiff (PAS) stain has limited utility in morphologically challenging cases of BE, particularly in the distinction of pseudogoblet cells from true goblet cells, and is not indicated as a reflexive test. Other ancillary stains need further study before they can routinely be applied to the diagnosis of BE and BE-associated dysplasia. We believe that these recommendations will provide helpful information to pathologists, gastroenterologists and others involved in the diagnosis and management of patients with BE.

**ACKNOWLEDGMENTS**
The authors thank Dr. Kenneth P. Batts, Dr. John R. Goldblum, Dr. Gregory Y. Lauwers, and Dr. Elizabeth Montgomery for their critical review of the manuscript and constructive comments, and Dr. Marie Robert for her oversight of the project.
<table>
<thead>
<tr>
<th>Group</th>
<th>Year of publication</th>
<th>Endoscopic requirement</th>
<th>Minimal length required</th>
<th>Goblet cells required</th>
<th>Specifically mentions cancer risk</th>
</tr>
</thead>
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<tr>
<td>ACG</td>
<td>2002, 2008</td>
<td>stated</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>BSG</td>
<td>2006</td>
<td>stated</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>AGA</td>
<td>2011</td>
<td>implied</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>BSG</td>
<td>2014</td>
<td>stated</td>
<td>Yes, ±1cm</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>ACG</td>
<td>2016</td>
<td>stated</td>
<td>Yes, ±1cm</td>
<td>yes</td>
<td>no</td>
</tr>
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</table>

**Table 1.** Comparison of recent written criteria for the diagnosis of Barrett’s mucosa.

ACG: American College of Gastroenterology; BSG: British Society of Gastroenterology; AGA: American Gastroenterological Association
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Objective</th>
<th>P53 IHC Scoring Method</th>
<th>Major Findings</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younes M, 1997</td>
<td>RL</td>
<td>P53 IHC as a marker for risk of progression in BE</td>
<td>Positive when intensity similar to staining in HT29 colon cancer cell line</td>
<td>At least one biopsy p53+ in 9/61 (15%)</td>
<td>Median follow up only 26 months</td>
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<td></td>
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<td>5/9 (56%) p53+ patients progressed to HGD or EAC on follow up</td>
<td>All progressors had at least one prior biopsy diagnosed as LGD or indefinite for dysplasia</td>
</tr>
<tr>
<td>Bian Y-S, 2001</td>
<td>CS</td>
<td>Concordance between TP53 mutation and p53 IHC expression in BE</td>
<td>Positive when &gt;10% nuclei positive in clusters or scattered throughout the tissue sample</td>
<td>TP53 mutations in 77% HGD, 20% LGD</td>
<td>Small sample size</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>IHC positive in 85% HGD and 71% LGD</td>
<td>Used macrodissected samples (n=77) from esophagectomies with cancer instead of longitudinal surveillance biopsies</td>
</tr>
<tr>
<td>Weston AP 2001</td>
<td>RL</td>
<td>p53 IHC as predictor of progression risk in BE with LGD</td>
<td>Percentage of positive nuclei in glandular area of interest scored by digitized image analysis</td>
<td>5/48 LGD patients showed progression</td>
<td>Only 1 definite carcinoma outcome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3/5 progressors positive for p53 (in 8.3-58.1% nuclei)</td>
<td>Study restricted to LGD cases only; some LGD illustrations provided raise suspicion for HGD</td>
</tr>
<tr>
<td>Skacel M, 2002</td>
<td>RL</td>
<td>Impact of p53 IHC on IOV and progression risk in LGD</td>
<td>&quot;only when staining occurred in the atypical area&quot;</td>
<td>9/16 (56%) LGD p53 positive</td>
<td>Very small sample size</td>
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<td>7/8 progressors p53 positive compared to 2/6 non-progressors</td>
<td>Mean follow up only 23 months</td>
</tr>
<tr>
<td>Murray L, 2006</td>
<td>RCC</td>
<td>P53 IHC as marker of disease progression in BE</td>
<td>Focal (&lt;10%) Diffuse (10-50%) Intense (&gt;50%)</td>
<td>Increase risk of progression only in cases with diffuse or intense staining</td>
<td>Mean follow up only 3.7 years</td>
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<td>Diffuse or intense staining present in only 32.4% of cases that progressed</td>
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<tr>
<td>Kaye PV, 2009</td>
<td>RL</td>
<td>Impact of p53 IHC on IOV in dysplasia diagnosis and patient outcome</td>
<td>Compared staining intensity in dysplastic foci with background non-dysplastic epithelium</td>
<td>Moderate overall agreement for dysplasia diagnosis</td>
<td>Unclear how IHC findings were incorporated into dysplasia diagnosis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Agreement improved with p53 IHC</td>
<td>Kappa for indefinite for dysplasia (0.06) and LGD (0.37) remained poor even after p53 IHC</td>
</tr>
<tr>
<td>Sikkema M, 2009</td>
<td>RCC</td>
<td>Predictive value of p53, Ki67 and aneuploidy as markers of progression risk in BE</td>
<td>Moderate-intense staining considered positive and graded as normal (&lt;15% positive nuclei), moderate (15-40%) or strong (&gt;40%) overexpression</td>
<td>&quot;Moderate&quot; p53 overexpression associated with increased risk for progression to HGD/EAC</td>
<td>Small number of cases; combined HGD and EAC used as outcome of interest</td>
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<td>32% biopsies from cases with progression had a prior diagnosis of LGD compared to 5% of control biopsies</td>
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<td>Moderate but not strong staining associated with progression; lacks biological plausibility</td>
</tr>
<tr>
<td>Bird-Lieberman EL, 2012</td>
<td>RNCC</td>
<td>Test biomarkers for risk of progression to HGD/EAC in BE</td>
<td>Positive when strong dark staining seen adjacent to background with no staining</td>
<td>LGD diagnosis by 2 expert GI pathologists significant predictor of progression to HGD/EAC</td>
<td>Unclear how many cancer outcomes included</td>
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<td></td>
<td>PS3 positivity associated with increased EAC risk but not combined HGD/EAC outcome</td>
<td>Difficult to explain why p53 expression would be associated with carcinoma but not combined HGD/EAC outcomes</td>
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<td>20.2% cases had indefinite or LGD diagnosis at baseline compared to 2.4% of controls</td>
</tr>
<tr>
<td>Kastelein F, 2013</td>
<td>RCC</td>
<td>p53 IHC as predictor of neoplastic progression in BE</td>
<td>At least one gland with overexpression or complete loss of expression</td>
<td>Aberrant p53 IHC in 11% biopsies with no dysplasia, 38% with LGD, 83% with HGD and 100% with EAC</td>
<td>Only 14 cases with cancer outcome</td>
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<td>Aberrant p53 more common in cases (49%) than controls (14%; RR=6.2)</td>
<td>All surveillance biopsies from patients with any dysplasia stained with p53 but only one set of random surveillance biopsies from patients without dysplasia</td>
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<td>Some LGD illustrations with aberrant p53 may be foveolar type HGD</td>
</tr>
<tr>
<td>Kaye PV, 2016</td>
<td>IOV</td>
<td>Assess reliability of dysplasia grading and p53 interpretation</td>
<td>&quot;Strong&quot; or &quot;absent&quot; staining considered aberrant</td>
<td>No aberrant staining in non-dysplasia cases compared to 21/22 LGD and all 15 HGD cases</td>
<td>Small number of cases in each group</td>
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<td></td>
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<td>Kappa improved from 0.47 to 0.55 after including p53 IHC findings</td>
<td>Unclear how p53 IHC findings were incorporated into dysplasia diagnosis and to separate LGD from HGD</td>
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</tbody>
</table>
**Figure Legends** [note to reviewers: jpeg photos have been inserted here for easy viewing; letters will be added to the final hi-res photos; please make recommendations with this in mind]

**Figure 1.** (A) Goblet cells are readily identified on this H&E stained biopsy that was taken from the distal esophagus. True goblet cells tend to be interspersed among foveolar cells and hence tend to be separated from one another. (B) Pseudogoblet cells are distended, goblet-shaped surface mucous cells that often occur in clusters and tend to contain grey-blue mucin on the H&E stain.

**Figure 2.** (A) An example of low grade dysplasia (H&E stain). (B) An example of high grade dysplasia (H&E stain).
**Figure 3.** (A) On an Alcian blue-PAS stain, the neutral mucin within foveolar and pseudogoblet cells stains bright pink or magenta; if goblet cells were present, they would stain bright blue. [plan to replace this photo with one containing goblet cells as well] (B) On an Alcian blue stain alone, in addition to true goblet cells (arrow), foveolar cells may also stain blue (“columnar blues”).
Figure 4. (A&B) Wild-type pattern of p53 expression in an example of BE without dysplasia. Scattered, faintly positive nuclei are present (A, H&E stain; B, p53 immunostain). (C&D) p53 overexpression in a case of high grade dysplasia. Strong and diffuse p53 staining correlates with *TP53* mutations (C, H&E stain; D, p53 immunostain). (E&F) This example of high grade dysplasia shows complete absence of p53 staining (“null pattern”), which also correlates with *TP53* mutations (E, H&E stain; F, p53 immunostain).
Figure 5. Challenges in interpreting the p53 stain. (A&B) Two wild-type (non-dysplastic) examples of BE showing scattered intensely staining nuclei with the p53 stain. (C) Diffuse but not uniform intense staining with p53. Does it represent wild-type or mutated TP53?
References

13. Takubo, K., et al., Differences in the definitions used for esophageal and gastric diseases in different countries: endoscopic definition of the esophagogastric junction, the precursor of Barrett's adenocarcinoma, the definition of Barrett's esophagus, and histologic criteria for mucosal adenocarcinoma or high-grade dysplasia. Digestion, 2009. 80(4): p. 248-57.


82. Kaye, P.V., et al., *Dysplasia in Barrett’s Oesophagus: p53 immunostaining is more reproducible then H&E diagnosis and improves overall reliability while grading is poorly reproducible.* Histopathology, 2016.


