Intestinal Metaplasia of the Esophagus or Esophagogastric Junction

Evidence of Distinct Clinical, Pathologic, and Histochemical Staining Features

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Abstract

Our purpose was to evaluate the clinical, histologic, and histochemical staining characteristics of intestinal metaplasia (IM) at an endoscopically normal-appearing esophagogastric junction (IM-EGJ) compared with IM in a columnar-lined esophagus (IM-CLE). A prospective study included 253 patients referred for elective upper gastrointestinal endoscopy. Biopsy specimens were obtained from 2 cm above and immediately distal to the squamocolumnar junction, the gastric corpus, and the antrum. Any red mucosa above the EGJ was sampled.

IM-CLE (prevalence, 5.5%) typically occurred in white male smokers with a long history of reflux symptoms. IM-EGJ (prevalence, 9.1%) was associated with corpus and antrum gastritis and with IM at these sites. IM-CLE usually (13/14 [93%]) was the incomplete type IM, whereas only 12 (52%) of 23 patients in the IM-EGJ group had incomplete IM.

IM-EGJ and IM-CLE should be considered as separate entities. Further research is needed to evaluate whether neoplastic progression of IM-EGJ is related to its mucin profile.

The predisposition of patients with a columnar-lined esophagus, traditionally called Barrett esophagus, to develop esophageal adenocarcinoma is well documented.1 The recognition that cancer complicating Barrett esophagus originates from intestinal-type columnar epithelium has led to redefinition of Barrett esophagus by the presence of intestinal metaplasia (IM) in the esophagus.2,3 Initially, histologic evidence of IM on biopsy specimens obtained from the lower esophagus was considered sufficiently distinctive to use its detection as the “gold standard” for a diagnosis of Barrett esophagus. Later on, it was shown that biopsy specimens immediately distal to the squamocolumnar junction also could reveal IM when Barrett esophagus was unsuspected endoscopically.4

The pathogenesis of IM at an endoscopically normal-appearing esophagogastric junction (EGJ) is subject to ongoing discussion. Some studies have indicated a relationship with gastroesophageal reflux disease,5-7 while others have implicated a role for Helicobacter pylori infection.8-10 Importantly, dysplasia and adenocarcinoma have been noted in association with IM just distal to a normally located squamocolumnar junction.10,11 The increasing frequency of adenocarcinoma of the EGJ, in parallel with the rising incidence of adenocarcinoma of the esophagus,12 further enhances the importance of identifying preceding pathologic conditions.

Histochemically, IM in a columnar-lined esophagus (IM-CLE) resembles incomplete IM of gastric mucosa.13-16 It differs from complete IM by the presence of mucus-secreting columnar cells that contain both neutral and acid mucins. As part of this study, we investigated the relative frequencies of incomplete vs complete IM at an endoscopically normal-appearing esophagogastric junction (IM-EGJ).
The objectives of this prospective biopsy study were to (1) assess the overall prevalence of IM-EGJ compared with IM-CLE in patients undergoing elective upper endoscopy; (2) evaluate the clinical, endoscopic, and histologic associations with special reference to features of gastroesophageal reflux disease (GERD) and gastric pathology; and (3) study the histochemical staining characteristics of IM-EGJ compared with IM-CLE.

Materials and Methods

Patients
Unselected adult patients (n = 260) scheduled for elective endoscopic examination of the upper gastrointestinal tract were asked to participate in the study, regardless of the indication for the procedure. Exclusion criteria were age younger than 18 years, a previous diagnosis of Barrett esophagus (irrespective of length) or upper gastrointestinal cancer, a history of upper gastrointestinal surgery, esophageal varices, coagulation disorders, in-hospital patients, pregnancy, and unwillingness or inability to give informed consent. Patients were prospectively recruited at the General Endoscopy Unit, Academic Medical Center, Amsterdam (n = 178) and at the General Endoscopy Unit, Kennemer Gasthuis, Haarlem (n = 82), the Netherlands. In both centers, the study was approved by the institutional medical ethical committee. All patients enrolled in the study gave written informed consent.

Questionnaire
At study entry, all patients completed a standardized questionnaire. Its structure was based on a review of the literature.17-19 Patients were asked to describe the main symptom that had resulted in current endoscopic examination. The present and past occurrence of GERD symptoms was surveyed. GERD symptoms were defined as heartburn or regurgitation.19 Patients were asked to report use of alcohol, history of cigarette smoking, and current medications with particular attention to the use of acid suppression therapy. Height and weight were recorded to calculate the patients’ body mass index.

Endoscopy and Biopsy Protocol
Endoscopies were performed in a standard manner using a videoendoscope. Documentation included the following: (1) the primary indication for endoscopy, (2) an endoscopic diagnosis of reflux esophagitis,20 (3) the presence of a hiatal hernia, and (4) the locations of the diaphragmatic impression, EGJ, and squamocolumnar junction in centimeters from the incisor teeth. The EGJ was identified as the most proximal margin of the gastric folds21,22 and the squamocolumnar junction as the demarcation between white (squamous-appearing) mucosa and red (columnar-appearing) mucosa. A normal appearing EGJ was diagnosed if an endoscopically unremarkable squamocolumnar junction coincided with the level of the EGJ. Any tongue-like or circumferential extension of red mucosa above the EGJ was classified as esophageal columnar lining, irrespective of its extent (defined as the distance from the EGJ to the squamocolumnar junction). Study protocol biopsy specimens were obtained at 4 standard locations (2 biopsy specimens per site): 2 cm above the squamocolumnar junction, immediately distal to the squamocolumnar junction, the gastric corpus, and antrum.

Pathologic Examination
All biopsy specimens were processed and evaluated at the Pathology Department, Academic Medical Center, Amsterdam. Each specimen was fixed in formalin, embedded in paraffin, sectioned at 3 levels, and stained routinely with H&E. Initial assessment of each set of biopsy specimens was performed by 1 of 5 staff pathologists (P.D., G.J.A.O., F.J.W.t.K., and others) who were aware of the study protocol. Slides of squamous epithelium were analyzed for histologic features of reflux esophagitis, ie, basal cell hyperplasia greater than 15% of the total thickness of the epithelium and extension of subepithelial papillae into the upper third of the epithelium.23 Also, the sole presence of intraepithelial eosinophils was considered an indicator of reflux esophagitis.24 Biopsy specimens from just below the squamocolumnar junction, the gastric corpus, and antrum were analyzed for the presence of IM, inflammation, and H pylori organisms. Goblet cells were used as criteria for
The type of gastric epithelium in biopsy specimens obtained just distal to the squamocolumnar junction was registered. Typical characteristics of cardiac mucosa included the coiled and loosely packed mucus glands containing scattered parietal cells. Slides of columnar epithelium from above the EGJ were reviewed for the presence of IM. A histologic diagnosis of dysplasia or cancer in any biopsy specimen was registered. At the end of the study, the biopsy material was submitted to a second review by one of the investigators (F.J.W.t.K.) who was blinded to the endoscopic data and to the interpretation of the first pathologist. For any discrepancies, final consensus was achieved by 2-observer assessment.

Subgroups of patients were identified using both endoscopic and histologic criteria. IM-EGJ was defined by the presence of IM just distal to an endoscopically unremarkable squamocolumnar junction located at the EGJ. Esophageal columnar epithelium irrespective of length but associated with IM above the endoscopically defined EGJ was categorized as IM-CLE. The reference group included all other patients who had no histologic evidence of IM at or above the EGJ. IM-EGJ and IM-CLE biopsy specimens were processed for additional staining techniques (see next 2 paragraphs) using unstained consecutive sections of tissue blocks.

**Mucin Histochemical Analysis**

Alcian blue at pH 2.5 (AB) was used to identify acid mucins in goblet cells and in columnar cells. Positive-staining columnar cells were analyzed exclusively in the surface epithelium lining the luminal part of the mucosa since they normally may be found in deep foveolar gastric epithelium. Alcian blue pH 2.5 and periodic acid-Schiff (PAS) was used to distinguish blue acid mucins from magenta neutral mucins (PAS-positive), and high iron diamine combined with alcian blue, pH 2.5, was used to differentiate between brown-black sulfated acid mucins (sulfomucins) and blue nonsulfated acid mucins (sialomucins). Based on mucin histochemical staining results, IM was classified as complete or incomplete. Complete or type I IM was characterized by the presence of goblet cells secreting acid mucins and adjacent nonsecretory columnar cells resembling normal absorptive enterocytes. Goblet cell metaplasia in association with secretory columnar mucous cells, rather than mature absorptive cells, was considered indicative of incomplete IM. Incomplete IM was further subdivided based on the absence (type II) or presence (type III) of sulfomucins in the columnar mucous cells.

**Immunohistochemical Analysis**

Immunohistochemical examinations were performed to demonstrate any endocrine cells (using antichromogranin polyclonal antibody, DAKO A430, Carpinteria, CA), to detect gastrin-producing cells (using抗gastrin polyclonal antibody, DAKO A568), and to seek pancreatic metaplasia (using antilipase monoclonal antibody, Chemicon 1453, Temecula, CA). Staining in more than 5% of cells relative to the total number of cells was considered positive. Expression of cytokeratins was evaluated by cytokeratin 7 immunostaining (clone OV-TL 12/30, BioGenex MU 255-UC, San Ramon, CA) and cytokeratin 20 immunostaining (clone IT-K, 20.8, BioGenex MU 315-UC). Both cytokeratin 7 and cytokeratin 20 are markers of glandular differentiation. Cytokeratin reactivity patterns were assessed with particular attention to deep gland involvement. In a previous study, Barrett esophagus exhibited only superficial cytokeratin 20 staining (with diffuse strong cytokeratin 7 staining), whereas gastric IM was associated with cytokeratin 20 staining in superficial and deep glands (and weak or absent cytokeratin 7 staining). All staining procedures of study biopsy specimens were combined with processing of negative and positive control specimens using appropriate human tissues.

**Statistical Analysis**

The data were analyzed using SPSS 7.5 for Windows 95 (SPSS, Chicago, IL). For continuous variables, comparisons between groups were made using the Mann-Whitney U test. Comparison of categoric variables was performed using the chi-square or the Fisher exact test when appropriate. Statistical significance was defined as $P < .05$ (2-tailed).

**Results**

**Prevalence of IM**

Of the 260 patients who consented to participate, for 7 the set of protocol biopsy specimens was incomplete, leaving 253 patients eligible for analysis. Their median age was 49 years (range, 21-87 years). The male/female ratio was 1:1. By endoscopic evaluation, 198 patients had a normal-appearing EGJ. Of these patients, 23 had IM in protocol biopsy specimens just distal to the squamocolumnar junction. In 55 patients, red mucosa extended cephalad from the endoscopically defined EGJ, with evidence of IM above the EGJ in 14 patients. Among these patients, the extent of esophageal columnar lining was less than 3 cm in 9 patients and measured 3 cm or more in 5 patients. Overall, the prevalence of IM-EGJ was 9.1% (23 of 253) and that of IM-CLE 5.5% (14 of 253). The 216 remaining patients (85.4%) who had no evidence of IM at or above the EGJ constituted the reference group.
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Demographic, Clinical, and Endoscopic Characteristics

Men and women were distributed equally among both IM-EGJ and reference group patients. In contrast, patients in the IM-CLE group were predominantly male (10/14 [71%]), although this trend did not reach statistical significance. Primary indications for endoscopy included upper abdominal pain or dyspepsia (46%), GERD symptoms (24%), follow-up of *H. pylori* eradication therapy (11%), nausea (5%), anemia (3%), and miscellaneous reasons including diarrhea, dysphagia, and atypical chest pain (11%). There was no association between any of the indications and either IM subgroup. When specifically asked, a majority of patients in all subgroups had at some time experienced typical GERD symptoms (70%-93%), but in IM-CLE, the duration of these symptoms was significantly longer, and current use of antireflux medications was significantly more common.

Frequencies of endoscopically diagnosed esophagitis were comparable among groups, and so was the distribution of its severity scores. Exclusion of patients receiving acid suppression therapy had no effect on the endoscopic prevalence of esophagitis. By definition, the EJ region appeared otherwise unremarkable in all patients in the IM-EGJ group. In the IM-CLE group, red mucosa above the EJ was tongue-like in 4 patients and circular in 10 patients. One patient with IM-CLE had a tumor in a 9-cm columnar-lined segment, which was diagnosed histologically as adenocarcinoma.

Histologic Characteristics

Biopsy specimens at 2 cm above a normally located squamocolumnar junction often revealed inflammatory changes in squamous epithelium, regardless of the current use of acid suppression. Cardiac-type mucosa was present invariably in IM-containing biopsy specimens distal to the squamocolumnar junction. In contrast, only 45.8% of the reference group had cardiac-type epithelium in biopsy specimens distal to the squamocolumnar junction. Fundic type epithelium was identified in the remaining patients. Data on gastric inflammation and *H. pylori* status were analyzed only for patients who did not undergo endoscopy for evaluation of *H. pylori* eradication therapy (n = 225). Corpus (18/20 [90%]) and antrum gastritis (20/20 [100%]) were significantly more frequent in patients in the IM-EGJ group compared with the reference group and the IM-CLE group. Thirty percent of patients in the IM-EGJ group (7/23) also had IM elsewhere in the stomach. This contrasted with 8.3% of the reference group (18/216; *P* = .005). Gastric IM was found in 3 patients in the IM-CLE group (21%; *P* = 0.21 compared with the reference group). Differences in *H. pylori* status between study groups did not reach statistical significance.

Dysplasia or cancer was not found in any of the patients with IM-EGJ or in patients in the reference group, but was diagnosed in 2 (14%) of 14 patients with IM-CLE. As mentioned, one of them had adenocarcinoma in a long columnar-lined segment. The other patient had less than 3 cm esophageal columnar lining, devoid of any suspected endoscopic lesions but associated with biopsy-proven low-grade dysplasia.

### Mucin Staining Characteristics

Generally, IM biopsy specimens distal to the squamocolumnar junction contained a variable amount of AB-positive cells, indicating the presence of acid mucins. Goblet cells produced acid mucins, either alone or in combination with...
interventing acid mucin–secreting columnar cells. Different patterns of acid mucin secretion in IM-EGJ and IM-CLE emerged [Table 3] and [Image II]. AB-positive surface columnar mucous cells were present in 12 (52%) of 23 patients with IM-EGJ but were almost invariably found in patients with IM-CLE (13/14 [93%]; P = .013). In all these cases, the epithelium was designated incomplete IM. The remaining patients had complete IM, since AB-positive columnar cells were absent (5 IM-EGJ) or were present in the base of foveolae only (6 IM-EGJ and 1 IM-CLE). With high iron diamine combined with alcian blue, pH 2.5, there was no significant difference in the frequency of sulfomucin-positive surface columnar cells. Irrespective of cell type, sulfomucin positivity tended to be less frequent in IM-EGJ (11/23 [48%]) than in IM-CLE (11/14 [79%]; P = .090), largely owing to a significant difference in the frequency of sulfomucin-secreting goblet cells (P = .040). The 2 newly identified patients with dysplasia or cancer in IM-CLE both had incomplete-type IM.

**Immunostaining Characteristics**

IM-EGJ biopsy specimens were indistinguishable immunohistochemically from IM-CLE biopsy specimens using antichromogranin, antilipase, or cytokeratin staining (Table 3). In all but 1 patient, cytokeratin 7 immunoreactivity involved both superficial and deep glands. No categoric differences in staining intensity were observed. Gastrin-producing cells were detected in areas of IM-CLE (3/14 [21%]), but never in association with IM-EGJ. The patient with adenocarcinoma was among the patients with gastrin reactivity in esophageal columnar biopsy specimens. Overall, immunohistochemical results did not relate to mucin staining characteristics of IM at or above the EGJ.
Mucin histochemical staining with alcian blue at pH 2.5 (A and B), alcian blue at pH 2.5 and periodic acid–Schiff (C and D), and alcian blue at pH 2.5 and high iron diamine (E and F) showing incomplete intestinal metaplasia in a biopsy specimen derived from a columnar-lined esophagus (A, C, and E) and complete intestinal metaplasia in a biopsy specimen obtained at an endoscopically normal-appearing esophagogastric junction (B, D, and F). Alcian blue positivity indicates the presence of acid mucins. In combination with periodic acid–Schiff, it distinguishes acid mucins (blue) from neutral mucins (magenta) and, in combination with high iron diamine, it differentiates between nonsulfated (blue) and sulfated acid mucins (brown-black) (all sections, original magnification ×80).
Discussion

Reported prevalence rates of intestinal metaplasia at a normal-appearing EGJ vary from 6% to 23%.4-10,31,32 The variation may stem from differences in patient selection, biopsy site, use of special stains, or, perhaps most important, from differences in the definition used. In our study, IM-EGJ was defined as goblet cell metaplasia detected in H&E-stained biopsy specimens obtained from an area just distal to an endoscopically unremarkable squamocolumnar junction located at the same level as the proximal margin of the gastric folds. By using these criteria, the prevalence of IM-EGJ in the present study was 9.1%. A similar frequency (10.3%) was recently reported on evaluation of the entire EGJ in an unselected autopsy population.33

Patients with IM distal to a proximally displaced squamocolumnar junction regardless of the distance to the EGJ were categorized as having IM in a columnar-lined esophagus. Conceptually, IM-CLE can be called Barrett esophagus, provided that this latter term refers to the histologic diagnosis of goblet cell metaplasia anywhere within the esophagus.5 It is current use to subdivide Barrett esophagus into short-segment and traditional or long-segment Barrett esophagus. With an arbitrary distance of 3 cm applied to our data, the prevalence of short-segment Barrett esophagus (<3 cm) was 3.6% (9/253) and that of long-segment Barrett esophagus (3 cm or more), 2.0% (5/253). Other investigators who also evaluated unselected patients undergoing routine endoscopy have reported similar frequencies of newly diagnosed long-segment Barrett esophagus (1%-4%).4,10,31,32 Prevalence figures of short-segment Barrett esophagus tend to be higher in the literature (6%-18%),4,10,31,32,34 compared with our findings. Some of these figures included patients with IM-EGJ, patients with 3-cm IM-CLE, or only patients with endoscopic lesions suggestive of short-segment Barrett esophagus. If these were removed, the reported prevalence of short-segment Barrett esophagus would drop to 2% to 7%.10,31,32 Clearly, there is no universal definition of short-segment Barrett esophagus, nor is it certain whether short-segment and long-segment Barrett esophagus should be regarded as distinct conditions.35,36

The present findings are consistent with the literature in that patients with IM-CLE were predominantly white male smokers who reported a significantly longer duration of GERD symptoms compared with a reference group.10 Current use of acid suppression therapy was most frequent among patients with IM-CLE. The data confirm that IM-CLE is a reflux-mediated esophageal disorder with a specific demographic group at risk. In contrast, IM-EGJ was as common in men as in women who had no increased history of GERD compared with the reference group.

Although some investigators have reported that GERD has an important role in the cause of IM-EGJ,5-7 our data disprove this contention, thereby confirming other study results.8-10 The role of GERD may best be studied by objective measurements also, rather than by clinical observations only. For this purpose, we sought histologic evidence of reflux esophagitis but encountered its previously reported limitations. Basal cell hyperplasia and lengthening of the papillae have been described in about 60% of biopsies obtained from the distal 3 cm of the esophagus in asymptomatic individuals.37 In our study, IM-EGJ and reference group patients showed a similar prevalence of (nonspecific) reactive changes in the lower esophagus. In patients with a proximally displaced squamocolumnar junction, squamous epithelial changes tended to be less prevalent. Of note, reactive changes are more likely to indicate reflux if present in biopsy specimens from above the distal 3 cm of the esophagus.38

Recent autopsy data have suggested that cardiac-type epithelium would represent an early histologic manifestation of GERD,39 but compelling evidence to support this concept is not yet available. Other autopsy findings have indicated that cardiac mucosa is present from birth as a normal structure.40 It can be expected that living-related data as to the finding of cardiac epithelium are less precise than autopsy data. The site of EGJ biopsy sampling may be critically important. In this context, we reviewed all EGJ biopsy specimens for histologic evidence of the squamocolumnar junction. The epithelial junction was included in as many IM-EGJ patients (8/23 [35%]) as reference group patients (65/216 [30.1%]), indicating a high level of constancy in the site of EGJ biopsy sampling. It is then particularly fascinating to document a clear difference in the finding of cardiac mucosa between the 2 groups (23/23 [100%] and 99/216 [45.8%], respectively). Cardiac-type epithelium may thus be a prerequisite for IM to develop in the EGJ region. This observation highlights the need to further explore the origin of this junctional mucosa.

Distal gastric biopsy sites revealed no significant differences between IM-CLE patients and the reference group. In sharp contrast, features of gastric pathology were significantly increased in patients with IM-EGJ. The study results, in line with those obtained in an autopsy series,33 indicate a close association between IM-EGJ, gastric inflammation, and IM in the rest of the stomach. It is commonly believed that gastric IM arises from an inflammatory response to H pylori colonization.41 Literature data on H pylori infection mostly originate from gastric corpus and antrum studies. Some investigations have suggested that IM at the EGJ also is a sequela of H pylori infection.8-10,42 We could not confirm a direct association, but the results are supportive of the concept that IM-EGJ evolves from a
similar mucosal process, as does IM at other gastric sites. Prospective studies on the natural history of *H pylori* at the EGJ are awaited.

Standard histologic evaluation fails to reliably differentiate between IM in the esophagus and IM in native cardiac mucosa. In our study, immunohistochemical stains were of little or no help in identifying whether IM occurred in the esophagus or in the most proximal part of the stomach. It has been reported that IM-CLE (defined as long-segment Barrett esophagus) and IM-EGJ show distinct patterns of cytokeratin 7 and cytokeratin 20 immunostaining, but our findings and those of other authors are not confirmatory. Studies on pancreatic metaplasia are scarce, but its presence has been described both in endoscopically defined Barrett esophagus and in random samples from the EGJ area (with and without IM). Accordingly, we found lipase reactivity both in association with IM-CLE and in association with IM-EGJ, with no significant difference. Gastrin reactivity was observed exclusively in IM biopsy specimens from the esophagus. It is known that the antral-like glands in the cardiac region do not contain gastrin-producing cells, which distinguishes them from their counterparts in the antrum. Apparently, the endocrine cell population in intestinalized columnar epithelium above the EGJ may involve gastrin-producing cells. The significance of this distinctive histologic finding warrants further study, especially with regard to the growth-stimulating features of gastrin and its precursors.

Long-term follow-up studies on the risk of neoplastic progression associated with IM-EGJ are just emerging. It seems as though the malignant potential of IM-EGJ is lower than that of IM-CLE. Our findings indicate that the alleged differences in malignant potential may follow distinct mucin profiles. The frequency of acid mucin–secreting columnar cells in surface epithelium of IM-CLE was 93% (13/14). This is in line with previous data indicating that IM in the esophagus resembles incomplete gastric IM. Among IM-EGJ patients, only 52% (12/23) were characterized to have incomplete IM. We wish to emphasize that the AB-positive columnar cells were present in the surface and not foveolar epithelium, and that the presence of goblet cells was mandatory for a diagnosis of IM. This is important because some gastric pit cells may be AB-positive in normal gastric mucosa, and acid mucin–secreting columnar cells may occur at the EGJ in the absence of goblet cells. The significance of acid mucins in metaplastic surface columnar cells is unclear. Their dense distribution in malignant IM-CLE may raise considerations regarding a role of these cells in neoplastic transformation. As for IM in the distal stomach, it has been shown that incomplete IM has a closer association with cancer development than IM in general. Perhaps that IM subtyping at the EGJ implies similar neoplastic consequences.

Our data indicate that IM-CLE and IM-EGJ should be regarded as separate entities. IM-CLE demonstrated its known association with GERD, whereas IM-EGJ was significantly more likely in patients with gastritis and IM in the distal stomach. It suggests an intermediate role of *H pylori*, but the data were not conclusive. Distinct frequencies of complete vs incomplete IM may help to explain a lower malignant potential of IM-EGJ compared with that of IM-CLE. The neoplastic progression risk of IM-EGJ should be explored relative to its mucin profile.

**References**


