

Robert E. Petras, M.D., FASCP, FACG
Associate Professor of Pathology
Northeastern Ohio Universities
College of Medicine
National Director for Gastrointestinal Pathology Services
AmeriPath, Inc.

CASE 3

BARRETT'S ESOPHAGUS: DYSPLASIA AND CARCINOMA

Barrett's esophagus, the eponym given to columnar epithelium-lined esophagus, is acquired through chronic gastroesophageal reflux (1,2). Traditionally, it was defined as the presence of columnar epithelium lining the tubular esophagus above the level of the LES (1). For purposes of cancer surveillance, the American College of Gastroenterology (ACG) now defines Barrett's esophagus as an endoscopic change in esophageal epithelium of any length that contains intestinal metaplasia (3,4).

Barrett's esophagus would be little more than a medical curiosity if not for its complications: ulcer, stricture, bleeding, and carcinoma. It is the association with carcinoma that has brought gastroesophageal reflux disease and Barrett's esophagus to so much attention (5). Although the exact magnitude of the cancer risk is unknown, the high prevalence and dismal outcome of carcinoma complicating Barrett's esophagus (6,7) have caused the ACG to recommend that patients with longstanding reflux symptoms have endoscopic examination to detect Barrett's esophagus (4). Once Barrett's esophagus is discovered, such patients should undergo endoscopic surveillance.

DIAGNOSIS OF BARRETT'S ESOPHAGUS

The Clinical Diagnosis of Barrett's Esophagus

Endoscopy remains the mainstay for diagnosing Barrett's esophagus (2). In general, the color (orange-red) and appearance (velvety) of Barrett's esophagus as seen through the endoscope is similar to that of gastric mucosa. Barrett's esophagus can be circumferential or tongue-like extensions of orange-red mucosa into the tubular esophagus (8). Occasionally, Barrett's esophagus can present as an island of orange-red mucosa entirely surrounded by the more pale pink to gray-white squamous epithelium of the esophagus. Some endoscopists augment endoscopic visualization with the use of vital stains such as methylene blue (9,10). Since other conditions such as a hiatal hernia, especially one occurring in the setting of severe gastroesophageal reflux, can sometimes mimic Barrett's esophagus endoscopically, the endoscopist's impression of Barrett's esophagus must be confirmed histologically (3,4).

The Histologic Diagnosis of Barrett's Esophagus

Three epithelial types were described in traditionally defined Barrett's esophagus and include junctional epithelium (gastric cardia-like), gastric fundic-type epithelium, and specialized columnar epithelium (incomplete intestinal metaplasia) (11). The junctional epithelium is composed of glands and pits that resemble the gastric cardia except for some atrophy and inflammation. The glands are composed of mucus-secreting cells (1,2,11). Chief, parietal,

Paneth's, enterochromaffin, and goblet cells are not encountered in junctional epithelium. Gastric fundic-type epithelium, encountered in some cases of traditionally-defined Barrett's esophagus, is virtually identical to that of the normal gastric body except that, again, there is usually some degree of inflammation and atrophy. Specialized columnar epithelium (incomplete intestinal metaplasia) is a distinctive epithelial type that is virtually unique to and considered diagnostic for Barrett's esophagus. Furthermore, this epithelial type (intestinal metaplasia) defines the cancer surveillance group (See below) (3,4).

Specialized columnar epithelium histologically can occur in a flat or villous configuration and consists of goblet cells and columnar cells. The goblet cells contain mucin that stains positively both with periodic acid-Schiff and with Alcian blue at pH 2.5. These mucins are most often a combination of sialomucins and sulfated mucins (12,13). The columnar cells between goblet cells most often resemble gastric foveolar epithelium or rarely intestinal absorptive cells. The cells lack absorptive capability or ultrastructural features of true intestinal absorptive cells and, therefore, the term "incomplete intestinal metaplasia" has been applied (14). Specialized columnar epithelium can also contain Paneth's cells and enterochromaffin cells (1,2,11) and can occasionally overlie simple mucus-type glands, or even gastric body-like glands.

The principal differential diagnostic consideration is a gastric heterotopia. Endoscopists encounter patches of ectopic gastric tissue, appearing as orange-red islands of abnormal mucosa surrounded by normal pink to white squamous esophageal mucosa in approximately 4% to 10% of patients who undergo upper endoscopy (15). These foci of gastric tissue occur in the cervical esophagus, are often referred to as inlet patches, and are thought to represent embryonic rests. Histologically, they can be similar to Barrett's esophagus and they are distinguished from Barrett's clinically by their cervical location, their separation from the stomach by intact esophageal squamous mucosa, and lack of association with reflux.

Problems with the Histological Diagnosis of Barrett's Esophagus

Intestinal metaplasia of the stomach can be histologically indistinguishable from specialized columnar epithelium of Barrett's esophagus (16). So-called "short-segment" Barrett's esophagus (intestinal metaplasia in the distal esophagus measuring <3 cm) can be difficult to distinguish from intestinal metaplasia of the cardia at or near a normally placed esophagogastric junction (3). The ACG suggests that this distinction be made at endoscopy (3,4). That said, although intestinal metaplasia at a normally placed esophagogastric junction may not technically be Barrett's esophagus (because it is not in the anatomic esophagus), it is possible that this epithelial type at that location increases the risk of developing adenocarcinoma of the gastric cardia or gastroesophageal junction (3,17). There are several lines of reasoning that associate intestinal metaplasia at the gastroesophageal junction with carcinoma of the gastroesophageal junction, lower esophagus and gastric cardia including: a) the prevalence of intestinal metaplasia at the gastroesophageal junction is proportional to the length of specialized columnar epithelium in Barrett's esophagus (18,19); b) carcinomas of the cardia and Barrett's associated carcinoma share common morphology, epidemiology, risk factors, frequency of gastroesophageal reflux and prognosis (see below), c) carcinoma of the cardia and Barrett's-associated carcinoma have shown a similar increased incidence (3,4,19-23). A proposed classification and clinical approach is outlined in Table 1.

TABLE 1* CLASSIFICATION AND CLINICAL APPROACH FOR COLUMNAR CELLS IN THE ESOPHAGUS OR INTESTINAL METPLASIA AT THE GASTROESOPHAGEAL JUNCTION

<u>Classification</u>	<u>Association with GERD**</u>	<u>Association with Carcinoma</u>	<u>Endoscopic Surveillance</u>
Barrett's esophagus with specialized columnar epithelium	Variable/Yes	Yes	Yes
Columnar epithelium of esophagus without specialized columnar epithelium	Yes	Unlikely	Probably Not
Intestinal metaplasia at gastroesophageal junction	Unclear	Probable	Unclear

* Modified from Reference 19. **GERD = gastroesophageal reflux disease.

Reiterating, Barrett's esophagus is now defined as a change of esophageal epithelium of any length, recognize at endoscopy that is proved by biopsy to contain intestinal metaplasia (3,4). My current practice is to confirm intestinal metaplasia by using an Alcian blue/PAS combination stain with a hemotoxylin counterstain. The updated ACG practice guidelines encourage use of at least an Alcian blue stain citing that its use decreases the change of missing goblet cells or of misinterpreting cells with prominent cytoplasmic vacuoles as goblet cells (4).

If one uses a routine Alcian blue/PAS stain in this setting, one must be aware of the potential Alcian blue positive staining pitfalls that are not considered to be specialized columnar epithelium of Barrett's esophagus. These include the submucosal glands of the esophagus and their secretions, positive staining in gastric type glands or pancreatic acinar metaplasia, the regenerative zone of gastric type epithelium, the multilayered epithelium and the so-called columnar blues. It is postulated that multilayered epithelium and columnar blues may be precursor cells of specialized columnar epithelium but without goblet cell morphology, the alcian blue positive staining of the multilayered epithelium and columnar blues is not considered Barrett's esophagus.

CARCINOMA AND CANCER SURVEILLANCE IN BARRETT'S ESOPHAGUS

Cancer Risk and Surveillance

Patients with Barrett's esophagus are at increased risk for esophageal adenocarcinoma (1-4,24). The exact magnitude of the risk is unknown and some suggest that the risk could be overestimated because of publication bias (25). Most prevalence rates for carcinoma complicating Barrett's esophagus come from referral institutions and range from 10%-15% (6,7,26). Cancer prevalence represents patients in a population already with carcinoma. The true problem in assessing the need for cancer surveillance involves patients with Barrett's esophagus who do not yet have carcinoma. What is their risk of developing carcinoma (incidence) and does that risk justify the cost of a cancer surveillance program? In prospective studies, Robertson et al reported an incidence of 1786 cases of Barrett's associated carcinoma per 100,000 population per year (27) and Hameeteman et al reported 1920 cases per 100,000 per year (28), rates that are 350 times and 125 times the rate of esophageal carcinoma in the general population respectively.

Though most agree that Barrett's esophagus places patients at risk for esophageal adenocarcinoma, no consensus has emerged as to whether the increased risk justifies the cost of a cancer surveillance program. Van der Veen et al concluded that systematic endoscopic surveillance was not indicated in patients with Barrett's epithelium, citing that there was no

difference in survival between patients with Barrett's esophagus and a control population (29). In a subsequent study of the same cohort with eight additional years of follow-up, eight additional patients had developed esophageal adenocarcinoma (30). This represented 1 carcinoma per 180 patient years or a forty-fold increased risk. Despite this fact and the 50% greater death rate in the Barrett's esophagus group vs. controls, the authors concluded that too few patients actually died of Barrett's esophagus-associated carcinoma (only two deaths), and therefore, formal surveillance would have been of little benefit.

Though controversial, in the absence of a definitive study to the contrary, it is prudent to place all patients with Barrett's esophagus into a cancer surveillance program. The American College of Gastroenterology recommends cancer surveillance for Barrett's esophagus with the surveillance goal being prevention of carcinoma or the detection of carcinoma in an early and potentially curable phase (4). The marker used as the end point for cancer surveillance programs is identification of epithelial dysplasia in a biopsy specimen.

Dysplasia, the presumed precancerous epithelial lesion, has been regularly recognized in esophageal specimens adjacent to and distant from Barrett's-associated adenocarcinomas (1,24). Circumstantial evidence suggests that dysplasia may not only be a marker for carcinoma, but may itself be the early carcinomatous change that can progress to invasive carcinoma (24). Although the circumstantial evidence for the dysplasia-carcinoma sequence is compelling, the progression of dysplasia to carcinoma is still largely unproven and the time course unknown. The potential benefits (largely unknown) of removing a dysplastic esophagus must be weighed against the relatively high mortality associated with esophagectomy (estimated to be 5-15%) and the dismal outcome in patients who present with invasive adenocarcinoma of the esophagus (34% survival at two years and 14.5% survival at five years) (31).

Dysplasia in Barrett's Esophagus: Histopathologic Diagnosis, Significance, and Proposed Patient Management

Dysplasia is recognized histologically and criteria for identifying these changes in ulcerative colitis are applied in studying Barrett's epithelium (2,24). A reaffirmation of criteria with numerous illustrations has been published (32). The term dysplasia in Barrett's esophagus should only be used to describe a change that is unequivocally neoplastic. As with inflammatory bowel disease, dysplasia in Barrett's epithelium can be closely mimicked by reparative epithelial changes associated with active inflammation and ulcer.

Dysplasia and repair are associated with nuclear enlargement and hyperchromasia, increased mitotic figures, and decreased intracellular mucin. However, some histologic features favor repair over dysplasia. The nuclei of repair are often round to oval with smooth external contours, are evenly spaced, do not overlap, contain granular chromatin with single or multiple chromocenters/nucleoli, and are remarkably similar to one another in both size and appearance. In contrast to dysplasia, the nuclear to cytoplasmic size ratio of reparative cells is often decreased, especially in cells adjacent to ulcerated areas. Nearby active inflammation helps to confirm a diagnosis of repair. Features that favor dysplasia over repair are: a) variable nuclear hyperchromasia associated with pleomorphism, b) irregular nuclear contours, c) marked nuclear stratification with crowding and overlap, d) loss of nuclear polarity, e) nuclear and architectural abnormalities that are visible at low magnification (33), and involvement of the surface epithelium. Some Barrett's-associated dysplasias can look similar to colonic or small intestinal adenomas (32-34), however, in my experience the majority do not.

Dysplasia has been reported to occur in all three types of epithelia seen in traditionally defined Barrett's esophagus. However, it is certainly more frequent in areas of specialized columnar epithelium (33) and it is unlikely that cancer ever occurs except in patients with specialized columnar epithelium (3,4,19). It is frequently difficult or impossible to ascertain epithelial types in mucosa totally replaced by dysplasia or carcinoma (33).

Reiterating, most use a modification of the Inflammatory Bowel Disease-Dysplasia Morphology Study Group Classification in Barrett's epithelium (1,35). Under this three-tiered system, biopsy findings are classified as negative for dysplasia, positive for dysplasia, or indefinite for dysplasia. Biopsy specimens interpreted as positive for dysplasia are further subdivided as low-grade or high-grade dysplasia based upon the degree of cytologic change present. In low-grade glandular dysplasia, the abnormal nuclei are limited to the basal half of the cells. In high-grade glandular dysplasia, more severe cytologic and architectural alterations are present. Hyperchromasia and pleomorphism are more marked. Nuclear crowding and stratification are often present. Nuclei may be found in the luminal half of the cells. No distinction is made between high-grade dysplasia and carcinoma in situ in this system. If equivocal changes are present, they are usually due to epithelial repair associated with active inflammation. In this setting the specimen is best classified as indefinite for dysplasia. In my opinion, high-grade glandular dysplasia can be reliably detected by an experienced surgical pathologist, but because of the marked interobserver variation reported in diagnosing low grade glandular dysplasia and indefinite for dysplasia (32,36,37), and similar outcome (3,38) I follow the guidelines of Reid et al (36) and have adopted similar management for either diagnosis.

The histologic grade of dysplasia has clinical significance (3,4,38,39). Infiltrating carcinoma is a rare event in patients with Barrett's esophagus initially negative for dysplasia (0-3%). In contrast, 60% of patients with initial high-grade dysplasia have developed or already have infiltrating carcinoma (21,35,39). The results are intermediate for low-grade dysplasia and indefinite for dysplasia (10-28% for each) (37,40,41). One study stands out in stark contrast by observing a much lower cancer incidence rate in high-grade dysplasia (16%) (42). This study has been criticized for its possible "overreads" because 70% of their patients had at least low-grade dysplasia, in contrast to most other studies in which the prevalence of low-grade dysplasia is only about 5% (39).

During surveillance endoscopy, four quadrant biopsy specimens at 1-2 cm increments are obtained throughout the entire length of the Barrett's epithelium (2-4,39,43). Patients negative for dysplasia can safely continue regular surveillance (q 1-2 years). The ACG suggests that after 2 negative surveillance endoscopies, that the interval can be increased to 3 years (4). Investigators recommend shorter term follow-up for "indefinite" and "low-grade" dysplasia. The ACG now suggests 1 year (4) although I prefer their former recommendation of 6 months (3). Management of high-grade dysplasia remains controversial. Some recommend continued surveillance for some patients (3,4) whereas the majority recommend esophagectomy for the surgically fit candidate if life expectancy exceeds 10 years (39). Since the operative mortality and morbidity of esophagectomy is high, it is prudent to confirm a diagnosis of high-grade dysplasia before moving on to esophagectomy. Immediate re-endoscopy with multiple biopsies (2,4,42,43) should be performed. This re-biopsy approach has the advantage that intramucosal or invasive carcinoma may be detected with careful endoscopic re-examination and extensive re-biopsy, thus making the decision for esophagectomy easier. The ACG recommends that HGD be confirmed by an "expert" pathologist (4). If after an original diagnosis of high grade dysplasia the follow-up endoscopy with biopsy is negative, then the original specimens should be re-reviewed. If high grade dysplasia is again confirmed, some form of intervention is recommended (39).

Investigators at the Mayo Clinic reported that focal high-grade dysplasia (single focus of <5 crypts) progressed at a relatively low rate (14%) vs. their more diffuse high-grade dysplasia cases (56%) (44). Based on this, the ACG recommended that patients with focal high-grade dysplasia be followed with intensive endoscopic surveillance (e.g., q 3 months) (4). Other studies found no difference in outcome between focal and diffuse high-grade dysplasia suggesting that this differential management approach should be abandoned (39). My approach to patient management is summarized in Table 2.

TABLE 2
DYSPLASIA IN BARRETT'S EPITHELIUM: MANAGEMENT PLAN BASED UPON
HISTOLOGIC INTERPRETATION*

<u>Histologic Interpretation</u>	<u>Management</u>
Negative	Yearly or every other year surveillance; after 2 negative endoscopies, interval can be increased to 2-3 years
Indefinite for dysplasia or biopsy in positive: low-grade dysplasia	Medical therapy for reflux, repeat 6 months – if negative x 2 go to yearly surveillance and recommendations similar to negative (see above)
If indefinite/low-grade dysplasia persists, dysplasia progresses or regresses	continue q 6 month surveillance until
Positive: high-grade dysplasia	Immediate re-endoscopy with biopsy/confirm (see text) then consider esophagectomy or other intervention

*Modified from other proposed management plans, see references 1, 2, 3, 4, 36, and 42.

Investigators at the University of Washington favor intense endoscopic surveillance for high-grade dysplasia and recommend esophagectomy only when intramucosal adenocarcinoma has been detected (45). The operative mortality for esophagectomy in their series was high. Although the results of their surveillance are considered acceptable, the biopsy protocol is so demanding and expensive that many believe that it can not be applied outside of a research setting. Furthermore, advanced cancers with metastasis have developed in 5% of patients followed by surveillance only for high-grade (39,40,42,46). Interobserver variation in histologic diagnosis (high-grade dysplasia versus intramucosal carcinoma) may limit their approach (46a).

Dysplasia is relatively rare in patients with Barrett's esophagus but data suggest that when biopsy specimens are positive for high-grade dysplasia, there is considerable risk that infiltrating carcinoma is already present. We reported our experience with esophagectomy for high grade dysplasia without endoscopic abnormality. We discovered a 40% prevalence of intramucosal adenocarcinoma in the resection specimens (35). This proportion has been consistent in the surgical literature (39). This fact along with the relatively low mortality rate for esophagectomy in places with experience allow us to recommend esophagectomy for high grade dysplasia in

patients physically sound enough to survive such an operation (35). Others have made similar recommendations (39).

While it is tempting to conclude that patients with early carcinoma detected by surveillance endoscopy have benefited from early resection, initial enthusiasm at these apparent successes must be tempered by the realization that in at least one series the operative-related death rate was 25% (45). In addition, one must critically consider the patients who underwent surgery for high-grade dysplasia in whom only high-grade dysplasia was identified in the resection specimens. There is currently no conclusive evidence that they would have ever developed invasive carcinoma and if they did, the time course from dysplasia to carcinoma is unknown. Preliminary data suggest that there is a more favorable survival in patients participating in surveillance endoscopy programs than in those patients who are not (3,4).

Other options for treatment of dysplasia and early cancer in Barrett's esophagus are usually reserved for high surgical risk patients and include photodynamic therapy, endoscopic ablation, and endoscopic mucosal resection (4,39,47).

Restoration of squamous mucosa after laser (or other) ablation of Barrett's epithelium in an achlorhydric environment has been described (48,49). This type of treatment for Barrett's esophagus could obviate the need for surveillance by eliminating the cancer risk. However, long term follow up studies are required to detect any effect on the incidence of carcinoma. Furthermore, lifelong therapy with proton pump inhibitors may be required to prevent regrowth of Barrett's epithelium. The long-term side-effects of this drug in humans are unknown but appear to be minimal. Another potential problem is that partially ablated mucosa can heal with squamous epithelium overlying buried specialized columnar epithelium. There are reports of carcinoma developing from these buried metaplastic tissues (39).

Dysplasia in Barrett's Esophagus: The Role of Other Pathological Techniques

Mucin histochemistry has been extensively investigated in Barrett's esophagus and dysplasia (12,14,24,50). Some retrospective analyses have shown an association between adenocarcinoma and the presence of sulfated acid mucins in the non-goblet cells of the specialized columnar epithelium (12). However, others have concluded that the presence of sulfated mucins in these cells is so common that it is of no predictive value as a marker for dysplasia or carcinoma (14,24,51). Heightened cancer risk has also been described with loss of o-acetylated mucin, aberrant expression of blood group antigens, and abnormalities of sucrase - isomaltase, but these are not used clinically (24,52,53,54).

Reid et al have reported their experience with deoxyribonucleic acid (DNA) analysis by flow cytometry in patients with Barrett's esophagus (40,55,56,57). In these studies, carcinoma and dysplasia in biopsy specimens were highly correlated DNA cell cycle abnormalities. Furthermore, there appeared to be progression of DNA abnormalities with increasing epithelial dysplasia. Investigators from this same institution have also demonstrated a strong association between multiple different DNA aneuploid cell populations and adenocarcinoma in Barrett's esophagus. Others, however, (58) have demonstrated discordance between DNA aneuploidy, dysplasia, and carcinoma. It is possible that flow cytometry could be a useful adjunct to standard histologic assessment in cancer surveillance of patients with Barrett's esophagus, but more study is needed. Since some examples of Barrett's associated adenocarcinoma and dysplasia lack DNA abnormalities by flow cytometry (40,57) it is clear that this technique could not be used alone in a cancer surveillance program. The role of DNA analysis could be in the identification of a subgroup of Barrett's patients requiring less frequent surveillance (42). Few, if any, patients with

histology negative for dysplasia and normal DNA content have progressed to adenocarcinoma . In these patients, one could argue that surveillance intervals could be extended to up to 5 years. At the moment, DNA content analysis must be considered a research tool and is rarely used clinically.

Chromosomal imbalances by comparative genomic hybridization (59) and abnormalities of various genes have been described in Barrett's associated adenocarcinoma including p53, APC, DCC, and Rb (60-64). p53 is one of the more commonly studied gene loci because abnormalities of p53 gene usually produce an altered protein that can be studied using immunocytochemistry. Abnormal p53 protein has been found in few or no specimens classified as negative for dysplasia. In contrast, abnormal p53 expression has been identified in many low-grade dysplasia cases and in approximately two thirds of the high grade dysplasias and carcinomas examined. Although aberrant p53 expression may be an objective marker for neoplastic progression, it cannot be used alone in a surveillance program and the current clinical utility is unknown. That said, p53 and Ki67 staining can be used to help diagnose low-grade dysplasia (65,66). Similarly, abnormal expression of C-erbB2, H-ras, C-myc, TGF , EGF, EGFr, have been reported, but are not used in clinical management (24).

Brush cytology can help in the diagnosis and management of patients with Barrett's epithelium by recognizing specialized columnar epithelium or carcinoma (67,68). Since brush cytology is done via the endoscope, biopsy is performed as well and cytology plays only a complimentary role. Non-endoscopically directed balloon cytology for cancer surveillance in Barrett's esophagus has been described (68). The feasibility of doing molecular studies on fluid-based cytologic preparations has been proved and could also be an adjunct in surveillance (69,70).

The ACG states that dysplasia is the best current indication of the risk of cancer in Barrett's esophagus. The ACG also concludes that a marker other than dysplasia in identifying a high risk group on which to perform surveillance has not yet been established (3).

CLINICAL HISTORY

67 year-old woman with Barrett's esophagus. R/O dysplasia.

DESCRIPTION OF SLIDE AND DISCUSSION

The number of mitoses figures are more than is usually encountered in Barrett's esophagus, even those examples of Barrett's esophagus with ulceration, active inflammation and marked regeneration. Many of the mitoses figures are arrested in metaphase characterized by the "ring" morphology. These are characteristic changes associated with colchicine (71). Upon questioning the gastroenterologist, this patient was receiving colchicine as therapy for primary biliary cirrhosis. In addition to the effects on mitoses, colchicine has been associated with increased apoptosis, epithelial stratification, and loss of polarity that can mimic epithelial dysplasia (71,72), changes present in this biopsy specimen.

DIAGNOSIS

Specialized columnar epithelium (intestinal metaplasia) consistent with Barrett's esophagus showing cytopathologic effects of colchicine mimicking low-grade epithelial dysplasia.

REFERENCES

1. Spechler SJ and Goyal RK. Barrett's esophagus. N Engl J Med 315:362-371, 1986.

2. Petras RE, Sivak MV Jr, Rice T. Barrett's esophagus: A review of the pathologist's role in diagnosis and management. *Pathol Annual* 26:1-32, 1991.
3. Sampliner RE, et al. Practice guidelines on the diagnosis, surveillance, and therapy of Barrett's esophagus. *Amer J Gastroenterol* 93(7):1028-1031, 1998.
4. Sampliner RE and the practice parameters committee of the American College of Gastroenterology. Updated practice guidelines on the diagnosis, surveillance and therapy of Barrett's esophagus. *Amer J Gastroenterol* 97:1888-1895, 2002.
5. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 340:825-31, 1999.
6. Spechler SJ, Robbins AH, Rubins HB et al. Adenocarcinoma in Barrett's esophagus: An over-rated risk? *Gastroenterology* 87:927-933, 1984.
7. Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. *N Engl J Med* 313:857-859, 1985.
8. Herlihy KJ, Orlando RC, Bryson JC, Bozynski EM, Carney CN, Powell DW. Barrett's esophagus: clinical, endoscopic, histologic, manometric, and electrical potential difference characteristics. *Gastroenterology* 86:436-443, 1984.
9. Canto MIF, Setrakian S, Willis J, Chak A, Petras R, Powe NR, Sivak MV, Jr. Methylene blue-directed biopsies improve detection of intestinal metaplasia and dysplasia in Barrett's esophagus. *Gastrointest Endosc* 51:560-568, 2000.
10. Canto MIF, Setrakian S, Willis JE, Chak A, Petras RE, Sivak MV. Methylene blue staining of dysplastic and nondysplastic Barrett's esophagus: An in vivo and ex vivo study. *Endosc* 33:391-400, 2001.
11. Paull A, Trier JS, Dalton MD. The histologic spectrum of Barrett's esophagus. *N Engl J Med* 295:476-480, 1976.
12. Jass JR. Mucin histochemistry of the columnar epithelium of the oesophagus: A retrospective study. *J Clin Pathol* 34:866-870, 1981.
13. Zwas F, Shields HM, Doos WG et al. Scanning electron microscopy of Barrett's epithelium and its correlation with light microscopy and mucin stains. *Gastroenterol* 90:1932-41, 1986.
14. Rothery GA, Patterson JE, Stoddard CJ, Day DW. Histological and histochemical changes in the columnar-lined (Barrett's) oesophagus. *Gut* 27:1062-1068, 1986.
15. Jacobs E, Dehou MF. Heterotopic gastric mucosa in the upper esophagus: A prospective study of 33 cases and review of literature. *Endoscopy* 29:710-715, 1997.
16. Jass JR. Role of intestinal metaplasia in the histogenesis of gastric carcinoma. *J of Clin Pathol* 33:801-810, 1980.
17. Cameron AJ, Lomboy CT, Para M, Carpenter HA. Adenocarcinoma of the esophagogastric junction and Barrett's esophagus. *Gastroenterology* 109:1541-1546, 1995.
18. Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH, Goyal RK. Prevalence of metaplasia at the gastro-esophageal junction. *Lancet* 344:1533-1536, 1994.
19. Spechler SJ, Goyal RK. The Columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. *Gastroenterology* 110:614-621, 1996.
20. Schnell TG, Sontag SJ, Chejfec G. Adenocarcinoma arising in tongues or short segments of Barrett's esophagus. *Digestive Disease and Science* 37:137-143, 1992.
21. Hamilton SR, Smith RRL, Cameron JL. Prevalence in characteristics of Barrett esophagus in patients with adenocarcinoma of the esophagus or esophagogastric junction. *Human Pathology* 19:942-948, 1988.
22. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA* 265:1287-1289, 1991.
23. Blot WJ, Devesa SS, Fraumeni JF Jr. Continuing climb in rates of esophageal adenocarcinoma: An update. *JAMA* 270:1320 1993.

24. Haggitt RC. Barrett's esophagus, dysplasia, and adenocarcinoma. *Human Pathol* 25:982-993, 1994.
25. Shaheen NJ, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterol* 119:333-338, 2000.
26. Achkar E, Carey W. The cost of surveillance for adenocarcinoma complicating Barrett's esophagus. *Amer J Gastroenterol* 83:291-294, 1988.
27. Robertson CS, Mayberry JF, Nicholson DA, James PD, and Atkinson M. Value of endoscopic surveillance in the detection of neoplastic change in Barrett's oesophagus. *Brit J Surg* 75:760-763, 1988.
28. Hameeteman W, Tytgat GNJ, Houthoff HJ, van den Tweel JG. Barrett's esophagus: Development of dysplasia and adenocarcinoma. *Gastroenterol* 96:1249-56, 1989.
29. Van der Veen AH, Dees J, Blankenstein JD, Van Blankenstein M. Adenocarcinoma in Barrett's esophagus: an over-rated risk. *Gut* 30:14-18, 1989.
30. van der Burgh A, Dees J, Hop WCJ, Van Blankenstein M. Oesophageal cancers in an uncommon cause of death in patients with Barrett's esophagus. *Gut* 39:5-8, 1996.
31. Sanfey H, Hamilton SR, Smith RRL, Cameron JL. Carcinoma arising in Barrett's esophagus. *Surg Gynecol Obstet* 161:570-574, 1985.
32. Montgomery E, Bronner MP, Goldblum JR, Greenson JK, Haber MM, Hart J, Lamps LW, Lavwers GY, Lazenby AJ, Lewin DN, Robert ME, Toledano AY, Shyr Y, Washington K. Reproducibility of the diagnosis of dysplasia in Barrett's esophagus: A reaffirmation. *Hum Pathol* 32:368-378, 2001.
33. Hamilton SR, Smith RRL. The relationship between columnar epithelial dysplasia and invasive adenocarcinoma arising in Barrett's esophagus. *Amer J Clin Pathol* 87:301-312, 1987.
34. Lee RG. Adenomas arising in Barrett's esophagus. *Am J Clin Pathol* 85:629-632, 1986.
35. Rice T, Falk G, Achkar E, Petras R. Surgical management of high-grade dysplasia in Barrett's esophagus. *Amer J Gastroenterology* 88:1832-1836, 1993.
36. Reid BJ, Haggitt RC, Rubin CE et al. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Human Pathol* 19:166-178, 1988.
37. Skacel M, Petras RE, Gramlich TL, Sigel JF, Richter JE, Goldblum JR. The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression. *Amer J Gastroenterol* 95:3383-3387, 2000.
38. Montgomery E, Goldblum JR, Greenson JK, Haber MM, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Washington K, Zahurak ML, Hart J. Dysplasia as a predictive marker for invasive carcinoma in Barrett's esophagus: A follow-up study based on 138 cases from a diagnostic variability study. *Human Pathol* 32:379-380, 2001.
39. Spechler SJ. Dysplasia in Barrett's esophagus: Limitations of current management strategies. *Am J Gastroenterol* 100:927-935, 2005.
40. Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: Baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 95:1669-1676, 2000.
41. Weston AP, Banerjee SK, Sharma P, et al. p53 Protein overexpression in low-grade dysplasia (LGD) in Barrett's esophagus: Immunohistochemical marker predictive of progression. *Am J Gastroenterol* 96:1355-1362, 2001.
42. Schnell TG, Sontag SJ, Chejfec G, Aranha G, Metz A, O'Connell S, Seidel UJ, Sonnenberg A. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 120:1607-1619, 2001.
43. Reid BJ, Weinstein WM, Lewin KJ et al. Endoscopic biopsies can detect high-grade dysplasia or early adenocarcinoma in Barrett's esophagus without grossly recognizable neoplastic lesions. *Gastroenterology* 94:81-90, 1988.

44. Buttar NS, Wang KK, Sebo TJ, et al. Extent of high-grade dysplasia in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology* 120:1630-1639, 2001.
45. Levine DS, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. *Gastroenterology* 105:42-50, 1993.
46. Weston AP, Sharma P, Topalovski M, Richards R, Cherian R, Dixon A. Long-term follow-up of Barrett's high-grade dysplasia. *Am J Gastroenterol* 95:1888-1893, 2000.
- 46a. Ormsby AH, Petras RE, Henricks WH, Rice TW, Rybicki LA, Richter JE, Goldblum JR. Observer variation in the diagnosis of superficial oesophageal adenocarcinoma. *GUT* 51:671-676, 2002.
47. Ell C, May A, Gossner L, Pech O, Gunter E, Mayer G, Henrich R, Vieth M, Muller H, Seitz G, Stolte M. Endoscopic mucosal resection of early cancer and high-grade dysplasia in Barrett's esophagus. *Gastroenterol* 118:670-677, 2000.
48. Berenson MM, Johnson TD, Markowitz NR, Buchi KN, Samowitz WS. Restoration of squamous mucosa after ablation of Barrett's esophageal epithelium. *Gastroenterology* 104:1686-1691, 1993.
49. Spechler SJ. Laser photoablation of Barrett's epithelium: Burning issues about burning tissues. *Gastroenterology* 104:1855-1858, 1993.
50. Haggitt RC, Reid BJ, Rubin CE et al. Barrett's esophagus: Correlation between mucin histochemistry, flow cytometry, and histologic diagnosis predicting increased cancer risk. *Amer J Pathol* 131:53-61, 1988.
51. Atkinson M. Barrett's esophagus - To screen or not to screen? *Gut* 30:2-5, 1989.
52. Lapertosa G, Baraccini P, Fulcheri E, et al. Mucin histochemical analysis in the interpretation of Barrett's Esophagus. Results of a multi-center study. *Amer J Clin Pathol* 98:61-66, 1992.
53. Lauwers GY, Melamed J, Rojas-Corona RR. Blood group antigens in Barrett's Esophagus and associated adenocarcinomas. *Modern Pathol* 6:588-591, 1993.
54. Wu GD, Beer DG, Moore JH, et al. Sucrase-isomaltase gene expression in Barrett's Esophagus and adenocarcinoma. *Gastroenterology* 105:837-844, 1993.
55. Reid BJ, Haggitt RC, Rubin CE, Rabinovitch RS. Barrett's esophagus Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. *Gastroenterology* 93:1-11, 1987.
56. Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC, Rabinovitch PS. Flow cytometric and histological progression to malignancy in Barrett's esophagus: Prospective endoscopic surveillance of a cohort. *Gastroenterology* 102:1212-1219, 1992.
57. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett's esophagus is associated with genomic instability. *Lab Invest* 1988;60:65-71.
58. Fennerty MB, Sampliner RE, Way D, Riddell R, Steinbronn K, Garewal HS. Discordance between flow cytometric abnormalities and dysplasia in Barrett's esophagus. *Gastroenterology* 97:815-820, 1989.
59. Walch AK, Zitzelsberger HF, Bruch J, Keller G, Angermeir D, Aubele MM, Mueller J, Stein H, Braselmann H, Siewert JR, Höfler H, Werner M. Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia-dysplasia-carcinoma sequence. *Am J Pathol* 156:555-566, 2000.
60. Blount PL, Ramel S, Raskind WH, et al. 17p allelic deletions and p53 protein over expression in Barrett's adenocarcinoma. *Cancer Research* 51:5482-5486, 1991.
61. Boynton RF, Blount PL, Yin J, et al. Loss of heterozygosity involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers. *Proceedings of National Academy Sci USA*. 89:3385-3388, 1992.

62. Boynton RF, Huang Y, Blount PL, et al. Frequent loss of heterozygosity at the retinoblastoma locus in human esophageal cancers. *Cancer Research* 51:5766-5769, 1991.
63. Huang Y, Boynton RF, Blount PL, et al. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. *Cancer Research* 52:6525-6530, 1992.
64. Rice TW, Goldblum JR, Falk GW, Tubbs RR, Kirby TJ, Casey G. P53 immunoreactivity in Barrett's metaplasia, dysplasia, and carcinoma. *J. Thoracic Cardiovasc Surg* 108:1132-1137, 1994.
65. Skacel M, Petras R, Rybicki L, et al. p53 expression in low-grade dysplasia in Barrett's esophagus: correlation with interobserver agreement and disease progression. *Am J Gastroenterol* 97:2508-2513, 2002.
66. Lorinc E, Jakobsson B, Landberg G, Veress B. ki67 and p53 immunohistochemistry reduces interobserver variation in assessment of Barrett's oesophagus. *Histopathology* 46:642-648, 2005.
67. Geisinger KR, Teot LA, Richter JE. A comparative cytopathologic, histologic study of atypia, dysplasia, and adenocarcinoma in Barrett's esophagus. *Cancer* 69:8-16, 1992.
68. Falk GW, Chittajallo R, Goldblum JR, Biscotti CV, Geisinger KR, Petras R, Birgisson S, Rice TW, Richter JF. Surveillance of Barrett's esophagus for dysplasia and carcinoma with balloon cytology. *Gastroenterology* 112:1787-1797, 1997.
69. Falk GW, Skacel M, Gramlich TL, Casey G, Goldblum JR, Tubbs RR. Fluorescence *in situ* hybridization of cytologic specimens from Barrett's esophagus: a pilot feasibility study. *Gastrointestinal Endoscopy* 60:280-284, 2004.
70. Fahmy M, Skacel M, Gramlich TL, Brainard JA, Rice TW, Goldblum JR, Connor JT, Casey G, Legator MS, Tubbs RR, Falk GW. Chromosomal gains and genomic loss of p53 and p16 genes in Barrett's esophagus detected by fluorescence *in situ* hybridization of cytology specimens. *Modern Pathology* 17:588-596, 2004.
71. Iacobuzio-Donahue CA, Lee EL, Abraham SC, et al. Colchicine Toxicity: Distinct morphologic findings in gastrointestinal biopsies. *Amer J Surg Path* 25:1067-1073, 2001.
72. Torbenson M, Montgomery EA, Iacobuzio-Donahue C, et al. Colchicine effect in a colonic hyperplastic polyp: a lesion mimicking serrated adenoma. *Arch Pathol Lab Med* 126:615-617, 2002.

CASE 4

RADIATION AND CHEMOTHERAPY-ASSOCIATED GASTRIC ULCERS VS GASTRIC CARCINOMA/DYSPLASIA

Regional forms of chemotherapy and radiation therapy are used clinically with increasing frequency. Many employ hepatic arterial infusion and are used to treat primary and metastatic carcinoma involving the liver. A number of complications can occur with these forms of therapy (1-5), including gastric ulcers (1,6). These ulcer can closely mimic primary gastric carcinoma clinically, endoscopically, and sometimes in biopsy and gastric brush cytology specimens (1,6-8). Similar atypia has been seen in small intestinal epithelium in patients receiving this form of regional chemotherapy (6,9).

In order to identify features useful in distinguishing hepatic arterial infusion chemotherapy (HAIC)-associated atypia from gastric carcinoma, we reviewed gastric ulcers associated with HAIC. For comparison we used a control group of 20 patients with early gastric carcinoma of intestinal type, the lesion most likely to be confused with chemotherapy-associated ulcer. The chemotherapy-associated lesion showed a marked resemblance to irradiation effect. The Table

lists those features that were most helpful in distinguishing chemotherapeutic effect (and radiation effect) from carcinoma/dysplasia in histologic sections (6).

TABLE

DIFFERENTIAL FEATURES BETWEEN HAIC-ASSOCIATED ATYPIA AND EARLY GASTRIC ADENOCARCINOMA

<u>CARCINOMA/DYSPLASIA</u>	<u>HAIC ATYPIA</u>	<u>GASTRIC</u>
Mucosal architecture	Preserved	Distorted
Location of atypia	Glands	Foveolar
Cellular features	Bizarre Marked enlargement	Uniform anaplasia
N/C ratio	Low	High
Cytoplasmic eosinophilia	Present	Absent
Cytoplasmic vacuolization	Present	Absent
Mitotic figures	Few or none	Numerous
Atypia in granulation tissue	Present	Absent
Intestinal metaplasia	Usually absent	Present

Similar bizarre cytologic atypia may be seen in gastric brushing specimens in patients receiving HAIC (7,8). The atypical features of the epithelial cells resemble pronounced radiation effect. Atypical cells in gastric brushings can exist singly or in small clusters or flat sheets. Papillary formations or three-dimensional cellular aggregates were not observed in the specimens we studied. The most consistent feature of HAIC atypia was marked enlargement of the cytoplasm and nucleus. In spite of large size, the nuclear-cytoplasmic size ratio remained relatively low. The cytoplasm of the atypical cells was often foamy or vacuolated. The enlarged nuclei were vesicular, round to oval, and often eccentrically placed in the cytoplasm. Nuclear contours were smooth. Massive round or angulated nucleoli, either single or multiple, were characteristic. Binucleation and multinucleation were common.

CLINICAL HISTORY

The patient, a 46 year old man, presents with marked epigastric pain. Upper endoscopy reveals a very large gastric ulcer involving most of the anterior wall of the body and antrum of the stomach. The ulcer is described as clean-based with smooth edges.

DESCRIPTION OF SLIDE AND DISCUSSION

The biopsy specimen from this case demonstrates epithelial atypia mimicking dysplasia/adenocarcinoma but shows many of the features described with HAIC-associated atypia. These findings prompted a phone call to the referring gastroenterologist for a more detailed clinical history. Twenty days prior to his presentation with severe epigastric pain, the patient had received selective internal radiation therapy (SIRT) for metastatic colonic adenocarcinoma involving the liver. His hepatic artery had been injected with an infusion of 35 micron, radioactive yttrium-90 microspheres made of biocompatible resin (SIR-Spheres, SIRTex Medical Inc., Lake Forest, IL). This history helps explain not only the epithelial atypia but the presence of the small black microspheres within the mucosal capillaries.

SIRT can be a useful palliative measure for metastatic carcinoma in liver (10-14). By using selective hepatic arterial infusion, forty times the radiation dose can be delivered to the tumor (vs. conventional radiation techniques) while minimizing side effects. SIR-Spheres employ yttrium-90 which emits beta irradiation with a half-time of 64 hours. Reported side effects of this therapy include lethargy, anorexia, nausea, fever, pancreatitis and right upper quadrant pain. Gastrointestinal hemorrhage has been reported in less than 2% of patients (10, 12-14, 15,16). Rare examples of gastric and duodenal ulcer, cholecystitis and interstitial pneumonitis have been reported, some in association with embolization and migration of the radioactive microspheres into extrahepatic organs (12-14, 16-18).

Based on our experience with two patients with gastric ulcer caused by SIRT, the gastric lesion appears self limiting. With a relatively short half-time, the radiation dose to the tissues becomes negligible in 2-3 weeks. Proton pump inhibitors may be effective as an aid in healing. Our patient was treated with esomeprazole (40 mg bid) with relief of the epigastric pain in one week. Follow-up endoscopy done 6 weeks later revealed near complete healing of the ulcer.

The atypia seen in gastric brushings and biopsy specimens from patients treated with hepatic arterial infusion chemotherapy/radiation therapy may be prominent and alarming. Care must be taken to avoid misinterpretation of the findings as adenocarcinoma/dysplasia. Documentation of a clinical history of prior treatment with hepatic arterial infusion chemotherapy/radiation therapy should alert the pathologist to this potential pitfall in interpretation (8).

DIAGNOSIS

Gastric epithelial atypia associated with hepatic arterial infusion of SIR-Spheres mimicking gastric dysplasia/carcinoma.

REFERENCES

1. Weidner N, Smith JG, Lavanway JM. Peptic ulceration with marked epithelial atypia following hepatic arterial infusion chemotherapy. A lesion initially misinterpreted as carcinoma. *Amer J Surg Pathol* 7:261-268, 1983.
2. Nakhleh RE, Wesen C, Snover DC, Grace T. Venocclusive lesions of the central veins and portal vein radicals secondary to intraarterial 5-fluoro-2-deoxyuridine infusion. *Human Path* 20:1218- 1220, 1989.

3. Marymont JV, Dakhil SR, Travers H, Housholder DF. Chemical cholecystitis associated with hepatic arterial chemotherapy delivered by a permanently implanted pump. *Human Path* 16:986-990, 1985.
4. Hohn D, Melnick J, Stagg R, et al. Biliary sclerosis in patients receiving hepatic arterial infusion floxuridine. *J Clin Oncol* 3:98-102, 1985.
5. Haq MM, Valdes LG, Peterson DF, Gourley WK. Fibrosis of extrahepatic biliary system after continuous hepatic artery infusion of floxuridine through an implantable pump (Infusaid pump). *Cancer* 57:1281-1283, 1986.
6. Petras RE, Hart WR, Bukowski RM. Gastric epithelial atypia associated with hepatic arterial infusion chemotherapy. Its distinction from early gastric carcinoma. *Cancer* 56:745-750, 1985.
7. Choi HY, Takeda M. Gastric epithelial atypia following hepatic arterial infusion chemotherapy. *Diagnostic Cytopathology* 1:241, 1985.
8. Becker SN, Sass MA, Petras RE, Hart WR. Bizarre atypia in gastric brushings associated with hepatic arterial infusion chemotherapy. *ACTA Cytologica* 30:347-350, 1986.
9. Schuger L, Peretz T, Goldin E, Durst AL, Okon E. Duodenal epithelial atypia: A specific complication of hepatic arterial infusion chemotherapy. *Cancer* 61:663-666, 1988.
10. Gray B, Van Hazel G, Hope M, et al. Randomised trial of SIR-Spheres plus chemotherapy vs. chemotherapy alone for treating patients with liver metastases from primary large bowel cancer. *Ann Oncol* 12:1711-1720, 2001.
11. Van Hazel G, Blackwell A, Anderson J, et al. Randomised phase 2 trial of SIR-Spheres plus fluorouracil/leucovorin chemotherapy versus fluorouracil/leucovorin chemotherapy alone in advanced colorectal cancer. *J Surg Oncol* 88:78-85, 2004.
12. Popperl G, Helmberger T, Munzing W, et al. Selective internal radiation therapy with SIR- Spheres in patients with nonresectable liver tumors. *Cancer Biother Radiopharm* 20:200-208, 2005.
13. Lim L, Gibbs P, Yip D, et al. Prospective study of treatment with selective internal radiation therapy spheres in patients with unresectable primary or secondary hepatic malignancies. *Intern Med J* 35:222-227, 2005.
14. Lim L, Gibbs P, Yip D, et al. A prospective evaluation of treatment with Selective Internal Radiation Therapy (SIR-Spheres) in patients with unresectable liver metastases from colorectal cancer previously treated with 5-FU based chemotherapy. *BMC Cancer* 5:132-138, 2005.
15. Lau WY, Ho S, Leung TW, et al. Selective internal radiation therapy for nonresectable hepatocellular carcinoma with intraarterial infusion of 90yttrium microspheres. *International Journal of Radiation Oncology, Biology, Physics.* 40:583-592, 1998.
16. Herba MJ, Thirlwell MP. Radioembolization for hepatic metastases. *Seminars in Oncology* 29:152-159, 2002.
17. Leung TW, Lau WY, Ho SK, et al. Radiation pneumonitis after selective internal radiation treatment with intraarterial 90yttrium-microspheres for inoperable hepatic tumors. *International Journal of Radiation, Oncology, Biology, Physics* 33:919-924, 1995.
18. Thamboo T, Tan KB, Wang SC, et al. Extra-hepatic embolisation of Y-90 microspheres from selective internal radiation therapy (SIRT) of the liver. *Pathology* 35:351-353, 2003.