Goblet Cell Mimickers in Esophageal Biopsies Are Not Associated With an Increased Risk for Dysplasia

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Context.—Identification of intestinal-type goblet cells (ITGCs) in hematoxylin-eosin–stained sections of esophageal biopsies is essential for the diagnosis of Barrett metaplasia. However, we have seen cases diagnosed as Barrett metaplasia based solely on cells that pose morphologic similarity to ITGCs on hematoxylin-eosin staining or stain positive with Alcian blue.

Objective.—Determine the clinical significance of goblet cell mimickers.

Design.—Initial biopsies from 78 patients with original diagnosis of Barrett metaplasia were reviewed and classified into 3 categories: (1) ITGCs, (2) goblet cell mimickers, or (3) neither. Sections from available paraffin blocks were stained with Alcian blue at pH 2.5. The presence of the different types of cells and positive Alcian blue staining were correlated with each other and evaluated for their significance as predictors of progression to dysplasia.

Results.—Goblet cell mimickers were present in 35 cases and were associated with ITGCs in the same biopsy in 23 (66%) of these cases. Intestinal-type goblet cells were present in 56 cases, and the remaining 10 cases, although called Barrett on the original report, did not show either ITGCs or goblet cell mimickers. Only the presence of ITGCs was associated with significant risk for dysplasia (P = .008). Positive Alcian blue staining was not associated with a significant risk for dysplasia.

Conclusions.—Our results indicate that the diagnosis of Barrett metaplasia should be rendered with confidence only when ITGCs are identified on routine hematoxylin-eosin–stained sections.

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were examined. Dysplasia was graded as negative for dysplasia, indefinite for dysplasia (IND), low-grade dysplasia (LGD), and high-grade dysplasia, according to published guidelines with minor modification; IND was rendered on cases morphologically similar to LGD, without active inflammation, even when the cellular changes characteristic of dysplasia did not involve the surface epithelium. Because a diagnosis of IND was found to be associated with a significant risk of malignant progression, and of the known significant interobserver and intraobserver variability in separating IND from LGD, we combined them into 1 category, LGD/IND, as proposed by Reid et al.

Follow-up information was available from the pathology reports and patient records. Hematoxylin-eosin–stained slides from the initial biopsies were reviewed by 1 pathologist for morphologic classification as (1) ITGCs, (2) GCMs, or (3) neither ITGCs nor GCMs. Paraffin blocks with sufficient tissue were available on 66 initial biopsies. Sections of these blocks were stained with Alcian blue (AB) at pH 2.5 and reviewed by a second pathologist who was blinded to the results of morphologic evaluation and patient outcome. Progression for dysplasia was performed using the Kaplan-Meier method and the log-rank test for statistical significance, with appearance of any grade of dysplasia as the outcome measure (end point). Specificity and sensitivity and all statistical analysis was performed using GraphPad Prism version 4.0b for Macintosh (GraphPad Software, San Diego, Calif).

RESULTS

Thirteen cases had biopsies from the gastroesophageal junction, including 2 cases that had additional biopsies from the esophagus; 13 cases had biopsies from 2 or more sites (levels) in the esophagus, but not gastroesophageal junction; and 52 cases had biopsies submitted in a single vial. Of the latter group of cases, the single site/level from the esophagus was stated on the vial (such as esophagus, 32 cm) in 9 cases, and the remaining cases were submitted as “distal esophagus” or “esophagus.”

Using the classification originally proposed by Faul et al., there were 56 cases with “specialized columnar epithelium” with “intestinal-type goblet cells,” 16 “junctional type” with cardiac mucous gland cells, and 6 “atrophic gastric-fundic type.” None of the 6 cases with gastric fundic type mucosa had ITGCs or GCMs. Seven (54%) of the 13 gastroesophageal junction biopsies had GCMs compared with 35 (54%) of the 65 other cases (P > .99).

Twelve (15%) of the 78 cases had GCM1 without ITGCs, 56 (72%) had typical ITGCs, and 10 (13%) neither ITGCs nor GCMs. Examples of ITGCs, which on routine H&E sections are goblet-shaped cells filled with characteristic inhomogeneous blue mucin, are shown in Figure 1, A and B. Examples of GCMs are shown in Figure 2. Two types of GCM are recognized: GCM1, which are goblet-shaped cells often filled with light pink homogeneous material (Figure 2, A), and GCM2, which are columnar nongoblet cells that are filled with faint blue mucin (Figure 2, B).

Two cases had all 3 types, ITGCs, GCM1, and GCM2. Twenty-three cases had GCM1 and ITGCs, 3 cases had GCM2 and ITGCs, and 6 cases had GCM1 and GCM2 cells. Thirty cases had only ITGCs, and 6 had only GCM1 cells.

The relationships between the different cell types are shown in the Table. Column ITGC shows that of the 56 cases of ITGCs, 30 only had ITGCs, whereas 23 had ITGCs + GCM1 and 3 had ITGCs + GCM2. Column GCM1 shows that of the 35 cases that had GCM1, 23 were associated with ITGCs, 6 were not associated with any of the other types (only GCM1), 6 were associated with GCM2 cells, and none were found in the fundic or junctional/cardia epithelium. Column GCM2 shows that 3 of the 9 cases that had GCM2 also had ITGCs and 6 also had GCM1, but in no case were there only GCM2 cells.

Because GCM2 cells were only present in biopsies that also had GCM1 cells, these 2 cell types are grouped together as GCMs in the present study. Sixty-six percent of the biopsies showing GCM1 cells also contained typical ITGCs.

The association between the cell types present in esophageal biopsies with an initial diagnosis of “Barrett esophagus” without dysplasia, and the risk of progression to dysplasia is shown in Figure 3. The presence of ITGCs, but not of GCMs, in esophageal biopsies was associated with significant progression to dysplasia during follow-up (P = .008). Only 1 patient with GCMs on the initial biopsy was diagnosed with LGD/IND after 146 months of follow-up. By contrast, 21 patients with ITGCs developed dysplasia on follow-up, 18 LGD/IND, and 3 high-grade dysplasia.

To determine whether a diagnosis of Barrett esophagus based on positive staining with AB is associated with a significant risk of progression to dysplasia, all evaluable blocks from the cases entered in this study were cut and stained with AB. Examples of AB-positive biopsies are shown in Figure 4. Three types of positive staining are recognized: (1) surface columnar cells without goblet shape (Figure 4, A), (2) cells in deeper glands without goblet shape (Figure 4, B), and (3) ITGCs that also show inhomogeneous blue staining of the intracellular mucin (Figure 4, D). In some cases, all glands in a biopsy from the esophagogastric junction stain extensively with AB, without having a single goblet cell (Figure 4, C). There was no significant correlation between positive AB staining and progression to dysplasia (P = .54, Kaplan-Meier with log-rank test, figure not shown). Because the number of cases stained with AB was less than those entered in the study and originally analyzed for the association between ITGCs and progression to dysplasia, we reanalyzed only the subset of cases available for AB staining for the association between ITGCs on H&E sections and progression to dysplasia and found this association remained significant in this subset of biopsies (P = .01, Kaplan-Meier with log-rank test, figure not shown). As a predictor of progression to dysplasia, AB-positive staining has a sensitivity of 0.34, specificity of 0.89, positive predictive value of 0.95, and negative predictive value of 0.18 with a likelihood ratio of 3.05 (two-sided Fisher exact test, P = .25). In the same set of patients, ITGCs as a predictor for progression to dysplasia has a sensitivity of 0.38, specificity of 0.94, positive predictive value of 0.95, and negative predictive value of 0.33 with a likelihood ratio of 6.08 (two-sided Fisher exact test, P = .03).

Of the 12 cases with GCMs, 8 remained free of ITGCs after 47 to 98 months of follow-up, whereas 4 showed typical ITGCs in subsequent biopsies. One of the latter 4 patients had a biopsy showing Barrett LGD/IND after 146 months of follow-up, whereas the other 3 remained free of dysplasia at 60, 107, and 137 months of follow-up.

COMMENT

The incidence of esophageal adenocarcinoma (EA) in the United States has been rising since the 1970s. Currently, there is no proven nonsurgical treatment for EA, a malignancy associated with a poor survival that has not improved significantly in the past 20 years or so despite...
recent advances in cancer diagnosis and therapy. The precursor lesion for EA is BM resulting from chronic gastro-esophageal reflux. Patients diagnosed with BM are enrolled in endoscopic surveillance programs aimed at detecting EA at an early stage, because surgical resection of early-stage EA offers the best hope for survival. These patients are subjected to esophageal endoscopy and biopsy at regular intervals, determined by the degree of dysplasia in esophageal biopsies. A correct diagnosis of BM is essential not only to spare patients who do not have BM unnecessary life-long surveillance, anxiety, and fear of having esophageal cancer but also to avoid the possible inability to obtain medical insurance. Moreover, overdiagnosis of BM results in decreased cost-effectiveness of the surveillance programs.

The definition of BM has evolved through the years and has been refined to reflect the clinical significance and consequences of this diagnosis. In the early 1970s Paul et al recognized 3 histologic types of BM: (1) atrophic gastric-fundic type, (2) junctional or cardia-type, and (3) specialized columnar with ITGCs. However, later it became apparent that only the “specialized type” with goblet cells

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<th>Association Between Different Types of Cells in 78 Esophageal Biopsies With Original Diagnosis of “Barrett’s Esophagus”*</th>
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* ITGC, intestinal-type goblet cell; GCM1 and GCM2, goblet cell mimickers type 1 and type 2, respectively (see text).
is associated with a significant risk of EA, and experts in the field started to reserve this diagnosis exclusively for biopsies containing ITGCs.2,3 Finally, in 1998, with the publication of the practice guidelines by the American College of Gastroenterology, the presence of ITGCs became a requirement for the diagnosis of BM.1

The identification of ITGCs would appear to be straightforward on routinely processed and H&E-stained sections of esophageal biopsies. However, we have encountered in our practice a significant number of cases in which an incorrect diagnosis of BM was rendered, usually because of the presence of GCMs in sections of the esophageal biopsies. One of us (M.Y.) has seen in consultation 3 cases with GCMs initially diagnosed as Barrett, negative for dysplasia, in 1 month (August 2005). In this study, we have identified two types of GCMs. GCM1 are goblet-shaped cells that do not contain intestinal-type mucin. Instead, these cells contain homogeneous, often slightly pink, intracytoplasmic material. The only reference to these cells that we could find in the literature was in a recently published book on gastrointestinal pathology in which these cells are referred to as pseudogoblets.14 The second type, GCM2, are columnar cells that are not goblet-shaped and contain mucin that looks, on H&E-stained sections, similar to the light blue intestinal-type mucin. Unlike true ITGCs, GCMs were not associated with a significant risk of dysplasia, and hence of malignancy, in our study, when not associated with ITGCs in the same biopsy. Despite the fact that ITGCs are easily recognizable on H&E-stained sections, many pathologists still rely on the AB stain to “confirm” the diagnosis of BM. This seems also to be the practice in numerous publications, including recent ones. In our experience, and that of others, AB staining are considered positive for AB.15 By doing this, the authors are specifically limiting the definition of AB-positive staining, and hence BM, to cases with true ITGCs. In such cases, one can not help but wonder what exactly is the utility of AB staining, and whether it is necessary. However, in reality, many pathologists feel tempted to regard as BM the presence of columnar cells with positive dark blue AB staining, even if they are not typical goblet shaped, as we sometimes see in our consultation cases. AB-positive, but not goblet, cells can be found in the pit epithelium of normal gastric mucosa.16 When such AB-positive columnar cells are found in the surface epithelium, they are abnormal and were given the name metastatic Alcian blue positive cells by Offerer et al.16 It has been suggested that these cells could be precursors of ITGCs.17 In this study, we found no significant difference in the risk of dysplasia between patients with AB-positive and those with AB-negative esophageal biopsies.

Because no standard biopsy protocol was followed for most of the patients in this study, and the length of the Barrett segment was not given in many cases, the prevalence of GCMs cannot be determined. Although the results of our study are significant, we cannot recommend that patients with GCMs be followed differently than patients with ITGCs at this time. Our study has several limitations, including lack of standard biopsy protocol and absence of information on the length of the Barrett segment. Because of the significant number of cases that we found to have both ITGCs and GCMs in the same biopsy, it is possible that the 4 cases of GCMs that subsequently had ITGCs were false-negative for ITGCs on the initial biopsy and that if standard biopsy protocol with adequate sampling was done in these cases most, if not all, could have had ITGCs on the first biopsy. Alternatively, it is possible that GCMs are precursors of ITGCs, as previously suggested by Chen et al,17 and if the underlying disease is left untreated or if inadequately treated, then progression to true ITGCs will occur. Unfortunately, we cannot answer these questions in this study. Perhaps if an initial biopsy shows GCMs, and a repeat biopsy also evidences these patients are at no risk of malignant progression, but this awaits confirmation by other investigators.

Finally, we have seen cases stained elsewhere in which “empty” goblet-shaped cells are present in what appears to be clearly intestinal metaplasia. Obviously, differences in the method and material used, including the type of hematoxylin, in the staining protocol will affect the staining results. Inconsistent results between laboratories can be also seen with special stains.

CONCLUSION

ITGCs identified by microscopic examination of routine H&E-stained sections of esophageal biopsies, and without help from special stains, is a reliable marker for patients at risk for dysplasia and EA. The presence of ITGCs should probably be the only acceptable definition of BM. Pathologists should be aware of the presence of GCMs that, in the absence of ITGCs, do not seem to have a predictive value in terms of malignant progression.

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Figure 4. Patterns of positive Alcian blue staining in esophageal biopsies. A through C, Staining in non-Barrett epithelium. Goblet cell mimickers showing Alcian blue–positive columnar cells, some with dark staining, located in the surface epithelium (A) or deeper glands (B and C). D, Staining in Barrett metaplasia showing typical intestinal-type goblet cells with dark blue inhomogeneous staining of intracytoplasmic mucin (Alcian blue, pH 2.5, original magnification ×20).

References