Published in final edited form as:

Am J Surg Pathol. 2016 June ; 40(6): 827–835. doi:10.1097/PAS.0000000000000623.

Histologic Features Associated with Columnar-Lined Esophagus in Distal Esophageal and Gastroesophageal Junction (GEJ) Biopsies from GERD Patients: A Community-Based Population Study

Genevieve Soucy, MD¹, Lynn Onstad², Thomas L. Vaughan, MD^{2,3}, and Robert D. Odze, MD FRCPC⁴

¹Centre Hospitalier Universitaire de Montreal, Montreal, Quebec, Canada

²Fred Hutchinson Cancer Research Center, Seattle, WA

³University of Washington, Seattle, WA

⁴Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Abstract

Background—There are inherent problems with the endoscopic and pathologic criteria for columnar-lined esophagus (CLE). Furthermore, the clinical and biological significance of an irregular squamocolumnar junction (SCJ) is unclear. The aim of this study was to evaluate the association between histologic features in SCJ biopsies and CLE, and to gain insight into the significance of an irregular SCJ.

Design—The study was a cross-sectional analysis of 2176 mucosal biopsies of the SCJ from 544 patients in a large prospective community clinic-based study of GERD in Washington State. Biopsy samples were evaluated blindly for a wide variety of histologic features, such as the presence and type of mucosal glands, submucosal glands and ducts, goblet cells, multilayered epithelium (ME), inflammation, and buried columnar epithelium. Histologic findings were correlated with the endoscopic findings [normal Z-line, irregular Z-line or CLE], and evaluated by logistic regression and ROC analysis.

Results—Five histologic features were associated with CLE: pure mucous glands, ME, presence of goblet cells, 50% of crypts with goblet cells, and buried columnar epithelium. Pure oxyntic glands was inversely associated with CLE. The features most strongly related to CLE included biopsies with 50% of crypts with goblet cells, ME, and mucosal gland type (AUC=0.71; 95% CI=0.66-0.76). Patients with an irregular Z-line were histologically similar to those with CLE.

Conclusions—Certain histologic features in biopsies of the SCJ are associated with the presence of CLE. Irregularity of the Z-line is probably indicative of ultrashort segment CLE, instead of being a potential variation of normal.

Address correspondence and reprint requests to: Robert D. Odze, MD FRCPC, Chief of Gastrointestinal Pathology, Brigham and Women's Hospital, Boston, MA, Professor of Pathology, Harvard Medical School, Brigham and Women's Hospital, Pathology, 75 Francis St., Boston, MA 02115, rodze@partners.org, (p) (617) 732-7549, (f) (617) 278-6950.

Presented in part at the annual United States and Canadian Academy of Pathology meeting in 2012, Vancouver, Canada

Keywords

Barrett's esophagus; goblet cells; GERD; pathology; histology; mucosal glands; multilayered epithelium

Introduction

In the USA, Barrett's esophagus (BE) is defined as replacement of the normal squamous-lined esophagus by metaplastic columnar epithelium that is endoscopically detectable, and contains goblet cells on histology¹. This condition develops as a result of chronic gastroesophageal reflux disease (GERD), and is a key precursor to esophageal adenocarcinoma^{1,2}. Regardless, the timing and sequence of pathologic changes in patients with columnar-lined esophagus (CLE), independent of the presence or absence of goblet cells, is unknown^{3,4}.

There are inherent problems with the endoscopic and pathologic criteria used to define CLE⁵⁻⁷. For instance, endoscopically, it is difficult to identify very short or ultrashort segments of CLE, particularly in patients with a hiatus hernia⁵. Furthermore, the clinical and biological significance of patients with an irregular squamo-columnar junction (SCJ), termed the "Z-line," are unclear, since this condition has been poorly studied⁸⁻¹¹. It is unknown whether patients with an irregular Z-line at endoscopy represent an anatomic variation of normal, or, alternatively, a very early (ultra-short) segment CLE (<1 cm in length), likely secondary to GERD^{8,10,12}. This condition is quite common in the general population^{8,10}. Thus, it is often difficult for endoscopists and pathologists to be certain whether columnar mucosa within biopsies obtained from an irregular Z-line represents metaplastic columnar mucosa of the distal esophagus or normal mucosa of the gastric cardia^{6,7}. Biopsies from this region occasionally demonstrate histologic features indicative of esophageal columnar metaplasia, such as multilayered epithelium (ME), submucosal glands or ducts in patients who do not have endoscopic evidence of CLE, but the significance of these findings are also unclear^{6,13}.

Unfortunately, previous studies that have evaluated the histologic features of the SCJ in the context of BE, ultra-short BE, or in biopsies from the GEJ region, often suffer from observer bias and lack of endoscopic correlation^{3,6,9,14-18}. They also are often limited by sampling error, or are based on study populations at high risk for BE and, therefore, are not applicable to the general community. To date there have not been any studies that have evaluated histologic features commonly present in the distal esophagus/GEJ region, including goblet cells, in patients with GERD from the general population and related these to endoscopic features such as the presence of CLE and an irregular Z-line.

The primary aim of this study was to determine which, if any, histologic features in SCJ biopsies from a large prospective cohort of community clinic-based patients with GERD symptoms, are associated with the presence of CLE. Our second aim was to determine the histologic features of patients with an irregular Z-line, in order to help determine whether this a normal finding or one that is more closely related to CLE.

Materials and Methods

The study group consisted of 544 patients (age 20-80 years) from five community gastroenterology clinics who participated in a case-control study of GERD in Western Washington between October 1, 1997 and September 30, 2000 as described previously^{19,20}. Participants underwent an upper endoscopy to investigate chronic GERD symptoms (defined as heartburn, acid regurgitation, atypical chest pain, and/or belching.) Persons with previously-diagnosed BE were excluded. During endoscopy, physicians recorded the presence and length of any visible CLE (in cm) and the characteristics (straight or irregular) of the Z-line, when no CLE was noted. Patients were subsequently classified into one of three endoscopic categories based on the findings at endoscopy. These included 1. normal endoscopy (straight and linear Z-line with no endoscopic evidence of CLE), 2. irregular Z-line with no evidence of CLE, 3. endoscopic evidence of CLE. For the purposes of some statistical analyses, the CLE group was further characterized as CLE <2 cm or CLE ≥ 2 cm in length. At the time of endoscopy, CLE was determined to be present if one or more tongues of salmon pink-colored mucosa were present in the tubular esophagus proximal to the GEJ. The GEJ was defined as the most proximal extent of the gastric folds, as per previously published criteria¹¹. The extent of esophageal CLE was defined as the distance (in cm) between the GEJ and the neo-SCJ (Neo-Z line), which represents the most proximal extent of columnar-appearing mucosa in the esophagus. These categories were based on the extent of CLE, regardless of the presence or absence of goblet cells in biopsies.

During endoscopy, all patients had separate 4-quadrant biopsies obtained from just distal to the SCJ for the purposes of the study. These were fixed in 10% buffered formalin, processed routinely in paraffin, and stained with Hematoxylin and Eosin (H&E). All of the biopsies were evaluated, in a blinded fashion without knowledge of the endoscopic findings, by one of the authors (GS) for a variety of histologic features (listed below). Biopsies selected from random patients, representing about 5% of the total cohort, were evaluated by a second pathologist (RDO) for determination of interobserver variability. Interobserver variability was minimal (<1%), and any discrepancies in interpretation were resolved by discussion at a multiheaded microscope.

In total, we evaluated 2216 mucosal biopsies, representing 4 biopsies per patient. Of the 544 patients, 289 (53.1%) had both squamous and columnar mucosa in 1 of the biopsies, 255 (46.9%) had only columnar and zero had only squamous mucosa in their biopsies. In the columnar portion of mucosa, we evaluated the presence or absence of submucosal glands, and their ducts, ME (as previously described)²¹, pancreatic acinar tissue, the presence and type of glands that reside underneath surface/crypt mucinous columnar epithelium (pure mucous, pure oxyntic, mixture of mucous and oxyntic), the presence and grade of active (neutrophilic) inflammation and chronic (lymphoplasmacytic) inflammation (recorded as either mild, moderate, or severe) (Figure 1A-D). The presence (yes versus no) and extent of goblet cells were also evaluated. The extent of goblet cells was scored as follows: 0%, <10% of crypts, 10-49%, or ≥ 50% of crypts that contain goblet cells.

In squamous mucosa, when present, we evaluated the presence and degree of neutrophils and eosinophils (graded as mild, moderate, or severe); the presence of ulceration; the

presence of basal cell hyperplasia (BCH), defined as basal cells that extend >15% of the thickness of the mucosa; and the presence of lamina propria papillary hyperplasia (PH), defined as papillae that extend >50% of the thickness of the mucosa. Other features evaluated included the presence of foci of squamous epithelium surrounded on both sides by columnar epithelium (often seen endoscopically as a squamous island), and the presence of subsquamous “buried” epithelium. Subsquamous buried epithelium was also evaluated for its histologic type (pure mucous, pure oxyntic, mixed mucous and oxyntic), and for presence of goblet cells. For all histologic features evaluated, data was recorded per patient, rather than per biopsy. Thus, a patient was considered to be positive for a specific histologic feature if that feature was present in at least 1 of the 4 biopsies. For histologic features that were evaluated quantitatively, such as the percentage of crypts with goblet cells, the values were calculated based on evaluation and quantitation of all fragments of tissue in all four biopsies, from each patient.

The aims of our prior (original) study were to investigate the etiology of BE and specialized intestinal metaplasia (SIM) of the GEJ^{19,20}. To maximize study power in the detailed data collection phase of the study, we enrolled all patients who were found to have SIM (“cases”) and a random sample of approximately 50% of patients who were found to have no evidence of SIM (“GERD controls”). Since the statistical analyses performed in this current project focuses on a different outcome (ie. CLE rather than SIM), in this study we adjusted for the random sampling by upweighting the records representing data from the original study GERD controls. Thus, Table 1 in this paper refers to “N,” which represents the actual number of patients, and also “PS,” which represents the effective population size. Multivariate unconditional logistic regression was used to calculate adjusted odds ratios (aORs) and 95% confidence intervals (CI) using Stata /SE (version 14)²². Similar to our prior study, in this current study, both clinic and gender were controlled for in our analyses.

This study was approved by the institutional review boards of the Fred Hutchinson Cancer Research Center, Seattle, Washington, USA, and all of the participating clinics and hospitals, including the Brigham and Women's Hospital, Boston, MA.

Results

Of the 544 patients, 269 (49.4%) had a normal appearing Z-line (normal Z-line), 58 (10.7%) had an irregular Z-line and 217 (39.9%) had CLE. Of the patients with CLE, 120 (55.3%) had <2 cm, and 97 (44.7%) had greater than 2 cm, of CLE. Table 1 summarizes the demographic features of the patients in each endoscopic category. Most patients were of European ancestry, with a mean age ranging from 48 to 52 years across endoscopic groups. Males comprised 60% of those with abnormalities and only 48% of those without.

Table 2A summarizes the pathologic features in the columnar portions of the biopsies according to type of endoscopic abnormality. No statistically significant differences were noted between any of the endoscopic subgroups with regard to the presence of submucosal glands or submucosal ducts, the presence of pancreatic acinar metaplasia, or the presence or degree (data not shown) of neutrophilic or chronic inflammation in columnar mucosa.

In contrast to the above mentioned histologic features, we observed large differences with regard to the presence of ME, goblet cells, and the percentage of crypts with goblet cells, when we compared patients with CLE to those with a normal endoscopy ($p<0.001$, $p=0.002$, and $p<0.001$, respectively). Patients with CLE also had a significantly higher percentage of biopsies composed of pure mucous glands (24.9% vs 11.6%), and lower percentage with pure oxyntic glands (21.2% vs 34.0%) compared to patients with a normal Z-line ($p<0.001$). Interestingly, overall, 27.3% had pure oxyntic glands (without any mucous glands) in their SCJ biopsies. Of these, 4.8% also had goblet cells in overlying surface mucinous columnar epithelium. This value was significantly lower than the frequency of biopsies with goblet cells in overlying surface mucinous columnar epithelium in patients who had either pure mucous glands or a mixture of mucous and oxyntic glands (23.1%, $p<0.001$). Furthermore, in patients whose biopsies contained only pure oxyntic glands, no differences were noted in the presence or degree of goblet cells in the overlying surface mucinous columnar epithelium between patients without endoscopic abnormalities (normal Z-line) versus those with an irregular Z-line or CLE. Finally, compared to patients with a normal Z-line, those with CLE also showed a significantly increased frequency of squamous islands (SI) (24.2% vs. 34.4%, $p=0.02$), neutrophilic inflammation in SIs (6.5% vs 11.8%, $p=0.05$) and buried epithelium ($p=0.02$).

The pattern of histologic differences that we observed when we compared those with an irregular Z-line to those with a normal Z-line was generally similar to what we found in the CLE vs normal Z-line group comparisons (described above), with only a few exceptions as noted below. ME, squamous islands and buried epithelium were all significantly increased in biopsies from the patients with an irregular Z-line compared to patients with a normal Z-line. Additional features with differences that were suggestive but not statistically significant include an increased percentage of biopsies with goblet cells (19.4% vs 13.9%, $p=0.22$); and mucosal gland type, with a higher percentage of pure mucosal glands (17.4% vs 11.6%) and lower percentage of pure oxyntic glands (16.3% vs 34.0%) in patients with an irregular Z-line compared to patients with a normal Z-line ($p=0.08$). Table 2B summarizes the pathologic features in the squamous mucosa portion of the biopsies according to type of endoscopic abnormality. No differences were observed in any of the histologic features of the squamous mucosa by endoscopic abnormality.

Figure 2 summarizes the distribution of multi-level histologic features (e.g., gland type and percent of crypts with goblet cells) that were significantly different between the three endoscopic patient groups in univariate analysis.

Table 3 summarizes the results of logistic regression analysis that evaluated the presence or absence of histologic features that were significant upon univariate analysis regarding the risk of either CLE, or any endoscopic abnormality (irregular Z-line or CLE). These features included mucosal gland type, ME, the presence and extent of goblet cells, and the presence of buried epithelium. Although the presence of squamous islands was also significantly related to risk of CLE, this feature was not included in these analyses since all but one patient with squamous islands also had buried epithelium. In this analysis, variables were adjusted for both clinic and gender. The three features having the strongest associations with CLE were goblet cells present in 50% of crypts (OR=3.3, $p=0.001$), ME (OR=2.3,

$p=0.003$), and the finding of pure mucous glands ($OR=1.9$, $p=0.02$). The finding of pure oxyntic glands showed a lower risk of CLE ($OR=0.7$), but the value was not statistically significant ($p=0.11$). Squamous islands with buried epithelium was not a significant risk factor for CLE in this multivariate model. Similar OR values were obtained for these histologic features when evaluated for the risk of any endoscopic abnormality (either an irregular Z-line or CLE) in patients with GERD.

Based on this multivariate analysis, ROC curves were generated for the best single, or combination of, histologic features associated with CLE (Figure 3A) or of any abnormality (irregular Z-line or CLE) (Figure 3B). The risk model for CLE that included the three statistically significant variables from Table 3 (goblet cells in $\geq 50\%$ of crypts, ME and mucosal gland type) showed moderate discriminatory ability with an area under the curve (AUC) = 0.71 [(95% confidence interval (CI)=0.66-0.76.)] The AUC based on the single strongest feature (goblet cells in $\geq 50\%$ of crypts; $AUC=0.65$; 95% CI=0.60-0.70) and two strongest features combined (adding ME; $AUC=0.68$; 95% CI=0.63-0.73) are also presented in Figure 3A. Similarly, the same three histologic features showed significant, but slightly more modest, discriminatory value with regard to patients with either any endoscopic abnormality (irregular Z-line or CLE) with an area under the curve of $AUC=0.68$ (95% CI=0.64-0.73).

A subanalysis was performed to compare the frequency of histologic features in biopsies from CLE patients with <2 vs. ≥ 2 cm of CLE. In that comparison, a significant increase was observed in the percentage of biopsies containing any goblet cells (18.7 versus 29.8, $p=0.03$), and in the percentage of crypts that contained goblet cells ($p=0.01$). None of the other histologic features showed any differences between these two subgroups of CLE patients. Finally, there were no significant differences in either the presence, or degree, of any of the histologic features between patients with an irregular Z-line and those with <2 cm of CLE.

Discussion

We performed this study to determine if there are histologic features that are associated with CLE, and to gain insight into the biological significance of an irregular Z-line. We found five histologic features that were associated with CLE when detected in biopsies of the SCJ (or neo-SCJ) when analyzed individually. These included mucosal gland type, with pure mucosal gland being positively associated with CLE and pure oxyntic gland type being inversely associated with CLE, ME, presence and amount of crypts with goblet cells, and squamous islands with underlying buried columnar epithelium. In multivariate analyses, histologic features significantly associated with CLE included $\geq 50\%$ of crypts with goblet cells, ME, and mucosal gland type. In combination, these features had an AUC of 0.71. Our data in patients with an endoscopically irregular Z-line revealed histologic similarity to CLE, and in particular to patients with <2 cm of CLE, and also similar significant differences with the normal endoscopy group as with the CLE group. This suggests that irregularity of the Z-line is a condition probably indicative of very early, or ultra-short, segment, CLE instead of being a potential anatomic variation of normal. Overall, we conclude that certain histologic features in biopsies of the SCJ (or neo SCJ) are associated with the presence of CLE and of

patients with very early CLE prior to the development of endoscopically evident tongues of CLE.

Our review of the literature did not find any prior publications that have evaluated histologic features associated with, or predictive of, CLE according to the endoscopic categories that we used in this study. However, there have been several prior studies that have looked at either individual histologic features, such as the presence and frequency of goblet cells, or a combination of features, such as the type of mucosal glands and the presence and degree of inflammation, in GEJ biopsies of either GERD patients or those with BE^{6,14,15,17,21,23-29}. For instance, in one study by Srivastava et al in 2007, of 20 patients with BE compared to 20 patients without BE who had biopsies of the proximal stomach, several histologic features such as squamous epithelium overlying glands with intestinal metaplasia, hybrid glands, and esophageal glands or ducts, were significantly associated with BE rather than the gastric cardia⁶. However, in that study, goblet cells were not evaluated since they were part of the criterion for BE and the type of endoscopic abnormality (irregular Z-line versus CLE) was not evaluated. In contrast, in another study by Lenglinger et al in 2007, 114 consecutive patients with GERD were evaluated for the presence and degree of CLE and this was correlated with the findings in biopsies of the SCJ or proximal stomach¹⁵. However, the biopsies were only evaluated for intestinal metaplasia (goblet cells) and dysplasia. Regardless, their finding with regard to goblet cells was similar to ours, since they identified goblet cells in 17% of patients without CLE (normal Z-line) and they were significantly increased in patients with CLE (24.7%), and in those with <0.5 cm of CLE. In comparison, we observed that 13.9% of patients with a normal Z-line had goblet cells, and this feature increased to 19.4% and 23.5% in patients with an irregular Z-line and CLE, respectively. Multiple other studies have also shown that goblet cells occur in a small proportion of GEJ biopsies from patients without CLE and that, in general, the prevalence rate and degree of goblet cell metaplasia increases in direct proportion to the length of CLE^{17,28-31}.

In the USA and other parts of the world, the finding of goblet cells is required for a diagnosis of BE based on the assumption that this is a necessary feature indicating increased risk of cancer, although this is controversial^{1,32}. Our finding of goblet cells in only 23.5% of patients with CLE in this study (18.7% in patients with <2 cm CLE and, 29.8% in patients with ≥2 cm CLE) suggests that either many of our CLE patients were early in the development of esophageal columnar metaplasia prior to goblet cell metaplasia, or goblet cells were missed due to sampling error. The latter is unlikely, or minimal, given that in our study, biopsies were obtained from the neo-SCJ in patients with CLE, an area of the esophagus previously shown to contain the highest proportion of goblet cells. Furthermore, our data is similar to those of others in which, in general, less than 35% of patients with short-segment CLE have goblet cells identified in their esophageal biopsies²⁸.

Thus, not all patients with CLE have goblet cells, and conversely, patients without CLE may have goblet cells identified in the gastric side of the GEJ. These findings should be taken into account when evaluating patients for BE. For instance, biopsies of the distal esophagus/GEJ region should not be interpreted as “consistent with BE” simply on the basis of finding goblet cells, unless the biopsies were confirmed endoscopically to have been obtained from the esophagus. Interestingly, in our study, goblet cells occurred predominantly

in mucosa that contained either pure mucous glands, or a mixture of mucous and oxyntic glands. However, we also detected goblet cells in 4.8% of those with pure oxyntic glands, including patients with CLE. In fact, biopsies consisting of pure oxyntic glands were detected in 16.3% of patients with an irregular Z-line and in 21.2% of patients with CLE. This suggests that the finding of pure oxyntic glands in a biopsy of the GEJ region may be metaplastic in origin, and thus, its presence does not necessarily imply gastric location, as suggested by some authors^{3,15,17,26,29}.

The clinical significance and histologic features of the SCJ in patients with an irregular Z-line at endoscopy have been poorly studied, and thus remain poorly understood. Our data showing a similar type and frequency of histologic features in the irregular Z-line group compared to the <2.0 cm CLE group suggests that the former patients, may represent very early, or ultrashort, segment CLE rather than being a variant of normal. Based on our study, we cannot determine whether our patients with an irregular Z-line represent an “early” stage in the development and progression of CLE, or whether these patients represent a “mild” form of CLE, and are not at risk for further extension of disease. There have been several studies that have evaluated progression to BE in patients with reflux esophagitis^{33,34}, but only a few have evaluated extension of length of CLE in patients with ultra-short BE or intestinal metaplasia at the GEJ³⁵⁻³⁷. These studies suggest that only a small percentage of patients with IM in the GEJ region eventually progress to endoscopically evident CLE. Whether the patients who progress are those with an irregular Z-line, and the factors responsible for or associated with progression, remains unknown and requires further outcome studies.

Another interesting result is that we found that a small subset of patients with a normal Z-line revealed some histologic features in their SCJ biopsies that, historically, have been thought to be exclusively associated with CLE^{18,21,24,26}. For instance, submucosal glands or ducts, or ME, were present in 0.4%, 2.3%, and 9.2% of our normal endoscopy patients, respectively. Possible explanations for these findings include: a) Incorrect interpretation of a slightly irregular Z-line as normal by the endoscopist, in which case these patients may have ultrashort CLE, b) normal variability of the location of the Z-line in the general population of patients with GERD, and c) these histologic features may, in fact, be present normally in the most proximal aspect of the stomach, and thus, their presence do not always represent CLE.

Several prior studies have evaluated submucosal glands or ducts in the GEJ region^{24,26}. For instance, in 2000, Chandrasoma et al evaluated the entire GEJ in 18 random autopsies²⁴. In that study, submucosal glands were identified in 22% of patients, and they always occurred underneath mucosa consisting of either pure mucous glands, or a mixture of mucous and oxyntic glands. These findings support the possibility that a small proportion of the general population, possibly those with GERD, show submucosal glands or ducts in the GEJ region under normal circumstances. We also found pure mucous glands, or a mixture of mucous and oxyntic glands, in 11.6% and 49.8% of patients with a normal Z-line, respectively. This data supports the theory that mucous glands, or mixed mucous or oxyntic glands, are a normal component of the GEJ region, and their presence do not necessarily indicate that it is metaplastic in origin.

Multilayered epithelium has been postulated to represent an early or transitional type of epithelium in the development of CLE^{21,28}. Multiple prior studies have shown a strong association between ME and both reflux esophagitis and BE^{18,21,38,39}. Our finding of a significantly increased frequency of ME in patients with an irregular Z-line or CLE, compared to those with a normal-appearing Z-line, supports ME as a possible precursor in CLE. In fact, our findings of a significant increase in the prevalence rate of ME between patients with a normal Z-line versus those with an irregular Z-line (9.2% versus 25.5%, $p<0.001$), but no further increase between irregular Z-line patients and those with established CLE (21.8%, $p=0.56$), further supports ME as important in the early stage of CLE development. Further follow up studies would need to be performed in order to determine if patients with ME, but a normal Z-line, are at risk for progression to CLE.

Regarding inflammation, we found no significant difference in either the presence, or extent, of active or chronic inflammation between any of the three endoscopic categories of patients. In one prior study by Der et al in 2001, of 141 patients with GERD who had cardiac biopsies obtained from the GEJ, in contrast to our study, significant chronic inflammation was present in 100% of cases, and the degree of chronic inflammation correlated with higher esophageal acid exposure as quantitated by a 24 hour pH test²⁵. However, our criteria for “increased” chronic inflammation differed from this prior study, where chronic inflammation was considered to be increased if any chronic inflammatory cells were present. In our study, this finding was interpreted as baseline normal for biopsies of this region. Thus, despite the presumed role of inflammation in the pathogenesis of CLE, our results do not support the type or degree of inflammation as potential predictive markers of CLE in patients with GERD.

Our study has several limitations. First, we did not include biopsies from control patients without GERD, so distinctive histologic features for GERD could not be determined. Second, endoscopic procedures were performed by multiple endoscopists from different hospitals, which may have resulted in interobserver variability in categorizing patients endoscopically. However, this remains a potential source of error in all studies of the GEJ and CLE, even those that are prospective because of inherent variability in this procedure. Furthermore, we adjusted for “clinic” in our multivariate model of risk assessment of specific histologic features. The strengths of our study included use of a uniform and systematic evaluation of pathology by two experienced GI pathologists, the large population size of patients in each endoscopic category, and the uniform manner in which biopsies were obtained from the SCJ or neo-SCJ.

In summary, we have shown that several histologic features, including mucosal gland type, ME, and 50% of crypts containing goblet cells, are associated with the presence of CLE, or an irregular Z-line or CLE, when found in biopsies obtained from the SCJ or neo-SCJ. In combination, these features have moderate discriminatory ability ($AUC=0.71$) for the presence of CLE. Our findings also support the theory that patients with an irregular Z-line represent an early (or ultra-short) segment of CLE. Future, preferably prospective studies are needed to determine whether certain histologic features are predictive of extension, and/or neoplastic progression, of BE.

Acknowledgments

Funding provided by: NIH K05CA124911

References

1. American Gastroenterological Association. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American gastroenterological association medical position statement on the management of barrett's esophagus. *Gastroenterology*. 2011; 140(3):1084–1091. [PubMed: 21376940]
2. Bhat S, Coleman HG, Yousef F, et al. Risk of malignant progression in barrett's esophagus patients: Results from a large population-based study. *J Natl Cancer Inst*. 2011; 103(13):1049–1057. [PubMed: 21680910]
3. Chandrasoma PT, Der R, Dalton P, et al. Distribution and significance of epithelial types in columnar-lined esophagus. *Am J Surg Pathol*. 2001; 25(9):1188–1193. [PubMed: 11688579]
4. Hahn HP, Blount PL, Ayub K, et al. Intestinal differentiation in metaplastic, nongoblet columnar epithelium in the esophagus. *Am J Surg Pathol*. 2009; 33(7):1006–1015. [PubMed: 19363439]
5. Sharma P, Morales TG, Sampliner RE. Short segment barrett's esophagus--the need for standardization of the definition and of endoscopic criteria. *Am J Gastroenterol*. 1998; 93(7):1033–1036. [PubMed: 9672325]
6. Srivastava A, Odze RD, Lauwers GY, Redston M, Antonioli DA, Glickman JN. Morphologic features are useful in distinguishing barrett esophagus from carditis with intestinal metaplasia. *Am J Surg Pathol*. 2007; 31(11):1733–1741. [PubMed: 18059231]
7. Naini BV, Chak A, Ali MA, Odze RD. Barrett's oesophagus diagnostic criteria: Endoscopy and histology. *Best Pract Res Clin Gastroenterol*. 2015; 29(1):77–96. [PubMed: 25743458]
8. Kim JB, Shin SR, Shin WG, et al. Prevalence of minimal change lesions in patients with non-erosive reflux disease: A case-control study. *Digestion*. 2012; 85(4):288–294. [PubMed: 22572860]
9. Fitzgerald RC, di Pietro M, Ragnauth K, et al. British society of gastroenterology guidelines on the diagnosis and management of barrett's oesophagus. *Gut*. 2014; 63(1):7–42. [PubMed: 24165758]
10. Dickman R, Levi Z, Vilkin A, Zvidi I, Niv Y. Predictors of specialized intestinal metaplasia in patients with an incidental irregular Z line. *Eur J Gastroenterol Hepatol*. 2010; 22(2):135–138. [PubMed: 19907339]
11. Sharma P, Dent J, Armstrong D, et al. The development and validation of an endoscopic grading system for barrett's esophagus: The prague C & M criteria. *Gastroenterology*. 2006; 131(5):1392–1399. [PubMed: 17101315]
12. Leodolter A, Nocon M, Vieth M, et al. Progression of specialized intestinal metaplasia at the cardia to macroscopically evident barrett's esophagus: An entity of concern in the ProGERD study. *Scand J Gastroenterol*. 2012; 47(12):1429–1435. [PubMed: 23110405]
13. Odze RD. Barrett esophagus: Histology and pathology for the clinician. *Nat Rev Gastroenterol Hepatol*. 2009; 6(8):478–490. [PubMed: 19581906]
14. Kilgore SP, Ormsby AH, Gramlich TL, et al. The gastric cardia: Fact or fiction? *Am J Gastroenterol*. 2000; 95(4):921–924. [PubMed: 10763938]
15. Lenglinger J, Ringhofer C, Eisler M, et al. Histopathology of columnar-lined esophagus in patients with gastroesophageal reflux disease. *Wien Klin Wochenschr*. 2007; 119(13-14):405–411. [PubMed: 17671821]
16. Sarbia M, Donner A, Gabbert HE. Histopathology of the gastroesophageal junction: A study on 36 operation specimens. *Am J Surg Pathol*. 2002; 26(9):1207–1212. [PubMed: 12218577]
17. Chandrasoma PT, Lokuhetty DM, Demeester TR, et al. Definition of histopathologic changes in gastroesophageal reflux disease. *Am J Surg Pathol*. 2000; 24(3):344–351. [PubMed: 10716147]
18. Wiczorek TJ, Wang HH, Antonioli DA, Glickman JN, Odze RD. Pathologic features of reflux and helicobacter pylori-associated carditis: A comparative study. *Am J Surg Pathol*. 2003; 27(7):960–968. [PubMed: 12826888]
19. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of barrett's esophagus. *Gastroenterology*. 2007; 133(2):403–411. [PubMed: 17681161]

20. Edelstein ZR, Bronner MP, Rosen SN, Vaughan TL. Risk factors for barrett's esophagus among patients with gastroesophageal reflux disease: A community clinic-based case-control study. *Am J Gastroenterol.* 2009; 104(4):834–842.
21. Glickman JN, Chen YY, Wang HH, Antonioli DA, Odze RD. Phenotypic characteristics of a distinctive multilayered epithelium suggests that it is a precursor in the development of barrett's esophagus. *Am J Surg Pathol.* 2001; 25(5):569–578. [PubMed: 11342767]
22. StataCorp.. Stata statistical software: Release 14. StataCorp LP.; college station, TX: 2015.
23. Ringhofer C, Lenglinger J, Izay B, et al. Histopathology of the endoscopic esophagogastric junction in patients with gastroesophageal reflux disease. *Wien Klin Wochenschr.* 2008; 120(11-12):350–359. [PubMed: 18709523]
24. Chandrasoma PT, Der R, Ma Y, Dalton P, Taira M. Histology of the gastroesophageal junction: An autopsy study. *Am J Surg Pathol.* 2000; 24(3):402–409. [PubMed: 10716154]
25. Der R, Tsao-Wei DD, Demeester T, et al. Carditis: A manifestation of gastroesophageal reflux disease. *Am J Surg Pathol.* 2001; 25(2):245–252. [PubMed: 11176074]
26. Chandrasoma P, Makarewicz K, Wickramasinghe K, Ma Y, Demeester T. A proposal for a new validated histological definition of the gastroesophageal junction. *Hum Pathol.* 2006; 37(1):40–47. [PubMed: 16360414]
27. Chandrasoma P, Wijetunge S, Demeester SR, Hagen J, Demeester TR. The histologic squamo-oxyntic gap: An accurate and reproducible diagnostic marker of gastroesophageal reflux disease. *Am J Surg Pathol.* 2010; 34(11):1574–1581. [PubMed: 20871393]
28. Csendes A, Smok G, Burdiles P, et al. Prevalence of intestinal metaplasia according to the length of the specialized columnar epithelium lining the distal esophagus in patients with gastroesophageal reflux. *Dis Esophagus.* 2003; 16(1):24–28. [PubMed: 12581250]
29. Oberg S, Johansson J, Wenner J, et al. Endoscopic surveillance of columnar-lined esophagus: Frequency of intestinal metaplasia detection and impact of antireflux surgery. *Ann Surg.* 2001; 234(5):619–626. [PubMed: 11685024]
30. Chandrasoma PT, Der R, Ma Y, Peters J, Demeester T. Histologic classification of patients based on mapping biopsies of the gastroesophageal junction. *Am J Surg Pathol.* 2003; 27(7):929–936. [PubMed: 12826885]
31. Desai TK, Krishnan K, Samala N, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic barrett's oesophagus: A meta-analysis. *Gut.* 2012; 61(7):970–976. [PubMed: 21997553]
32. Srivastava A, Golden KL, Sanchez CA, et al. High goblet cell count is inversely associated with ploidy abnormalities and risk of adenocarcinoma in barrett's esophagus. *PLoS One.* 2015; 10(7):e0133403. [PubMed: 26230607]
33. Ronkainen J, Talley NJ, Storskrubb T, et al. Erosive esophagitis is a risk factor for barrett's esophagus: A community-based endoscopic follow-up study. *Am J Gastroenterol.* 2011; 106(11):1946–1952. [PubMed: 21946284]
34. Labenz J, Nocon M, Lind T, et al. Prospective follow-up data from the ProGERD study suggest that GERD is not a categorical disease. *Am J Gastroenterol.* 2006; 101(11):2457–2462. [PubMed: 17029609]
35. Cameron AJ, Lomboy CT. Barrett's esophagus: Age, prevalence, and extent of columnar epithelium. *Gastroenterology.* 1992; 103(4):1241–1245. [PubMed: 1397881]
36. Dekel R, Wakelin DE, Wendel C, et al. Progression or regression of barrett's esophagus--is it all in the eye of the beholder? *Am J Gastroenterol.* 2003; 98(12):2612–2615. [PubMed: 14687805]
37. Meining A, Ott R, Becker I, et al. The munich barrett follow up study: Suspicion of barrett's oesophagus based on either endoscopy or histology only--what is the clinical significance? *Gut.* 2004; 53(10):1402–1407. [PubMed: 15361485]
38. Langner C, Wolf EM, Plieschnegger W, et al. Multilayered epithelium at the gastroesophageal junction is a marker of gastroesophageal reflux disease: Data from a prospective central european multicenter study (histoGERD trial). *Virchows Arch.* 2014; 464(4):409–417. [PubMed: 24535701]
39. Shields HM, Rosenberg SJ, Zwas FR, Ransil BJ, Lembo AJ, Odze R. Prospective evaluation of multilayered epithelium in barrett's esophagus. *Am J Gastroenterol.* 2001; 96(12):3268–3273. [PubMed: 11774935]

Figure 1. Columnar-lined esophagus (CLE) showing mucosa with pure mucous glands (A), pure oxyntic glands (B), a mixture of mucous and oxyntic glands (C) and multilayered epithelium (D). (Hematoxylin and Eosin staining, magnification 20x).

Figure 2. Distribution of gland type (A) and percent crypts with goblet cells (B) by category of endoscopic abnormality: Normal, Irregular Z-line, and Columnar-lined esophagus (CLE).

Figure 3. ROC curves for columnar-lined esophagus (CLE) (A) and for CLE plus irregular Z-line (B). The curves represent the strongest single (goblet cells present in 50% of crypts; blue), pair (plus multilayered epithelium; red) and trio (plus gland type; green) of histologic features.

Table 1

Demographic Features of the GERD Patients According to Type of Endoscopic Abnormality

Demographic Feature	Endoscopic Abnormality			
	None	Irregular Z-line	CLE ¹	Total (Irregular + CLE)
	N=269	N=58	N=217	N=275
	PS ² =476	PS=98	PS=349	PS=447
	Percent ³			
Gender				
Male	48.1	59.2	60.5	60.2
Female	51.9	40.8	39.5	39.8
Mean Age (95% CI)	50.0 (48-52)	47.8 (44-52)	51.5 (50-53)	50.7 (49-52)
Race				
White	87.0	93.9	91.4	92.0
Black/African	5.7	0.0	2.9	2.2
Asian American	2.1	2.0	2.3	2.2
American Indian/Eskimo	1.0	1.0	0.0	0.2
Other/unknown	4.2	3.1	3.4	3.4

¹Columnar-lined esophagus²Population size used in weighted analysis³Based on population size

Table 2A

Presence and Frequency of Histologic Features of Columnar Mucosa in GERD Patients According to Type of Endoscopic Abnormality.

Clinical Feature	Endoscopic Abnormality				Irreg vs. Normal	CLE vs. Normal	Total vs. Normal	CLE vs. Irreg
	None	Irregular Z-line	CLE ¹	Total ² (Irreg + CLE)				
	Percent ³							
	P-values							
Submucosal gland	0.4	0.0	0.9	0.7	0.65	0.56	0.70	0.48
Submucosal duct	2.3	3.1	4.6	4.2	0.69	0.18	0.22	0.53
Mucosal gland	95.4	95.9	98.8	98.2	0.87	0.05	0.09	0.17
Type of gland								
Pure mucous gland	11.6	17.4	24.9	23.3	0.08	<0.001	<0.001	0.25
Pure oxyntic gland	34.0	16.3	21.2	20.1				
Mixed mucous/oxyntic	49.8	62.2	52.7	54.8				
No mucosal gland	4.6	4.1	1.2	1.8				
Multilayered epithelium	9.2	25.5	21.8	22.6	<0.001	<0.001	<0.001	0.56
Pancreatic acinar metaplasia	15.3	17.4	15.2	15.7	0.72	0.97	0.92	0.71
Goblet cells	13.9	19.4	23.5	22.6	0.22	0.002	0.002	0.45
% Crypts with goblet cells								
0%	86.1	80.6	76.5	77.4	0.16	<0.001	<0.001	0.37
<10%	7.6	9.2	6.3	6.9				
10-49%	3.2	2.0	4.6	4.0				
50-100%	3.2	8.2	12.6	11.6				
Neutrophilic inflammation	20.2	21.4	23.8	23.3	0.83	0.35	0.39	0.71
↑ Chronic inflammation	55.5	63.3	55.0	56.8	0.30	0.92	0.76	0.28
Squamous islands (SI)	24.2	38.8	34.4	35.4	0.03	0.02	0.006	0.55
Any abnormality in SI	6.5	3.1	12.3	10.3	0.31	0.03	0.12	0.04
Neutrophils	6.5	3.1	11.8	9.8	0.31	0.05	0.16	0.05
Ulceration	0.0	0.0	0.6	0.4	NA	0.24	0.30	0.60
Buried epithelium	24.2	38.8	33.8	34.9	0.03	0.02	0.008	0.50
Type of epithelium								
Mucous in BE	18.5	31.6	24.6	26.2	0.10	0.12	0.06	0.51
Oxyntic	1.9	0.0	2.6	2.0				
Mixed	2.9	5.1	3.4	3.8				
Goblet cells	0.8	2.0	3.2	2.9				
No buried epithelium	75.8	61.2	66.2	65.1				

¹ Columnar-lined esophagus

² Irregular Z-line

³ Based on weighted analysis

Table 2B

Presence and Frequency of Histologic Features of Squamous Mucosa in GERD Patients According to Type of Endoscopic Abnormality.

Clinical Feature	Endoscopic Abnormality						
	None	Irregular Z-line	CLE ¹	Total (Irreg ² + CLE)	Irreg vs. Normal	CLE vs. Normal	Total vs. Normal
	Percent ^{3,4}				P-values		
Squamous mucosa (SM) present	48.1	67.4	54.2	57.0	0.01	0.20	0.04
Any abnormality in SM	38.0	24.2	32.3	30.2	0.12	0.37	0.18
Type of abnormality in SM							
Neutrophils	28.0	18.2	25.9	23.9	0.21	0.73	0.45
Eosinophils	12.7	10.6	14.8	13.7	0.73	0.64	0.80
Ulcer	8.7	3.3	3.7	3.5	0.27	0.13	0.08
Basal cell hyperplasia	21.8	13.6	20.1	18.4	0.27	0.75	0.49
Papillary hyperplasia	16.2	7.6	10.0	9.4	0.20	0.18	0.10

¹ columnar-lined esophagus

² irregular Z-line

³ based on weighted analysis

⁴ for presence of squamous mucosa based on total population; for all other features based on those with squamous mucosa present

Table 3

Results of logistic regression analysis¹ of risk of endoscopic abnormality on key histologic features

Histologic feature	Risk of CLE ^{2,3}		Risk of irregular Z-line or CLE ⁴	
	OR (95% CI)	P	OR (95% CI)	P
Goblet cells in 50% of crypts	3.3 (1.6, 6.5)	0.001	2.9 (1.5, 5.4)	0.001
Multilayered epithelium	2.3 (1.3, 4.0)	0.003	2.4 (1.5, 4.1)	0.001
Mucosal gland type				
Non-pure	1	--	1	--
Pure mucous glands	1.9 (1.1, 3.2)	0.02	1.7 (1.0, 2.8)	0.05
Pure oxyntic glands	0.7 (0.4, 1.1)	0.11	0.7 (0.4, 1.1)	0.08

¹ Adjusted for clinic and gender

² Columnar-lined esophagus

³ Weighted analysis (N=486, population size 825)

⁴ Weighted analysis (N=544, population size 923)