

REVIEW

Barrett's oesophagus—a pathologist's view

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Barrett's oesophagus, a precancerous condition for oesophageal adenocarcinoma, detected on endoscopy and confirmed on histology, shows intestinal metaplasia of the lower oesophagus. The significance of microscopic foci of intestinal metaplasia at the gastro-oesophageal junction, corresponding either to so-called 'ultrashort' segment Barrett's oesophagus, or to carditis with intestinal metaplasia, is still a matter of debate. The surveillance of patients with Barrett's oesophagus is still based on systematic biopsy sampling of Barrett's mucosa on endoscopy, looking for dysplasia. Although well-established classifications of dysplasia are now used by most pathologists, there remain numerous

problems with this subjective marker (sampling, diagnostic reproducibility, natural history, etc). Therefore, many alternative biomarkers have been proposed, but only DNA aneuploidy, proliferation markers and p53 loss of heterozygosity/overexpression have been shown to be of some use at the present time. Some endoscopic improvements already allow a better selection of biopsies, and it may be that in future new technologies will allow 'virtual biopsies'. On the other hand, the role of pathologists now extends to the evaluation of new therapeutic modalities of early neoplastic lesions in Barrett's oesophagus, especially endoscopic mucosal resection.

Keywords: Barrett's oesophagus, dysplasia, intestinal metaplasia, oesophageal cancer

Abbreviations: BO, Barrett's oesophagus; CELLO, columnar epithelium-lined lower oesophagus; CK, cytokeratin; EMR, endoscopic mucosal resection; GOJ, gastro-oesophageal junction; GORD, gastro-oesophageal reflux disease; HGD, high-grade dysplasia; IM, intestinal metaplasia; LGD, low-grade dysplasia

Introduction

Barrett's oesophagus (BO) is defined as the replacement of the normal squamous epithelium of the lower oesophagus by metaplastic columnar epithelium.^{1–3} It is a consequence of prolonged gastro-oesophageal reflux disease (GORD). Also known as 'endobrachyo-oesophage' (in France) and as columnar epithelium-lined lower oesophagus (CELLO), it is increasing in incidence. It is a precancerous condition, as it predisposes to the development of oesophageal adenocarcinoma, a tumour with a rapidly increasing

incidence in most Western countries.⁴ BO is found in 1.6% of the general population⁵ and in 10% of those patients who undergo endoscopy for symptoms of GORD. Follow-up studies in surveillance programmes of patients with BO have demonstrated an incidence of adenocarcinoma ranging from one in 52 to one in 441 patient-years.

Pathologists play a central role in the diagnosis of BO. This diagnostic role has been a matter of perplexity for many pathologists, due to changes in the definition of BO in recent years, with the emergence of so-called short and even 'ultrashort' segment BO. After the diagnosis of BO is made, pathology is again at the centre of surveillance, as the only recognized marker of an increased risk of cancer is dysplasia, diagnosed on histological examination of endoscopic biopsy specimens.

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Along with these changes in diagnostic procedures, there have been major changes in therapeutic options, with the emergence of various types of non-surgical treatment of early neoplastic lesions, either ablative [mainly endoscopic mucosal resection (EMR)] or destructive.⁶ Again, pathologists play a central role in these new issues, especially when they have to report mucosectomy specimens.

It may be that in the near future new methods of surveillance will replace traditional endoscopy with biopsies. These new technologies already include endoscopic improvements (e.g. chromoendoscopy and narrow-band imaging) and in the future entirely new methods may allow 'virtual biopsies' (e.g. optical coherence tomography and Raman spectroscopy).⁷ These methods may induce major changes in the role of pathologists in BO.

Barrett's oesophagus: an endoscopic and histological definition and diagnosis

Glandular mucosa in the lower oesophagus presents as a red velvety mucosa over the gastro-oesophageal junction (GOJ). It can extend either circumferentially or as one or several tongues, and in some cases as a mixture of these two patterns (Figure 1). It was considered initially that this mucosa had to extend at least 30 mm over the GOJ to diagnose BO,¹ but this definition has changed, due to the recognition of short-segment BO measuring < 30 mm^{1,8,9} However, as it may be difficult to measure precisely a short-segment BO and to localize the metaplastic mucosa

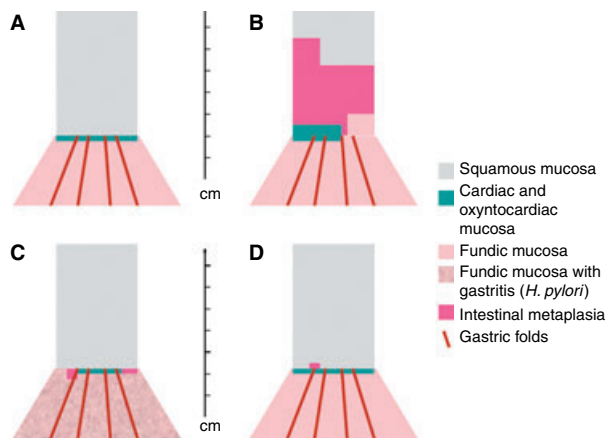


Figure 1. Schematic representation of normal gastro-oesophageal junction (A) and various types of intestinal metaplasia (B–D). B, Long-segment Barrett's oesophagus. C, Carditis with intestinal metaplasia of the cardiac mucosa. D, 'Ultrashort' segment Barrett's oesophagus.

and the GOJ, it is now recognized that the major diagnostic criteria of BO are histological.

It must be kept in mind that the current definition, needing histological confirmation of intestinal metaplasia (IM), is based on routine standard endoscopy with random biopsies. It may be that the improvements of endoscopic techniques will allow a form of 'endoscopic histology' and will therefore modify very much the current definitions of BO.¹⁰

DIAGNOSTIC HISTOLOGICAL FEATURES

Conventional histology

IM of the oesophagus, the so-called specialized epithelium, is the diagnostic feature of BO when it is located in the oesophagus and not in the upper part of the stomach.¹¹ This mucosa is considered as an incomplete form of IM, similar to type II and type III IM of the stomach. Morphologically, it frequently shows a villiform pattern. The epithelium is composed mainly of goblet cells interspersed between intermediate mucous cells, both in the surface and glandular epithelium (Figure 2A). Mature absorptive intestinal cells with a well-defined brush border are rare. Paneth cells may be present. Endocrine cells can be seen on special staining in the glands. On electron microscopy, the goblet cells have characteristic apical mucin granules and the columnar mucin cells have features intermediate between gastric mucous cells and intestinal absorptive cells.¹²

Together with the characteristic IM, two other types of mucosa can be present in BO, i.e. cardiac-type and fundic-type mucosa.¹¹ These mucosae usually show some inflammation and architectural changes. Except in children, IM is the most common type encountered in BO.¹³ However, its frequency on endoscopic biopsies varies with the length of BO and also with the number of endoscopic biopsy series performed.¹⁴ It was classically considered that the three types of mucosa had a zonal distribution from intestinal to cardiac to fundic mucosa joining the upper part of the stomach. However, mapping studies have demonstrated in most BO a patchwork of the three mucosal types, with a predominance of IM.^{15,16} Most authors consider that IM is present in all cases in adults if sufficient sampling over a prolonged time scale is carried out. Therefore, most definitions include this epithelium as an absolute diagnostic criterion.^{2,17–19} For example, it was stated by the American Gastroenterological Association Chicago workshop that 'oesophageal IM documented by histology is a prerequisite criterion for the diagnosis of BO'.¹⁹ This strict definition has been challenged recently by the

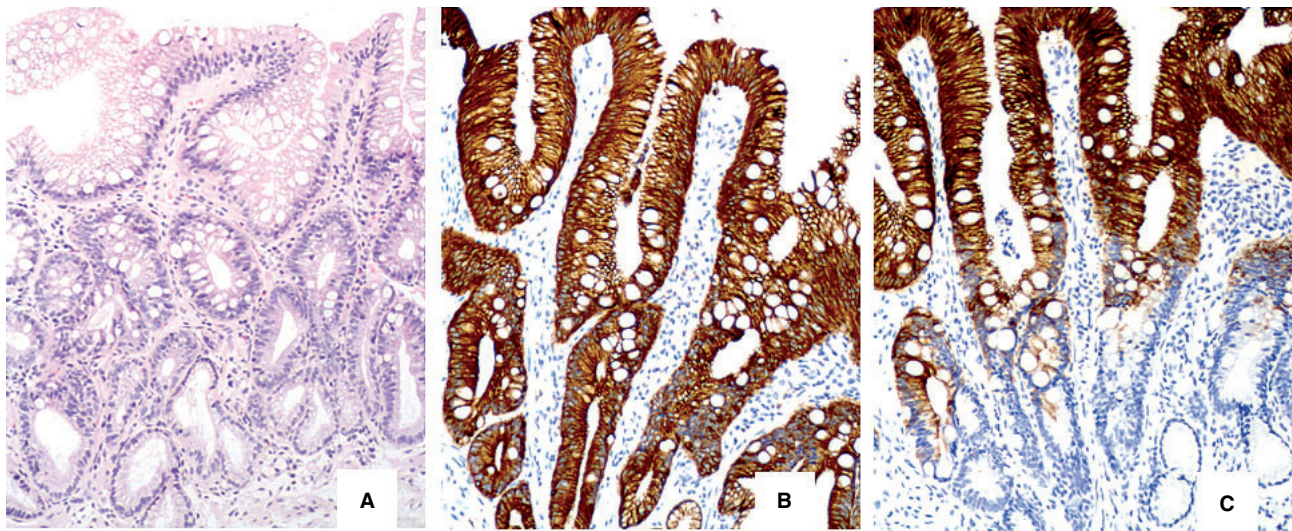


Figure 2. Typical Barrett's mucosa with intestinal metaplasia, so-called 'specialized mucosa'. **A**, The presence of large goblet cells filled with mucin is easily seen on standard H&E staining. **B,C**, Cytokeratin (CK) 'Barrett' pattern of intestinal metaplasia; CK7 is strongly and diffusely expressed (**B**), while CK20 is expressed at the surface epithelium and upper part of crypts (**C**).

'new British Society of Gastroenterology (BSG) guidelines for the diagnosis and management of Barrett's oesophagus',³ which consider that 'the presence of areas of IM, although often present, is not a requirement for diagnosis'. The rationale behind this change was that sampling errors at the initial endoscopy may miss an area of IM, leading to the exclusion of patients from the surveillance programme purely due to inappropriate biopsy sampling. This divergence may have major consequences when comparing results of clinical trials between countries in the future.

Mucin histochemistry

Both columnar mucinous cells and goblet cells produce mucins that can be characterized with mucin histochemistry. The columnar cells may produce neutral mucins, similar to gastric surface epithelial cells, and/or acidic mucins, typical of intestinal mucosa. Therefore, these cells can stain red (neutral mucins), blue (acidic mucins) or magenta (neutral and acidic mucins) on a combined periodic acid–Schiff–alcian blue stain.^{20,21} Some authors have suggested that the presence of acidic mucins (blue on alcian blue) is a characteristic feature of BO in the absence of typical goblet cells; however, this theory has been disputed by other studies that show alcian blue-positive columnar cells in gastric cardiac surface or neck cells in patients with neither metaplasia of the lower oesophagus nor GORD.^{22,23}

Therefore, the only characteristic feature of 'specialized' intestinal Barrett's mucosa is the presence of goblet

cells (Figure 2A). These cells are usually easily visualized on routinely stained sections. However, these goblet cells in all cases produce acidic mucins. Accordingly, it has been proposed that systematic staining of biopsies of the GOJ with alcian blue could be of interest to demonstrate rare positive goblet cells, which may indicate short-segment BO. Acidic mucins can be divided into sialomucins and sulphomucins. On a combined high iron diamine–alcian blue stain, sialomucins stain blue and sulphomucins stain brown-black. In specialized Barrett's mucosa, goblet cells usually contain both sulphomucins and sialomucins. The presence of sulphomucins in columnar cells is a characteristic feature of type III IM of the stomach, a lesion with a premalignant potential. In BO, it is very common to have sialomucin-containing columnar cells, a feature that shows that this pattern cannot be used to delineate a population at high risk of malignancy.^{20,21}

Immunohistochemistry

As immunohistochemistry is now routinely used in pathology departments, numerous studies have tried to find sensitive and specific markers of intestinal-type mucosa in the oesophagus. These markers include the MUC antigens and other mucin components, and different cytokeratin (CK) subtypes.

Cytokeratins are the intermediate filaments characteristic of epithelial cells. They are expressed in 20 distinct forms, with highly variable patterns along the type of epithelium. As the characteristic pattern is conserved in most carcinomas, CK20, a marker of

intestinal differentiation, and CK7, a marker of ductal differentiation, are used in the diagnosis of poorly differentiated carcinomas. Regarding BO, it has been proposed that there is a unique pattern of CK7–CK20 expression of Barrett's IM, with strong CK7 staining both at the surface and in deep glands, and weak superficial CK20 positivity.²⁴ However, although CK7 strong staining is a constant feature, it appears that CK20 is often relatively strongly expressed in Barrett's mucosa, possibly because the sensitivity of immunohistochemical techniques has recently increased greatly (Figure 2B,C). This sensitive and specific Barrett CK pattern has been observed in both long- and short-segment BO, and even in 'ultrashort' segment BO.^{25,26} However, some groups have not obtained the same results with these antibodies, and there is currently debate regarding the contribution of CK immunohistochemistry in the diagnosis of Barrett's mucosa.^{27–29} Regarding the preneoplastic significance of CK immunoreactivity, it seems unlikely that this pattern will be of great use, as it probably gives results very similar to those obtained with mucin histochemistry, with lack of sensitivity for preneoplasia. Similar results have been obtained with antibodies reacting with intestinal goblet cells, such as Das1 antibody.³⁰

Other antibodies have also been used to characterize IM in BO, directed against MUC mucin gene products, especially MUC1 and MUC2 (an intestinal mucin). These studies have demonstrated aberrant expression of MUC2 in Barrett's intestinal mucosa, lost when the cells become neoplastic. MUC1 was absent in metaplastic and dysplastic epithelium, but was expressed in carcinomas, which suggests that it could differentiate dysplasia from carcinoma in mucosal biopsy specimens.^{31,32}

ULTRASHORT BARRETT'S OESOPHAGUS OR INTESTINAL METAPLASIA OF THE GASTRIC CARDIA

It has been shown by many studies that IM is present in the GOJ region in up to one-third of patients without endoscopic evidence of BO (for review^{33,34}). Although expert pathologists strongly dispute the normal histological features of the GOJ (native or metaplastic 'cardiac' mucosa, oxyntocardiac mucosa?),^{34–36} it is indisputable that IM is an abnormal feature on biopsy specimens taken in this region. When there are very short segments of IM and no clear endoscopic aspect of columnar-lined oesophagus, does this metaplastic epithelium originate from the oesophagus (so-called ultrashort BO) or from the stomach (IM of the gastric cardia)? This question may be of importance, as it is highly probable that the risk of malignancy is higher

for GORD-induced IM in the oesophagus than for *Helicobacter pylori*-induced IM in the stomach.³⁷ Although a number of clinical, endoscopic, histological, histochemical and immunohistochemical features may orientate towards a GORD-related or *H. pylori*-associated IM,³⁴ there are no absolute criteria to distinguish between these two conditions, except for the presence of oesophageal mucosal or submucosal glands in a biopsy with IM, that confirms that the specimen is from the tubular oesophagus (Figure 1).³³ However, one may wonder whether this point is of clinical relevance at the present time, as most guidelines do not recommend to biopsy the GOJ when there are no endoscopic findings suggestive of BO.

Morphological markers of cancer risk in Barrett's oesophagus

As the major risk of patients with BO is of developing oesophageal adenocarcinoma, there has been considerable interest in defining a subgroup of high-risk patients in whom effective surveillance can be undertaken. Barrett's adenocarcinoma develops through a multistep process with progressive worsening of a precursor lesion called 'dysplasia'. At present, the morphological identification of dysplasia in endoscopic mucosal biopsy specimens is the standard method of detecting patients at increased risk of cancer and is used to delineate this population.³⁸

DEFINITION OF DYSPLASIA (INTRAEPITHELIAL NEOPLASIA)

Dysplasia is a purely morphological term. It is defined microscopically as replacement of the intestinal epithelium by 'an unequivocally, but as yet non-invasive, epithelium'.³⁹ Although this definition was initially proposed for premalignant changes developed in inflammatory bowel disease, it has been progressively extended to the entire gastrointestinal tract, including BO.⁴⁰ The term 'dysplasia' can also indicate a congenital (hereditary or not) lesion. Thus, this term tends to be replaced by the term 'intraepithelial neoplasia' widely used in other organ systems and recommended in BO by the World Health Organization⁴¹ and by two recent consensus reports.^{17,18} 'Non-invasive' means that the lesion is confined within the basement membrane of the gland within which it arises. Thus, dysplasia has to be distinguished from invasive cancer, especially in its early or superficial form with an invasion limited to the lamina propria. The definition of dysplasia also excludes all reactive changes.

DIAGNOSIS AND CLASSIFICATION OF DYSPLASIA

There are essentially two classifications of dysplasia in BO: the three-tiered classification of mild, moderate and severe dysplasia, and the adaptation of Riddell's classification for dysplasia arising in inflammatory bowel disease.³⁹ The latter distinguishes five categories: negative for dysplasia; indefinite for dysplasia; low-grade dysplasia (LGD); high-grade dysplasia (HGD); and invasive cancer. LGD includes the mild and moderate categories of the three-grade system and HGD, severe dysplasia. The three-tiered classification of mild, moderate and severe dysplasia, although still in use in some centres, is obsolete because it creates more intra- and interobserver variability than the two-grade system.

The criteria used to grade dysplasia are based on the severity of both cytological and architectural abnormalities (Table 1). Architectural changes include glandular distortion and crowding. Papillary extensions may be present into gland lumen, and villiform configuration of the mucosal surface can be observed. Cytological changes include nuclear alterations such as

variation in size and shape, nuclear and/or nucleolar enlargement, increased nuclear–cytoplasmic ratio, hyperchromatism and increased number of abnormal mitoses.

The distinction between LGD and HGD depends on the distribution of nuclei within the cells, LGD being characterized by nuclei that remain confined to the basal half of the cells and HGD by nuclei that are stratified haphazardly between the basal and apical halves.⁴² Most authors consider that these changes have to involve the mucosal surface to ascertain the diagnosis of dysplasia.^{38,39,43,44} Indeed, the presence of surface maturation in an atypical crypt lesion is a feature which would normally help pathologists exclude a diagnosis of dysplasia in favour of a diagnosis of crypt regeneration. However, in a recent study, Lomo *et al.* have characterized a significant pathological change, 'dysplasia-like atypia limited to the bases of the crypts, without involvement of the surface epithelium'.⁴⁵ According to them, these lesions, previously considered indefinite for dysplasia by some authors, may be a true subtype of dysplasia and may represent a histological marker of increased risk of progression

Table 1. Main diagnostic features of neoplastic lesions in Barrett's oesophagus, categorized according to the Vienna classification of epithelial neoplasia of the digestive tract⁵⁵

	Terminology in Vienna classification	Diagnostic features, remarks
Category 1	Negative for dysplasia	Architecture within normal limits. No nuclear abnormalities, except focal nuclear stratification. Greater nuclear alterations acceptable when associated with inflammation, erosion, or ulceration
Category 2	Indefinite for dysplasia	Architecture may be moderately distorted. Nuclear abnormalities less marked than those seen in dysplasia. Changes too marked for negative but not sufficient for the diagnosis of dysplasia
Category 3	Low-grade dysplasia	Architectural and cytological changes severe enough to suggest neoplastic transformation. Diagnosis of high-grade or low-grade based on the severity of changes. Alterations are especially noteworthy if they involve the mucosal surface The categories 'non-invasive carcinoma' and 'suspicion of invasive carcinoma' are not included in most other classifications
Category 4	4.1 High-grade dysplasia 4.2 Non-invasive carcinoma (carcinoma <i>in situ</i>) 4.3 Suspicion of invasive carcinoma	
Category 5	Invasive neoplasia 5.1 Intramucosal carcinoma 5.2 Submucosal carcinoma or beyond	

to cancer. This hypothesis was supported by a high association with conventional dysplasia and/or adenocarcinoma, associated with evidence of proliferative and molecular abnormalities. Thus, they proposed that pathologists sign out these lesions as 'indefinite for dysplasia with basal crypt dysplasia-like atypia'.

The term carcinoma *in situ* (or intraepithelial carcinoma) is not used in the Riddell's classification, as it is considered indistinguishable from HGD. In intramucosal carcinoma, neoplastic cells have penetrated through the basement membrane and infiltrate into the lamina propria, leading to a small risk of regional lymph node metastasis.

Unlike inflammatory bowel disease-associated colonic dysplastic lesions, BO-related dysplasia is mainly flat and often arises in glands that retain their normal configuration and lack nuclear stratification. Elevated or pedunculated lesions can occur, but are uncommon. They can be multifocal. They have been called dysplasia-associated lesion or mass, the equivalent of elevated dysplastic lesions in inflammatory bowel disease. It has been shown that the presence of these polypoid lesions was an indicator of a high risk of cancer.^{46,47} Because of the flat nature of dysplasia in BO, its detection depends critically on adequate sampling of the mucosa by the endoscopist. Current practice guidelines recommend that four quadrant biopsy specimens be taken at 10-mm intervals at closely timed intervals ('Seattle' protocol).⁴⁸

Many non-biopsy endoscopic methods are currently under evaluation to identify IM and dysplasia (for review¹⁰). Although encouraging results have been obtained with techniques such as chromoendoscopy and narrow-band imaging,⁴⁹ which may allow a reduction in the number of biopsies, further evaluation is necessary before these techniques are applicable in routine practice.¹⁹ Encouraging results have also been obtained with techniques such as light-induced fluorescence endoscopy, light-scattering spectroscopy, Raman spectroscopy, etc.,^{50,51} but many technical issues still have to be answered before they can be used in clinical settings.

DIAGNOSTIC REPRODUCIBILITY OF DYSPLASIA— THE VIENNA CLASSIFICATION

Most studies comparing diagnoses of dysplasia among different pathologists have concluded that there is significant intra- and interobserver variability.^{44,52–54} The categories at the lower end of the histological spectrum (indefinite for dysplasia and LGD) show the lowest κ values (marker for the variability which does account for agreement that occurs by chance alone)

and, thus, the highest variability. These results emphasize the need to obtain a second opinion in difficult cases, especially when a therapeutic decision has to be made. The discrepancies are even more considerable when diagnoses made by Western and by Japanese pathologists are compared. This point is crucial when analysing the Japanese literature on early neoplastic lesions of the gastrointestinal tract. As BO is rare in Japan, this problem may be less important for this lesion than for gastric and intestinal dysplasia. Nevertheless, in a study of 21 oesophageal lesions examined at the World Congress of Gastroenterology in Vienna, almost all lesions were classified as carcinoma by pathologists with a Japanese viewpoint, and only 10–67% of the same lesions by those with a Western viewpoint.⁵⁵ In Western countries, architectural features, particularly poor maturation and excessive crowding, are mostly taken into account for a diagnosis of dysplasia, whereas in Japan cytological features are predominant. After reaching a consensus, this international panel of pathologists proposed the Vienna classification to minimize disagreement (Table 1). This classification has still to be tested prospectively in large series of patients.

NATURAL HISTORY OF DYSPLASIA

The existence of a precursor lesion of Barrett's adenocarcinoma offers a window of opportunity for early detection and cure. A convenient model proposes that Barrett's adenocarcinoma follows a progression from IM to indefinite dysplasia, LGD, HGD and invasive carcinoma. HGD, the immediate precursor lesion to Barrett's adenocarcinoma, is also a marker of synchronous or metachronous adenocarcinoma, as in most surgical series up to 40% of BO resected for HGD have an occult adenocarcinoma.^{56,57} The frequency of these unsuspected cancers varied with the endoscopic and biopsy protocol, with very few cancers detected when patients were followed with the 'Seattle' protocol.⁴⁸ The natural history of HGD is somewhat controversial and is still very difficult to predict for one individual patient.⁴³ HGD can progress rapidly to adenocarcinoma,^{58,59} but it can also persist for many years with no such progression.^{60,61}

The distinction between unifocal and multifocal HGD has been emphasized by some authors, with a high rate of progression from unifocal to multifocal HGD or invasive carcinoma,^{46,58} a result challenged by Dar *et al.*⁶² Even less is known about the natural history of LGD. One of the explanations could be the high degree of interobserver variability in establishing this diagnosis. It was considered traditionally that LGD was in

most cases a very slowly progressing lesion. In most series, there was even a high rate of apparent regression from LGD to non-dysplastic mucosa. This last phenomenon has several potential explanations: initial overdiagnosis of LGD, due to the difficulty in differentiating reactive from dysplastic changes, sampling variability, or real neoplastic regression. However, this general opinion about the benign course of LGD has been challenged by some studies.^{63,64} Interestingly, in one of these studies, all cases were reviewed blindly by three gastrointestinal pathologists.⁶⁴ When all three agreed on the initial diagnosis of LGD, four of five patients progressed to a more severe lesion, when none of the eight patients with no agreement on the initial diagnosis progressed.

Thus, a thorough understanding of pathogenesis, natural history and biological significance of dysplasia is crucial to the optimal management of BO-related dysplasia. The subdivision of dysplasia into low-grade and high-grade has consequences for treatment due to the different risks of malignancy. It is generally accepted that a diagnosis of HGD is an indication for resection, by either surgery or endoscopy. At the present time, LGD implies close follow-up.

Molecular pathology of neoplastic transformation of Barrett's mucosa

In addition and parallel to the morphological sequence of events leading from metaplasia to carcinoma in Barrett's mucosa, chromosomal changes and accompanying genetic alterations occur, with ensuing abnormalities in gene expression and cell cycle regulation. Although the frequency and timing of these alterations are not as well established as in colorectal carcinogenesis, some authors have proposed a molecular cancer progression scheme of BO.⁶⁵⁻⁶⁸ Some of these changes may be used as criteria for recognizing patients at high risk of developing cancer.

The most common genetic alteration in BO is inactivation of the *p16INK4A/CDKN2A* tumour suppressor gene (chromosome 9p21). *p16INK4A/CDKN2A* is a cell cycle-regulatory gene which inhibits CDK4 and CDK6, preventing phosphorylation of Rb, thereby inhibiting cells from entering S phase. Inactivation of both alleles of *CDKN2A* appears to be an early event causing clonal expansion.⁶⁸ p16 has been shown to be inactivated through mutation or deletion in Barrett's adenocarcinoma.⁶⁹

The recently described CpG island methylator phenotype (CIMP), a new distinct pathway of tumorigenesis characterized by methylation of multiple CpG

islands, seems to have a role in the evolution of Barrett's adenocarcinoma.^{70,71} The study of promoter hypermethylation in BO using multiple target genes including *p16*, *APC*, *TIMP3* (a tumour suppressor gene related to invasion), *TERT* (immortalization), *RUNX3* and *HPPI1*, may indicate independent risk factors for the progression to HGD or invasive adenocarcinoma.⁷⁰

Loss of p53 is an important event in BO progression. It has been shown that *p53* gene mutations are occasionally found in metaplastic non-dysplastic mucosa and in LGD, and that the frequency of mutations increases dramatically in HGD and adenocarcinoma, reaching 80% of cases in some series, with an even higher frequency of loss of heterozygosity (LOH) at the p53 locus. There is evidence that P16INK4/CDKN2A lesions precede p53 lesions in the vast majority of cases.⁷² After inactivation of p53, tetraploid (4N) and aneuploid (any other formulation of chromosomes than 2N and 4N) clones tend to develop.

CLINICAL IMPLICATIONS

As mentioned, significant problems have emerged when using dysplasia as an exclusive marker for increased cancer risk in patients with BO. Thus, molecular biomarkers predicting increased risk of progression are needed for improved risk assessment.⁷³ Only DNA aneuploidy and p53 expression have been investigated in prospective studies involving relatively large numbers of patients. DNA aneuploidy, demonstrated by flow cytometry, has been evaluated in prospective trials representing phase IV biomarker development studies and is now being used in some centres to adjust frequency of surveillance in clinical practice.⁷⁴ However, this marker is not used in most centres, probably because of technical difficulties.

A single, large prospective study has shown that 17p LOH is a good predictor of progression to HGD and carcinoma, suggesting that p53 is a potential biomarker in patients with BO.^{75,76} Several studies have suggested a role of p53 immunohistochemical analysis in the selection of patients with LGD who will progress to HGD or adenocarcinoma. However, most of these studies were retrospective and involved only limited numbers of patients. In a recent case-control study of several potential biomarkers (expression of p53, cyclin D1, cyclooxygenase 2, β -catenin) in patients with and without malignancy in BO, only staining of p53 at the initial biopsies was significantly associated with the risk of malignant progression.⁷⁶ However, sensitivity was only 32.4%, preventing this

Table 2. Main molecular events involved in the neoplastic transformation of Barrett's mucosa and their potential use as biomarkers of an increased risk of cancer

Events	Type of change	Clinical use	Comments
Increased proliferation by Immunohistochemistry	Ki67 increased expression	+/-	Surface expression strengthens a diagnosis of dysplasia
Flow cytometry	Increased G ₂ -M phase	+/-	Limited technical availability
DNA-ploidy	Early DNA aneuploidy due to genomic instability	+	Limited technical availability
Cell-cycle regulators			
p16	Early LOH, hypermethylation of second allele	-	Most common event, no large-scale studies
Cyclin D1	Increased expression in cancer	-	Frequent, no large-scale studies
GF and GF receptors TGF- α , EGF	Increased expression in cancer	-	No prospective studies
EGFR	Frequent amplification in cancer	-	
c-erbB2	Less common overexpression than EGFR	-	
Tumour suppressor genes			
p53	Frequent LOH and mutation in HGD and cancer LOH	+/-	Positive large prospective study. Not available in most centres
	Abnormal protein expression	+	Recognized marker of an increased risk, but low sensitivity
APC	Early LOH and promoter methylation	-	

GF, Growth factor; LOH, loss of heterozygosity; TGF, transforming growth factor; EGF, epidermal growth factor; HGD, high-grade dysplasia.

marker from being applied as a criterion for endoscopic surveillance.

Increased p53 expression is accompanied by increased Ki67 labelling. Interestingly, the proliferative compartment stained by Ki67 increases in size and expands from the base of the crypts towards the surface epithelium.⁷⁷ However, due to the large overlap in Ki67 expression between sets of lesions that have been classified by grade of dysplasia, this marker is not used in routine clinical practice.

Many other markers have been suggested as being of interest to define precisely a subgroup in patients with BO with increased risk of cancer, including CDC2/CDK1, p16, cyclin D1, etc.⁷⁸ However, none of these markers has been studied in large prospective studies. Even for p53 and DNA aneuploidy, there is no proof that the use of these markers will reduce cancer incidence in various populations. These results regard-

ing the main biomarkers studied in BO are summarized in Table 2.

Non-surgical treatment of Barrett's oesophagus: impact for the pathologist

Until recently, pathologists dealing with BO had to examine only two types of histological specimen: (i) endoscopic biopsies, either systematically sampled during screening endoscopy or directed by tumours or any mucosal irregularity; (ii) oesophagectomy specimens in patients with adenocarcinoma or HGD. New mucosal ablative or destructive techniques can now be used primarily to treat dysplasia and early oesophageal adenocarcinoma. Some techniques do not allow any histological study, as their goal is to induce complete destruction of the metaplastic and neoplastic tissue.

These techniques include photodynamic therapy, laser therapy, multipolar electrocoagulation, argon plasma coagulation, radiofrequency ablation and cryotherapy.⁷⁹ EMR is an ablative technique which removes mucosa by resecting through the submucosa. A tissue specimen is obtained that has to be evaluated for histological diagnosis and staging. Although the same criteria are used as in biopsies and surgical specimens, some specific points merit attention for the pathologist receiving EMR specimens. Ideally, the specimens should be oriented, pinned and stretched on cardboard in the endoscopy unit. Painting of the base and margins is useful, as tumour extension to the deep margin indicates surgery and remnants of the neoplastic epithelium at the lateral margins indicate re-excision or postoperative ablation (most often by photodynamic therapy or laser coagulation). It has been shown in some series that there was a high rate of microscopically incomplete resection (up to 96% of cases in the series by Mino-Kenudson *et al.*)⁸⁰ and therefore continued endoscopic surveillance is mandatory in all patients. However, it may be that repeated 'stepwise' EMR is more effective for complete removal of neoplastic Barrett's mucosa.⁸¹ The presence of a double layer of muscularis mucosa is almost constant in Barrett's oesophagus (Figure 3) and has important consequences when examining EMR specimens. It is usually considered that the deeper layer is the original muscularis mucosa and the most superficial layer is newly formed. Therefore, only when the invasion extends beyond the deeper layer is a diagnosis of submucosal carcinoma (T1b or T1sm) justified.¹⁸ Other parameters important to assess in order to indicate more extensive surgery in case of an adenocarcinoma are tumour differentiation and vascular invasion. A

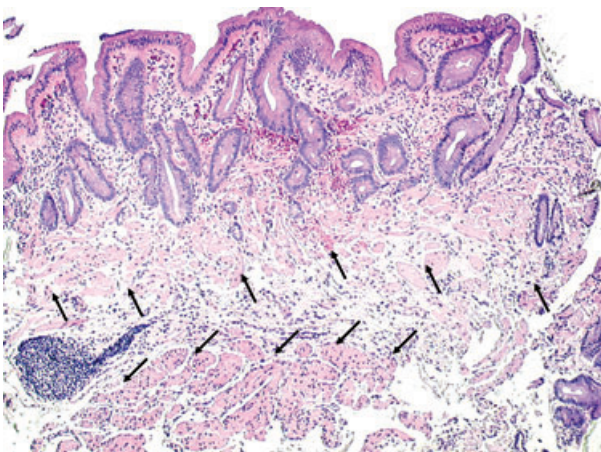


Figure 3. The characteristic double muscularis mucosae (arrows) of Barrett's mucosa can be seen in some cases on biopsies (H&E).

recent report has suggested that frozen section analysis of EMR specimens may be of help in real-time management of BO with HGD and/or adenocarcinoma.⁸² Histological examination of EMR specimens is also of value in diagnosing precisely the degree of dysplasia and searching for occult carcinoma; it has been shown that EMR is superior to biopsy for this purpose, with discrepancies most often showing a higher grade on EMR than on biopsy specimens.⁸³

After local therapy, surveillance is mandatory and raises additional problems for the pathologist. Although some cases of complete histological regression of Barrett's mucosa have been documented,⁸⁴ the major problem is the occurrence of subsquamous specialized intestinal mucosa (so-called buried Barrett's glands) that can be seen with all therapeutic modalities, including treatment with proton pump inhibitors that induce partial replacement of columnar epithelium with squamous epithelium (Figure 4).⁸⁵ The frequency of buried glands varies among different series but may be as high as 51% of cases. These glands may be difficult to identify on small endoscopic biopsies. Although their exact significance is still debated, they have the

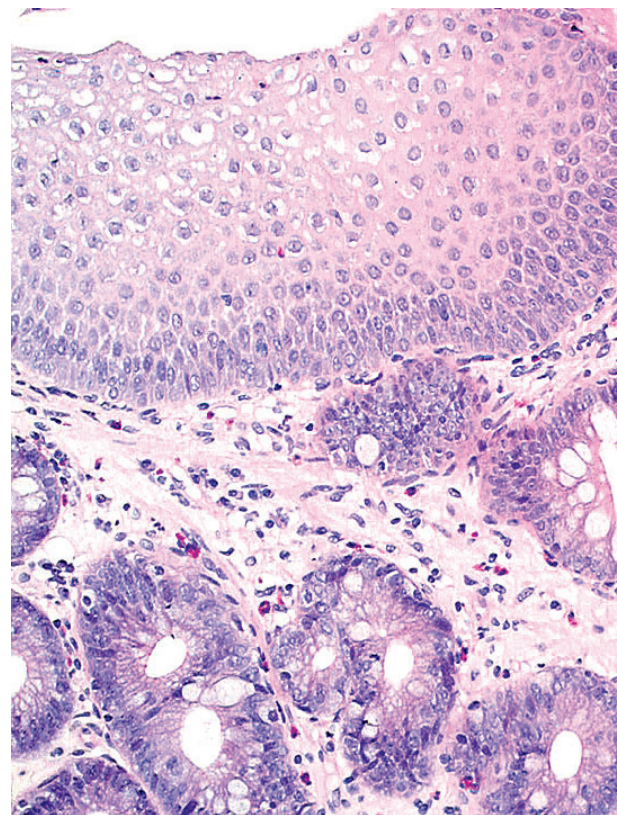


Figure 4. Biopsy of Barrett's mucosa after endoscopic laser therapy. Buried glands with intestinal metaplasia, indefinite for dysplasia, can be seen under the surface squamous epithelium (H&E).

potential to progress through stages of dysplasia to cancer, which is in keeping with the presence of various genetic alterations.⁸⁶ On the other hand, it has been suggested that these buried glands may be 'protected' in some way from exposure to the luminal contents of the oesophagus, which could explain their lower proliferative capacity.⁸⁷ Clinical and experimental studies have shown that the luminal content provides the stimulus for proliferation and dedifferentiation, and therefore plays a major role in Barrett's carcinogenesis.⁸⁸ The high frequency of these residual glands beneath squamous epithelium has two consequences: (i) practical, i.e. the requirement for histological confirmation of complete re-epithelialization with sufficiently deep biopsies; (ii) theoretical—a contribution to the open debate on the cells of origin of BO and re-epithelialized squamous islands, which are essentially unknown. There is evidence to support the possibility that pluripotent (mesenchymal?) stem cells could differentiate into columnar or squamous cells depending on environmental factors.

In conclusion, BO is a condition in which pathologists have a very important role in diagnosis, surveillance and treatment. It must be emphasized that this role has changed recently due to the use of new therapeutic techniques with new types of histological specimens, and may change even more in the near future, as many new imaging techniques are emerging, either in combination with classical endoscopes, or comprising entirely new techniques. These techniques may greatly improve the endoscopic recognition of tissue or even cellular abnormalities, and therefore avoid random biopsy sampling. Pathologists also have a role to play in the development of these techniques.

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