

Rodger C. Haggitt Gastrointestinal Pathology Society Companion Meeting

USCAP 2007

Gastroesophageal Junction Pathology:
Diagnostic and Controversial Issues

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Introduction

The gastroesophageal junction (GEJ) is a poorly defined anatomic area that represents the junction between the distal esophagus and the proximal stomach (cardia). The GEJ is an area that is commonly exposed to the injurious effects of gastroesophageal reflux disease (GERD) and *H.pylori* infection^{1,2}. These are the two most common pathologic disorders affecting this region. In fact, both of these disorders may result in chronic inflammation and the subsequent development of intestinal metaplasia (IM), which increases the risk of neoplasia³. Over the last several decades, there has been a dramatic rise in the incidence of adenocarcinoma of the GEJ region, which is presumed to be related to GERD, *H.pylori* or both⁴.

This lecture will focus on the clinical, pathologic, and pathogenetic aspects of GERD and *H.pylori*-induced inflammation of the GEJ region. For the purpose of this review, the GEJ is considered the mucosal area involving the distal 1 cm of esophagus and the proximal 1 cm of stomach. Unfortunately, there are many limitations in performing studies on diseases of the anatomic region^{1,5}. This is, in part, related to problems in evaluating anatomic landmarks of the GEJ endoscopically, inadequate standardization of endoscopic and pathologic methods, and inconsistent use of definitions and terminology used to define the histology and pathology of this region. For instance, there is a lack of consistency in the literature with regard to patient populations studied, methods of endoscopy utilized, sites of tissue sampling, and the use of adjunctive tests in defining IM in mucosal biopsies. Nevertheless, the information in this review article represents an evidence-based analysis, and provides information that pathologists may use to differentiate GERD from *H.pylori* infection, and determine the cause of IM, in the GEJ region.

Histology of the Gastroesophageal Junction

The esophagus is lined by non-keratinizing stratified squamous epithelium, and contains scattered mucous glands within the lamina propria similar to those that reside in the gastric cardia⁶. In addition, the esophagus contains multiple linear rows of salivary gland-like mixed mucous/serous glands in the submucosa which empty their contents into the lumen by a duct system lined partly by cuboidal, and partly by squamous, epithelium.

A mild periductal infiltrate of mononuclear cells is commonly present surrounding the submucosal glands and ducts. The stomach is lined by mucinous columnar epithelium and contains either pure oxyntic glands, or pure mucous glands, in the deep portion of the mucosa of the corpus and antrum/pylorus, respectively. A mixture of oxyntic and mucous glands is often present at the junction between these two gastric compartments. The true anatomic GEJ corresponds to the most proximal aspect of the gastric folds, which represents an endoscopically apparent transition point in the most individuals⁷. Unfortunately, this anatomic landmark is often obscured in patients with hiatus hernia, which is a major cause of GERD. In “normal” individuals, the anatomic GEJ also corresponds to the histologic transition point between the esophageal squamous epithelium and the gastric mucinous columnar epithelium. This transition point is termed

the “Z”-line⁶. However, many, if not most, adults, particularly those with either physiologic or pathologic GERD, have a proximally displaced “irregular” Z-line indicating that the histologic squamocolumnar junction (SCJ) is located above the anatomic GEJ. The histologic characteristics of the short segments of columnar mucosa located above the anatomic GEJ in these individuals are similar to the gastric cardia (see below), being composed of either pure mucous glands or mixed mucous/oxyntic glands, and leads to difficulty in distinguishing columnar metaplasia of the distal esophagus from the true gastric cardia in biopsies from the GEJ region^{8,9}. As discussed further below, this distinction is clinically important since columnar metaplasia of the esophagus is caused by GERD and represents a non-intestinalized type of Barrett’s esophagus (BE) and a precursor for the intestinal type of BE, the latter of which has recently been defined by the presence of goblet cells^{5,10}.

In contrast, the true gastric cardia is defined as the area of mucosa located distal to the anatomic GEJ and proximal to the portion of stomach composed entirely of oxyntic glands (corpus). Inflammation of this anatomic region is termed “carditis”, and is most often caused by *H.pylori* infection although there is accumulating evidence to suggest that GERD may cause carditis as well^{1,3,11,12}. Unfortunately, endoscopists often mistakenly use the term “cardia” to indicate the location of a mucosal biopsy from the GEJ region, even though, in some cases, the biopsy may have actually been obtained from the distal esophagus^{3,8}. Thus, it is often incumbent upon the pathologist to determine the precise location of a particular biopsy of the GEJ region, particularly if it contains IM, which is a major risk factor for the subsequent development of dysplasia and carcinoma. Thus, IM in the GEJ region may represent several diagnostic possibilities; ultrashort segment BE (which is defined as columnar metaplasia of distal esophagus, with goblet cells, of <1.0 cm in length) or chronic carditis with IM. These two conditions have a different etiology, natural history and treatment and are discussed further below^{2,13,14}.

There is controversy regarding the origin and histologic features of the true gastric cardia^{1,11,15-17}. Some authors believe that the cardia is normally composed, at birth, of surface mucinous columnar epithelium and underlying oxyntic glands identical to the gastric corpus, whereas other maintain that the true anatomic cardia is normally composed of mucinous columnar epithelium with underlying mucous glands, or mixed mucous and oxyntic glands (mucous/oxyntic glands). Proponents of the former theory believe that the mere presence of mucous, or mixed mucous/oxyntic, glands in the mucosa from the GEJ region indicates that it is metaplastic in origin and developed as a result of GERD^{16,18}. Nevertheless, the results of several studies in neonates, infants and young pediatric patients, all patient groups that have a low incidence of GERD and *H.pylori* infection, have shown that the true gastric cardia is composed of either pure mucous, or mixed mucous/oxyntic, glands underlying mucinous columnar epithelium in most, if not all, individuals^{11,15,17,19,20}. The area of mucosa distal to the GEJ occupied by this type of epithelium ranges from 1-4 mm in length¹⁵. For instance, in a study of 30 consecutive pediatric autopsies by Kilgore et al, mucous glands were present on the gastric side of the anatomic GEJ in 100% of cases¹⁵. The mean length of mucosa composed of mucous glands was 1.8 mm. Similar findings were reported by Glickman et al in a biopsy study of 74 pediatric patients in which pure mucous glands were identified

in the true gastric cardia in 81%, and mixed mucous/oxyntic glands in the remaining 19% of patients¹⁷. Interestingly, a very small portion of patients show only pure oxyntic glands in the “cardia” region^{16,21}. However, this finding is often focal and does not usually involve the entire circumference of the true gastric cardia.

There is also evidence to suggest that the length of mucosa composed of either pure mucous glands, or mixed mucous/oxyntic glands, increases with age, and is presumed to be related to ongoing “physiologic” GERD^{15,17,21-24}. In fact, the increase in length is due, most often, to columnar metaplasia of the distal esophagus above the anatomic GEJ, although in some circumstances (ex. autoimmune gastritis, *H.pylori* infection), “cardia-type” mucosa, as described above, may increase in length by distal extension into the proximal corpus^{25,26}. In fact, a short segment of esophageal columnar mucosa, often only a few mm’s in length, is a relatively common finding among patients who present for upper GI endoscopy in major hospital centers¹. Although, theoretically, this represents BE, a new definition of BE has recently been adopted that refers only to those patients who have superimposed IM, characterized by the presence of goblet cells, since it is this latter type of epithelium that is a risk for neoplastic progression¹⁰. Unfortunately, this definition has serious limitations, given that IM is often focal and, thus, may be missed due to sampling error²⁷.

Etiology of Inflammation of GEJ Region (“GEJitis”)

Gastroesophageal reflux disease and *H.pylori* infection are the major etiologic factors in the development of inflammation and IM of the GEJ region^{1,2}. In some individuals, both these etiologic agents may, in fact, act synergistically to cause inflammation^{24,28,29}. The possibility that other, as yet unidentified, etiologic factors, such as NSAIDs, may be responsible for inflammation in the GEJ region needs to be considered, but alternative etiologies have not been investigated thoroughly.

GERD

GERD induced chronic carditis and/or columnar metaplasia of the distal esophagus occurs more often in white males, in early to mid adult life, and is more common in patients who consume alcohol and tobacco, compared to patients with *H.pylori*-induced carditis². Endoscopically, the presence of a hiatus hernia, and/or the presence of an irregular, proximally located, Z-line relative to the anatomic GEJ, with or without signs of gastric type mucosa extending into the distal esophagus, and evidence of esophagitis, are endoscopic features in support of GERD as the etiology of inflammation in the GEJ region.

Pathologically, GERD patients often show only a mild degree of mononuclear inflammation within the lamina propria^{16,22}. However, acute inflammation, in the form of cryptitis, crypt abscesses or infiltration of the surface epithelium, either with or without surface erosion, may be present in some cases as well. Eosinophils are often a prominent component of the inflammatory infiltrate similar to that which occurs in the squamous epithelium in patients with GERD-induced non-erosive esophagitis²². Eosinophils may infiltrate the columnar epithelium in the gastric cardia as well. A dense infiltrate of

plasma cells, associated with reactive lymphoid follicles, are uncommon in GERD unless there is superimposed *H.pylori* infection. In a biopsy study by Wieczorek et al of 30 patients with known GERD induced inflammation, and 25 patients with known *H.pylori*-induced inflammation, of the GEJ region, GERD patients showed a significantly lower degree of plasma cells, neutrophils, and overall inflammation, but significantly more eosinophils, in comparison to *H.pylori* cases in which neutrophils, lymphocytes, plasma cells, and reactive lymphoid aggregates were not prevalent³⁰. In addition to gastric-type mucinous epithelium and underlying mucous, or mixed mucous/oxyntic, glands, a variety of “hybrid” cells that contain both gastric and intestinal morphologic features, and “pseudogoblet” cells (representing distended foveolar cells), may be seen in biopsies from GEJ as well³¹⁻³³. However, the specificity of those other cell types for esophageal columnar metaplasia versus native cardia epithelium is currently unknown.

In addition, the squamous mucosa in patients with GERD often shows reactive changes characterized by basal cell hyperplasia, elongation of the rete pegs and congestion of the lamina propria^{2,30}. Evidence of active esophagitis, characterized by eosinophilic and/or neutrophilic inflammation, may be present in more severe cases as well. In GERD patients who have a proximally displaced Z-line and, thus, columnar metaplasia of the distal esophagus, the lamina propria of the metaplastic columnar epithelium may look identical to that seen in the true gastric cardia. However, other features may be present that can help identify the epithelium as esophageal in origin. These include submucosal glands or ducts, and multilayered epithelium (ME), and are discussed further below in the differential diagnosis section³⁴.

H.pylori

The clinical, endoscopic and pathologic features of *H.pylori*-induced chronic carditis are often distinct from those seen in GERD, but there is overlap^{2,30}. Clinically, *H.pylori* patients are usually older in age and have a more equal male to female ratio than GERD patients. Endoscopically, the Z-line usually approximates closely the proximal aspect of the gastric folds in non-GERD patients, and hiatus hernias are less common. In *H.pylori* patients, the cardia often shows similar pathologic features to that seen in *H.pylori* antritis, characterized by a variable increase in the amount of lymphocytes and plasma cells in the lamina propria, neutrophilic cryptitis, and reactive lymphoid aggregates³⁰. In contrast to GERD, eosinophils are usually absent or only few in number.

In addition, patients with *H.pylori* carditis, but without GERD, reveal normal non-inflamed esophageal squamous epithelium. Use of special stains is recommended for detection of *H.pylori* organisms^{35,36}. However, since *H.pylori* organisms do not normally colonize intestinal type epithelium, they may be few in number in biopsies that contain IM. An exception to this finding occurs in patients who have been previously treated with a proton pump inhibitor, in which case there is some evidence to suggest that the organisms migrate proximally in the stomach^{37,38}.

Intestinal Metaplasia of the GEJ

Prevalence;

Nearly one-third of patients who present for upper GI endoscopy and without endoscopic evidence of BE, reveal IM in the GEJ region^{1,39}. Not surprisingly, the changes of detecting IM has been shown to increase proportionally with the number of biopsies obtained at endoscopy⁴⁰. In fact, there is some circumstantial evidence to suggest that the prevalence of IM is higher in patients who have longer lengths of mucosa composed of pure cardia type glands, and, therefore, may be higher in patients with GERD compared to those with *H.pylori*^{16,18,21}. For instance, in a study by Chandrasoma et al of 959 patients in whom biopsies were obtained from the GEJ region, 70% of those who had between 1-2 cm of mucosa composed of mucous glands, or mixed mucous/oxynitic glands, in the GEJ region had IM in comparison to only 15% of patients who had <1 cm of “cardia-type” mucosa¹⁸. Similarly, several studies have shown an association between the finding of IM in biopsies of the GEJ region and male gender, white race, and higher patient age, features representative of a GERD clinical profile⁴¹⁻⁴³.

Interestingly, IM detected in cardia biopsies from *H.pylori* patients is usually composed of a mixture of the incomplete and complete types in contrast to GERD-induced carditis and columnar metaplasia of the distal esophagus in which IM is usually of the incomplete type^{3,34,42,44}. The type and pattern of IM at the GEJ may explain the differences in dysplasia and cancer risk in patients with IM in the cardia related to *H.pylori* versus those with metaplastic columnar epithelium with IM in the distal esophagus related to GERD (see below)

Pathogenesis;

It is widely believed that chronic inflammation is the underlying stimulus for the development of IM in the GEJ, regardless of the etiology^{3,22,30,45}. In general, most cardia biopsies contain, at minimum, a mild degree of chronic inflammation which is normally increased in comparison to the corpus or antrum, particularly in *H.pylori* negative patients³⁰. In fact, a positive correlation was noted between the degree of chronic inflammation and the presence of IM in a biopsy study by Goldstein et al⁴⁶. In GERD patients, it is commonly believed that chronic reflux leads to inflammation and ulceration of the native squamous epithelium, which if persistent, may lead to IM. Squamous epithelium converts first to cardia-type columnar epithelium composed of mucinous columnar epithelium and mucous or mixed mucous/oxynitic glands, prior to intestinalization^{17,32,45}. Thus, with ongoing injury, and chronic inflammation, mucinous columnar epithelium converts to an intestinal phenotype as a result of secondary metaplastic reaction. Intestinal metaplasia related to GERD-induced metaplasia is usually of the incomplete type, which is composed of a mixture of acid-mucin containing goblet cells and gastric-type mucinous columnar epithelium. As mentioned below, the type and extent of IM is one of several factors that may help pathologists distinguish mucosal biopsies of the distal esophagus from the gastric cardia.

Unfortunately, the precise cell of origin of metaplastic columnar epithelium in the esophagus is unknown^{2,34}. However, possible sites of multipotential stem cells includes the basal layer of the native squamous epithelium, esophageal mucosa and/or submucosal glands and ducts, gastric cardia epithelium, congenital rests of gastric or intestinal

epithelium in the esophagus and/or the subepithelium mesenchyme of the esophagus^{34,27-50}. Interestingly, recent studies suggest that metaplastic and dysplastic epithelium in the stomach may derived from bone marrow cells, but this theory has never been investigated in the esophagus⁵¹. Nevertheless, based on a series of experiments by Gillen et al and Li et al, it is now firmly believed that metaplastic epithelium in the esophagus is derived from cells that are, in fact, intrinsic to the esophagus rather than the stomach^{50, 52}. There is also some evidence to suggest that esophageal mucosal ducts harbor stems that can differentiate into columnar epithelium^{34,47}.

Some authors suggest that the squamous to columnar cell transition in the distal esophagus occurs via an intermediate, or transitional phase prior to intestinalization^{17,30,34,53}. In 1993, Shields et al reported the presence of a distinctive type of multilayered epithelium (ME) that shows morphologic and cytochemical characteristics of both squamous and columnar epithelium⁵⁴. These investigators hypothesized that acid-induced ulceration of squamous epithelium exposes an as yet unidentified stem cell, which upon repeated acid damage, is stimulated to differentiate toward a columnar phenotype after passing through an intermediate ME phase. Multilayered epithelium is a biologically active epithelium that is phenotypically similar to fully developed BE³⁴. This epithelium has been shown by Glickman et al to contain a high capacity for cell proliferation and differentiation³⁴. It has also been shown to be highly associated with BE in a prospective biopsy study by Shields et al⁵³. Multilayered epithelium was also recently shown to be strongly associated with GERD-induced inflammation of the GEJ region in a biopsy study by Wieczorek et al³⁰. In their study, 30% of patients with GERD induced inflammation of the GEJ region had ME compared to only 4% (1 patient) of *H.pylori* patients (and this patient also had a hiatus hernia!). Thus, regardless of the putative role of ME in the development of columnar metaplasia in the esophagus, the presence of ME in a GEJ biopsy is considered highly specific for GERD and, thus, probably represents a specific histologic biomarker of GERD-induced metaplastic columnar epithelium in the distal esophagus⁵⁵. Multilayered epithelium is usually detected at the SCJ and often in the vicinity of the openings of the submucosal gland ducts, observations supporting the theory that the submucosal gland duct epithelium may contain stem cells that give rise to metaplastic columnar epithelium^{34,53,55}.

In contrast, IM that develops in the true gastric cardia secondary to chronic *H.pylori* infection represents a columnar to columnar (goblet cell) metaplastic reaction. It is currently unclear whether GERD-induced inflammation in the true gastric cardia can lead to IM in this manner, although there is some evidence in favor of this mechanism^{21,16,56}. Unfortunately, the pathogenesis of IM in the stomach has been poorly studied. Nevertheless, studies in the distal stomach have confirmed that chronic inflammation is a required precursor of IM and, thus, it is presumed that the same pathogenetic sequence occurred in the cardia of *H.pylori* infected patients, but this remains to be proven^{2,57}. In the stomach, the factors responsible for the conversion of mucinous columnar epithelium to intestinal-type epithelium are unknown. However, the cell or origin is presumed to reside in the deep foveolar or stem cell region of the gastric mucosa. In contrast to GERD-induced IM in the esophagus, IM in the cardia due to *H.pylori* infection is usually composed of a mixture of incomplete and complete-type IM.

Natural History;

It is commonly believed that IM is the mucosal field upon which adenocarcinoma develops, both in the esophagus and stomach, regardless of the specific etiology^{1,41,61}. In one study, 86% of adenocarcinomas that developed in the GEJ were associated with IM in adjacent, non-tumorous, mucosa¹³. With regard to adenocarcinomas of the GEJ region, evidence linking adenocarcinoma of the distal esophagus to GERD-induced IM is stronger than the data linking IM to the development of true cardia cancers^{14,59}. It is likely that most cancers develop through an IM-dysplasia-carcinoma sequence. Unfortunately, the true cancer risk for patients with IM in the distal esophagus secondary to GERD versus those with IM in true gastric cardia secondary to *H.pylori* is, essentially, unknown. In one study by Sharma et al, the dysplasia risk was shown to be greater in patients with short-segment BE versus those with IM of the gastric cardia only¹⁴. In that study, the prevalence rate of dysplasia in patients with short-segment BE versus those with carditis and IM was 11.3% versus 1.3%, with an incidence rates of 4.6% and 1.5%, respectively. In another study by the same lead author, the incidence of dysplasia in patients with short-segment BE was 5.7% per year⁵⁹. However, in both of these studies, the risk of neoplasia was not defined specifically for patients with either ultrashort BE, or for those columnar metaplasia of the distal esophagus but without IM. Thus, although difficult to interpret, IM in metaplastic columnar epithelium in the distal esophagus (i.e. ultrashort or short-segment BE) probably has a higher likelihood of progression to malignancy in comparison to patients with I in the true gastric cardia related to *H.pylori* infection. Clearly, this issue requires further investigation.

Differential Diagnosis of *H.pylori* Versus GERD in GEJ Biopsies

As noted above, it is important to differentiate true carditis from columnar metaplasia of the distal esophagus, given that the etiology, pathogenesis, natural history, and possibly, risk of malignancy is different between these two pathologic conditions. Since, as mentioned above, it is difficult for endoscopists to know the precise anatomic location of a biopsy in the GEJ region (i.e. whether it is located above or below the anatomic GEJ) it is incumbent upon pathologists to help establish the precise location, and etiology of inflammation, of inflammatory conditions in mucosal biopsies from the GEJ region, whenever possible. Pathologically, the finding of esophageal mucosal or submucosal glands or ducts, or ME, in a biopsy from the GEJ is helpful to confirm that a particular biopsy is from the tubular esophagus, and if mucinous columnar epithelium is present, then it is reasonable to conclude that it is metaplastic in origin². Other pathologic features may be helpful in this differential as well. For instance, in a recent study by Srivastava et al published in abstract form, a wide variety of objective morphologic parameters were evaluated in SCJ biopsies from the GEJ region in 20 patients with BE and 20 with carditis with IM⁶⁰. In that study, the presence of crypt atrophy, crypt disarray, the presence and extent of incomplete IM (comprising >50% of crypts), the finding of squamous epithelium overlying crypts with IM, ME, esophageal gland/ducts, or the presence of hybrid glands (deep glands composed of IM in their most superficial aspect), were all features highly associated with BE in biopsies obtained from the GEJ

region. In fact, squamous mucosa overlying crypts with IM, hybrid glands, and esophageal gland/ducts were each 100% specific for BE. Another recent study by that same research group confirmed that there is good agreement among GI pathologists for distinguishing BE from carditis with IM using the morphologic features outlined above (unpublished information).

The findings in esophageal squamous epithelium and in the remainder of the stomach (antrum, corpus) may also be helpful if biopsies are obtained from these locations. For instance, most, if not all cases, of *H.pylori* carditis also show *H.pylori* antritis and/or funditis^{36,36}. Conversely, the presence of active esophagitis in the squamous epithelium of the esophagus, combined with the finding of a normal antrum or corpus is strong evidence in favor of GERD as the cause of “GEJitis”³⁰. As mentioned above, *H.pylori* stains should be performed on all biopsies from the GEJ region since positivity can help confirm the diagnosis readily.

Some studies have suggested that there are, in fact, differences in mucin histochemistry between esophageal columnar epithelium, or goblet cells, derived from squamous epithelium and either normal or inflamed gastric cardia type epithelium^{32,45,61-63}. However, many of these studies suffer from limitations discussed previously in the introduction section of this paper. Nevertheless, in a recent well-controlled and rigidly defined mucosal biopsy study by Glickman et al, immunohistochemical expression of MUC-1 and MUC-6 were shown to be highly associated with BE-associated IM in contrast to IM in the cardia related to *H.pylori* infection⁶³. In their study, combined MUC-1 and MUC-6 staining in goblet cells was 90% specific for intestinalized epithelium related to GERD, or BE. Unfortunately, the low sensitivity of these stains probably limits their use in clinical practice.

Basic mucin histochemical stains, such as PAS (neutral mucins), Alcian blue (acid mucins) and high-iron diamine [acidic (colonic-type) sulphomucins], have not been shown to be helpful in distinguishing esophageal-derived columnar epithelium from the gastric cardia^{32,33,64,65}. Both non-goblet and goblet cells may be positive with any, or all, of these stains regardless of the site of origin. Some have advocated the use of Alcian blue to distinguish distended foveolar cells (“pseudogoblet” cells) (light staining) from true goblet cells (dark staining), regardless of their location. Unfortunately, studies have documented that both of these cells types may stain either weakly or strongly with Alcian blue, regardless of whether they are from metaplastic columnar epithelium in the distal esophagus or from the proximal cardia^{33,64,65}. In a study by Chen et al, high-iron diamine positivity in non-goblet columnar cells in mucosal biopsies from the GEJ region were shown to be highly associated with IM and, as such, the authors speculated that sulphomucin containing non-goblet cells are a specific marker of IM³². However, sulphomucin containing goblet cells were detected in both the esophagus and stomach in that study. The utility of other mucin histochemical markers such as DAS-1, CDX-2, HEP and CD10 have all been proposed as markers of IM in the esophagus, but non have been shown to be useful in this particular differential diagnosis⁶⁶⁻⁶⁹.

The pattern of cytokeratin 7 and 20 (CK7/20) immunostaining has recently been reported to help distinguish IM in the distal esophagus from IM in the true gastric cardia⁸. In 1999, Ormsby et al identified a distinctive type of CK7/20 immunostaining pattern in BE, consisting of diffuse strong CK7 staining of the surface and gland

epithelium, and superficial weak columnar staining with CK20, and termed this the “BE CK7/20 staining pattern”⁷⁰. The authors of that study found that the BE staining pattern was highly sensitive and specific for BE compared to gastric IM. Unfortunately, several other investigators have not been able to confirm the findings by Ormsy et al, and instead, have shown that the CK 7/20 immunostaining profile in biopsies from the GEJ or cardia often show a BE pattern as well^{8,45,71,72}. For instance, in a biopsy study by Glickman et al, patients with BE, both long and short segment types, as well as those with IM of the GEJ secondary to either *H.pylori* or GERD, showed a similar high degree of BE CK7/20 staining pattern (91% and 90%, respectively)⁷¹. In addition, there are many limitations to the use of CK7/20 immunostaining in evaluating biopsies from the GEJ region. These include differences in technical aspects of staining which create different staining results, observer variability in interpretation of a BE staining pattern, and variability depending on the types of samples utilized (biopsies versus resection specimens)⁸. For instance, in another inter-institutional observer variability study by Glickman et al, a different pattern of CK7/20 staining was observed in mucosal biopsies that were fixed in formalin versus those fixed in Hollandaise solutions³⁷. In addition, investigators from two different institutions showed significant disagreement on recognition of a BE staining pattern. Another major limitation of the CK 7/20 immunostaining technique is that it requires an appropriate amount of mucosa with IM to be present in order for the staining reaction to be interpreted reliably, at this present in time, a feature that is rarely present in small mucosal biopsies from the GEJ region. Thus, use of CK7/20 immunostaining is not recommended on daily clinical practice to separate esophageal from gastric IM on small biopsies from the GEJ region.

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