Dysplasia and early neoplasia in Barrett's oesophagus

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Abstract

Over the last 20–30 years, oesophageal adenocarcinoma has increased six-fold in the west, the majority complicating Barrett's. The greatest risk is associated with higher grades of dysplasia. Although there is ongoing research into molecular alterations, which may be helpful in predicting progression to cancer, the main predictive indicator remains the histological identification and grade of dysplasia. Significant inter and intraobserver variability in the diagnosis of dysplasia is well documented and atypia can be seen in other settings including inflammation. Given the screening and management implications for the patient, a robust diagnosis is essential, such that agreement between two pathologists with an interest in gastrointestinal pathology is of paramount importance, together with regular communication between pathologists and clinicians.

This article reviews the literature and attempts to address some of the areas of diagnostic difficulty.

Keywords adenocarcinoma; Barrett's oesophagus; dysplasia; inflammation; oesophagus

In 1906 the pathologist Tileston first described "peptic ulcer of the oesophagus", with oesophageal epithelium around ulceration resembling that of the stomach but it wasn't until 1957 that Norman Barrett described the "lower oesophagus lined by columnar epithelium".

Barrett's oesophagus is now defined as metaplastic change from squamous to columnar-lined epithelium, visible endoscopically and confirmed histologically (Figure 1).

Epidemiology of Barrett's and associated neoplasia

Over the last 20–30 years oesophageal adenocarcinoma has increased six-fold in the West and possibly also in the East, where traditionally squamous cell carcinoma predominates. This increase apparently mirrors a similar increasing incidence of Barrett's oesophagus, the true rate of the latter is confounded by increasing recognition by endoscopists and variable referral practices for symptomatic heartburn and dyspepsia.[1] Prevalence is given as 1.5–10%, depending on whether or not there is a history of 'heartburn' in the population studied.[2] It is estimated that >80% of Barrett's oesophagus remains undiagnosed. Therefore, most Barrett's-associated adenocarcinomas will present *de novo*. For those patients with diagnosed Barrett's oesophagus the risk of progression is estimated to be between 0.2 and 0.4% which is lower than previously thought.[3–5] The variation in estimates is likely to be due to the different definitions. Endoscopically visible segments with confirmed intestinal metaplasia harbour the highest risk.

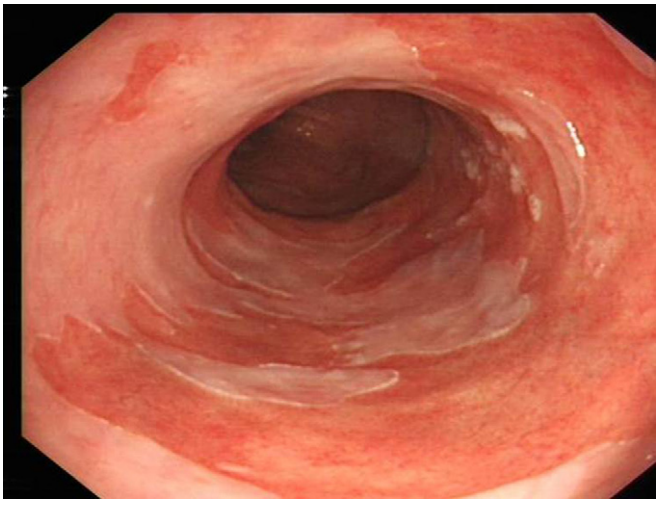

Figure 1 Barrett oesophagus.

Risk factors

Barrett's oesophagus occurs on a background of chronic gastro-oesophageal reflux disease,[6] and heartburn symptoms are an independent risk factor for oesophageal adenocarcinoma. The carcinoma increase may be related to the obesity epidemic, either through direct physical effects increasing reflux or indirectly through a metabolic syndrome.[7,8] Smoking is not a significant risk factor and modest alcohol consumption may have a protective effect.[9] The reasons for a male preponderance are unclear but an increased incidence in post-menopausal women raises questions about hormones and iron status.[10] Although dietary factors are difficult to study, a diet rich in fruit and vegetables appears to be protective[11]; dietary nitrates may increase the local oesophageal concentrations of potentially carcinogenic nitric oxide.[12] Any role for *H. pylori* infection remains unclear.

Molecular associations

Barrett's oesophagus is characterized by significant heterogeneity at the molecular level. The genetic alterations elucidated in progression to adenocarcinoma are predominantly in tumour suppressor genes and a profound increase in copy number. Loss of one functional p16 (CDKN2A) allele occurs prior to the onset of dysplasia in over 85% of cases,[13] generally caused by promoter methylation, less frequently by mutation. The mutation spectrum is consistent with oxidative damage and chronic inflammation.[14] This early clone expands until loss of the second allele occurs by loss of heterozygosity (LOH), thus creating a p16 null clone.[13,15] These changes appear to be initiating events, with

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no alterations in proliferation, the cells remaining diploid. TP53 is another well-known tumour suppressor gene, playing a controlling role in cell growth and protecting against accumulation of genetic errors.[16] Loss of p53 occurs by promoter CpG island methylation, mutation or LOH generally within the p16 null clones and results in an increased progression rate to cancer, with a relative risk of 16 compared to those with no loss. Depending on the mechanism for TP53 inactivation there may be nuclear accumulation of non-functional p53 protein, demonstrable by immunohistochemistry, with such immunostaining giving an odds ratio for adenocarcinoma development of 11.7 (95% CI 1.93, 71.4).[17] As dysplasia advances there are increased DNA tetraploid fractions followed by aneuploidy. Widespread cytogenetic abnormalities and TP53 LOH further increase future cancer risk.[18] At advanced stages there are often multiple different clones present and a higher degree of clonal diversity is associated with an increased risk of adenocarcinoma development.[16] The International Cancer genome project will for the first time enable a more accurate catalogue of the sequence of associated changes.[19]

As well as these DNA copy number changes, sequence alterations in the regulatory mechanisms, such as methylation and microRNAs which are important in gene silencing, are increasingly recognized to occur.[20,21] Inflammatory changes in the stroma may also be important in determining the likelihood of progression,[22] compatible with the idea that the inflammatory microenvironment can be considered the seventh cancer hallmark.[23] None of these molecular changes have yet entered clinical practice as biomarkers, although immunohistochemical p53 positivity is being used by some. Not all p53 aberrations will lead to protein accumulation and hence sensitivity is lacking.[24] Robust biomarker panels as adjuncts to dysplasia categorization would be most informative in view of increasing evidence for low progression rates.[25]

Endoscopic definition

There is international consensus that a clinical diagnosis of Barrett's oesophagus depends on an endoscopically visible segment, so-called ultra-short Barrett's (intestinal metaplasia on biopsy with no visible segment) being of uncertain clinical significance. The segment should be described using the Prague C, M criteria, C referring to circumferential and M to the maximal length. Due to inter and intraobserver variation in measuring this, segments of <2 cm are considered to be of dubious significance[26] although a cut off for surveillance is currently controversial.

Histopathology definition

In North America and parts of Europe, a histopathological diagnosis of Barrett's depends upon visible intestinal metaplasia (goblet cells)[27] but not in the UK.[28]

The American Gastroenterology Association have considered goblet cells a prerequisite since intestinal metaplasia was thought to have the highest risk of malignant progression and therefore, surveillance is restricted to these cases.[27,29,30] Rate of goblet cell identification is related to several issues: length of Barrett's, location of biopsies (more goblet cells in proximal oesophagus), number of biopsies, patient age and gender.[31] Recent studies have shown that the same frequency of DNA abnormalities in metaplastic epithelium with and without goblet cells.[29,32,33] Non-goblet glandular epithelium may occur prior to the formation of goblet cells, since it has been shown to have expression of intestinal immunohistochemical markers such as CDX-2, DAS-1, MUC2 and villin.[34,35]

Although revised British Society of Gastroenterology guidelines are in progress and due for publication shortly, the current guidelines[28] state that a diagnosis of "columnar-lined oesophagus", depends on the histopathologist being aware of the precise biopsy site, (distance from the incisor teeth and relation to gastro-oesophageal junction) and uses the following categories

- **Biopsies diagnostic for columnar-lined oesophagus (CLO)**

These comprise native oesophageal structures, with or without intestinal metaplasia.

- **Biopsies, corroborative of an endoscopic diagnosis of CLO, if taken from the anatomical oesophagus**

These biopsies show intestinal metaplasia.

- **Biopsies in keeping with, but not specific for CLO, if taken from the anatomical oesophagus**

Glandular epithelium present without intestinal metaplasia.

- **Biopsies without evidence of CLO**

Squamous mucosa only.

Microscopy

The columnar epithelium may be villiform or flat and contains a non-organoid collection of mucinous or oxyntic cells. Goblet cells, paneth cells or endocrine cells may also be present. Some chronic inflammatory cells are often admixed. Native oesophageal structures, typically ducts, may be included. It is important not to misinterpret pseudogoblet cells, which are widespread in Barrett's. These contain neutral mucin. If there is uncertainty, alcian blue will stain the goblet cells.

Dysplasia

Adenocarcinoma arises through a metaplasia-dysplasia-carcinoma sequence. The presence of any grade of dysplasia, based on strict criteria applied by specialist pathologists, using consensus reporting, is associated with a significant risk of disease progression.[3,4]

Low grade dysplasia (LGD) can persist for a long time or even regress — Weston et al showed 10% LGD progression, 25% persistence and 65% regression,[36] others have reported progression of less than 1% per patient year.[37,38] Because of significant interobserver variability in the diagnosis of LGD, it is difficult to be sure of it's natural history[37,39,40] although, recent studies based on pathologists agreement, show that cases of LGD are more likely to progress, estimating 13.4% per patient per year.[4]

High grade dysplasia (HGD) is associated with an increased risk of developing adenocarcinoma. Previous studies have reported 0–73% (mean 39.9%) of patients with a pre-operative diagnosis of HGD having adenocarcinoma on resections, however a large meta-analysis has revealed this figure to be 12.7%[41] A more recent randomized control trial suggests that 19% of cases with HGD progress per year.[42] With improvements in resolution of endoscopes and the taking of multiple biopsies there should be less chance of missing prevalent carcinoma.