

# Barrett's oesophagus: from metaplasia to dysplasia and cancer

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Barrett's oesophagus is a premalignant condition that predisposes to the development of oesophageal adenocarcinoma. It is detected on endoscopy and confirmed histologically by the presence in the lower oesophagus of a metaplastic mucosa, the so-called specialised epithelium, which resembles incomplete intestinal metaplasia in the stomach. These similarities with incomplete intestinal metaplasia are present on histology, mucin histochemistry, and immunohistochemistry with various differentiation markers (cytokeratins and MUC antigens). On morphology, the carcinogenetic process of Barrett's mucosa progresses through increasing grades of epithelial dysplasia. Dysplasia, a synonym of intraepithelial neoplasia, is the only marker that can be used at the present time to delineate a population of patients at high risk of cancer. Among the numerous molecular events that have been shown to play a role in the neoplastic transformation of Barrett's mucosa, only changes in DNA ploidy, increased proliferation, and alterations of the p53 gene have been suggested to be of potential help in the surveillance of patients.

accumulation of genetic abnormalities in cells, from normal cells to invasive malignant cells.<sup>4–6</sup> Some of these genetic changes have been proposed as an adjunct to morphology for the screening and surveillance of patients with Barrett's oesophagus.<sup>7–8</sup>

## MORPHOLOGY OF BARRETT'S OESOPHAGUS

### Macroscopic features

Glandular mucosa in the lower oesophagus presents as a red velvety mucosa over the gastro-oesophageal junction. It can extend either circumferentially or as one or several tongues, and in some cases as a mixture of these two patterns. Until recently, it was considered that this mucosa had to extend at least 30 mm over the gastro-oesophageal junction to diagnose Barrett's oesophagus. But this definition has changed, owing to the recognition of short segment Barrett's oesophagus measuring less than 30 mm.<sup>9–10</sup> However, as it may be difficult to measure precisely a short segment Barrett's oesophagus and to localise the metaplastic mucosa and the gastro-oesophageal junction, it is now well admitted that the major diagnostic criteria of Barrett's oesophagus is histological. The significance of intestinal metaplasia discovered on biopsies taken from an endoscopically normal junction (sometimes considered as an "ultrashort" Barrett's oesophagus) remains controversial, and will not be discussed in this text.

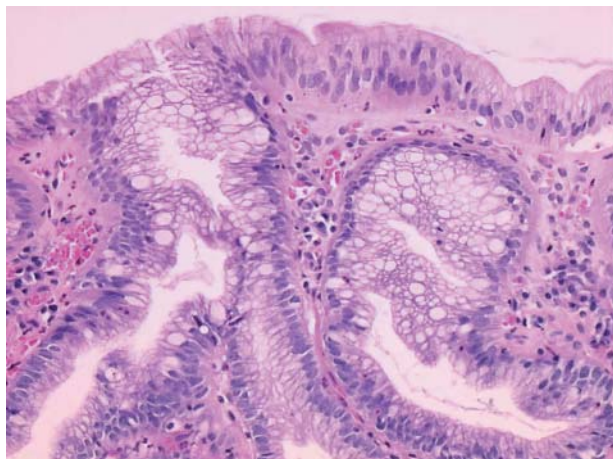
### Histological features

Intestinal metaplasia of the oesophagus, the specialised epithelium, is the diagnostic feature of Barrett's oesophagus when it is located in the oesophagus and not in the upper part of the stomach.<sup>11</sup> This mucosa is considered an incomplete form of intestinal metaplasia, similar to type II and type III intestinal metaplasia in 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 (fig 1). Mature absorptive intestinal cells with a well defined brush border are rare. Paneth cells may be present, but they are as rare as in incomplete intestinal metaplasia of the gastric mucosa. Endocrine cells can be seen on special stainings in the glands. On electron microscopy, the goblet cells have characteristic apical mucin granules, and the columnar mucin cells have features intermediate

Barrett's oesophagus, or columnar lined oesophagus, is an acquired condition that results from chronic gastro-oesophageal reflux. It is characterised by the metaplastic replacement of the normal squamous epithelium of the lower oesophagus by columnar epithelium. The diagnosis of Barrett's oesophagus is made on endoscopy, but it has to be confirmed by the histological examination of biopsies, which show the characteristic incomplete intestinal metaplasia (also called "specialised" mucosa).<sup>1</sup> Intestinal metaplasia is present in all cases in adults if sufficient sampling over a prolonged timescale is carried out. Barrett's oesophagus is a premalignant condition that predisposes to the development of oesophageal adenocarcinoma, a tumour with an increasing frequency in most Western countries.<sup>2</sup> It has been well demonstrated in surveillance studies that adenocarcinoma develops through a multi-step morphological pathway. This process is characterised by increasing grades of dysplasia (intraepithelial neoplasia), the precursor of invasive adenocarcinoma.<sup>3</sup> Parallel to the metaplasia–dysplasia–adenocarcinoma sequence, numerous studies have demonstrated the

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**Abbreviations:** CK, cytokeratin; HGD, high grade dysplasia; LGD, low grade dysplasia



**Figure 1** Barrett's mucosa with incomplete intestinal metaplasia (specialised mucosa). The epithelium is composed of goblet cells interspersed between intermediate mucous cells, both in the surface and glandular epithelium (haematoxylin–eosin, original magnification  $\times 400$ ).

between gastric mucous cells and intestinal absorptive cells.<sup>12</sup>

Together with the characteristic intestinal mucosa, two other types of mucosa can be present in Barrett's oesophagus, that is, cardiac type and fundic type mucosa.<sup>11</sup> These mucosae usually show some inflammation and architectural changes. Except in children, intestinal type epithelium is the most common type encountered in Barrett's oesophagus.<sup>13</sup> However, its frequency on endoscopic biopsies varies upon the length of Barrett's oesophagus and also with the number of endoscopic biopsy series performed.<sup>14</sup> 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, some mapping studies have demonstrated in most Barrett's oesophagus a patchwork of the three mucosal types, with a predominance of the intestinal mucosa.<sup>15–16</sup>

### Mucin histochemistry

Both columnar mucinous cells and goblet cells produce mucins that can be characterised using mucin histochemistry. The columnar cells may produce neutral mucins, similarly 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 PAS–alcian blue stain.<sup>17–18</sup> Some authors have suggested that the presence of acidic mucins (blue on alcian blue) is a characteristic feature of Barrett's oesophagus in the absence of typical goblet cells; however, this theory has been disputed by other studies that showed alcian blue positive columnar cells in gastric cardiac surface or neck cells in patients with neither metaplasia of the lower oesophagus nor gastro-oesophageal reflux disease.<sup>19–20</sup>

The only characteristic feature of intestinal Barrett's mucosa is the presence of goblet cells. These cells are usually easily visualised on routinely stained sections. However, these goblet cells produce in all cases acidic mucins. Accordingly, it has been proposed that systematic staining of biopsies of the gastro-oesophageal junction with alcian blue could be of interest to demonstrate rare positive goblet cells, which may indicate short segment Barrett's oesophagus. Acidic mucins can be divided into sialomucins and sulfomucins. On a combined high iron diamine–alcian blue stain, sialomucins stain blue, and sulfomucins stain brown–black. In specialised Barrett's mucosa, goblet cells usually

contain both sulfomucins and sialomucins. The presence of sulfomucins in columnar cells is a characteristic feature of type III intestinal metaplasia of the stomach, a lesion with a premalignant potential. In Barrett's oesophagus, 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.<sup>17–18</sup>

### Immunohistochemistry

As immunohistochemistry is now routinely used in almost all 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.

CKs 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 routinely used in the diagnosis of poorly differentiated carcinomas. Regarding Barrett's oesophagus, it has been proposed that there is a unique pattern of CK7–CK20 expression of Barrett's intestinal metaplasia, with a strong CK7 staining both at the surface and in deep glands, and a weak superficial CK20 positivity.<sup>21</sup> This sensitive and specific Barrett CK pattern has been observed in both long and short segment Barrett's oesophagus, and even in ultrashort segment Barrett's oesophagus.<sup>22–23</sup> However, some groups did not obtain the same kind of results with these antibodies, and there is currently a debate regarding the contribution of CK immunohistochemistry in the diagnosis of Barrett's mucosa.<sup>24–26</sup> Regarding the pre-neoplastic 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 a lack of sensitivity for pre-neoplasia. Similar results have been obtained with antibodies reacting with intestinal goblet cells, such as Das1 antibody.<sup>27</sup>

Other antibodies have also been used to characterise intestinal metaplasia of Barrett's oesophagus, 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 biopsies.<sup>28–29</sup>

### MORPHOLOGICAL MARKERS OF CANCER RISK IN BARRETT'S OESOPHAGUS

Because the major risk of patients with Barrett's oesophagus is to develop an oesophageal adenocarcinoma, there has been considerable interest in defining a subgroup of high risk patients in whom an effective surveillance can be undertaken. At the present time, only morphological markers, especially epithelial dysplasia, are used to delineate this population.<sup>30</sup>

#### Definition of dysplasia (intraepithelial neoplasia)

Dysplasia is a purely morphological term. Although it could be considered from an etymological point of view as an ambiguous and vague term, meaning malformation, it is used by gastrointestinal pathologists to design premalignant lesions. Dysplasia has been defined by Riddell *et al*<sup>31</sup> as an unequivocal neoplastic epithelium strictly confined within the basement membrane of the gland from which it arises. Although this definition was initially proposed for



pre-malignant changes developed on inflammatory bowel disease, it has been progressively extended to the entire gastrointestinal tract, including Barrett's oesophagus.<sup>32</sup> Dysplasia as a pre-malignant lesion is strictly synonymous to intraepithelial neoplasia, a term in use in most organs including the gynaecological tract, and that has been recommended in Barrett's oesophagus by the World Health Organization<sup>33</sup> and by two recent consensus reports.<sup>34 35</sup> Dysplasia has to be distinguished on both ends of the morphological spectrum of changes, from regenerative non-neoplastic modifications, often called atypia, and from invasive cancer, especially in its early or superficial form with an invasion limited to the lamina propria.

### Diagnosis and classification of dysplasia

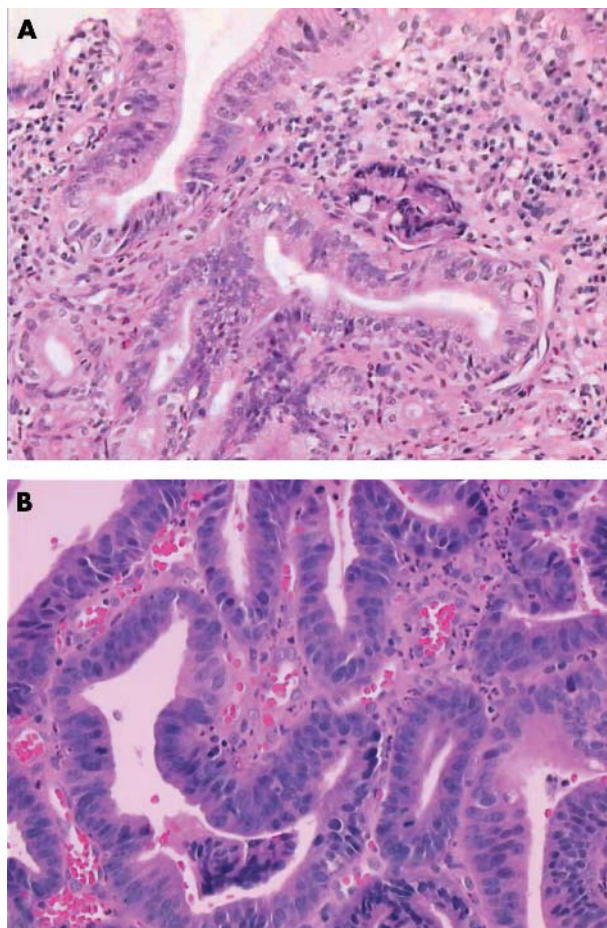
As a morphological entity diagnosed in routine work, dysplasia has to be recognised on endoscopic biopsies with routinely stained sections. It comprises both architectural and cytological abnormalities. Architectural changes include glandular distortion and crowding. Papillary extensions may be present in 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 to cytoplasmic ratio, hyperchromatism, and increased number of abnormal mitoses. Most authors consider that these changes have to involve the mucosal surface to ascertain the diagnosis of dysplasia.<sup>30 31 36 37</sup>

Based on the degree of the abnormalities present, dysplasia is classified into grades of increasing severity. Although a three tiered classification (mild–moderate–severe) is still in use in some centres, most pathologists use a two tiered system that distinguishes between low grade dysplasia (LGD) and high grade dysplasia (HGD) (fig 2). In this two grade system, LGD includes the mild and moderate categories of the three grades system. The Riddell's classification of dysplasia also includes a category of mucosa indefinite for dysplasia.<sup>31</sup> 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. The main morphological criteria for the diagnosis of dysplasia are presented in table 1.

### Diagnostic reproducibility of dysplasia—The Vienna classification

It has been shown for a long time that there is intra and inter-observer variation in the diagnosis of dysplasia in Barrett's oesophagus. Given the progressive and subtle changes that occur from non-dysplastic to LGD to HGD, it is not surprising that this variation exists. Among the various studies published in the literature,<sup>37–41</sup> some series have enrolled expert senior pathologists, and others have implicated general pathologists. In a recently published "expert" study, the diagnoses made by 12 senior gastrointestinal pathologists on 125 biopsies were compared.<sup>37</sup> When a four grade system was employed (non-dysplastic/indefinite and low grade/high grade/cancer), the kappa index was low (0.43). Kappa improved (0.66) when a simplified classification was used (non-dysplastic/indefinite and low grade/high grade and cancer). In a study involving 20 general pathologists in the USA, there was very large variation on diagnoses of non-dysplastic mucosa, LGD, and HGD.<sup>40</sup> These results emphasise the need to obtain a second opinion on difficult cases, especially when a therapeutic decision has to be made.

The diagnostic differences are even more considerable when diagnoses made by Western pathologists and those



**Figure 2** Dysplasia in Barrett's mucosa, with both architectural and cytological abnormalities of low grade (A) and high grade (B) (haematoxylin-eosin, original magnification  $\times 400$ ).

made by Japanese pathologists are compared. This point is crucial when analysing the Japanese literature on early neoplastic lesions of the gastrointestinal tract. As Barrett's oesophagus is rare in Japan, this problem may be less important for this lesion as 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.<sup>42</sup> After reaching a consensus, this international panel of pathologists proposed a classification to minimise disagreement. This "Vienna classification" is presented in table 2. The main advantages of this five tier system may be to propose clear surveillance and therapeutic consequences for the various diagnostic categories. However, this classification still has to be tested prospectively in a large series of patients.

### Natural history of dysplasia

When patients included in surveillance cohorts are considered, it has been well established that the presence of dysplasia indicates an increased risk of carcinoma. However, the natural history of this lesion is still very difficult to predict for one individual patient.<sup>36</sup>

### High grade dysplasia

HGD is the nearest precursor of adenocarcinoma, as shown by its presence around the cancer on surgical specimens, and before the cancer in surveillance programmes. It must be remembered that dysplasia detected on endoscopic biopsies

**Table 1** Histological criteria for grading dysplasia in Barrett's oesophagus

	Criteria
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.
Indefinite	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.
Positive for dysplasia	Architectural and cytological changes severe enough to suggest neoplastic transformation. Diagnosis of high grade or low grade based on the severity of changes: high grade dysplasia is diagnosed if either architectural and/or cytological abnormalities are sufficiently prominent. Alterations are especially noteworthy if they involve the mucosal surface.
Intramucosal carcinoma	Carcinoma has penetrated through the basement membrane of the glands into the lamina propria but not yet invaded the submucosa.

Adapted from Geboes and Van Eyken,<sup>30</sup> Riddell *et al.*,<sup>31</sup> Montgomery *et al.*<sup>37</sup>

is also frequently a marker of synchronous carcinoma, as in most surgical series up to 40% of Barrett's oesophagus resected for HGD have an occult adenocarcinoma.<sup>43–44</sup> The frequency of these unsuspected cancers varied upon the endoscopic and bioptic protocol, with very few cancers detected when patients were followed using the "Seattle" protocol (four quadrant biopsies at 1 cm intervals) at closely timed intervals.<sup>45</sup>

The natural history of HGD is still a matter of debate. In two large series that included 145 patients with HGD, although the risk of malignant transformation was relatively high, the majority of patients did not progress to adenocarcinoma after several years of follow up<sup>46–47</sup>; among patients without carcinoma after 1 year of searching after the initial diagnosis of HGD, 25% and 16% of patients developed carcinoma after a mean surveillance period of 2.5 years and 7.3 years, respectively. When considering these series, it can be concluded that HGD does not progress to adenocarcinoma in the majority of patients within some years, and that non-surgical procedures (surveillance and endoscopic treatments) can be considered as reasonable options in those patients, a statement that is still very much debated in the literature.<sup>36</sup> It is interesting to note that in one of these two series, there was an unusually high proportion of Barrett's oesophagus patients with LGD (737 of 1099, 67%), and the histological diagnoses were made during a period of 20 years by one experienced pathologist.<sup>46</sup>

Recently, the distinction between unifocal and multifocal HGD has been emphasised by some authors, with a high rate of progression from unifocal to multifocal HGD or invasive carcinoma (8 of 15 patients within a mean follow up period of 37 months),<sup>48</sup> a result confirmed by another study that demonstrated a risk of malignant progression increased by 3.7 when diffuse HGD was present,<sup>49</sup> but recently challenged by Dar *et al.*<sup>50</sup> It has also been shown that the presence of

endoscopic polypoid lesions (an equivalent of DALM (Dysplasia Associated Lesion or Mass) in inflammatory bowel disease) was an indicator of high risk of cancer.<sup>48–51</sup>

### Low grade dysplasia

The natural history of LGD is even less known. This could be at least partially due to the poor diagnostic reproducibility of this lesion. It was considered traditionally that LGD was a very slowly progressing lesion in most cases. 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 recent studies. In a study based on multicentre pathological recruitment among 26 cases with a diagnosis of LGD, 4 patients (15%) developed HGD and 4 (15%) an adenocarcinoma, 2–65 months after the initial diagnosis of LGD.<sup>3</sup> In another study, 7 patients (28%) developed HGD (5 patients) or an adenocarcinoma (2 patients) after a mean follow up of 26 months (range 2–43 months) after the diagnosis of LGD.<sup>52</sup> Very interestingly, in this latter study all cases were reviewed blindly by 3 gastrointestinal pathologists. When all 3 pathologists agreed on the initial diagnosis of LGD, 4 of 5 patients progressed to a more severe lesion, when none of the 8 patients with no agreement for the initial diagnosis progressed.

### 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 Barrett's oesophagus.<sup>4–5–53–54</sup> Some of these changes may be used as criteria for recognising Barrett's oesophagus patients with a high risk for developing cancer.

It has been proposed by Hanahan and Weinberg<sup>55</sup> that there are six major changes for a cell to become malignant: cell provides growth signals, ignores growth inhibitory signals, avoids apoptosis, replicates without limit, sustains angiogenesis, and invades and proliferates. Morales *et al* illustrated recently that these alterations are present during the carcinogenesis of Barrett's oesophagus.<sup>53</sup>

Table 3 summarises the main molecular events that have been shown to play a role in the neoplastic transformation of Barrett's mucosa. Only those changes that have been

**Table 2** Vienna classification of epithelial neoplasia of the digestive tract<sup>42</sup>

Category	Classification
1	Negative for neoplasia/dysplasia
2	Indefinite for neoplasia/dysplasia
3	Non-invasive low grade neoplasia Low grade adenoma/dysplasia
4	Non-invasive high grade neoplasia
4.1	High grade adenoma/dysplasia
4.1	Non-invasive carcinoma (carcinoma in situ)
4.1	Suspicion of invasive carcinoma
5	Invasive neoplasia
5.1	Intramucosal carcinoma
5.2	Submucosal carcinoma or beyond

**Table 3** Molecular events involved in the neoplastic transformation of Barrett's mucosa and their potential clinical use

Events	Type of change, comment	Diagnostic use
<b>Increased proliferation by</b>		
immunohistochemistry	Ki67 expression at the surface in HGD	++
flow cytometry	Increased G2-M phase	+
<b>Cell cycle regulators</b>		
p16 (CDKN2A)	Early LOH, late hypermethylation of 2 <sup>nd</sup> allele	-
cyclins D1 and E	Increased expression in cancer	-
growth factors (GF) and GF receptors		
TGF $\alpha$ , EGF	Increased expression in cancer	-
EGFR	Frequent amplification in cancer	-
c-erbB2	Less common overexpression as EGFR	-
<b>Tumour suppressor genes</b>		
p53	Frequent mutation in HGD and cancer	++
APC	Early LOH and promoter methylation	-
Rb	Uncommon direct implication	-
<b>Cell adhesion</b>		
E-cadherin	Decreased expression in cancer	+/-
$\beta$ catenin	Decreased expression and nuclear shift	+/-
<b>COX-2</b>	Increased expression, results in increased angiogenesis and decreased apoptosis	?
<b>Telomerase</b>	Increased expression parallel to dysplasia	-
<b>DNA ploidy</b>	Early DNA aneuploidy	+
<b>Bcl2/bax</b>	Disturbed balance	?
<b>Microsatellite instability</b>	Very uncommon in cancer	-

suggested to be of potential help in the surveillance of patients will be presented more in detail, that is changes in DNA ploidy, increased proliferation, and alterations of p53 gene and protein.

#### Changes in DNA content: DNA aneuploidy

Cells that contain any other formulation of chromosomes than 2N (diploid) and 4N (tetraploid) are said to be aneuploid. These cells can be detected by flow cytometry, which shows in some tumours and dysplastic tissues DNA aneuploid clones. The technique is based on staining a suspension of single cells with quantitative fluorescent DNA dye, with a detection system that shows an amount of fluorescence proportional to the amount of DNA in each cell.

Aneuploidy does not correlate with any single mutation of the genes listed in table 3, but reflects large DNA changes due to genomic instability. Over 90% of HGD and adenocarcinomas developed in Barrett's oesophagus are DNA aneuploid, and there is a significant relation between the presence of DNA aneuploid population and the progression from non-dysplastic Barrett's intestinal mucosa to dysplasia and adenocarcinoma. As flow cytometry is able to detect a subset of patients with unremarkable biopsies (non-dysplastic or indefinite for dysplasia) but who have DNA content abnormalities identical to those observed in HGD and carcinoma, it has been suggested that this technique may be useful in screening patients with Barrett's oesophagus. This theoretical interest is maximal in patients with LGD, a lesion with an undetermined natural history. The Seattle group has shown in prospective studies that patients with DNA aneuploid cells or increased DNA tetraploid populations have an increased risk of developing HGD or carcinoma.<sup>56</sup> In a more recent study, the same group showed that among patients with non-dysplastic, indefinite for dysplasia, or LGD mucosa, the risk of cancer was strongly related to the presence of DNA aneuploidy or increased 4N populations.<sup>57</sup> However, although some studies have confirmed that DNA aneuploidy is a prognostic factor for malignant transformation in Barrett's oesophagus,<sup>58</sup> other groups have reported frequent discordance between histology and DNA ploidy,<sup>59</sup> which may be due to technical issues. This could partially explain why this technique is still not widely diffused in clinical routine practice.

#### Increased proliferation

Most genetic changes that occur during the carcinogenesis of Barrett's oesophagus affect genes involved in the regulation of cell cycle (table 3), with an ensuing increased proliferation. Initially, this hyperproliferative state was demonstrated by studies with tritiated thymidine, and later with BrDU. These studies showed an increased S phase in Barrett's metaplastic mucosa, especially when dysplasia was present. DNA flow cytometry also allows us to study the cell cycle, and a number of studies have shown that the number of cells into the S phase and into G2M phases (DNA tetraploid cells) was a predictor of dysplasia.

During the past 15 years, numerous studies have used two markers of cell proliferation that can be evaluated by immunohistochemistry on routinely processed oesophageal biopsies (review in<sup>16</sup>); proliferating cell nuclear antigen (PCNA) and Ki67 (usually stained with the monoclonal antibody MIB1). PCNA is an indicator of cell cycle progression at the G1/S transition, and Ki67 is expressed in proliferating cells (G1, S, G2, and M phases). Numerous studies have shown an increased proportion of cells stained by both antibodies parallel to the progression of histological changes from metaplasia to increasing grades of dysplasia. Interestingly, the proliferative compartment stained by Ki67 increases in size and expands from the base of the crypts towards the surface epithelium.<sup>60</sup> However, due to large overlaps of Ki67 stainings between groups defined along the severity of histological lesions, this marker is not really used in clinical routine practice.

The mechanisms of increased proliferation in Barrett's mucosa and the possibilities of therapeutic control are issues of great importance. Several studies have shown both *ex vivo* and *in vitro* that acid plays a major role in this increased proliferation.<sup>61-62</sup> The impact of effective acid suppression on the development of dysplasia and cancer requires prospective information.

#### Alterations of p53

Mutations of the p53 gene are the most common genetic alteration in human cancer. The p53 gene encodes p53 protein, a major transcription factor that facilitates cell cycle arrest, DNA repair, and apoptosis. It has been shown by molecular biology 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 at the p53 locus. Moreover, these allelic losses at 17p usually occur before the loss of 5q (bearing the APC locus), a result that suggests that p53 mutation is a relatively early event in Barrett carcinogenesis. The prolonged half life of the mutant p53 protein with an ensuing increase cellular p53 concentration make indirect visualisation of p53 gene mutation by immunohistochemistry possible. Numerous immunohistochemical studies have shown a very low percentage of p53 over expression in non-dysplastic mucosa (5%), increasing to 10–20% in LGD, and to more than 60% in HGD and carcinoma (reviewed in<sup>16</sup>). Most of these studies have been performed retrospectively, making it very difficult to determine the interest of p53 immunohistochemistry in the surveillance of patients with Barrett's oesophagus. However, some prospective studies have been published recently. In two studies involving 97 patients, 9 patients developed HGD or malignancy, including 8 patients among 13 patients with at least one biopsy p53 positive, and only one patient among the 84 patients without p53 over expression.<sup>63–64</sup> Recently, Weston *et al* confirmed these result and showed in patients with LGD an increased risk of progression to HGD or cancer in case of p53 over expression.<sup>65</sup>

These results suggest that the study of p53 expression by immunohistochemistry is of interest in the surveillance of patients with Barrett's oesophagus, especially in those patients with a mucosa indefinite for dysplasia or with LGD.

## CONCLUSIONS

Barrett's oesophagus is now clearly recognised as a pre-neoplastic condition, which is diagnosed endoscopically with an histological confirmation, showing in most cases incomplete intestinal metaplasia, the so-called specialised mucosa. A diagnosis of dysplasia in Barrett's oesophagus has major clinical and therapeutic consequences, although numerous studies have demonstrated that it is not perfectly reproducible. Numerous markers that have been proposed in complement, most often issued from the improved knowledge of genetic and molecular processes involved in the carcinogenesis of Barrett's oesophagus. At the present time, only immunohistochemistry with antibodies directed against p53 protein and proliferation markers, and DNA flow cytometry, can be of some help. It is probable that in the near future techniques such as global gene expression profiling with DNA microarrays and proteomics will be of help in this field.<sup>66</sup>

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