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Prevalence of Subsquamous Intestinal Metaplasia in Endoscopic Mucosal Resection Specimens of Patients with Barrett's Esophagus

Dissertation

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Abbreviations

ACG: American College of Gastroenterology

APC: Argon Plasma Photocoagulation.

ASGE: American Society of Gastroenterology

BCDA: basal crypt dysplasia-like atypia

BE: Barrett Esophagus.

CBE-EMR: Complete Barrett Eradication-Endoscopic Mucosal Resection

CLE: Columner lined epithelium.

DGVS: Deutsche Gesellschaft für Verdauungs- und Stoffwechselkrankheiten

EAC: esophageal adenocarcinoma

ESD: Endoscopic Submucosal Dissection.

EUS: Endoscopic Ultrasound.

GEJ: Gastroesophageal junction.

GERD: Gastro Esophageal Reflux Disease.

HADS: Hospital anxiety and depression scale

HGD: High Grade Dysplasia.

HGIN: High Grade Intraepithelial Neoplasia.

IMC: Intramucosal Carcinoma.

IND: Indefinite for Dysplasia.

LGD: Low Grade Dysplasia

LGIN: Low Grade Intraepithelial Neoplasia.

LOH: loss of heterozygosity

MIE: Minimally invasive esophagectomy

MRI: Magnetic Resonance Tomography.

NEG: Negative for Dysplasia.

NO: Nitric Oxide.

NO3: Nitrates

NSE: Neosquamous epithelium.

PDT: Photodynamic therapy.

PET: Positron Emission Tomography.

Prague C: Maximal Circumferential length of Barrett.

Prague M: Maximal longitudinal length of Barrett.

RFA: Radiofrequency Ablation.

SQBE: Subsquamous Barrett Esophagus.

TNF-α: Tumor necrosis factor-alpha

Hypothesis

Barrett´s esophagus is a premalignant condition for esophageal adenocarcinoma, a cancer with one of the fastest rising incidence rates over the past decade and a highly lethal malignancy once it is symptomatic (Fock and Ang 2011). Endoscopic mucosal resection offers realistic alternative to esophagectomy in expert centers (Spechler et al. 2011). Endoscopic resection and/or ablation of Barrett esophagus (BE) is directed by the endoscopic visual impression of pinkish Barrett versus whitish esophageal mucosa; microscopically however, BE may extend underneath the normal squamous epithelium of the esophagus and may therefore not be visible and hence escape screening or endoscopic therapy if not intentionally included in the area to be targeted. Key to improving the prognosis of patients with adenocarcinoma is being able to completely eradicate the area of intestinal metaplasia to avoid recurrence; as dysplasia may still develop and cancer may remain unrecognized in its early curable stage. Recurrence of visible Barrett's esophagus reported in variable percentages after different ablation techniques (van Vilsteren et al. 2011), (Seewald et al. 2003), (Giovannini et al. 2004) (Chennat et al. 2009a), (Badreddine et al. 2010) and (Ganz et al. 2008). Despite successful endoscopic therapies for BE with HGD or IMC, significant rates of buried BE glands under squamous postprocedural reepithelialization reported (Mino-Kenudson et al. 2007), (Haggitt 1994), (Biddlestone et al. 1998) and (Barham et al. 1997). Considerable controversy and debate continues about whether these ‘‘buried glands’’ represent a neoablative phenomenon or existed before endoscopic therapy. It is plausible that subsquamous intestinal metaplasia that was not completely resected may at least in part explain this observation. The magnitude of subsquamous intestinal metaplasia in Barrett's esophagus is unknown; a recent study showed such an extension in 28% of 47 cases

(Chennat et al. 2009b). This study aims to determine the prevalence and proximal extent of buried BE underneath squamous epithelium in a larger cohort with Barrett´s esophagus who underwent endoscopic mucosectomy via CBE-EMR for BE with and without neoplasia.

1. Introduction

1.1. Overview of the disease

Barrett's esophagus (BE) is the replacement of the normal squamous lining of the distal esophagus by columnar mucosa. Barrett's esophagus (BE) is the only known precursor of esophageal adenocarcinoma with tumors arising through an inflammation–metaplasia–dysplasia–carcinoma sequence, a tumor whose incidence is rapidly increasing and now accounts for more than half of esophageal cancers in the West (Parkin 2002). The esophageal cancer risk for patients with BE has been estimated to be > 40 times greater than the general population (Shaheen et al. 2000), with ~ 10 % of BE patients progressing to adenocarcinoma. However, both publication (Shaheen et al. 2000) as well as referral bias (Pohl et al. 2008) may lead to an overestimation of the risk of BE.

Perhaps no tumor in the past 10 years has undergone such changes in epidemiology, diagnosis, and treatment as adenocarcinoma of the esophagus (Barrett's carcinoma). The previous generation of textbooks gave it an extremely poor prognosis; now it has become an oncological disease that can be diagnosed at an early stage and treated with a high probability of cure. The main contributor to this change has been the introduction of high-resolution video endoscopy and endosonography (endoscopic ultrasonography, EUS), which have made it possible to identify neoplasia early and to stage and treat it accurately. Local endoscopic treatment of early Barrett's carcinoma (pT1m, L0, V0, G1/2) is now an established standard treatment in Germany (DGVS 2005).

1.2. Epidemiology and Pathogenesis

Esophageal carcinoma is still a rare tumor entity in Germany, with an incidence of 5000 new cases per year. However, in the past 30 years the

incidence of adenocarcinoma of the esophagus has been rising more sharply than that of any other tumor in the western world (Pohl and Welch 2005). In Germany, the Bavarian cancer registry for the Regensburg region showed a rise in adenocarcinomas as a percentage of esophageal carcinomas from 9% to 31% between 1992 and 2004. In the USA already more than 50% of malignant tumors of the esophagus are histologically adenocarcinomas, which have thus overtaken squamous cell carcinoma of the esophagus (which used to predominate) in frequency (Brown et al. 2008). In addition, there is a tendency to diagnose Barrett's carcinomas earlier, so that in some centers T1 tumors already account for more than 50% of the whole.

With regards to pathogenesis, it is rather certain is that gastric acid reflux, with heartburn as the main symptom, is the main risk factor for development of Barrett's carcinoma (Lagergren et al. 1998). Barrett's carcinoma development usually occurs over the course of years and involves a sequence of metaplasia–dysplasia (low grade–high grade neoplasia) carcinoma leading to malignant differentiation (Spechler 2002) A US study some years ago showed that at the stage of high-grade dysplasia (without visible lesions), more than 70% of patients do not change over a follow-up time of 7 years (Schnell et al. 2001). The metaplastic columnar cells of Barrett's esophagus are in some ways a favorable adaptation to chronic reflux since they appear to be more resistant to reflux-induced injury than the native squamous cells. Unfortunately, esophageal columnar metaplasia predisposes to the development of adenocarcinoma (Morales et al. 2002). Several physiologic abnormalities contribute to the severity of GERD in patients with long segment Barrett's esophagus (*table 1*). As a result, patients who have long segment Barrett's esophagus are predisposed to reflux highly caustic gastric contents (often without warning symptoms) into an

esophagus whose ability to protect itself is compromised by defective clearance mechanisms and diminished secretion of growth factors. Given the propensity for severe GERD in patients with long segment Barrett's esophagus, it was initially assumed that the metaplasia progressed in extent over the years as columnar epithelium replaced more and more reflux-damaged squamous epithelium. However, for reasons that are unclear, such progression is observed only rarely (Cameron and Lomboy 1998). In most cases, Barrett's esophagus appears to develop to its full extent over a short period of time (ie, <1 year), with little or no subsequent progression. Why this occurs is not well understood. Patients with short segment Barrett's esophagus often have few or no symptoms and signs of GERD.

Table 1: GERD and Barrett's

Abnormality	Contribution to GERD
Gastric acid hypersecretion with or without duodenogastric reflux	Gastric contents available for reflux are highly caustic to the esophagus due to high concentrations of acid and, with duodenogastric reflux, bile
Extreme hypotension of the lower esophageal sphincter	Impairment in primary antireflux barrier
Poor esophageal contractility	Reduced ability to clear esophagus of refluxed material
Diminished esophageal pain sensitivity	Reduced warning of esophageal injury which can also decrease compliance with antireflux therapy
Decreased salivary secretion of epidermal growth factor	May delay healing of esophagus

The development of intestinal metaplasia in patients with short-segment disease may be due to exposure to noxious agents that accumulate at the gastroesophageal junction (GEJ). After meals, there is a pocket of acid at the GEJ that escapes the buffering effects of ingested food (Fletcher et al. 2001). This postprandial acid pocket has a mean length of 2 cm, beginning in the most proximal stomach and extending more than 1 cm above the squamo-columnar junction (Z-line) into the distal esophagus. In

healthy volunteers, the very distal esophagus (5 mm above the Z-line) is exposed to acid for more than 10 percent of the day (Fletcher et al. 2004). Potential consequences of such persistent acid exposure include not only acid-peptic injury, but also exposure to high concentrations of nitric oxide (NO) generated from dietary nitrates (NO3) in green, leafy vegetables. Most ingested nitrate is absorbed by the small intestine and excreted unchanged in the urine, but approximately 25 percent is concentrated by the salivary glands and secreted into the mouth where bacteria on the tongue reduce the recycled nitrate to nitrite (NO2). When swallowed nitrite encounters acidic gastric juice, the nitrite is converted rapidly to nitric oxide (NO). After nitrate ingestion, high levels of NO have been demonstrated at the GEJ (Iijima et al. 2002). NO can be genotoxic and, potentially, carcinogenic. Thus, the GEJ is exposed repeatedly to acid, pepsin, NO, and other noxious agents in gastric juice that can lead to chronic inflammation and metaplasia. The lifelong carcinoma risk of a patient with Barrett's esophagus is not known, but a yearly carcinoma incidence of 0.5% per year may be assumed, which would mean that 5% of patients with Barrett's esophagus would develop carcinoma within 10 years (Sharma et al. 2009). After excluding both prevalent cancers and high-grade dysplasia, a recent systematic review and meta-analysis of EAC and HGD incidence rates in BE patients, reported an incidence of EAC and HGD combined of 10.2/1000 person years (Sikkema et al. 2010). The incidence of EAC alone was 6.3/1000 person years and the incidence of HGD alone was found to be 4.0/1000 person years (Sikkema et al. 2010). The reported wide ranges of observed intervals between BE diagnosis and incident EAC may either be due to large individual differences in progression rates or to large differences in the stage of diagnoses of BE.

As practically no reliable long-term predictors of malignant progression have been identified, there is usually no indication at diagnosis which BE patients will survive to incident EAC (Ong et al, 2010). On the other hand, studies of large groups of BE patients referred by general practitioners have generated some insights into the patterns of BE incidence, such as its age specific increase and the delayed BE onset of about 20 years in females (van Blankenstein et al. 2005) (van Soest et al. 2005) (Derakhshan and McColl 2009). In 1992 Cameron and Lomboy estimated that BE developed at a mean age of 40 years, whereas the mean age at EAC diagnosis was 64 years. They concluded from this observation that the average incubation period from BE to EAC was more than 20 years (Cameron and Lomboy 1992). A mathematical model based on the observed EAC incidence in a Danish cohort, required all EAC cases to have acquired BE before the age of 45. This also suggested an incubation period of several decades (Van Blankenstein et al. 2007). Unfortunately the unrecorded onset of BE precludes measuring its duration by direct observation. Currently the only available surrogate for the date of BE onset is the date of BE diagnosis. Recently, the Rotterdam BE follow-up cohort revealed a long incubation period between onset of BE and development of HGD/EAC, in patients without HGD/EAC at baseline as illustrated by 24 patients diagnosed with BE at a young age and followed for a mean period of 25.5 years. Their tumor-free survival established a minimum incubation period, suggesting a true incubation period of three decades or more (den Hoed et al. 2011). A possible overestimation of cancer risk due to publication and referral bias has already been mentioned (Shaheen et al. 2000), (Pohl et al. 2008).

The risk of progressing to esophageal adenocarcinoma is determined by development of genomic instability and dynamic clonal evolution in the distal esophagus modulated by host and environmental risk and protective

factors (figure 1), including inherited genotype. The challenge for investigators of Barrett's esophagus lies in integrating knowledge about genomic instability and clonal evolution into clinical management to increase the lifespan and quality of life of individuals with this condition (Reid et al. 2010). In the future, the ability to perform individualized risk stratification using genetic markers would be desirable.

There is consensus that male sex constitutes a risk factor (M:F = 7:1) for developing adenocarcinoma of the esophagus. In addition, familial increased incidence of Barrett's esophagus and Barrett's adenocarcinoma has been observed (Chak et al. 2009). One more definitely established association is that between Barrett's adenocarcinoma and overweight, especially abdominal obesity (Hampel et al. 2005). The combination of overweight and reflux symptoms is associated with a higher risk. Adipose tissue is a dynamic endocrine organ. Adipocytes secrete numerous hormones or 'adipokines' that exhibit mitogenic activity such as leptin (Somasundar et al. 2003) (Beales and Ogunwobi 2007) (Ogunwobi et al. 2006) (Francois et al. 2008) (Kendall et al. 2008), adiponectin (Kelesidis et al. 2006) (Ogunwobi et al. 2008) (Wang et al. 2008) (Konturek et al. 2008) (Rubenstein et al. 2008) (Yildirim et al. 2009) , interleukin-6 (IL-6) (Dvorakova et al. 2004) (Moons et al. 2005) and tumour necrosis factor-alpha (TNF-α) (Eksteen et al. 2001) (Tselepis et al.2002) . It has been postulated that these hormones may mediate the progression of Barrett's oesophagus to cancer (table 2) (Winzer et al. 2010).

Nicotine abuse, on the other hand, plays only a subordinate role in the pathogenesis of esophageal carcinoma, and alcohol consumption has no notable role (Wu et al. 2001).

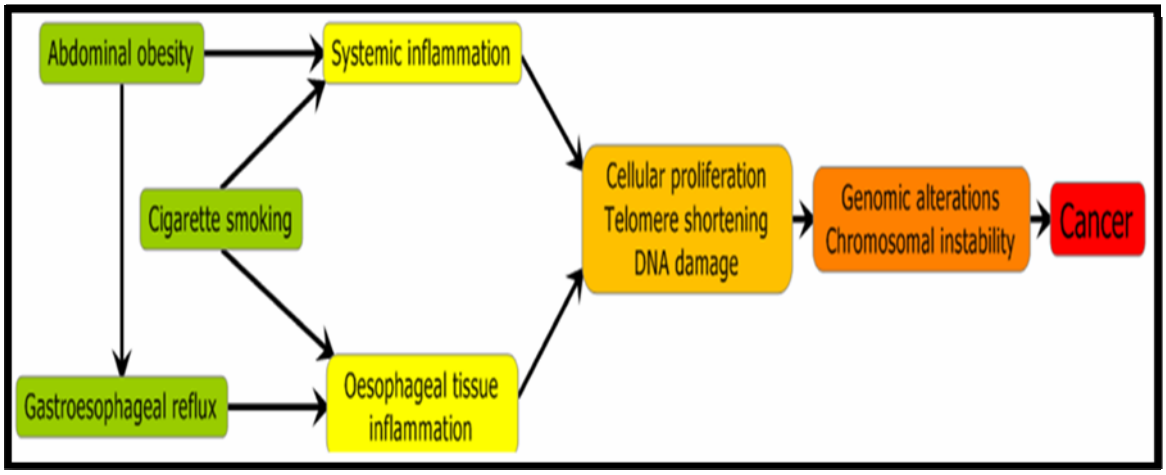


Figure1: Pathogenesis of Inflammation and oesophageal adenocarcinoma**.**

Table 2: Biomarkers associated with the progression of Barrett's oesophagus to oesophageal adenocarcinoma.

Biomarker	**Direction**	**Putative mechanism of promoting oesophageal adenocarcinoma**
Gastro-oesophageal reflux frequency and severity	Increased	Chronic inflammation and damage to oesophageal epithelium
Central obesity	Increased	Systemic metabolic dysfunction Increased reflux of gastric acid into the lower oesophagus via increased intra-abdominal pressure and/or hiatus hernia
Leptin	Increased	Mitogenic Angiogenic Anti-apoptotic
Adiponectin	Decreased	Increased insulin resistance Pro-inflammatory Anti-apoptotic
Inflammatory mediators: C-reactive protein TNF-α Interleukin-6	Increased	Mitogenic Angiogenic Increased differentiation Anti-apoptotic Decreased DNA repair
Insulin	Increased	Mitogenic Anti-apoptotic Increased leptin Increased tumour necrosis factor-α Decreased adiponectin

1.3. The Impact of Barrett's Esophagus on the Individual Patient

Although the risk of esophageal cancer or death from Barrett's esophagus is low for an individual, the impact of this diagnosis on patients is great because mortality from esophageal cancer remains high and psychological and financial consequences have been documented. A review of the literature reveals some contradictory results for studies assessing the impact of a diagnosis of Barrett's esophagus on life expectancy. Some have suggested that patients with Barrett's oesophagus have increased (Solaymani-Dodaran et al. 2005), similar (Eckardt et al. 2001) or decreased (Anderson et al. 2003) mortality rates compared with the general population. In a population-based study in Northern Ireland, Anderson et al compared mortality rates for subjects with Barrett's esophagus with those for age- and sex-matched subjects in the general population. The investigators found no significant differences in overall mortality rates between the 2 groups (Anderson et al. 2003). Although deaths from esophageal cancer were more common in the group with Barrett's esophagus, the total frequency of such deaths was so low that it had little effect on overall mortality. Another study that compared survival for subjects with Barrett's esophagus with survival for 2 control groups (the general population and patients with Schatzki's rings) also found no difference in life expectancy among the groups (Eckardt et al. 2001). In contrast, Moayyedi et al. in the United Kingdom found increased mortality for subjects who had Barrett's esophagus compared with age- and sex-matched subjects in the general population (Moayyedi et al. 2008). Interestingly, however, the excess mortality in the patients with Barrett's esophagus was primarily due to extraesophageal diseases such as bronchopneumonia and ischemic heart disease. The excess deaths from cardiovascular problems may be related to the association of Barrett's esophagus with obesity, which also is a risk factor for

cardiovascular disease. Smokers and those with high BMI have an increased overall mortality in population studies (Prescott et al. 1998), (Bender et al. 1999), this may explain the association between Barrett's esophagus and 'all-cause' mortality. This emphasizes that we should look beyond the increased risk of esophageal adenocarcinoma when managing our Barrett's patients. It would be sensible to encourage smoking cessation and weight loss when appropriate.

Patients with Barrett's esophagus report a poorer quality of life than individuals in the general population (Eloubeidi et al. 2000) (Kulig et al. 2003). It is unclear whether this is due to anxiety about cancer, discomfort due to GERD symptoms, or other factors. However, it is likely that the quality of life for patients with Barrett's esophagus varies with a number of important factors, such as disease duration and the number of surveillance endoscopies performed. Attempts to quantify quality of life in Barrett's esophagus using health state utilities have repeatedly shown diminished utility for life with this condition. The negative impact on utility varies with the degree of dysplasia in Barrett's epithelium and has been reported to be as low as 0.77 for patients with high-grade dysplasia (Gerson et al. 2007).

By generic and organ-specific quality of life measures, subjects with Barrett's esophagus repeatedly have been shown to have substantially lower scores than population norms. A diagnosis of Barrett's esophagus especially in presence of dysplasia appears to cause psychological stress, emotional burden (Shaheen et al. 2008) and may be associated with substantial, but incompletely understood, additional costs such as increased life and health insurance premiums (AGA 2011). In a study of BE patients undergoing endoscopic surveillance, the BE patients had higher anxiety scores than the general population at multiple time points

on the HADS (Hospital anxiety and depression scale) (Essink-Bot et al. 2007).

1.4. Diagnosis and staging

1.4.1. Endoscopic Diagnosis

Examination using high definition video endoscopy is the primary and central diagnostic technique (*figure 2a,1b*,3a). Additional techniques such as real chromoendoscopy with the use of contrast agents to accentuate surface topography (contrast staining), and/or identify specific epithelia by vital staining (absorptive staining), or chemical reactions (reactive staining e.g., with dilute acetic acid) and virtual chromoendoscopy are intended to enable diagnosis of early neoplasia at the millimeter level by improving the visualization of surface structures (*figure 3*b) (*table 3*) but neither of which is evidence-based as yet. The value of these procedures is currently under investigation.

Together with the endoscopic aspect histological confirmation by biopsy remains the standard. The use of so called endomicroscopy (microscopic evaluation *in vivo*) is purely experimental at the present time. Effective communication between the gastroenterologist and pathologist is crucial to the diagnosis, risk assessment, and management of BE. The depth of wall infiltration by the tumor and the para-esophageal lymph nodes are evaluated by EUS, which is superior to other imaging techniques for this purpose (Pech et al. 2006). Nevertheless, despite initial high accuracy rates (Puli et al. 2008), poorer staging results have been reported for early disease, to differentiate mucosal from submucosal infiltration (May et al. 2004) and also in advanced tumors in clinical routine (Kutup et al. 2007). Complementary procedures such as elastography or contrast EUS are still at the development stage. Should EUS raise suspicion of local lymph node metastasis in a patient with a T1 or T2 tumor, the diagnosis should be confirmed histologically/cytologically by means of EUS-guided fine-

needle aspiration, since proof of lymphatic tumor involvement would change the treatment strategy.

Table 3: Chromoscopy for Barrett Oesophagus.

Stain	Type	Use
Methylene blue	Vital stain	Surveillance...identifies intestinal metaplasia and possibility dysplasia.
Toluidine blue	Vital stain	Surveillance...identifies columnar mucosa.
Lugol's solution	Vital stain	Screening...accentuates squamocolumnar border and highlights small islands and tongues of columnar mucosa.
Acetic acid	Contrast stain	Screening...accentuates squamocolumnar border and highlights small islands and tongues of columnar mucosa
Indigo carmine	Contrast stain	Surveillance...identifies intestinal metaplasia and dysplasia when used with magnification endoscopy,

Other imaging procedures:

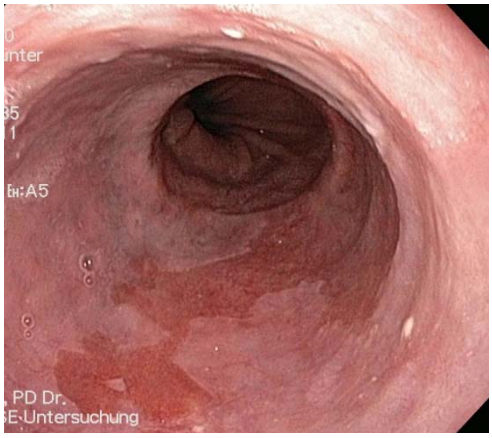
The diagnostic work-up should include multiple array thoracic computed tomography (CT) including the upper abdomen, and abdominal ultrasonography. Neither magnetic resonance imaging (MRI) nor positron emission tomography (PET) or PET-CT imaging has been shown at present to be adequate as standard investigations in Barrett's adenocarcinoma.

1.4.2. Histopathological Diagnosis:

Normal anatomy and histology of the esophagus

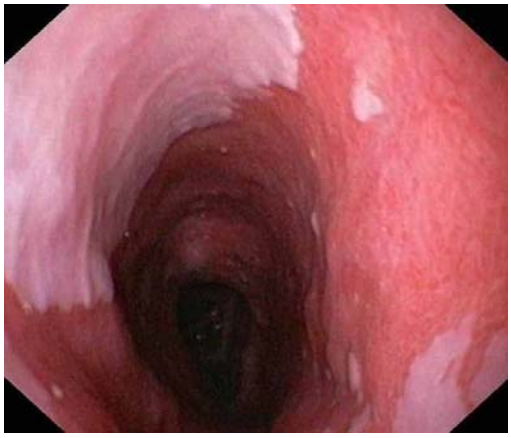
The esophagus is normally lined by stratified squamous epithelium. Scattered compact submucosal glands and their associated squamous-lined ducts are also characteristic features of this organ. Historically, it was believed that the distal 1-2 cm of the “normal” esophagus was lined by columnar mucosa. However, contemporary evidence points to the contrary. In fact, it is now widely believed that most, if not all, columnar mucosa proximal to the anatomic gastroesophageal junction (GEJ) is abnormal (metaplastic) and is attributable to chronic gastroesophageal

reflux (Hayward 1961), (Weinstein and Ippoliti 1996), (Chandrasoma et al. 2000) and (Chandrasoma et al. 2003).



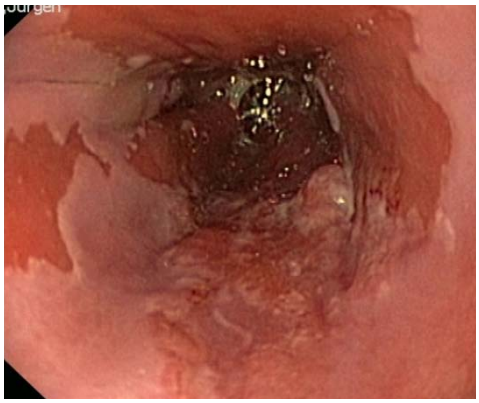
2a

Figure 2a: Long Barrett tongue.

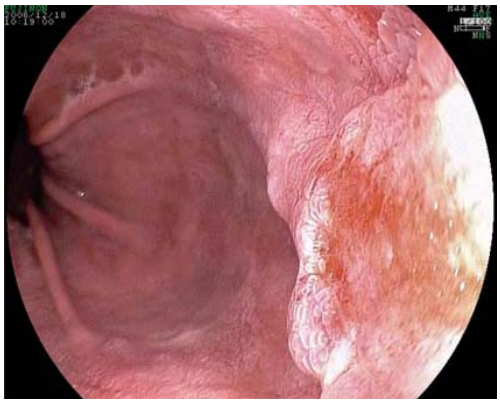


2b

2b: Long Barrett segment.



3a



3b

Figure 3a: Early neoplasia with mucosal irregularities within Barrett's oesophagus... **3b** Early neoplasia by Chromoendoscopy using acetic acid.

The most proximal portion of the stomach is often referred to as the gastric “cardia” (Kilgore et al. 2000), (Glickman et al. 2002) and (Derdoy et al. 2003). This narrow region of mucosa is typically composed of surface foveolar cells and either pure mucous glands or glands with mixed mucous and parietal cells. The origin of this type of mucosa is a subject of debate. Some authorities believe it to be always esophageal and, thus, metaplastic, whereas others believe it is normally present at birth. Regardless, it is accepted that the length of “cardia-type” mucosa increases (extends proximally with age, probably as a reflection of

physiological reflux. Because the “cardia” is an ill-defined structure of questionable etiology, it is preferable to abandon this confusing term in favor of the term “proximal stomach.” The “proximal stomach” transitions to the body of the stomach, which is composed of pure oxyntic-type glands (ie, mixture of parietal and chief cells). Accurate detection of abnormal (metaplastic) columnar mucosa in the distal esophagus is incumbent on precise localization of the anatomic GEJ. Unfortunately, identification of this critical landmark is fraught with difficulty and controversy (Bellizzi and Odze 2010). For instance, various definitions of the GEJ exist, two of which are used most commonly in clinical practice. In Japan, the GEJ is defined by the distal-most limit of the palisading longitudinal blood vessels, which correspond to veins in the lamina propria of the distal esophagus in histologic tissue sections (Takubo et al. 2008), (Takubo et al. 2009), (Sharma et al. 2006a) and (Ogiya et al. 2008). Palisading vessels may be confused for other types of vascular patterns in the proximal stomach, and they are frequently difficult to identify in patients with esophagitis. In contrast, in the United States and in many other parts of the world, the GEJ is defined by the most proximal extent of the gastric folds (Sharma et al. 2006b), (Sampliner 2002), (Sharma et al. 2004) and (Wang and Sampliner 2008). Unfortunately, identification of this landmark is also difficult because it may vary with respirations and procedure-related air insufflations (figure 4). Nevertheless, use of the proximal limit of the gastric folds as the “definition” of the GEJ has been incorporated into the Prague C & M Criteria (figure 5), an international effort to develop and validate an endoscopic grading system for Barrett’s esophagus (BE). In this scheme, recognition of the GEJ was accomplished with “almost perfect reliability” (Sharma et al. 2006b). However, even in established BE, the

measurements by endoscopy and that by stepwise 1 cm biopsies may vary considerably (Egger et al. 2004).

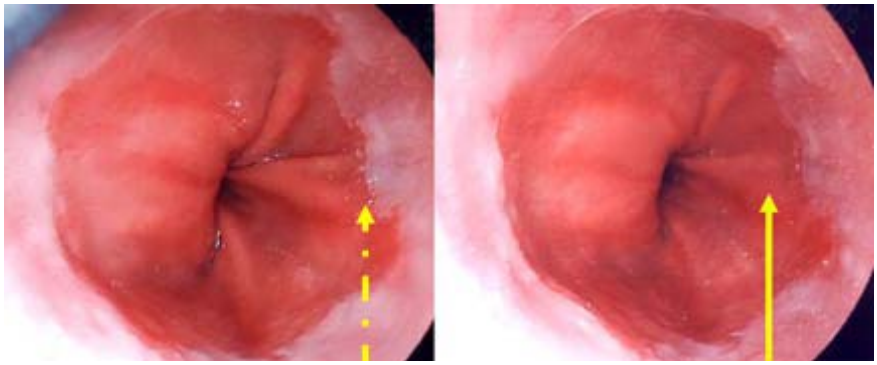


Figure 4.Endoscopic image of Barrett's oesophagus. The two pictures are from the same patient but were taken five seconds apart. The panel on the right shows correct air insufflation during endoscopy, whereas the panel on the left shows the oesophagus suboptimally distended. As a consequence, the picture on the left may be misdiagnosed by inexperienced endoscopists as a hiatal hernia, because the folds in the oesophageal lining extend to the gastro-oesophageal junction (dashed arrow). The panel on the right indicates circumferential Barrett's oesophagus, which can easily be seen above the folds of the hiatal hernia (solid arrow).

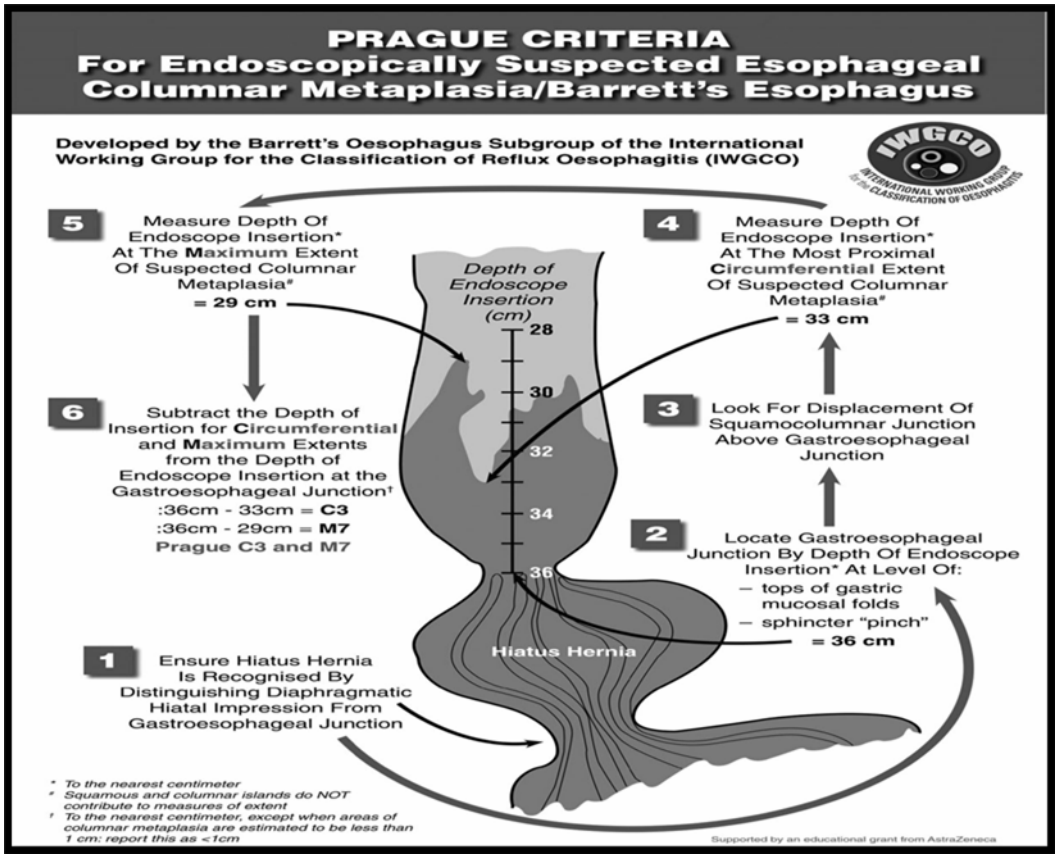


Figure 5: Prague Classification.

Definition of Barrett's esophagus

Fundamentally, BE represents replacement of normal squamous epithelium of the distal esophagus by metaplastic columnar epithelium. Unfortunately, the definition of BE varies worldwide. The main difference concerns the requirement for histologic confirmation of columnar mucosa with goblet cells. In Japan, columnar-lined esophagus (CLE) is diagnosed when columnar mucosa (salmon-colored, velvety mucosa distinct from the normal pearlescent squamous mucosa) is identified endoscopically in the distal esophagus; histologic confirmation is not required (Takubo et al. 2008), (Takubo et al. 2009) and (Ogiya et al. 2008). According to the British Society of Gastroenterology, BE represents an endoscopically apparent area of columnar mucosa proximal to the GEJ, proven on histologic examination; the most recent guideline has dropped the requirement for the demonstration of intestinal metaplasia (IM) (ie, goblet cells) (Playford 2006). Biopsies allow distinction of metaplastic columnar mucosa from endoscopic mimics, such as esophagitis. In contrast, in the United States, a diagnosis of BE is dependent on the finding of endoscopic evidence of columnar mucosa proximal to the anatomic GEJ and histologic confirmation of IM (ie, goblet cells) (Sharma et al. 2004) and (Wang and Sampliner 2008). Traditionally, three types of columnar epithelia have been described in Barrett's esophagus (Paull et al. 1976): *Cardiac epithelium*, which has a foveolar (pitted) surface and glands that are lined almost exclusively by mucus-secreting cells; these cells resemble those in the gastric cardia. *Gastric fundic-type epithelium* which has a foveolar surface lined by mucus-secreting cells, and a deeper glandular layer that contains chief and parietal cells; these cells resemble those in the gastric fundus. *Specialized intestinal metaplasia* (also called specialized columnar epithelium), which has intestinal-type crypts lined by mucus-secreting

columnar cells and goblet cells. Specialized intestinal metaplasia has intestinal features such as goblet cells and villi that readily distinguish it from normal gastric and esophageal mucosae. It is the most common histologic type found in Barrett's esophagus, and the only one that has a clear malignant potential (*table 4*) (Spechler 2002)), although this has recently been questioned again (Takubo et al. 2009). Most authorities insist on the demonstration of specialized intestinal metaplasia to confirm an endoscopic diagnosis of Barrett's esophagus. Historically, BE has been divided into long-segment (>3 cm), short-segment (1-3 cm), and ultra short-segment (<1 cm) categories. However, these are no longer recognized as distinct entities. For instance, the American Gastroenterological Association refers to the distinction of long- from short-segment BE as "arbitrary and not clinically valid" (Sharma et al. 2004).

Table 4: Classification of Barrett's

Classification	Association with GERD	Association with carcinoma	Endoscopic surveillance
Columnar lined esophagus **with** specialized intestinal metaplasia	Variable	Yes	Yes
Columnar lined esophagus **without** specialized intestinal metaplasia	Variable	Unlikely	Probably not
Specialized intestinal metaplasia at the esophagogastric junction	Variable	Probable	Unclear

Histology of Barrett's esophagus

Barrett epithelium contains a mosaic of cell types, including those normally seen in the stomach (ie, surface and glandular mucinous cells and parietal cells), intestine (ie, goblet cells and less frequently enterocytes, endocrine cells, and Paneth cells), and even the pancreas (i.e, acinar cells). In addition, a variety of cells with features intermediate between gastric and intestinal phenotype, such as "multilayered

epithelium," are present as well (Paull et al. 1976) and (Offner et al. 1996). In fact, goblet cells are often not the predominant cell type and may be difficult to identify. These must be distinguished from "pseudogoblet" cells, which superficially resemble goblet cells due to the presence of apical mucous, but in contrast to the latter, tend to occur in concentrated rows within surface epithelium, are barrel-shaped, and contain pale, neutral mucin. Unfortunately, pathologists often have trouble distinguishing these cell types, and histochemical stains are not useful in this distinction. Representative images of BE are presented in *figure 6*.

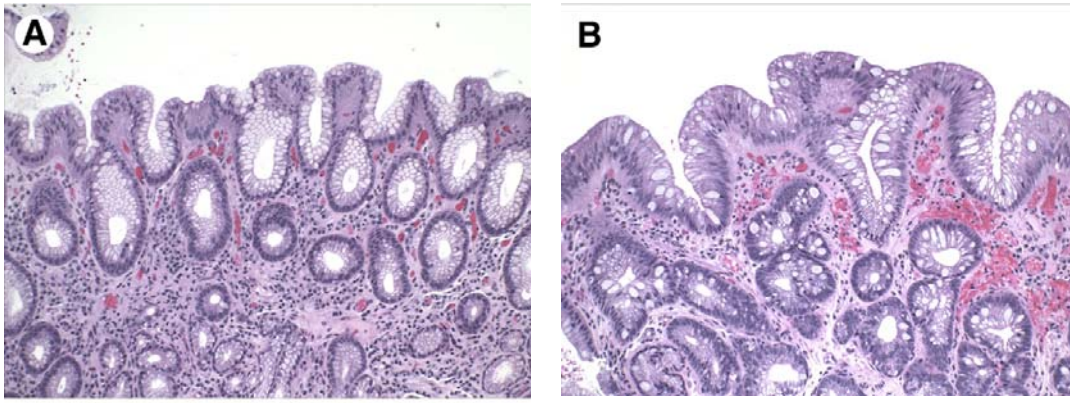


Figure 6 (A) BE, characterized by columnar epithelium with mucous cells and pseudogoblet cells. Mucous glands, and one gland with an isolated goblet cell, are identified in the deep lamina propria. There is increased inflammation in the lamina propria as well. (B) BE, characterized by columnar epithelium with numerous goblet cells. In this example of nondysplastic BE, the bases of the crypts, and the glands, show architectural distortion, branching, and a slight back-to-back configuration. Nuclear atypia is present in the bases of the crypts, with increased mitotic figures, but these changes do not reach the threshold for dysplasia.

Although BE is generally thought of as an epithelial disorder, most cases also exhibit stromal alterations (Rubio and Riddell 1988), (Takubo et al. 1991), (Abraham et al. 2007) and (Lewis et al. 2008). These alterations include duplication and fragmentation of the muscularis mucosae (MM), increase in the number of blood vessels and lymphatics, and changes in the constituent inflammatory cells. Duplication of the MM results in two layers, one being newly formed and superficial, and the other being deep (original MM). Thus, the new, superficial MM, which forms at the base of metaplastic crypts, divides the mucosa into essentially four

compartments: (1) inner (native) lamina propria, (2) inner (neo) MM, (3) outer (neo) lamina propria, and (4) deep (native) MM. At present, invasion by carcinoma into any of these compartments is considered "intramucosal carcinoma," although the implications with regard to risk of lymph node metastasis are a subject of ongoing research interest. These stromal alterations are depicted in *figure 7*.

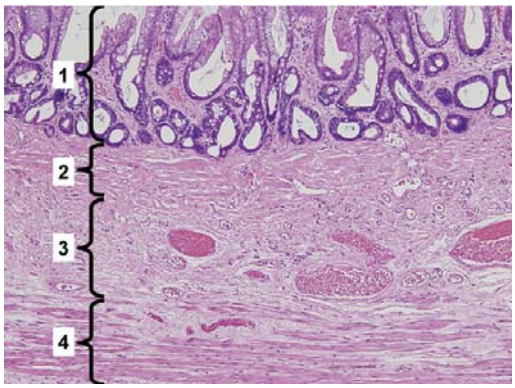


Figure 7: Stromal alterations in BE. Formation of a new, superficial MM at the base of the metaplastic glands, results in the division of Barrett mucosa into four compartments: (1) epithelium and investing inner lamina propria, (2) inner (neo) MM, (3) outer (neo) lamina propria, and (4) deep (native) MM.

Should goblet cells be required for a diagnosis of Barrett's esophagus?

Goblet cells have long been held as the "defining" cell type of BE. This is based largely, and historically, on their frequent presence in resection specimens from patients with adenocarcinomas of the esophagus or GEJ (Haggitt et al. 1978), (Skinner et al. 1983), (Smith et al. 1984), (Paraf et al. 1995), (Cameron et al. 1995), (van Sandick et al. 2000) and (Chandrasoma et al. 2007). However, there are a number of problems with this definition that require closer evaluation. These include the following: (1) detection of goblet cells is prone to sampling and interpretive error; (2) nongoblet columnar epithelium is intestinalized and genetically abnormal; (3) dysplasia and cancer may arise in nongoblet columnar epithelium; and (4) goblet cells are fully differentiated, without proliferative capability and thus, at best, represent a surrogate biomarker of mucosa at risk for cancer, rather than the cancer "cell of origin." The density of goblet cells in any segment of CLE (and our ability to detect

them) is dependent on a variety of factors, such as patient age, length of the columnar-lined segment, number and location in which biopsies are obtained, among others (Kim et al. 1994), (Oberg et al. 2001), (Chandrasoma et al 2001), (Jones et al. 2002) and (Harrison et al. 2007). For instance, Oberg and coworkers detected IM (ie, goblet cells) in 30.5% of patients with 1-2 cm of CLE, compared with 88.9% in patients with _6 cm of CLE at index endoscopy. After 6 endoscopies, the likelihood of detecting goblet cells in patients with 1-2cm segments of CLE increased to 63.6% (Oberg et al. 2001). Jones and coworkers performed repeat endoscopies on 43 patients in whom short-segment BE was suspected endoscopically, but in whom an initial biopsy failed to reveal goblet cells; biopsies from 10/43 patients (23%) demonstrated goblet cells at repeat endoscopy (Jones et al. 2002). Harrison and coworkers, based on an analysis of 1646 biopsies from 125 consecutive patients with apparent CLE, recommended that a minimum of 8 biopsies should be obtained to detect goblet cells. In that study, goblet cells were identified in 68% of patients when a mean of 8 biopsies were obtained versus 34.7% when a mean of 4 were evaluated (Harrison et al. 2007). Some studies suggest that the goblet cell density is greatest near the proximal neo-squamocolumnar junction. For instance, Chandrasoma and coworkers detected goblet cells in 100% of patients when biopsies were obtained from the proximal aspect of the columnar mucosa, compared with 69% of patients when biopsies were obtained from the distal CLE (Chandrasoma et al. 2001). Non goblet columnar epithelium in patients with CLE has recently been shown to possess “intestinal” features and to exhibit molecular abnormalities similar to those seen in fully established BE (ie, with goblet cells) (Chaves et al. 2007), (Hahn et al. 2009) and (Liu et al. 2009). For instance, Hahn and coworkers examined metaplastic non goblet epithelium in patients either without (*n* =30) or with (*n* =59) goblet

cells for immunohistochemical expression of markers of intestinal differentiation, such as DAS-1, villin, and CDX-2. Patients with metaplastic non goblet epithelium demonstrated reactivity for these markers in 30%, 17%, and 43%, of cases, respectively, whereas patients with goblet cells demonstrated reactivity in 90%, 95%, and 98% of cases (Hahn et al. 2009). Liu and coworkers demonstrated DNA content abnormalities in nongoblet columnar epithelium in patients either with or without goblet cells. The cell of origin of BE and BE-related neoplasia is unknown, but recent evidence suggests that progenitor cells are located in the crypt bases. However, these cells are difficult to recognize histologically and biochemically and do not represent goblet cells, which are more often located in the superficial aspects of the crypts and surface epithelium, regions of mucosa with little or no proliferative capability (Kelty et al. 2007).

1.5. Neoplasia in Barrett's esophagus

1.5.1. Classification

At present, histologic grading of dysplasia represents the "gold standard" method of estimating cancer risk in patients with BE (Sharma et al. 2004), (Wang et al. 2008), (Weston et al. 1999), (Weston et al. 2000), (Reid et al. 2000), (Skacel et al. 2000), (Montgomery et al. 2001a), (Montgomery et al. 2001b), (Buttar et al. 2001b), (Schnell et al. 2001), (Weston et al. 2001), (Skacel et al. 2002) (Weston et al. 2004), (Dulai et al. 2005), (Sharma et al. 2006a), (Srivastava et al. 2007b) and (Kaye et al. 2009). In fact, the specific histologic criteria were originally adapted from a study in inflammatory bowel disease (Reid et al. 1988) and (Riddell et al. 1983). Clinically relevant diagnostic categories include negative for dysplasia (NEG), indefinite for dysplasia (IND), positive for dysplasia [either low-grade (LGD) or high-grade (HGD)], intramucosal adenocarcinoma (IMC), and invasive adenocarcinoma, the latter of which

implies infiltration of tumor into the submucosal, or deeper, layers of the bowel wall. Recent consensus (WHO classification) has replaced dysplasia by intraepithelial neoplasia (LGIN/HGIN) (Hamilton and Aaltonen 2000) but dysplasia is still used frequently and especially in the US.

There is significant discrepancy in the criteria used to diagnose "adenocarcinoma" between Western and Japanese pathologists. For instance, in the West, a diagnosis of adenocarcinoma is reserved for cases in which there is definitive histologic evidence that neoplastic epithelium has breached the glandular basement membrane, whereas in Japan, greater emphasis is given to cytologic, rather than architectural, atypia. Thus, in that country, "adenocarcinoma" may be diagnosed in the absence of histologic documentation of tissue invasion (Schlemper et al. 2001), This difference in philosophy has led to considerable difficulty in interpretation of Japanese data by Western physicians, and vice versa. The "Vienna classification of gastrointestinal neoplasia" was devised to bridge this gap. Diagnostic categories in this system are largely similar to the above "Western" ones, with the caveat that LGD is, instead, referred to as "noninvasive low-grade neoplasia" and HGD is, instead, referred to as "noninvasive high-grade neoplasia." This latter group emphasizes that Japanese pathologists may consider a certain lesion malignant based on cytology alone (ie, carcinoma in situ), and it also recognizes that certain cases may be suspicious for carcinoma, but the diagnosis cannot be established with certainty (Schlemper et al. 2000). A side-by-side comparison of the diagnostic terminology used in the traditional Western and Revised Vienna systems is presented in *table 5*.

Table 5: Comparison of Western and revised Vienna classification schemes for dysplasia in BE

Western	Vienna
Negative for dysplasia	Negative for neoplasia
Indefinite for dysplasia	Indefinite for neoplasia
LGD	Mucosal low-grade neoplasia (low-grade adenoma/dysplasia)
HGD	Mucosal high-grade neoplasia: high-grade adenoma/dysplasia noninvasive carcinoma (carcinoma in situ) suspicious for invasive carcinoma
Intramucosal carcinoma	Intramucosal carcinoma
Frankly invasive carcinoma	Submucosal invasion of neoplasia

1.5.2. Pathology of precursor lesions

Non dysplastic BE reveals a normal "baseline" level of regenerative changes that are considered "NEG." It shows, in general, an absence of atypical cytologic or architectural features characteristic of dysplasia. Regenerating epithelial cells normally demonstrate a progressive increase in mucin content (with a reduction in nuclear/cytoplasmic ratio), from the bases of the glands to the mucosal surface. This phenomenon is referred to as "surface maturation" and is a fundamental feature of regenerating epithelium. Metaplastic epithelium may also demonstrate slight baseline architectural distortion, such as occasional branching and budding of crypts, atrophy, irregularity, and mitotic activity (Reid et al. 1988) and (Srivastava et al. 2007a). The latter is usually limited to the basal "regenerative" zone of the crypts and not the surface epithelium as commonly seen in dysplasia. Unfortunately, in cases with active inflammation (ie, neutrophils, erosion, or ulceration), regenerating epithelium may appear quite "atypical" and, in some cases, difficult to distinguish from dysplasia. In these situations, the term "indefinite for dysplasia" is used. In diagnostically challenging cases, clues to an accurate "benign" diagnosis include restriction of the area of epithelial atypia to mucosa with the most severe active inflammation and ulceration, and a gradual diminution of epithelial atypia with the

surrounding non inflamed mucosa, but these are relatively subjective features and, thus, are not always useful. “Indefinite for dysplasia” does not represent a discrete biologic entity. Instead, it represents a “provisional” diagnosis for biopsies in which the pathologist is uncertain whether or not dysplasia (generally low-grade) is present. This uncertainty is usually due to the effects of active inflammation, erosion, or ulceration on the cytologic and, in some cases, architectural features, which can simulate those seen in dysplasia. Occasionally, this diagnosis may also be assigned to biopsies in which technical artifacts (eg, thick or overstained sections, lack of surface epithelium) preclude accurate assessment of dysplasia. However, there is wide variation in the frequency of use of this category in clinical practice, which is often related to the pathologist’s individual experience with dysplasia specimens in BE (Kaye et al. 2009). For cases in which a diagnosis of IND is rendered, it is recommended that both the clinician and pathologist have a clear understanding of the reason for uncertainty and plan further management accordingly. Repeat biopsy is typically recommended within a 3-6 month period after aggressive anti reflux treatment, to reassess the atypical area after the inflammation has subsided. For cases that are considered IND due to technical artifact, immediate rebiopsy may be indicated (Odze 2006). There is recent evidence to suggest that dysplasia arises from stem cells in the bases of the crypts and progresses, with time, to involve the full length of the crypts. Morphologically, dysplasia is defined as “unequivocal neoplastic epithelium confined to the basement membrane” (Riddell et al. 1983). In reality, the cytology of LGD in BE frequently mirrors that of conventional colonic adenomas, which is referred to as “adenomatous” dysplasia (Odze 2006). In these cases, nuclei are enlarged, elongated, hyperchromatic, and stratified, although they are generally confined to the basal half of the cell cytoplasm. The

cytoplasm is typically mucin-depleted and shows an increased nuclear/cytoplasmic ratio; goblet cells are generally inconspicuous. These changes involve the crypts and surface epithelium (ie, lack of surface maturation). Glands may demonstrate slight crowding and show other mild architectural abnormalities, such as atrophy, dilatation, and branching.

In contrast, by convention, HGD exhibits a greater degree of cytologic and/or architectural aberration. Characteristic architectural changes include increased budding, branching, and crowding, villiform surface configuration, and the presence of intraluminal bridges or papillae. Cytologic features of HGD include marked nuclear pleomorphism (ie, variation in nuclear size and shape), loss of polarity (ie, loss of normal nuclear orientation, in which the long axis of the nucleus is perpendicular to the basement membrane and basally oriented), and full-thickness nuclear stratification. Mitotic figures, especially atypical ones, are often present and may involve the surface epithelium. Unfortunately, distinction of LGD from HGD is fraught with variability due to several reasons. (1) The extent of HGD needed to upgrade a biopsy from LGD has never been determined. (2) Dysplasia develops along a linear scale and not as discrete stepwise increments. (3) Some types of dysplasia (eg, "non-adenomatous/foveolar") do not have typical features and may be difficult to differentiate from nondysplastic epithelium (Rucker-Schmidt et al. 2009). Representative images of LGD and HGD are presented in *figure 8*.

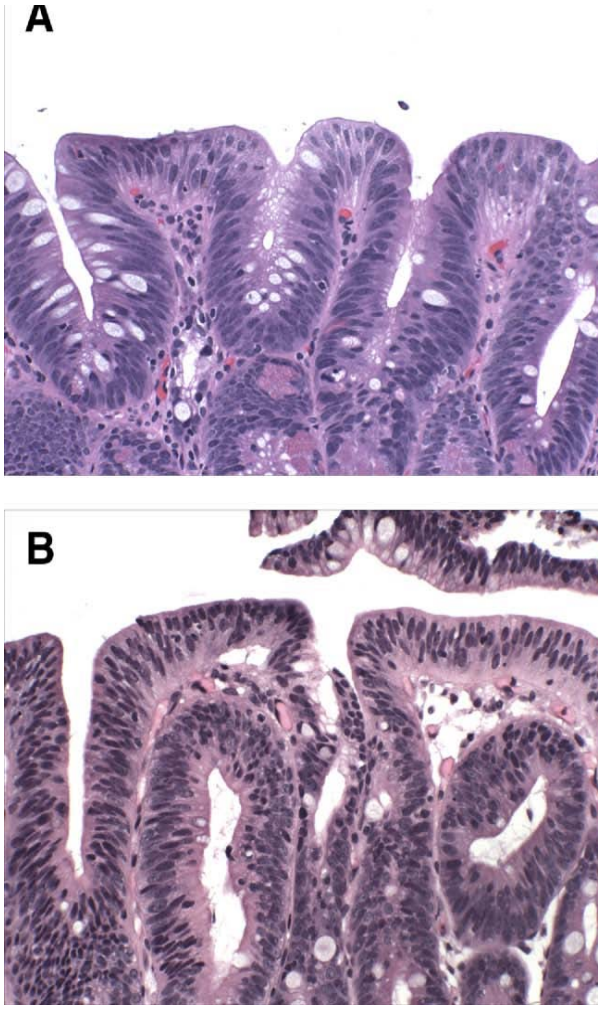


Figure 8 (A) LGD in BE. The epithelium is composed of enlarged, hyperchromatic, and pseudostratified nuclei, but the nuclei are limited, for the most part, to the basal half of the cell cytoplasm. There is no evidence of surface maturation. Overall, the architecture is preserved. (B) HGD in BE. In contrast to LGD, this epithelium shows a greater degree of cytologic and architectural atypia. The nuclei are larger, show more significant loss of polarity, increased mitoses at all levels of the crypts, and a more compact back-to-back crypt pattern.

Intramucosal adenocarcinoma is defined as a neoplasm that has breached the basement membrane and infiltrates the lamina propria or MM but has not invaded the submucosa. The category includes tumors within the lamina propria and those having invaded into, but not through, the MM. In the esophagus, this lesion is associated with a small risk of regional lymph node metastasis and, as such, is staged as T1a (Westerterp et al. 2005) and (Liu et al. 2005). IMC is diagnosed when single or small clusters of malignant cells infiltrate the lamina propria. Carcinomas that invade into the submucosa are considered submucosal invasive carcinoma. In these cases, the risk of lymph node metastases increases dramatically with depth of invasion (Westerterp et al. 2005)**.**

1.5.3. Interobserver variability

There is significant interobserver variation in the assessment of dysplasia in BE (Reid et al. 1988), (Montgomery et al. 2001), (Kaye et al. 2009), (Alikhan et al. 1999), (Kerkhof et al. 2007) and (Downs-Kelly et al. 2008).This is principally related to the following. (1) Reactive changes, particularly in the setting of active inflammation, overlap with those seen in dysplasia. (2) Dysplastic changes develop along a morphologic continuum, such that the distinction of LGD from HGD is, in reality, artificial. (3) There are no objective criteria to distinguish HGD from IMC (Ormsby et al. 2002). (4) Endoscopic biopsies virtually never sample the submucosa; thus, it is difficult to determine depth of invasion in these samples. (5) Although community-based pathologists evaluate most BE specimens, their experience with BE neoplasia is highly variable. Reid and coworkers performed the first observer variation study of dysplasia in BE in 1988 (Reid et al. 1988). After development of consensus criteria, 8 gastrointestinal (GI) pathologists blindly reviewed 70 slides. They achieved 86% agreement in distinguishing HGD or IMC from all other diagnoses. However, agreement for other diagnostic categories varied from poor to average. Subsequently, Montgomery and coworkers confirmed that interobserver reproducibility is greatest at the extreme ends of the morphologic spectrum of dysplasia and carcinoma (Montgomery et al. 2001). Ormsby et al. reported that on evaluating resection specimens and after implementation of uniform histological criteria, even experienced gastrointestinal pathologists frequently disagree on a diagnosis of high grade dysplasia versus intramucosal adenocarcinoma. Treatment strategies based on the histological distinction of high grade dysplasia from intramucosal adenocarcinoma using limited biopsy specimens should be re-evaluated (Ormsby et al.

2002). Downs-Kelly and coworkers reported only moderate agreement for a diagnosis of HGD among GI pathologists (Downs-Kelly et al. 2008). Finally, Alikhan and coworkers evaluated agreement among community pathologists to diagnose and grade BE biopsies (Alikhan et al. 1999). Five slides, including 2 cases of non goblet columnar mucosa and 1 each of NEG, LGD, and HGD, were evaluated by 20 community pathologists. For the 2 cases of non goblet columnar epithelium, 58% of the pathologists misinterpreted this finding as "BE," despite the absence of goblet cells. All 20 pathologists recognized that a BE NEG biopsy represented "BE," but agreement for a diagnosis of dysplasia was extremely poor: NEG (35%), IND (10%), "moderate" dysplasia (15%), LGD (35%), HGD (0%), carcinoma (5%).

1.5.4. Natural history of Barrett's-associated dysplasia

Estimates of cancer occurrence in patients with dysplasia vary widely, from 3% at 5 years for patients with LGD to 59% at 5 years for patients with HGD (Reid et al. 2000) and (Sharma et al. 2006a). The wide variation in reported rates of progression is attributable to many factors, such as differences in patient populations studied, studies that combine prevalent and incident dysplasia in the analyses, variability in the frequency of surveillance and biopsy protocols, and pathologists' diagnostic variability, among others. There is controversy regarding the biologic potential and natural history of LGD. A recent study by Sharma and coworkers, in fact, suggested that most patients with LGD actually show "regression," which implies that follow-up biopsies after a diagnosis of LGD was made revealed no evidence of dysplasia (Sharma et al. 2006a). In that study, 618 patients were followed for a combined total of 2546 patient years, with a mean follow-up of 4.1 years, and only 12 patients developed cancer. The incidence of LGD was 4.3% per year,

and of the 156 patients with this diagnosis, 66% revealed no evidence of dysplasia upon follow-up, 21% showed persistent LGD, and 13% showed progression to HGD or cancer. There is evidence to suggest that the level of diagnostic agreement among pathologists correlates positively with outcome of LGD. For instance, Skacel and coworkers reported progression rates to cancer in 8% of patients with LGD diagnosed by a single pathologist (Skacel et al. 2002). However, LGD cases diagnosed and agreed on by 2 study pathologists showed a progression rate of 41%, and those agreed on by all 3 study pathologists showed a progression rate of 80%. Similar results were found by Kaye and coworkers (Kaye et al. 2009). In that study, the 10-year progression rate to esophagectomy or carcinoma-related death was significantly higher when several pathologists agreed on a diagnosis of LGD. In a study by Srivastava and coworkers, 14 of 31 patients (45%) with a maximum index diagnosis of LGD confirmed, and agreed on by 3 GI pathologists, progressed to adenocarcinoma in the follow-up interval (Srivastava et al. 2007b). These findings suggest that a consensus diagnosis by at least 2 pathologists improves risk assessment of patients with BE and emphasizes the importance of the American College of Gastroenterology recommendations that all dysplasia diagnoses be confirmed by an expert GI pathologist prior to institution of patient management (Wang et al. 2008). The natural history of patients with HGD is better understood. For instance, the risk of cancer in patients with a biopsy diagnosis of HGD ranges from 16% to 59% within 5 years (Reid et al. 2000) and (Schnell et al. 2001). In 1 study of 1099 BE patients by Schnell and coworkers, 16% of patients with HGD developed cancer during follow-up surveillance (Schnell et al. 2001). In another study by Reid and coworkers, the 5-year risk of cancer in patients with prevalent HGD was 59% compared with only 31% for patients with incident HGD (Reid et al. 2000). Thus, due to

the high rates of progression, patients with HGD are considered candidates for immediate and definitive therapy.

1.5.5. Predictors of progression

Because morphologic evaluation of dysplasia is fraught with a high degree of interobserver variability, many investigators have sought alternative, more objective methods to assess risk of cancer progression in BE. These include the relevance of the gross (endoscopic) appearance of the dysplastic lesions, the extent of dysplasia, and a variety of immunohistochemical and molecular markers. Some studies have even shown an association between the presence of a hiatus hernia and the length of the BE segment as risk factors for progression to cancer (Weston et al. 2004), (Iftikhar et al. 1992), (Menke-Pluymers et al. 1993), (Weston et al. 1997) and (Rudolph et al. 2000).

Endoscopically, dysplasia in association with nodules, ulcers, or strictures has been shown to be associated with an increased risk of synchronous, or metachronous, adenocarcinoma. In a study by Buttar and coworkers, 60% of patients with dysplastic nodules developed adenocarcinoma compared with only 23% of patients without nodules at endoscopy (Buttar et al. 2001b). In another study by Montgomery and coworkers, dysplasia associated with frank ulceration increased the chance of detecting adenocarcinoma at the time of esophageal resection (Montgomery et al. 2002). Although never systematically studied, the presence of strictures increases a clinician's suspicion for adenocarcinoma.

Finally, some dysplastic lesions in BE may grow as exophytic, well-circumscribed, sessile or stalked polypoid lesions that, historically, have been mistermed "adenomas" of the esophagus. Thurberg and coworkers

studied these lesions, finding that adenoma-like polypoid dysplastic lesions in BE showed a high association with HGD and adenocarcinoma within the polyp and adjacent flat mucosa (Thurberg et al. 1999). Thus, in this circumstance, endoscopic polypectomy is inadequate treatment.

Cancer in BE develops within a field of clonally aberrant cells that expand to involve wide areas of mucosa (Wong et al. 2001), (Maley et al. 2004) and (Maley et al. 2006). Therefore, it is not surprising that several studies have shown a strong correlation between the extent of dysplasia and the risk adenocarcinoma. In a long-term, prospective follow-up case-control study of 77 BE patients, 44 of whom eventually developed adenocarcinoma, the extent of dysplasia was strongly associated with development of adenocarcinoma (Srivastava et al. 2007b). Interestingly, in that study, extent of LGD had more prognostic impact than extent of HGD. Two other studies that evaluated extent of dysplasia in individual biopsy specimens showed contrasting results, but, overall, suggested that the finding of diffuse HGD, characterized by dysplasia in more than 1 biopsy at different levels of the esophagus, or involving >5 crypts in 1 biopsy sample, was associated with subsequent adenocarcinoma, or adenocarcinoma at the time of resection (Buttar et al. 2001b) and (Dar et al. 2003).

At present, there are no clinical guidelines offered with regard to evaluation of extent of dysplasia for the purpose of stratifying patients into low- and high-risk groups. Non-endoscopy-, non morphology-based biomarkers are a subject of ongoing intense research. Unfortunately, although many potential immunohistochemical and molecular biomarkers have been studied, few have been validated as markers of cancer progression in phase 3 or phase 4 prospective trials. Immunostaining for

p53 has been widely studied, but the results have been variable (Younes et al. 1993) (Gimenez et al. 1998) (Skacel et al. 2002) (Weston et al. 2004) (Lorinc et al. 2005). In general, the frequency of positive immunostaining for p53 has been shown to correlate with higher grades of dysplasia, and, in some cases, is associated with an increased risk of cancer. However, p53 may be detected in biopsies that are histologically NEG, and this marker's immunostain suffers from a high rate of both false positives and false negatives. DNA content abnormalities and genetic alterations in *p53* and *p16* represent the most promising biomarkers studied to date (Reid et al. 1992) (Reid et al. 1993) (Galipeau et al. 1999) (Reid et al. 2000) (Wong et al. 2001) (Rabinovitch et al. 2001) (Reid et al.2001) (Maley et al. 2004). Reid and coworkers have shown that patients with diploid baseline biopsies showed a significantly lower rate of cancer progression compared with patients with either aneuploidy or an increased 4N fraction (tetraploidy) (Reid et al. 2000). Some studies show that a combination of biomarkers, such as DNA content and LOH of *p53* and *p16*, are more sensitive and specific indicators of progression then of any of these markers alone. In a recent study by Wang and coworkers in 2009, promoter methylation of both the *p16* and *APC* genes was associated with a significantly higher rate of progression to HGD or cancer compared with BE patients without either of these abnormalities (odds ratio 15.0) (Wang et al. 2009). Nevertheless, at present, morphologic identification of dysplasia remains the gold standard method of assessing risk of cancer progression in patients with BE.

1.6. Management Guidelines for Barrett Esophagus

Because of the malignant potential of biopsy-proven BE, several guidelines have been established regarding clinical and surgical

management (*table* 6) (Sharma et al. 2004) (Hirota et al. 2006) (Wang and Sampliner 2008). When BE without dysplasia (ie, metaplasia) is found on biopsy, conservative management is advocated, with the cornerstones of therapy being symptom control (as with acid suppression therapy and lifestyle modification) and periodic endoscopic surveillance to rule out progression of disease. The current surveillance guidelines recommend 2 follow-up endoscopies with biopsy within 1 year of the diagnosis of BE and follow-up every 3 to 5 years thereafter. Surveillance endoscopy is also the mainstay of management for BE with LGD, with repeat endoscopy initially recommended by the American College of Gastroenterology (ACG) and American Society for Gastrointestinal Endoscopy (ASGE) guidelines at 6 months to exclude the presence of HGD missed on prior biopsy. Annual follow-up endoscopies with biopsy are recommended thereafter as long as dysplasia persists. If regression is noted, surveillance every 3 to 5 years is recommended as with nondysplastic BE. The American Gastroenterological association (AGA) guidelines are more conservative, as noted in table 6, with insufficient literature at present to support one guideline over another (Hudson et al. 2011).

Unfortunately, there is greater discrepancy regarding the management of BE with HGD, the predecessor lesion to EAC. Historically, the gold standard in patients with HGD has been surgical esophagectomy when possible. Such a recommendation has been based largely on the concern that when HGD is present, possible areas of occult adenocarcinoma may be missed on endoscopic biopsies because of sampling error or observer variability. There is also concern that these areas largely contribute to the high rates of progression from HGD to invasive cancer over time. An often-quoted number among surgical literature is the notable histologic presence of occult cancer in 40% of esophagectomy specimens obtained

from patients with HGD (Tschanz 2005). However, recent literature suggests that the presence of clinically significant cancer (ie, cancer invading the submucosa that instills high rates of nodal metastasis and prompt surgical attention) may be lower than previously thought. In a 2009 study, the rate of submucosal invasion of occult adenocarcinoma in esophagectomy specimens of patients with BE and HGD was 6.7% (Wang et al. 2009). Likewise, a systemic review of 14 studies from which many of the current guidelines for surgery are derived noted that when dysplasia that only involved the lamina propria (and not the submucosa) was excluded, the prevalence was reduced from 38% to 12.7% (Konda et al. 2008). This becomes important as intramucosal (T1a) adenocarcinoma has been shown to respond to endoscopic resection and ablation and may not absolutely require a surgical resection.

Given the potential reduction in the prevalence of occult, clinically significant submucosal cancer (T1b) by 80% of that previously reported, greater interest has emerged regarding endoscopic alternatives to surgical management of BE with HGD or IMC. Although a lack of randomized controlled trials comparing esophagectomy and endoscopic modalities limits full appreciation to date, (table 6) demonstrates that the latter options are considered viable alternatives to the management of BE with HGD and early noninvasive adenocarcinoma (T1a) when surgery unwanted or difficult. However, despite promising observational studies relating to EMR and endoscopic ablative therapy, the gold standard presently remains surgical esophagectomy, and should be portrayed as such to patients in discussion regarding options for the management of Barrett's neoplasia (Hudson et al. 2011).

Table 6: Management Guidelines for Barrett's Esophagus:

Category	**ACG** (Wang &Sampliner 2008)	**ASGE** (Hirota et al. 2006)	**AGA** (Sharma et al. 2004)
BE without dysplasia	2 endoscopies with biopsy within 1 year with follow-up endoscopy every 3 years	Follow-up annually until 2 consecutive examinations within 1 year confirming no dysplasia. Then proceed with surveillance every 3 years.	Follow-up endoscopy at 1 year. If no dysplasia, defer further surveillance to once every 5 years or until morbidity/mortality felt to limit further value of surveillance.
BE with LGD	Follow-up endoscopy in 6 months. If no dysplasia, then yearly follow-up EGDs until no dysplasia seen on 2 consecutive endoscopies.	Repeat EGD in 6 months with biopsy. If LGD confirmed, continue surveillance every 12 months as long as dysplasia persists.	Follow-up EGD with biopsy in 1 year. If LGD confirmed by 2 pathologists, reexamine annually (or every years if discrepancy in pathologist opinion).
BE with HGD	Repeat EGD with biopsy within 3 months. If mucosal irregularity, proceed to EMR. Continue endoscopic surveillance every 3 months or to further intervention (eg, adjuvant ablation or esophagectomy).	Proceed to surgical intervention if patient desires, or surveillance endoscopy if not. Surveillance EGD every 3 months for = 1 year, then subsequent lengthening per endoscopist preference EMR or ablative treatments to be considered for persistent HGD.	Proceed to surgical esophagectomy or endoscopic endoscopic therapy (EMR, ablation). Surveillance recommended every 3 months thereafter with intervention (eg, ablation, surgical resection) as needed.

1.7. Pathology related to endoscopic therapy

Given a lower concomitant risk of “invasive” cancer than previously thought, and given advances in endoscopic technology, patients with HGD are increasingly managed with a variety of complementary endoscopic techniques, including endoscopic mucosal resection (EMR), photodynamic therapy (PDT), and radiofrequency ablation (RFA). This approach has been clinically validated. In two retrospective cohort studies, Prasad and coworkers demonstrated equivalent overall survival in patients with HGD or IMC who were treated endoscopically versus those

who were managed only with esophagectomy (Prasad et al. 2007b) and (Prasad et al. 2009).

1.7.1. Ablation therapy

Thermal ablation techniques (such as argon plasma coagulation and radiofrequency ablation) are not primary treatment modalities for early Barrett's carcinoma, and neither is photodynamic therapy (licensed in the USA), because they make histological assessment of the tumor, and hence any risk assessment, impossible.

Endoscopic ablation techniques, such as laser, argon plasma coagulation, PDT, and RFA, have shown variable success rates at eliminating BE and/or associated neoplastic epithelium. For instance, a meta-analysis by Wani and coworkers showed remarkably lower incidence rates of LGD and HGD in ablation-treated BE patients compared with controls (Wani et al. 2009). Recently, a large prospective multi-institutional trial of RFA showed a marked decrease in incidence of neoplasia upon long-term outcome (Shaheen et al. 2009).

Because of ablation, patients often develop islands of re-epithelialized squamous mucosa, commonly referred to as “neosquamous epithelium” (NSE). NSE may also develop in patients treated with high-dose proton pump inhibitors (PPI), but without ablation (Biddlestone et al. 1998), (Hornick et al. 2005) and (Sharma et al. 1998). The prevalence of NSE following ablation is even higher, approaching 100% following PDT or RFA (Biddlestone et al. 1998) (Ban et al. 2004) (Barham et al. 1997) (Sharma et al. 2007). NSE may be apparent grossly or microscopically. Histologically, NSE appears similar to pre-ablated (“normal”) squamous epithelium, being composed of basal cells at the base of epithelium and

mature squamous cells at the surface. Fortunately, NSE has been shown to be void of the molecular aberrations characteristic of BE. For instance, in 1 study by Pouw and coworkers, 100% of pre-RFA BE patients showed abnormal Ki-67, p53, and FISH assays (for chromosome 1, 9, *p16*, and *p53*), but post-RFA NSE showed none of these abnormalities, in all (100%) cases (Pouw et al. 2009). In a study by Paulson and coworkers, post-PPI NSE and adjacent BE were micro dissected and evaluated from 20 BE patients by PCR for *p16* and *p53* abnormalities. In that study, 95% of NSE specimens showed both wild-type *p16* and *p53* despite the presence of mutations in 1 or both of these genes in adjacent non dysplastic BE (Paulson et al. 2006). These findings, as well as those from others, suggest strongly that NSE has no malignant potential and represents a successful outcome of ablation.

Although the pathogenesis of NSE is currently unknown, stem cells have been proposed to arise from squamous epithelium at the neo-squamocolumnar junction, Barrett's columnar epithelium, or even the esophageal glands or ducts, for which there is abundant morphologic evidence (Biddlestone et al. 1998) (Paulson et al. 2006) (Glickman et al. 2001) (Berenson et al. 1993) (Leedham et al. 2008). Recent studies also suggest that NSE may be derived from as yet unidentified mesenchymal cells or from bone marrow derived cells, but these theories have not been tested (Sarosi et al. 2008) (Thiery 2003). All of these tissues have also been proposed for the source of stem cells responsible for the development of BE as well.

The significance of NSE, from the clinical perspective, is that residual Barrett's epithelium and/or dysplasia may persist underneath NSE and thus remain invisible to the endoscopist's eye. Presumably, this may

allow “buried” BE to progress to carcinoma covertly, and, in fact, several of these cases have been reported (Van Laethem et al. 2000). The prevalence rate of buried Barrett’s or buried dysplasia is variable and dependent on the type of endoscopic therapy. For instance, frequencies range from 0% in RFA-treated patients to 51% in post-PDT treated patients (Sharma et al. 1998) (Ban et al. 2004) (Sharma et al. 2007) (Sharma et al. 2008) table 7 and table 8.

However, in most studies (table 9), the frequency of buried dysplasia is less than the frequency of buried Barrett’s epithelium. In the majority of cases, buried neoplasia was detected within 18 months of ablation. Therefore, it is not clear whether the neoplasms developed from non-neoplastic glands that were buried by the ablation, or from neoplastic glands that either was already subsquamous before ablation or that were buried by the ablation procedure (Gray et al. 2011).

Endoscopic ablation can bury metaplastic glands with neoplastic potential but, even without ablation, buried metaplasia often is found in areas where Barrett’s epithelium abuts squamous epithelium. However, available reports do not provide crucial information on the adequacy of biopsy specimens and, therefore, the frequency and importance of buried metaplasia after endoscopic ablation remain unclear (Gray et al. 2011).

Table 7: Frequency of buried metaplasia following radiofrequency ablation for Barrett's esophagus

Report first author	Degree of neoplasia	Number of patients	Number of biopsies	Mandated surveillance biopsy protocol	Biopsy depth	Duration of follow-up	Percentage of patients found to have buried metaplasia
Herrero *et al.* *2011*	EC, HGD, LGD	24	1,272	Yes	NR	Median 29 months	12.5 % (at NSQ junction)
van Vilsteren *et al. 2011*	HGD, EC	22	NR	Yes	NR	Median 24 months	0 %
Fleischer *et al.* 2010	No dysplasia	50	1,473	Yes	85 % LP or deeper	5 Years	0 %
Lyday *et al.* 2010	No dysplasia, IND,LGD, HGD	338 in < 1 year cohort 137 in > 1 year cohort	NR	No	NR	Median 9 months Median 20 months	0 % in both cohorts
Pouw *et al.* 2010	HGD or EC	24	1,201	Yes	NR	Median 22 months	0 %
Eldaif *et al.* 2009	No dysplasia, LGD	27	NR	No	NR	8 Weeks	0 %
Pouw *et al.* 2009	HGD or EC	16	385	Yes	37 – 51 % LP or deeper	Median 26 months	0 %
Shaheen *et al.* 2009	LGD, HGD	84	9,517	Yes	NR	12 Months	5.1 %
Sharma *et al.* 2009	LGD, HGD	62	> 2,500	Yes	NR	Median 24 months	0 %
Vassiliou *et al.* 2010	All grades including no dysplasia	15	NR	Yes	NR	Median 20.3 months	0 %
Ganz *et al.* 2008	HGD	142	NR	No	NR	12 Months	0 %
Gondrie *et al.* 2008a	HGD, IMC	12	363	Yes	NR	Median 14 months	0 %
Gondrie *et al.* 2008b	HGD, IMC	11	473	Yes	NR	Median 19 months	0 %
Hernandez *et al.* 2008	No dysplasia, LGD, HGD	10	247	Yes	NR	3–38 Months	10 %
Pouw *et al.* 2008	HGD, EC	44	44 1,475	Yes	NR	Median 21 months	2.7 %
Sharma *et al.* 2008	No dysplasia, LGD	10	886	Yes	NR	2 Years	0 %
Roorda *et al.* 2007	LGD, HGD	13	NR	Yes	NR	Mean 12 months	0 %
Sharma *et al.* 2007	No dysplasia	100	4,306	Yes	NR	12 Months	0 %

EC, early cancer; HGD, high-grade dysplasia; IMC, intramucosal cancer; IND, indefinite for dysplasia; LGD, low-grade dysplasia; LP, lamina propria. Note: A number of reports share the same authors, and we cannot exclude the possibility that some patients were included in more than one series.

Table 8: Reported frequency of buried metaplasia after photodynamic therapy for Barrett's esophagus

Report first author	Degree of Neoplasia	Number of patients	Number of biopsies	Mandated surveillance biopsy protocol	Biopsy depth	Duration of follow-up	Percent found to have buried metaplasia
Bronner *et al.* 2009	HGD	138 treated 70 controls	23,498 treated 10,160 controls	Yes	NR	5 Years	30 % in treatment group 33 % in control group
Hornick *et al.* 2008	HGD, EC, IA	12	NR	No	NR	3–38 Months	25 %
Schembre *et al.* 2008	HGD, IMC	42 (PDTarm)	NR	Yes	NR	Median 20 months	11.9 %
Mino-Kenudson *et al.* 2007	HGD, superficial carcinoma, or IA	52	1302	Yes	NR	Mean 29.3 months	17.3 % with buried metaplasia 25 % with buried neoplasia (HGD, LGD, or AC)
Fourolis and Thorpe 2006	HGD, IMC, or T2 AdenoCa	31	NR	Surveillance mandated, biopsy protocol NR	NR	Median 14 months	20 % (5 of 25 with HGD or IMC before treatment)
Ragunath *et al.* 2005	HGD, LGD	13	NR	Yes	NR	12 Months	7.7 %
Peters *et al.* 2005	HGD, EC	28	NR	Yes	NR	Median 19 months	25 %
Ban *et al.* 2004	HGD, EC	33	478	Yes	NR	Mean 16.7 months	51.5 %
Etienne *et al.* 2004	HGD, IMC	12	NR	Yes	NR	Mean 34 months	0 %
Hage *et al.* 2004	No dysplasia, LGD	24	NR	Yes	NR	Median 12 months	4.2 %
Kelty *et al.* 2004a	No dysplasia	25	NR	Yes	NR	4 Weeks	24 %
Kelty *et al.* 2004b	No dysplasia	34	NR	Yes	NR	Median 12 months	24 %
Wolfsen *et al.* 2004	HGD and IMC	102	NR	Yes	NR	Median 1.6 years	4 %
Overholt *et al.* 2003	LGD, HGD, EC	102	NR	Yes	NR	Mean 50.65 months	3 %
Wolfsen *et al.* 2002	HGD EC	48	NR	Yes	NR	Median 18.5 months	2.1 %
Buttar *et al.* 2001	IMC, IA	17	NR	Yes	NR	Mean 13 months	0 %
Ackroyd *et al.* 2000a	LGD	18	NR	Yes	NR	2 Years	0 %
Ackroyd *et al.* 2000b	LGD	40	NR	Surveillance mandated, biopsy protocol NR	NR	12 Months	0 %
Overholt *et al.* 1999	HGD or EC	100	NR	Yes	NR	Mean 19 months	5 %
Gossner *et al.* 1998	HGD or EC	32	NR	Yes	NR	NR	6.25 %
Barr *et al.* 1996	HGD	5	NR	" Multiple biopsies " Q 2 months	NR	26–44 Months	40 %
Overholt and Panjehpour 1996	EC, HGD, LGD, No dysplasia	45	NR	Yes	NR	6 – 66 Months	4.4 %

EC, early cancer; HGD, high-grade dysplasia; IA, invasive adenocarcinoma; IMC, intramucosal carcinoma; LGD, low-grade dysplasia; NR, not reported. Note: A number of reports share the same authors, and we cannot exclude the possibility that some patients were included in more than one series.

Table 9: Published cases of neoplasia in buried metaplasia

Report first author	Patient age (years)	Endoscopic therapy	Baseline degree of neoplasia	Location of buried neoplasia	Degree of buried neoplasia	Time after ablation	Outcome
Shand *et al.* *2001*	67	APC	LGD	NR	Metastases (liver)	4 months	Unresectable tumor
Van Laethem *et al.* 2000	68	APC	No dysplasia	Squamo-columnar junction	TisN0	18 months	EMR, no recurrence at 12 months
Mino-Kenudson *et al.* 2007	NR	PDT	HGD / superficial adenocarcinoma	Variable	IA (2) IMC (4) HGD (6) LGD (1)	2–25 months	8 of 13 responded to another session of endoscopic therapy
Overholt *et al.* 2003	NR	PDT	HGD	Near NSQ-columnar junction in 2 of 3 patients	NR	6 months, 46 months, 52 months	1 retreated, no recurrence at 3 years 1 retreated, no recurrence at 1 year 1brachytherapy, local recurrence at 6 months
Ban *et al.* 2004	NR	PDT	HGD / superfi cial adenocarcinoma	NR	IA (3) IMC (2) HGD (7)	NR	NR
Ragunath *et al.* 2005	55	PDT	LGD	NR	T1N0	4 months	Esophagectomy
Overholt *et al.* 1999	NR	PDT	HGD	Center of treated area	IMC	6 months	Repeat PDT; no recurrence at 24 months
Bonavina *et al.* 1999	NR	Laser ablation	No dysplasia	NR	T1N0	6 months	Esophagectomy
Wolfsen *et al.* 2002	NR	PDT	T1N0M0 adenocarcinoma	At site of prior lesion	T1N0	NR	Esophagectomy

APC, argon plasma coagulation; EMR, endoscopic mucosal resection; HGD, high-grade dysplasia; IA, invasive adenocarcinoma; IMC, intramucosal carcinoma; LGD, low-grade dysplasia; NR, not reported; NSQ, neosquamous; PDT, photodynamic therapy.

Histologically, buried Barrett's may be composed of both mucous or intestinalized glands and are histologically similar to both pre- and postablation nonburied Barrett's epithelium. Unfortunately, buried dysplasia is difficult to interpret because the features that pathologist use to evaluate grade of dysplasia, such as involvement of the full length of the crypt and the presence or absence of surface maturation, cannot be evaluated easily in buried glands covered by NSE. A photomicrograph of buried BE is shown in figure 9.

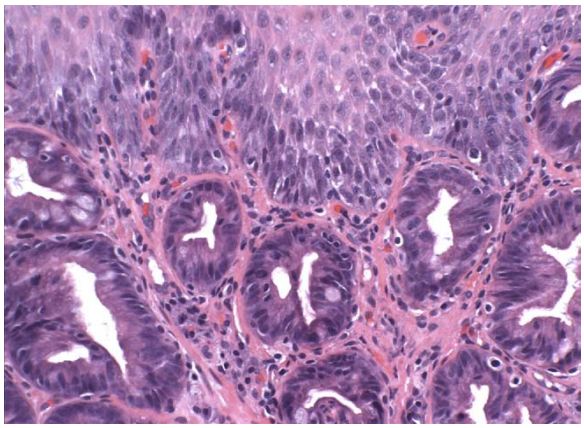


Figure 9: Residual buried Barrett's post-photodynamic ablation. Underlying the squamous epithelium are crypts with goblet cells. Evaluation of dysplasia in buried Barrett's is difficult because of the lack of the ability of the pathologist to evaluate the full length of the crypt, and surface epithelium, which connects the buried crypts to the luminal surface.

The biologic potential of buried BE is the subject of ongoing investigation. In one study by Hornick and coworkers, post-PPI-treated buried BE showed a significantly lower Ki-67 crypt proliferation rate compared with nonburied BE (Hornick et al. 2005). However, the frequency of p53 and cyclin D1 over expression was similar. Interestingly, in that study, buried BE not exposed to the lumen showed significantly lower crypt proliferation capability than buried BE that showed open connection to the lumen of the esophagus. In another study in 2008 by the same research group, Hornick and coworkers showed that post-PDT buried BE also showed significantly lower crypt proliferation rates and significantly fewer DNA alterations, determined by high fidelity image cytometry on micro dissected crypt cells (Hornick et al. 2008). These findings suggest that buried BE post-PDT may have less biologic potential than nonburied BE, but this requires further prospective study. Residual nonburied BE post ablation has been more extensively studied and, unfortunately, has revealed persistent proliferative and molecular abnormalities, suggesting it retains malignant potential. Most preliminary data also suggest that residual nonburied and buried dysplasia, post

ablation, continues to be at risk for malignant progression as well (Krishnadath et al. 2000). For dysplasia, success rates for evaluation are variable (40%-100%) and procedure-dependent. Downgrading of dysplasia is common, but molecular abnormalities have been shown to persist and progress to adenocarcinoma.

1.7.2. Endoscopic mucosal resection

If endoscopy shows a T1 tumor (table 10), the guidelines of the German Association for Digestive and Metabolic Diseases (DGVS) recommend performing a diagnostic endoscopic resection (evidence grade B). In this, the tumor-bearing mucosa including the submucosa beneath it is completely resected.

T stage esophageal cancer

Primary tumor (T)*	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	High-grade dysplasia•
T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
T1a	Tumor invades lamina propria or muscularis mucosae
T1b	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor invades adventitia
T4	Tumor invades adjacent structures
T4a	Resectable tumor invading pleura, pericardium, or diaphragm
T4b	Unresectable tumor invading other adjacent structures, such as aorta, trachea, etc.

Note: cTNM is the clinical classification, pTNM is the pathologic classification.
* At least maximal dimension of the tumor must be recorded and multiple tumors require the T(m) suffix.
• High-grade dysplasia includes all noninvasive neoplastic epithelia that was formerly called carcinoma in situ, a diagnosis that is no longer used for columnar mucosae anywhere in the gastrointestinal tract.

If histological analysis of the resected sample shows a mucosal carcinoma without risk factors (no lymph node invasion, no venous infiltration by the tumor, R0 resection [basal margin]), the patient is considered treated with curative intent and requires regular endoscopic follow-up. In patients in whom submucosal infiltration and/or lymphatic or venous infiltration are shown, esophageal resection is recommended, since the expected rate of lymph node metastasization is 20% to 40%.

Endoscopic resection in the esophagus is carried out using the “suck and cut” technique figure 10. This method has limitations particularly in regard to en-bloc resection of large-surface neoplasias, and therefore multiple pieces are resected side by side, called “piecemeal”. The most commonly used techniques for piecemeal resection are the cap and the ligation techniques, both have been shown to yield equivalent results in two randomized trials (figures 11, 12, 13) (May et al. 2003) and (Peters et al. 2007).

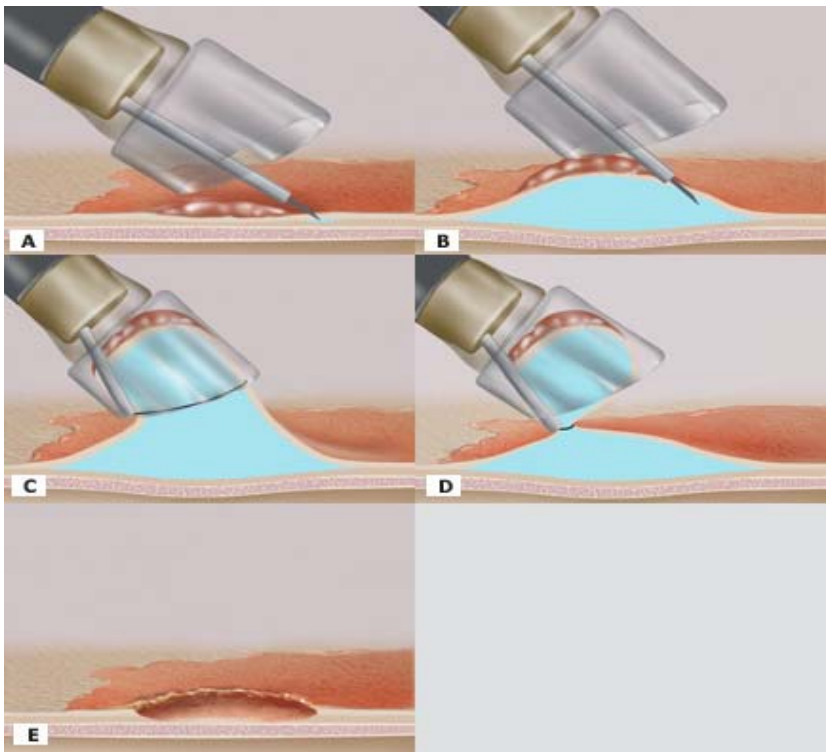


Figure 10 EMR Barretts: A) and B) A transparent cap is attached to the distal tip of the endoscope and the target lesion is lifted by injection of a fluid, usually diluted epinephrine (1:100.000), into the submucosal layer, using a standard sclerotherapy needle. C) and D) After removal of the needle, a crescent shaped snare is positioned into a distal ridge within the cap. The lesion is sucked into the cap thus creating a pseudo-polyp that is immediately captured by forcefully closing the pre-positioned EMR-snare. E) The lesion is removed using electrocoagulation.

The new endoscopic enbloc resection technique of "endoscopic submucosal dissection (ESD)" has been developed in Japan mainly for gastric cancer (Oda et al. 2006) and (Gotoda et al. 2006).This technique,

however, makes high demands in terms of time, personnel, and equipment, is associated with more procedural risks, and has not yet become established in Europe (Oka et al. 2006) and (Imagawa et al. 2006). Furthermore, in Barrett esophagus with often poorly demarcated and multiple neoplastic lesions of various stages, ESD is probably less well suited.

In contrast to ablative therapy, EMR is a procedure designed to remove mucosa and superficial submucosal tissue, which allows for more accurate histologic evaluation and grading of dysplasia, and determination of location and depth of invasion by adenocarcinoma when present. As a result, EMR has emerged as a valuable adjunctive diagnostic method and is often used in conjunction with (some form of) ablative therapy. The role of EMR as a therapeutic procedure to remove HGD and/or IMC is controversial and requires further study. Initially, EMR was used to treat focal areas of HGD or IMC arising in the setting of BE. However, one major drawback of focal resection was the high rate of synchronous and recurrent lesions noted by various groups, which ranged from 14% to 47%. This rate tended to increase with longer durations of observation (Buttar et al. 2001a), (Ell C et al. 2000), (May et al. 2002a), (May et al. 2002b), (Nijhawan and Wang 2000), (Pech et al. 2003), (Larghi et al. 2005) and (Mino-Kenudson et al. 2005).

In response to this limitation, certain investigators are performing complete Barrett's eradication EMR (CBE-EMR) with the aim of removing all Barrett's epithelium to thereby reduce the risk of synchronous and metachronous lesions (Peters et al. 2006), (Seewald et al. 2003), (Giovannini et al. 2004) and (Larghi et al. 2007).

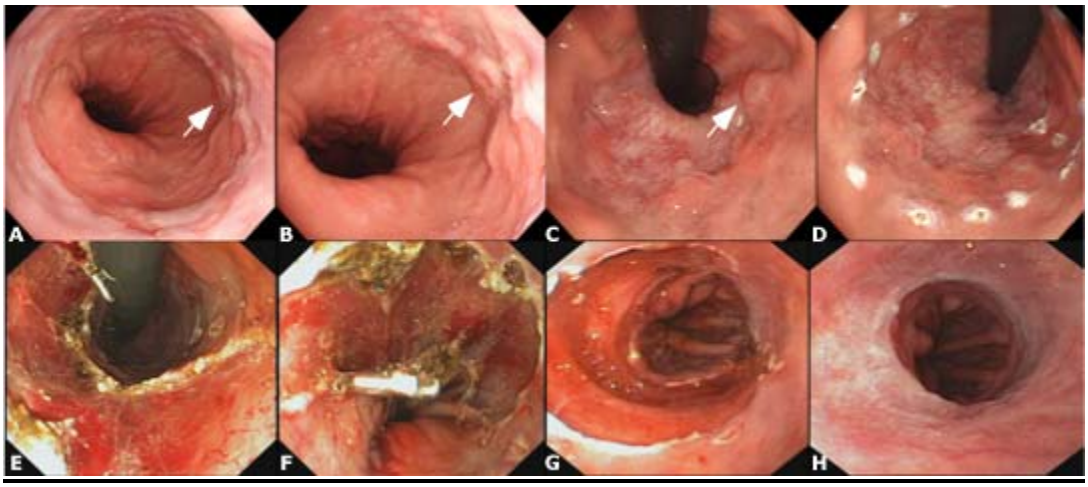


Figure 11:Radical stepwise EMR Endosc A 2 cm long segment of Barrett's esophagus is visible with a raised area subsequently found to correspond to a mucosal cancer (panels A to C). Electrocoagulation markers were placed to delineate the lesion (panel D) after which it was resected with the cap technique (panel E). A bleeding site was treated by placement of a hemoclip (panel F). Histologic evaluation of the resection specimen showed a mucosal cancer. The patient subsequently underwent stepwise complete resection of the residual Barrett's epithelium (panel G) resulting in complete neosquamous reepithelialization (panel H).

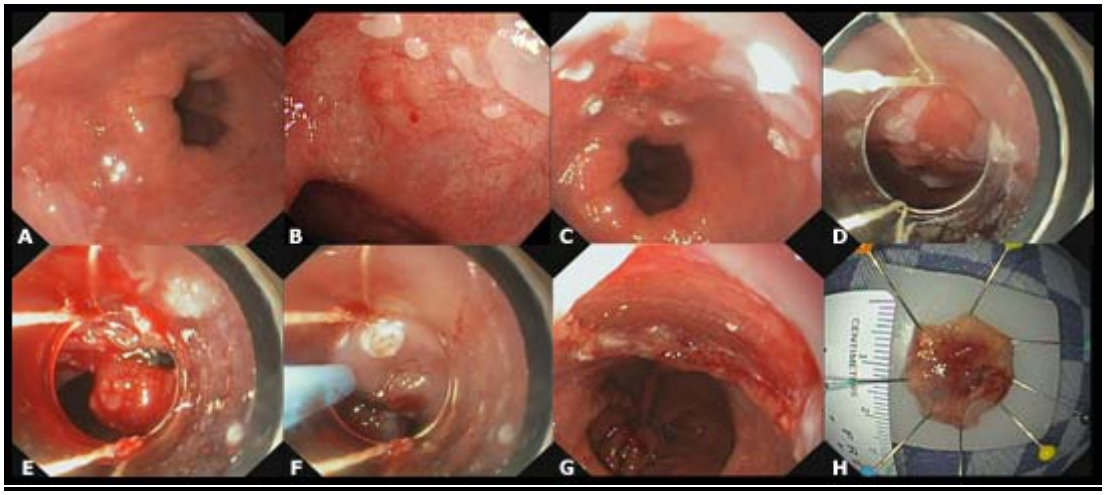


Figure 12: EMR HGIN Barrett's Endosc A) Overview of a 3 cm long segment of Barrett's esophagus. B) There is a subtle lesion in the center of the endoscopic image. C) View after placement of electrocoagulation markers to delineate the lesion. D) A transparent cap with six rubber bands at its outside (identical to a variceal six-shooter) has been attached to the distal tip of the endoscope (Wilson-Cook Medical, Limerick, Ireland). The two wires to which the rubber bands are connected pass through the working channel of the endoscope and are connected to the handle that allows release of the bands. E) The area of interest is suctioned into the cap, without prior submucosal injection, followed by the release of one of the black rubber bands. F) The modified handle of the MBM device allows passage of a hexagonal polypectomy snare alongside the wires of the ligator. The snare is closed either above or below the rubber band followed by cutting using electrocoagulation. G) The wound after resection. H) The specimen is retrieved for histological assessment. Histology showed HGIN. The lateral and deep resection margins were free of dysplasia.

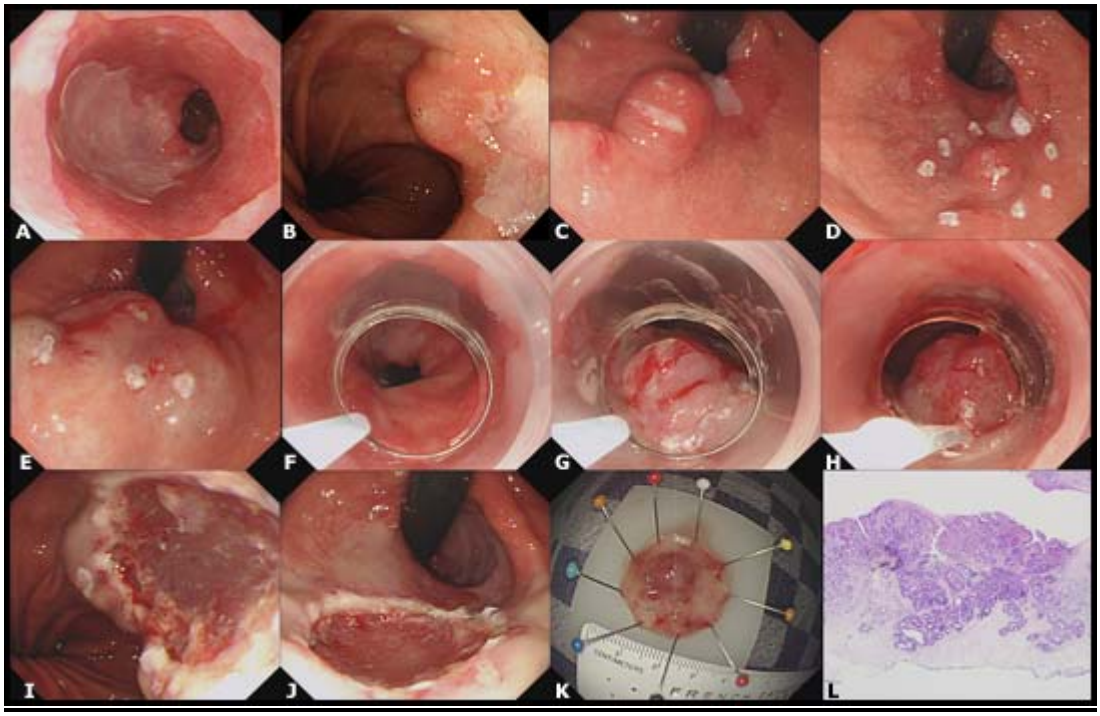


Figure 13: EMR early esoph cancer Endosc A) A 4-cm long segment of Barrett's esophagus with a large island of squamous mucosa in its center. B) A detailed view of a lesion at the 3 o'clock position. C) Same lesion shown in the retroflexed position. D) The lesion has been delineated by placing coagulation markers at its outer surface. E) The lesion has been elevated by injection of diluted epinephrine solution through a standard sclerotherapy needle. F) A transparent cap has been attached to the distal tip of the endoscope and a crescent shaped snare is positioned into the distal ridge of the cap. G) Using the coagulation markers for orientation, the lesion is identified and subsequently sucked into the cap. H) After closure of the snare, the resulting pseudo-polyp, including the lesion, is pushed outside the cap and removed using electrocoagulation. I) The created EMR wound shown in the antegrade position; there is still some mucosal swelling due the submucosal lifting. J) EMR wound shown in the retroflexed position, no markers can be identified indicating an endoscopically complete resection; note the mucosal whitening due to the vasoconstrictive effect of the epinephrine solution used for submucosal lifting. K) The EMR specimen is subsequently removed from the stomach using retrieval net and pinned down on paraffin to prevent shrinking and curling. L) Microscopic view of the specimen showing a well differentiated cancer infiltrating into the deeper layers of the muscularis mucosae, there is no infiltration into the submucosa.

Despite successful reports of endoscopic therapies for BE with HGD or IMC, the literature described significant rates of strictures although the relationship between size and circumferential extent of EMR for BE and stricturing is unknown (Katada et al. 2003). Alternatively, EMR of focal lesions could be combined with thermal ablation. Initially, using argon plasma coagulation and photodynamic therapy, buried BE glands were found under the squamous postprocedural reepithelialization (figure 14) (Van Laethem et al. 1998) (Haggitt 1994) (Biddlestone et al. 1998) (Barham et al. 1997) (Mino-Kenudson et al. 2007a). In one study, even two cases developed cancer on follow-up 12 and 18 months respectively

after a beamer therapy with successful initial reversal (Kahaleh et al. 2002). Considerable controversy and debate continues about whether these ‘‘buried glands’’ represent a neoablative phenomenon or existed before endoscopic therapy. The newest form of ablative therapy, namely radiofrequency ablation (table 7) seems to have a very low rate of buried glands, so it could become the new standard of combination therapy with EMR for complete BE ablation, although results have to withstand the test of time and spread into clinical practice.

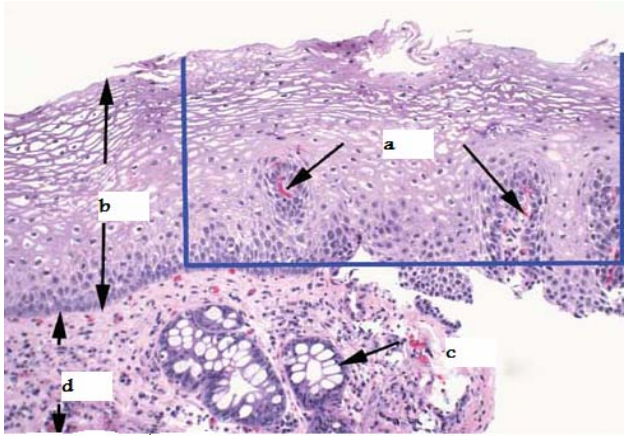


Figure 14:.Photomicrograph of a pinch biopsy specimen of neosquamous mucosa showing buried metaplastic glands in the subepithelial lamina propria (H & E, magnification × 40). a) Papillae (with lamina propria), b) Squamous Epithelium, c) Buried metaplastic glands, d) Lamina propria.The blue lines delimit a hypothetical biopsy specimen that contains only the epithelial layer. Note that this hypothetical specimen includes papillae, and so could be categorized as containing “lamina propria.” This categorization belies the true depth of the hypothetical biopsy specimen, because such a specimen is not informative for buried metaplasia.

Currently, EMR is recommended for BE patients with LGD, HGD, or IMC in which the size of the tumor is <20 mm and other risk factors, such as lymphovascular invasion, venous invasion, or poorly differentiated morphology, are not present (Ell et al. 2007). Intermediate indications include BE patients with IMC >20 mm in size, or those with multifocal carcinoma, but without other high-risk pathologic factors or invasion beyond the superficial submucosa (“SM1”). The role of the pathologist evaluating EMR specimens is to determine an accurate dysplasia grade and depth of invasion of carcinoma, when present. Also

important are determination of the presence or absence of lymphovascular invasion and the status of the lateral and deep tissue margins, the latter of which have been shown to represent an important negative prognostic parameter (Mino-Kenudson et al. 2005), (Peters et al. 2006), (Seewald et al. 2003), (Giovannini et al. 2004), (Larghi et al. 2007), (Mino-Kenudson et al. 2007b), (Haggitt et al. 1994), (Biddlestone et al. 1998), (Barham et al. 1997), (Ell et al. 2007), (Prasad et al. 2007a) and (Vieth et al. 2004). For instance, when adenocarcinoma is noted at a surgical margin, the risk of recurrence approaches 50%. In one large series of gastric EMR specimens, the rate of tumor recurrence in patients with a positive circumferential margin was 33%-50% (Lauwers et al. 2004). Evaluation of depth of invasion in EMR specimens is important because the rate of lymph node metastasis has been shown to correlate with depth of invasion. For instance, the rate of lymph node metastasis of carcinomas that invade the lamina propria ranges from 0% to 10%, but increases to 8%-10% and 20%-36% for tumors that invade the deep lamina propria (located between the newly developed superficial MM and the original deep MM) and submucosa, respectively (figure 15) (Holscher et al. 1997), (Abraham et al. 2007), (van Sandick et al. 2000), (Westerterp et al. 2005) and (Liu et al. 2005). Holscher et al. found no patient with mucosal adenocarcinoma had lymph node metastases, whereas 16% with submucosal infiltration showed lymph node involvement. van Sandick et al. reported no lymph node metastasis in case of intramucosal cancer whereas in submucosal cancer, lymph node metastases were present in 30% of cases (van Sandick et al. 2000). Westerterp and coworkers recorded that only one of the 79 T1m1-3/sm1 tumors (1%) showed lymph node metastases as compared with 18 out of 41 T1sm2-3 tumors (44%) (Westerterp et al. 2005). Liu et al. classified the T1 tumors into four groups based on the depth of invasion: T1a, invading into lamina propria;

T1b, into muscularis mucosae; T1c, into superficial submucosa; and T1d, into deep submucosa. The depth of tumor invasion was significantly associated with the presence of lymph node metastasis (36% in T1d, 8% in T1c, 12% in T1b, and 0% in T1a; $P < 0.001$) (Liu et al. 2005). In a study by Abraham et al. the rate of lymph node metastases was 10% with invasion into duplicated MM which suggests that these tumors can behave aggressively despite their technically intramucosal location (Abraham et al. 2007).

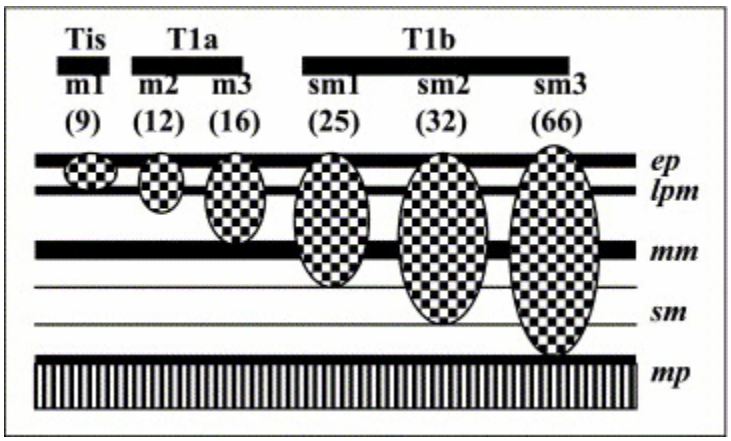


Figure 15: Proportion of patients without recurrence of Barrett's esophageal cancer according to depth of invasion after esophagectomy (Westerterp et al. 2005)

EMR specimens increase the pathologist's ability to determine more precisely the depth of invasion, while also improving diagnostic accuracy of dysplasia, relative to mucosal biopsies. For instance, in a study by Mino-Kenudson and coworkers in 2005, 37% of cases of BE with dysplasia showed a change of dysplasia grade in pre-EMR biopsies compared with EMR specimens (Mino-Kenudson et al. 2005). In that study, biopsies under-reported the grade of neoplasia in 21% of cases and over-reported the grade in 16%. In a study by Larghi and coworkers, 24% of BE patients with a diagnosis of HGD in biopsy specimens showed an increase in grade to IMC, and 40% of patients with IMC had their stage

increased to submucosal invasive carcinoma by evaluation of EMR specimens (Larghi et al. 2005).

Mino-Kenudson et al. also showed greatly improved diagnostic agreement between pathologists when evaluating dysplasia in EMR specimens compared with biopsies (Mino-Kenudson et al. 2007b). The use of EMR as a treatment alternative to esophagectomy for dysplasia/superficial carcinoma is controversial and currently under investigation. For instance, in 1 European series of 100 patients with low-risk adenocarcinoma (well to moderately differentiated, ≤ 20 mm in diameter, and tumor confined to mucosa without angiolymphatic invasion or ulceration), a recurrence rate of only 11%, and a mortality rate of 0%, was found after a mean follow-up of 3 years when patients were treated by EMR alone (figure 16) (Ell et al. 2007).

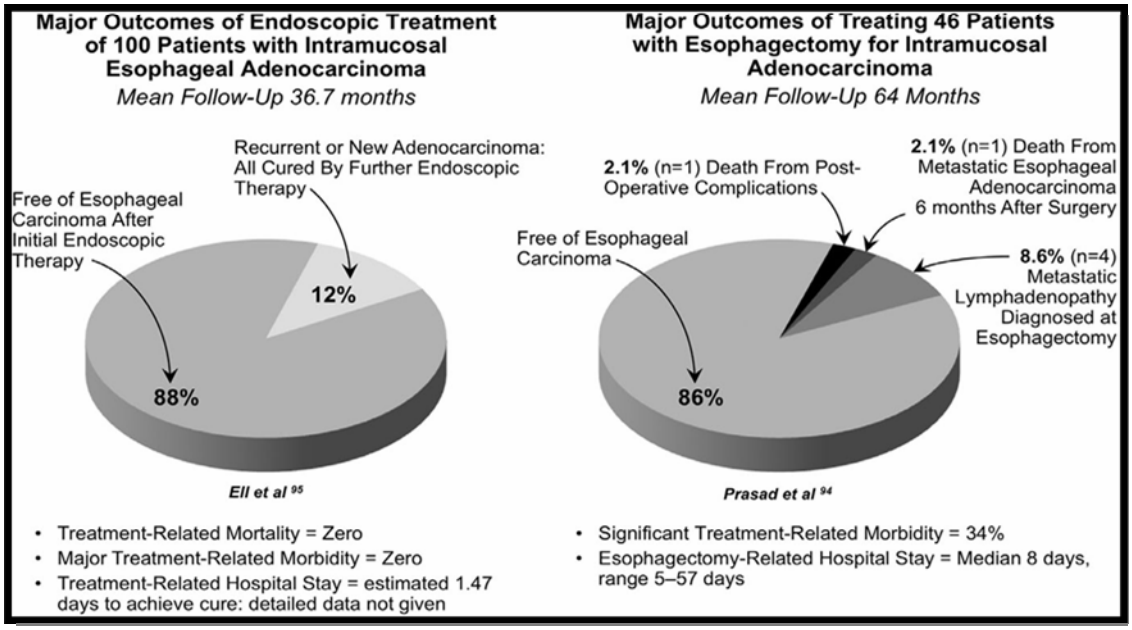


Figure 16: Major outcomes related to expert esophagectomy or expert endoscopic therapy of esophageal adenocarcinoma, assessed as intramucosal at the time of primary therapy, from two published series (Prasad et al. 2009) and (Ell et al. 2007). Comparisons between the non-randomized studies available are hazardous, because of many potential confounders. The follow-up duration of the endoscopic therapy study depicted is almost half that of the surgical data. Patient age (62.1, range 31–86 years) in the endoscopic therapy group was lower than in the surgical group (67.7 ± 1.4 SEM years). The 34% rate of significant treatment-related morbidity after esophagectomy was due to anastomotic leakage or stricturing, cardiopulmonary complications and feeding jejunostomy leakage. The reported outcomes of endoscopic therapy are so superior to those of esophagectomy, that they overcome concerns above possible confounding factors.

Unfortunately, the prevalence rate of positive margins in EMR specimens continues to be problematic and reaches up to 83% in some series (Mino-Kenudson et al. 2005), (Peters et al. 2006), (Seewald et al. 2003), (Giovannini et al. 2004), (Larghi et al. 2007), (Mino-Kenudson et al. 2007b), (Haggitt et al. 1994), (Biddlestone et al. 1998), (Barham et al. 1997), (Ell et al. 2007), (Prasad et al. 2007) and (Vieth et al. 2004). Other studies suggest that not only the presence, but also the location, of the positive margin (whether it is a lateral/circumferential margin or a deep margin) has significance with regard to recurrent neoplasia. For instance, in Mino-Kenudson and colleagues' study on the clinicopathologic features of 27 EMR specimens, whereas 56% of cases with a positive lateral and negative deep margin persisted/recurred, 86% with a positive deep margin persisted/recurred (Mino-Kenudson et al. 2005). The method of processing EMR specimens is important in order for pathologists to accurately evaluate the morphologic properties of the tumor. EMR specimens should be mounted cleanly on a wax block, stretched gently, and then fixed for at least 12 hours to produce well-oriented tissue samples. Proper inking of the lateral and deep margins should be performed, and tissue sections should be obtained at not more than 2-mm intervals, to optimize evaluation of the grade and stage of neoplasms, and to determine the presence or absence of vascular invasion. EMR specimens removed piecemeal are difficult to evaluate pathologically, resulting in decreased diagnostic accuracy and a higher rate of reported positive margins (Odze and Lauwers 2008). A photomicrograph of IMC in an EMR specimen is shown in figure 17. A summary of key diagnostic and therapeutic features of EMR is presented in table 11.

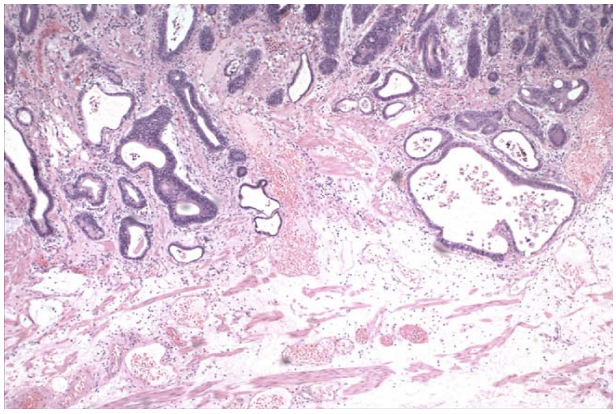


Figure 17: Superficial well-differentiated adenocarcinoma arising in BE with HGD. Most Barrett's patients show fragmentation and duplication of the MM. In this case, adenocarcinoma is infiltrating into, and through, the new superficially developed MM and into the neo-lamina propria. The original (deep) MM present as a thin fragmented layer at the bottom of the image. This tumor is still considered "intramucosal" adenocarcinoma because it has not yet penetrated the original (deep) MM.

Table 11: Diagnostic and therapeutic advantages and disadvantages of EMR

	Advantages	**Disadvantages**
Diagnostic features	a. Larger portion of tissue with visualization of stromal landmarks. b. Increased diagnostic accuracy and reproducibility.	a. Difficult to evaluate margins due to cautery Artifact.
Therapeutic features	a. Combined with mucosal ablative techniques may provide equivalent survival in select patients with HGD or IMC. b. Minimal morbidity. c. No mortality.	a. Frequent positive margins necessitating repeat EMR or esophagectomy. b. Limited role in patients with extensive disease. c. Not therapeutically useful for submucosal invasive carcinomas.

1.8. Esophagectomy:

1.8.1. High risk characteristics of Barrett's neoplasia

Endoscopic therapy has advantages in that it is organ preserving and does not have the same morbidity and mortality as surgery. However, not all cases are successful or appropriate for endoscopic therapy. Indications for esophagectomy include lymph node metastasis and failure of endoscopic therapy. Risk factors for submucosal invasion, lymph node metastasis, and failure of endoscopic treatment need to be incorporated into the management strategy of a patient with HGD and IMC. These risk factors

are evident in endoscopic appearance, pathological characteristics, and results of endoscopic treatment (Table 12) (Konda and Ferguson 2010).

Table12 High-risk characteristics associated with submucosal invasion, lymph node metastasis, or unsuccessful endoscopic therapy

Endoscopic characteristics
Long-segment Barrett's esophagus
Visible lesions with high risk endoscopic characteristics
Polypoid mass
Excavated lesions or ulcers
Evidence of lymph node involvement by EUS + FNA
Pathological characteristics
Multifocal HGD
Evidence of submucosal invasion
Deeper two thirds of the submucosa carries high risk of lymph node metastasis
Moderately or poorly differentiated tumor
Evidence of lymphatic channel invasion
Evidence of vascular invasion
Evidence of neural invasion
Treatment characteristics
Failure of ablation of remainder for Barrett's epithelium
Piecemeal endoscopic resection (as opposed to *en bloc* resection)
Longer time to achieve eradication

1.8.2. Advantages of esophagectomy

The strategy of performing esophagectomy for HGD or IMC not only cures the index condition, but also addresses occult cancer and prevents cancer death (Rice 2006). Although endoscopic treatment is an appropriate and cost-effective (Pohl et al. 2009) approach for the treatment of many patients with HGD and IMC, patients who are appropriate surgical candidates can benefit from esophagectomy. The surgical specimen enables accurate staging of disease to diagnose areas of occult cancer, and confirms treatment adequacy with negative margins and lymph nodes. Conventional approaches are transhiatal esophagectomy and transthoracic esophagectomy. Minimally invasive esophagectomy (MIE) techniques are growing in popularity because of their perceived benefits of reduced pain, lower incidence of postoperative

complications, and faster recovery. These MIE techniques include video-assisted thoracoscopy surgery with laparotomy or laparoscopy, laparoscopy with a right thoracotomy, or laparoscopic transhiatal resections. These procedures have been studied in mostly retrospective studies and conclusions are limited in terms of direct comparisons to open surgery due to lack of prospective randomized trials (Decker et al. 2009) (Biere et al. 2009). The issue of the morbidity and mortality of esophagectomy is the major concern for either open esophagectomy or MIE. Adverse outcomes include pulmonary complications, hemorrhage, anastomotic leakage, infections, and recurrent nerve palsy. Although one study based on a national Veteran's Affairs database has reported morbidity of almost 50% and mortality of 10% (Bailey et al. 2003), the expertise and volume of the center, the experience of the surgeon, the patient risk factors, and the indications for esophagectomy should be taken into account (Birkmeyer et al. 2003), (Reavis et al. 2008) and (Fernando et al. 2009). In institutions with expertise and high volumes, the mortality rate is 2%-3% (Law et al. 2010). It is also important to note that esophagectomy specifically for HGD has a different risk profile than that of esophagectomy for cancer. Comorbid diseases, debilitation from cancer and/or neoadjuvant therapy, and issues with locally advanced disease are not as predominant in patients with HGD. A pooled mortality rate of 1% was calculated among six studies that involved esophagectomy for HGD (Fernandoet al. 2009). Quality of life indicators for patients underwent esophagectomy for HGD and IMC are equivalent to those of the general population (Moraca et al. 2006).

1.8.3 Indications for esophagectomy in Barrett's HGD and IMC

Strong indications for esophagectomy include lymph node metastasis and failure of endoscopic therapy. Invasion of tumor into the submucosa is

still considered a strong indication for esophagectomy, although invasion into the superficial third of the submucosa does not carry the same lymph node metastasis risk as the deeper two thirds, and potentially could be treated endoscopically (Update on the paris classification of superficial neoplastic lesions in the digestive tract 2005) (Manner et al. 2008). Factors to consider in the management strategy for HGD and IMC include characteristics that are associated with lymph node metastasis, submucosal invasion, and failure of endoscopic therapy, as listed in table 12, and may serve as milder indications for esophagectomy. Excavated lesions (Paris classification 0-III) are not typically considered to be amenable to endoscopic therapy due to high suspicion of submucosal invasion, whereas protruding lesions (0-I) and depressed lesions (0-IIc) are a concern for submucosal invasion and should be approached with caution endoscopically (table 13).

Table 13: Relative risk of submucosal invasion associated with endoscopic appearance of lesions

Endoscopic appearance	**Paris classification**	**Relative risk of submucosal invasion**
Polypoid	0-Ip	Higher
Sessile	0-Is	Higher
Slightly raised	0-Ia	Low
Flat	0-Ib	Low
Slightly depressed	0-Ic	Higher
Excavated	0-III	Very high

These circumstances allow for endoscopic resection to serve as a diagnostic tool to stage the lesion accurately to determine if the lesion is amenable to endoscopic therapy. Multifocal high grade is a milder indication for esophagectomy than previously considered, due to the evolving options of ablative therapy. These risk factors, as listed in table 12, need to be weighed with patient characteristics, patient preferences,

available surgical expertise, available endoscopic expertise, and surgical approach options to decide if esophagectomy or endoscopic therapy is appropriate for each case.

2. Patients and Methods

2.1 Patients

The patients eligible for inclusion in this study were all those who underwent EMR for suspected or confirmed neoplastic BE with HGD or IMC at our institution between January 2000 and December 2010 and who had not undergone any prior endoscopic ablative therapy for this condition. Before CBE-EMR, all the patients underwent upper endoscopy with staging EUS. Only patients with findings consistent with disease confined to the mucosa (uT1m) after a detailed white-light endoscopic examination and without evidence of suspicious lymphadenopathy by EUS were considered for CBE-EMR. Upper endoscopy was performed by using either a standard or a high-definition upper endoscope when available (GIF-Q160, GI-H180; Olympus Hamburg), as well as narrow-band imaging (when available). Spraying of the esophageal mucosa with acetic acid was performed to further enhance mucosal-surface patterns. Before performing CBE-EMR, a full discussion with the patient and family (when permitted) was conducted concerning the risks and benefits of all possible managements, including esophagectomy, surveillance biopsies, and endoscopic ablative and resection therapies. Patient demographics and endoscopic findings (Barrett length, location and size of the neoplasia) were recorded.

2.2 Mucosectomy

The CBE-EMR was performed by the endoscopic cap suction snare method with a saline solution submucosal injection and/ or the endoscopic band ligation techniques were used. Mucosectomy was performed until all visible Barrett's epithelium was removed. Mucosectomy was extended to include the endoscopically determined

squamocolumnar junction and a small (approximately 1 cm) length of squamous esophagus proximally and 1 to 2 cm of the gastric cardia distally from the Barrett's epithelium. Immediately after resection, all mucosectomy specimens were retrieved from the patient and were preserved in formaldehyde.

2.3. Pathology

After fixation, all mucosectomy specimens were oriented by the pathologist with the submucosal side facing down. The deep margin of all EMR specimens was inked black and then the tissue was serially sectioned and totally embedded for histologic examination. Specimens had been routinely oriented before assessment so that the margin containing squamous epithelium was oriented at 12 o`clock. All resection specimens from this patient population which contained squamous epithelium (i.e. those from proximal resection margins) were retrospectively retrieved and reviewed blindly by 2 GI pathologists (A.H.M. and A.Q.) with expertise in BE in a double-blinded fashion. Each slide was reviewed for the presence or absence of BE with or without dysplasia or IMC located underneath the squamous epithelium. When possible, the length of the proximal extension of subsquamous BE was measured from specimens which contain full-thickness squamous epithelium at least at one side of the specimen. Patients for whom index mucosectomy pathology slides are unavailable will be excluded from the study.

2.4 Statistical analysis

Our study is the first large cohort of patients with endoscopically resected neoplastic BE. Our focus was to assess the extent and the prevalence of subsquamous BE and to compare it with a recent data published by

Chennat et al. on a smaller study group from USA center (Chennat et al. 2009b). Also, to estimate the presence of a possible significant differences of Length SQBE between non-neoplastic and neoplastic, in correlation with BE length.

3. Results

A total of 128 patients underwent CBE-EMR in the period between January 2000 and December 2010 at our Interdisciplinary endoscopy center in the University hospital Hamburg-Eppendorf. In 30 cases histopathology slides were unavailable (partially analyzed elsewhere) or incomplete, plus in one patient no specimens contained squamous epithelium founded; so these cases had been excluded. Pre-treated patients had been primarily excluded. Inclusion criteria was eligible only for 97 patients, the mean age was 65 years and the male: female ratio was 5,9:1. Data of included patients are shown in **Table 14**. The most severe histologic diagnoses included early (T1) cancers in 58 cases, HGIN in 15 cases and LGIN in 3 cases. No neoplasia was found in the remaining 21 cases, partially because it was the second EMR and the first one was not available in this patient (n=12) or the neoplastic histology could only be found in biopsies but not in the resection specimens.

Results of subsquamous Barrett epithelium (SQBE) are shown in **Table 15**. As can be seen, SQBE could be found in 95 out of 97 cases (**Figure 18 a,b,c**), with a considerable length of SQBE: Overall, almost a third had a SQBE extension of 5 mm or more, which was especially prevalent in neoplastic BE (50%). Looking at subsquamous extension of neoplastic tissue, tumor infiltration underneath the subsqamous epithelium spread 3 mm and more in 17.5% of cases (**Figure 19**).

442 specimens of the 97 study cases were analyzed and subsquamous BE was found in 42.5% of specimens or 97.9% of patients. Mean length of subsquamous BE extension was 3.52 mm (0.0-9.6 mm) with 27.8% of cases measuring 5 mm or more. In 56 cases (57.7%), subsquamous BE consisted entirely or partially of neoplasia (42 cancers, 12 HGIN, 2 LGIN) with a mean subsquamous tumor extension of 1.2 mm (range 0.0-5.2 mm). Forty-one of the total 97 patients had short-segment BE (≤ 3 cm

of Barrett's epithelium); with the mean length of the Barrett segment in the whole study group 4 cm (range 0.5-12 cm) and in 8 patients the Barrett length not recorded in the mucosectomy report propably those were referred with initial diagnostic findings from other clinics. Visible lesions were recorded and identifiable on white-light examination in 58 of the 97 total included patients with equal incidence proximally as well distally within the Barrett segment and in one patient with along 8 cm Barrett segment the neoplasia endoscopically occupied the whole length.

There are no statistically significant differences in the mean SQBE length between non-neoplastic vs neoplastic BE (3.65 vs. 3.48 mm) ($p = 0.686$, t-Test). The correlation coefficient between SQBE length and BE length is -0.244, which means that longer SQBE are associated with shorter BE ($p < 0.01$). For those patients with short BE (up to 3 cm) this correlation is no more statistically significant ($p = 0.272$), while for the group with long BE it is still ($p = 0.002$).

Table 14: Overview on patients (n=97) from whom specimens (n=442) were included in the analysis

Mean age (range)			65 (43-85)
Sex ratio m:f			83:14
Mean number of EMR pieces			4.6 (range 1-24)
Barrett length			
Mean/range (cm)			4 (range 0.5-12) *
Short Barrett (≤ 3 cm)			41
Long Barrett (> 3 cm)			48
Most severe histologic diagnosis			
T 1 carcinoma		58	
T1m			48 47 G1, 1 G2
T1sm			10
sm1		7	4 G1, 2 G2, 1 G3
sm2		3	all G2
HGIN			15
LGIN			3
No neoplasia		21	
Lesion location (n=58)**			
Proximal	23		
Middle	11		
Distal	23		
Whole length	1		

* Barrett length not reported in 8 cases.
** Only those cases in which a lesion was present and identifiable (not reported in 21, not present in 18).

Table 15: Overview on the results of subsquamous BE epithelium (SQBE)

General occurrence of SQBE		
% of all patients	95/97	97.9%
% of all specimens	188/442˚	42.5%
Histology of SQBE		
Non-neoplastic BE	40/95	42.1%
LGIN	2/95	2.1%
HGIN	12/95	12.6%
T 1 Carcinoma	41/95*	43.2%
Length of SQBE		
All cases (mean)	3.52 mm	range 0.0-9.6 mm
% of cases ≥ 5 mm	27/97	27.8%
Non-neoplastic BE (mean)	3.65 mm	range 0.0-7.3 mm
% of cases ≥ 5 mm	6/21	28.5%
Neoplastic BE (mean)	3.48 mm	range 0.0-9.6 mm
% of cases ≥ 3 mm	38/76*	50%
Correlation with BE length		
*All cases***		
Length of SQBE in BE ≤3cm	4.3 mm	range 0.2-9.6 mm
Length of SQBE in BE >3cm	2.7 mm	range 0.0-8.4 mm
Non-neoplastic BE		
Length of SQBE in BE ≤3cm	3.8 mm	range 0.3-6.8 mm
Length of SQBE in BE >3cm	2.7 mm	range 0.0-7.3 mm
Neoplastic BE		
Length of SQBE in BE ≤3cm	4.5 mm	range 0.2-9.6 mm
Length of SQBE in BE >3cm	2.7 mm	range 0.3-8.4 mm
Correlation of SQ neoplasia length with BE length		
Length of SQ neoplasia all cases	1.2 mm	range 0.0-5.2 mm
% of cases ≥ 3 mm	17/97	17.5%
BE length ≤3cm	1.5 mm	range 0.0-5.2 mm
BE length >3cm	0.97 mm	range 0.0-4.8 mm

Correlation with degree of neoplasia

No neoplasia (n=21)	3.7 mm	range 0.0-7.3 mm
HGIN (n=15)	3.3 mm	range 0.4-9.5 mm
LGIN (n=3)	2.8 mm	range 0.3-6.2 mm
Cancer (n=58)	3.4 mm	range 0.0-9.6 mm
T1 m (n=48)	3.3 mm	range 0.0-8.4 mm
T1sm (n=10)	4.0 mm	range 0.3-9.6 mm

* all cancer plus non-neoplastic BE, one patient has only SQ cancer 2.2mm excluded.
** BE length did not reported in 8 patients.
˚ only EMR specimens with squamous epithelium (188).

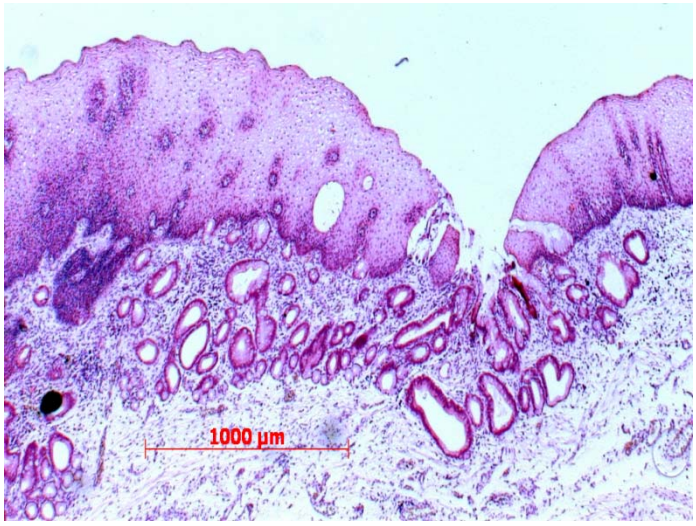


Figure (18a) Barrett epithelium reaches proximally underneath squamous epithelium of the distal esophagus (HE; 200x).

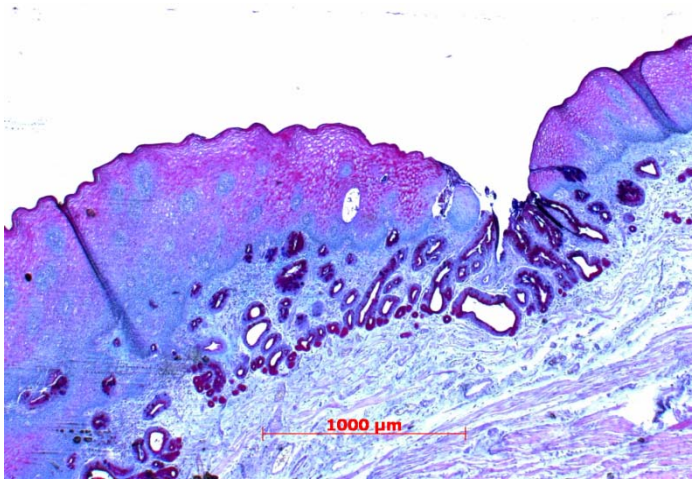


Figure (18b) Barrett epithelium underneth the squamous epithelium shows a positive alcian blue staining (200 x)

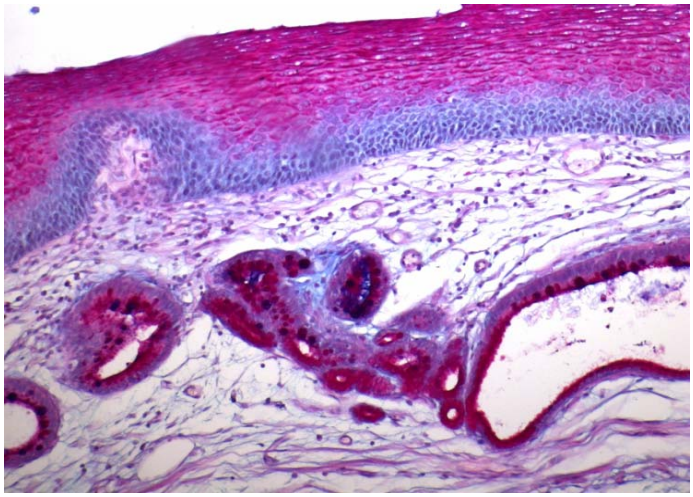


Figure (18c) Barrett epithelium underneth the squamous epithelium shows a positive alcian blue staining (200 x)

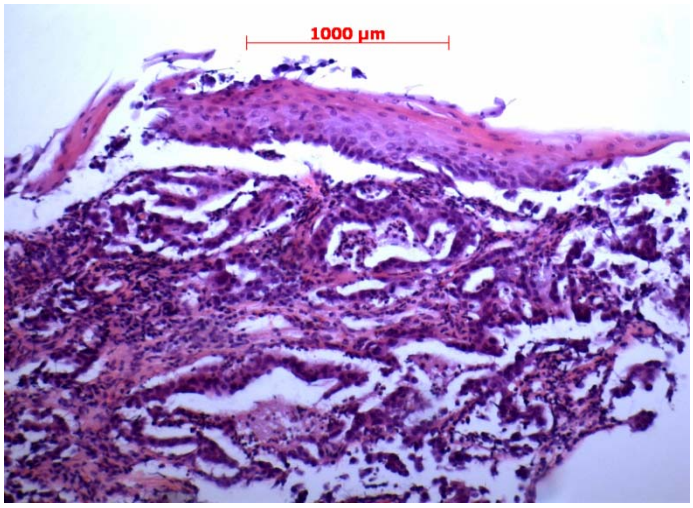


Figure 19. Barretts adenocarcinoma underneath squamous epithelium of the distal esophagus (HE; 100 x).

4. Discussion

4.1 Significance of subsquamous BE

Currently, minimally invasive endoscopic techniques for managing BE with HGD or IMC offer a realistic promising alternative to esophagectomy in expert centers.

Although the same clinical end point exists for endoscopic ablative therapies and for CBE-EMR, the latter has the added advantage of tissue acquisition, which allows for histopathologic examination of larger and deeper specimens. The results of our study suggest that Barrett's epithelium buried underneath squamous mucosa is not just a post ablation related phenomenon but rather an inherent issue in this population of patients with histologically advanced BE which supports the results by Chennat et al. (Chennat et al. 2009b). The presence of these buried glands may be explained by the variation between what is seen on endoscopic examination and what is truly present on histologic examination. The endoscopically determined squamocolumnar junction, although clearly demarcated in most patients on white-light examination, may not represent the actual histologic transition zone. Prior reports of BE with overlying squamous mucosa were noted in biopsy specimens of patients with BE who have solely been on medical therapy (ie, H2 blockers) without prior anti-reflux surgery. All specimens were taken with a biopsy forceps, and none had dysplasia noted in the buried BE.

A recent study revealed a significant prevalence of buried Barrett's epithelium with or without dysplasia under squamous mucosa (squamocolumnar junction) on initial mucosectomy specimens (Chennat et al. 2009b).

Regression of the endoscopically defined BE segment did not always equate with a lack of the presence of buried BE (Sampliner et al. 1988).

Another explanation of the buried BE phenomenon might be that chronic proton pump inhibitor usage decreases the acidity of reflux material and causes partial regression of BE, which allows for squamous overgrowth. In areas of prior mucosal acid or bile reflux related injury, or when prior surveillance biopsy specimens have been obtained, similar squamous re epithelialization might also have occurred. Macroscopic squamous islands identified on endoscopy have been systematically biopsied, which revealed the presence of underlying intestinal metaplasia without dysplasia, and pointed out the concern for a persistent hidden risk of malignant transformation. Thus, macroscopically identifiable squamous islands cannot reliably be used as a marker for regression of BE or definitive reduction in cancer risk (Sharma et al. 1998). Certain investigators believe that the buried BE epithelium has heightened protection against malignant transformation by acid or bile reflux because of the overlying squamous mucosa (Kelty and Ackroyd 2000) Buried glands have also been shown to have less-severe proliferative abnormalities, especially when no detectable connection to the esophageal lumen was found. This observed reduction in proliferation may be because of decreased exposure to transformative contents in the lumen or because of alteration in surface epithelial cells sloughing into the crypt lumen. Buried BE glands have also been noted to have fewer DNA content abnormalities, which suggests that they may have a lower neoplastic potential (Kelty and Ackroyd 2000) (Hornick et al. 2005) (Hornick et al. 2008). In contrast, several reports describe the development of subsquamous adenocarcinoma after treatment of advanced BE with either APC or PDT (Van Laethem et al. 2000) (Overholt et al. 2003).

4.2 Prevalence of subsquamous BE

Our observation of 97.9% (95/97 patients) overall prevalence rate of subsquamous BE presence on index mucosectomy presents a clinical conundrum related to the choice of non tissue acquiring endoscopic ablative therapy versus EMR. Barrett's epithelium was identified up to 9.6 mm proximal to the endoscopically apparent squamocolumnar junction. Both our prevalence rate and proximal subsquamous extension exceeds that assumed by Chennat et al. of a 28% (14/47 patients) and 5.6 mm consequentively (Chennat et al. 2009b). Therefore, strong consideration should be given to whether proximal margins must extend at least 1 cm proximal to the endoscopically determined squamocolumnar junction for all BE ablation therapies, including CBE-EMR. We reported also a higher point prevalence of 57.9% (55/95 patients) with neoplasia (2 LGIN, 12 HGIN, 41 Carcinoma) in subsquamous BE versus 21% (10/47patients) (HGD or IMC) by Chennat et al. (Chennat et al. 2009b). This difference also in comparative findings with the 2 prior mentioned respective studies from (Sampliner et al. 1988) and (Sharma et al. 1998) could be partially attributed to the use of EMR, with deeper tissue sections for more accurate staging. With the above-referenced articles highlighting concerns about the inaccuracy of using endoscopic markers for BE regression and/or cure, the technique of CBE-EMR holds added promise to provide more reliable histologic information that could guide therapy. Based on these findings, we modified our practice and ablation protocol accordingly.

The study was conducted in a retrospective, yet double blinded fashion. The two expert GI pathologists independently concurred with the qualitative and quantitative findings. However, both pathologists acknowledge that the measurement of an arbitrary linear distance of the buried Barrett's epithelium from the resection's squamous margin may

not realistically represent the true depth of subsquamous BE location. This is because EMR specimens are 3 dimensional and when specimen processing occurs for histopathologic review, the tissue is reformatted for a 2-dimensional view on the slide. During this process, the EMR specimens are not oriented in the tissue cassette during the serial sectioning process. Despite this potential inaccuracy in distance determination, the paramount issue is the finding of subsquamous Barrett's glands as much as 9.6 mm away from the histologic squamocolumnar junction, regardless of orientation.

5. Summary

Considerable debate continues around the clinical implications and the natural history of buried BE glands. Their presence on initial mucosectomy for BE with either HGD or IMC is an important finding that warrants further attention as endoscopic therapy becomes more mainstream in the management of this disease. Perhaps what lies beneath the surface makes a difference, and, based on this, surveillance biopsies and endoscopic ablative therapies for BE with HGD or IMC should extend to a minimal of 1 cm proximal to the endoscopically determined squamocolumnar junction.

6. ABSTRACT

Background: Endoscopic resection and/or ablation of Barrett esophagus (BE) is directed by the endoscopic visual impression of pinkish Barrett versus whitish esophageal mucosa; microscopically however, BE may extend underneath squamous epithelium.

Objective: To evaluate prevalence and extension of subsquamous BE in a large cohort of patients with endoscopically resected BE.

Design: Retrospective double-blinded review.

Setting: A tertiary-care academic referral center.

Methods: Resection specimens of 97 patients treated by endoscopic mucosal resection (EMR) for suspected or confirmed neoplastic BE (83 men, 14 women, mean age 65 years) were retrospective reanalyzed by two experienced GI histopathologist. Orientation of resected specimens pinned on cork was done prior to histopathological workup with squamous epithelium oriented proximally. Subsquamous extension was measured in all specimens which contained squamous epithelium. Endoscopic and other histopathologic data were also analyzed.

Results: 442 specimens of the 97 study cases were analyzed and subsquamous BE was found in 42.5% of specimens or 97.9% of patients. Mean length of subsquamous BE extension was 3.52 mm (0.0-9.6 mm) with 27.8% of cases measuring 5 mm or more. In 56 cases (57.7%), subsquamous BE consisted entirely or partially of neoplasia (42 cancers, 12 HGIN, 2 LGIN) with a mean subsquamous tumor extension of 1.2 mm mm (range 0.0-5.2 mm).

Conclusions: Subsquamous extension of BE including all forms of neoplasia is an almost regular event. A minimal safety margin of 1 cm has to be regularly kept in order to avoid BE and/or tumor recurrence

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10. Eidesstattliche Versicherung:

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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