Barrett's esophagus : The Metaplasia - Dysplasia - Carcinoma sequence : Morphological aspects

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Abstract

In the gastrointestinal tract, epithelial dysplasia is defined as an "unequivocal neoplastic transformation, confined within the boundaries of the basement membrane" or "the presence of unequivocally neoplastic cells that replace a variable proportion of the normal epithelium". It can be recognized by microscopy because of cytological and architectural changes. Reactive changes or equivocal changes should thus not be called "dysplasia". As dysplasia is confined within the basement membrane, it is a noninvasive neoplastic transformation. In the lower esophagus lined by columnar epithelium (Barrett's esophagus) dysplasia is classified as negative, indefinite or positive. Positive lesions are subdivided into low-grade and high-grade dysplasia according to the severity of the lesions. Carcinoma in situ (intraepithelial carcinoma) is included in the category of high-grade dysplasia. The presence of dysplasia can be recognized on biopsies and on cytological preparations. Several techniques have been introduced with the purpose to improve the diagnostic yield. These include special stains for the assessment of mucin, enzymehistochemistry and immunohistochemistry for tumor markers such as CEA and CA 19-9 and molecular techniques. Mucin histochemistry, enzymehistochemistry and immunohistochemistry for traditional markers have limited practical value. The nuclear presence of abnormal products such as mutant p53 can be identified using immunohistochemistry and appropriate antibodies. Flow cytometry can identify aneuploid cell populations and Fluorescent In Situ Hybridization (FISH) can identify chromosomal gains and losses. These techniques provide additional information but they identify other phenomena which do not necessarily appear at the same moment as dysplasia during the process of carcinogenesis. Application of these techniques can however certainly help to support a diagnosis of dysplasia while negative results do not necessarily disproof such a diagnosis.

The temporal course of the progression of dysplasia and the development of carcinoma is not well known and seems to be variable. Low-grade dysplasia may persist for long periods. A direct progression towards carcinoma has been noted although more often an increase in the severity of the dysplasia, before the development of carcinoma, was seen during the observation period. High-grade dysplasia can also persist for many months, sometimes even years without obvious evolution but it can also progress rapidly to carcinoma. (Acta gastroenterol. belg., **2000**, 63, **13-17**).

Key words : Barrett, esophagus, metaplasia, dysplasia.

Introduction

Barrett's esophagus is present in 0.8% to 2% of the patients undergoing upper gastrointestinal endoscopy and in 6 to 14% of patients in whom esophagitis is observed (1). According to some studies, 20% of patients with esophagitis will progress to Barrett's esophagus (2). About 20% of patients having endoscopy, with or without reflux symptoms, have histological evidence of intestinal metaplasia at the gastroesophageal junction (3). It is difficult to determine which patients should undergo endoscopy to screen for the presence of Barrett's esophagus. A high yield — low cost strategy was found for Caucasian males, over 50 yrs of age with heartburn longer than 5 yrs but the best strategy might be to do endoscopy in all Caucasians with heartburn (4). Two to 5% of patients with Barrett's metaplasia have a lifetime risk of Barrett's adenocarcinoma. The majority of clinical and experimental evidence supports the concept that adenocarcinoma in Barrett's esophagus develops as a stepwise progression from columnar epithelium through various stages of dysplasia to malignancy. This has been called "the metaplasia-dysplasia-adenocarcinoma sequence" (5). Hence it seems appropriate to look for dysplastic changes in the mucosa and to study the transition from metaplasia to dysplasia.

Barrett's metaplasia — Columnar epithelium lining the lower esophagus

Barrett's esophagus is a condition in which the normal squamous epithelium of the esophagus is replaced by a "metaplastic" columnar epithelium. The surface epithelium lining the lumen can be composed of "gastric type" mucous cells or "intestinal type" goblet cells. This can give rise to a mosaic pattern in which variable numbers of goblet cell are found as solitary cells or in small groups interspersed in between the gastric type cells. For the mucosa (surface and glandular epithelium) three different types of epithelium have been distinguished. These include a junctional or cardiac type, composed of acinar glands with mucosecreting cells and gastric type epithelial cells at the surface ; a fundic type mucosa with glands lined by parietal cells and gastric type epithelial cells at the surface and a specialized type or intestinal type with intermediate and goblet cells on the surface and in the glands (6). The number of epithelium types present in a patient may vary from only one to all three. The number and site of taking biopsy specimens will influence the detection of these. In several studies a "zonation" has been suggested with the specialized intestinal type of mucosa

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usually in a more proximal position close to the squamous epithelium and the fundic type mucosa more distally close to the cardia (7). This is however uncommon and usually the mucosa is heterogeneous and presents a mosaic pattern both at the surface and in its glandular part (8). An increase in the frequency of intestinal type epithelium with increasing length of the columnar- lined segment has been reported (9). In the majority of studies the intestinal type is seen most frequently.

Inflammation in Barrett's mucosa

Mucosal inflammation is common in Barrett's esophagus. Mononuclear cells as well as active inflammation characterized by the presence of neutrophils in the lamina propria and in the surface and glandular epithelium are often observed. The distribution of the inflammatory infiltrate has not been studied extensively but inflammation seems to be less common in the specialized "intestinal type" mucosa.

The following data were obtained in a series of 65 patients (31 male - 34 female ; mean age 51 +/- 1.8 yrs) with macroscopic Barrett's esophagus from whom 4 (3-8) biopsies were obtained for each patient. The features investigated included : a) the epithelial subtype in the surface (gastric type or mixed gastric and specialized) and glandular part (gastric - specialized - fundic types or mixed) ; b) the composition and intensity of the lamina propria infiltrate : lympho-plasmocytic ; mixed lympho-plasmocytic and granulocytes ; c) the distribution of the infiltrate : superficial or deep - proximal or distal ; and d) the relation between epithelial structures and the cellular infiltrate.

The glands were of the gastric type in 19 cases. A mixture of gastric type and intestinal type glands was found in 23 cases and in the remaining 23 cases all glands were of intestinal type. The surface epithelium was composed of purely gastric type epithelial cells in 14 cases (in 5 of these the glands were also gastric type but ducts of submucosal esophageal glands were present confirming the esophageal nature of the biopsies) of gastric and intestinal type cells in 42 cases (in 19 cases gastric type cells were predominating) and of intestinal type - specialized cells in 9 cases. In 5 patients surface and glands were purely of the intestinal-specialized type.

A lympho-plasmocytic infiltrate was present in all patients. The intensity was however variable. A transmucosal distribution was found in only 9/65. In 5/9 the glandular deeper part was composed of glands lined by specialized - intestinal type cells ; in 3 out of 9 the glands were a mixture of junctional - gastric type and intestinal type ; in 1 only gastric type glands were present. In 56 the infiltrate was limited to the superficial half of the lamina propria

Active inflammation, characterized by the presence of neutrophils was present in 34/65 cases. It was seen in 14/14 cases with gastric type surface epithelial cells, in 19/42 cases with a mosaic surface and in 1/9 with pure intestinal type metaplasia. In the 19 cases with a mosaic pattern showing inflammation, gastric type epithelial cells predominated in the surface. Neutrophils were mainly found in between surface epithelial cells.

Inflammation was mainly found in biopsies taken more proximal towards the gastroesophageal junction.

In general it seems that inflammation in Barrett's mucosa is usually mild and mainly located near the squamocolumnar junction. The cellular infiltrate is more commonly composed of mononuclear cells. Active inflammation is mainly observed in association with gastric type epithelial cells on the surface and is less common in intestinal type mucosa.

The etiology of the inflammation is unclear. Although Helicobacter pylori can colonize Barrett's mucosa, there is no convincing evidence to support the existence of an association between Helicobacter pylori and Barrett's esophagus (10,11). The inflammation is probably multifactorial in origin with gastroduodenal contents playing a major role and Helicobacter pylori infection being occasionally associated.

Pathogenesis of Barrett's metaplasia

The origin of the columnar metaplasia occurring in Barrett's esophagus is not precisely known. A congenital origin has been proposed but much more evidence supports the acquired nature. Barrett's metaplasia in the distal esophagus is most likely the result of severe and / or recurrent reflux of gastroduodenal contents into the esophagus with or without abnormal clearance of the refluxate. Reflux is responsible for epithelial cell damage and loss, associated with inflammation and balanced by esophageal defense mechanisms and healing. Yet assuming Barrett's metaplasia to be an acquired condition, the question arises where the columnar cell originates from. Several candidate cells have been considered such as epithelial cells of the gastric cardia mucosa, stem cells in the ducts of submucosal esophageal glands and basal stem cells of the squamous epithelium (12,13). The latter would present genuine metaplasia.

Epithelial cell healing involves proliferation of progenitor and / or stem cells depending on the extent of the damage. Stem cells are multipotent cells which means that they can produce cells which can differentiate into various directions. In reflux disease, healing of the esophageal epithelium occurs in an abnormal environment and this might also affect healing.

Gastroesophageal reflux initially affects the cells in the superficial compartment of the squamous epithelium. The regenerating inflamed epithelium contains immature squamous cells and these are sensitive to acid and bile damage (14). Through the loss of the superficial layers the functional stem cells in the basal zone at the tip of the papillae are in a relative superficial

position, making them more susceptible to the refluxate. These stem cells of the esophageal epithelium are considered to be pluripotent (5).

An acid environment might promote the production of acid resistant cells such as gastric type metaplasia or junctional type epithelium. The presence of bile in the gastroduodenal refluxate (and of Helicobacter pylori infection in the stomach) might promote a bile resistant lineage and the formation of intestinal or specialized metaplasia. A combination of both acid and bile would induce a mosaic pattern (15). This would explain why Barrett's mucosa is composed of a heterogeneous surface and glandular epithelium and an inflamed lamina propria.

Barrett's metaplasia is highly abnormal

Barrett's mucosa is an inflamed heterogeneous tissue with a disturbed architecture. This has several consequences. Active inflammation is associated with the production of metabolites inducing varying degrees of cell damage. Because of the heterogeneity, epithelial cell cohesion may be less well developed resulting in increased permeability. Epithelial cell cohesion normally depends upon the molecules of the cytoskeleton and the junctions. There are molecular differences between intestinal type and gastric type cells and hence cell-cell adhesion might be impaired. A reduced e-cadherin expression has indeed been observed in Barrett's columnar metaplasia (16). The proliferative compartment is less well delineated in the metaplastic epithelium (17,18). The expression of transforming growth factor beta-one is enhanced in the metaplastic epithelium of Barrett's esophagus. A variable augmentation of ras proteins (protooncogenes) has been shown to occur in Barrett's junctional and specialized epithelium, and, although rarely, accumulation of the p53 protein can occur in the nondysplastic metaplasia (19-21).

Barretts metaplasia can contain cytogenetically abnormal clones that occupy extensive regions of the Barrett's segment and persist for several years and aneuploidy has been observed in nondysplastic columnar epithelium (22). All these changes may well prepare the metaplasia for a dysplastic transformation which is one of the early steps in carcinogenesis (23).

Dysplasia in Barrett's metaplasia

Dysplasia is a morphological term which etymologically means "malformation". It is derived from classic Greek and composed of "dys" which means "bad" and "plasis" which means "form". The origin of a malformation can be variable. It can be a macroscopic or microscopic lesion and congenital (hereditary or not) or acquired. If acquired, the nature of the dysplastic transformation can be regenerative (due to healing and repair following damage), or neoplastic (degenerative). When used for microscopic epithelial changes of the gastrointestinal tract, dysplasia is defined as "Unequivocal, noninvasive (confined within the basement membrane), neoplastic transformation of the epithelium excluding all reactive changes" (24).

This definition stresses the nature and origin of the lesion while its identification relies upon the recognition of morphologic features resulting from cytological and architectural changes in routinely processed and haematoxylin and eosin stained sections. Biopsies from patients with Barrett's esophagus have to be examined for the presence of dysplasia. A distinction is made between three possible conditions. A biopsy can be negative for dysplasia (normal architecture and cells), indefinite (normal architecture, cytological alterations such as hyperchromatic nuclei) or positive (normal or variably altered architecture and cytological abnormalities). When positive, dysplasia is usually subdivided into low- and high-grade dysplasia. In low-grade dysplasia the architectural abnormalities are limited but cytological abnormalities are definitely present. The cells lining the surface and crypt are tall columnar cells showing lack of maturation. Mucus production is decreased. The nuclei are elongated, hyperchromatic and there is a tendency towards stratification. Mitotic figures can appear in the surface. In high-grade dysplasia the cytological and architectural abnormalities are more pronounced. Cellular and nuclear pleomorphism appear with loss of polarity and increased stratification and the glands are highly irregular. Intraepithelial carcinoma (carcinoma in situ), typically recognized by the presence of cribriformed glands and / or marked nuclear aberrations, is included in the category of high-grade dysplasia. Some authors make a distinction between three grades of severity for dysplasia : mild, moderate and severe. The first two are included in low-grade dysplasia in the two grade system, and severe dysplasia is included in high-grade dysplasia.

In 11 series of Barrett's esophagus without visible carcinoma, totaling 438 patients dysplasia was present in 10% of cases, with high-grade dysplasia occurring in only 2%. The mean incidence in 16 published series complicated by carcinoma was 78% (59 - 100) (25). The presence of dysplasia can be recognized on biopsies and on cytological preparations. A positive diagnosis of low-grade dypslasia is not always simple. Interobserver studies have shown that the agreement for the diagnosis of low-grade dysplasia in Barrett's esophagus is less good than for high-grade dysplasia (26). Several techniques have been introduced in order to improve the diagnostic yield. These include special stains for the assessment of mucin, enzymehistochemistry and immunohistochemistry for tumor markers such as CEA and CA 19-9 and molecular techniques. Mucin histochemistry, enzymehistochemistry and immunohistochemistry for traditional markers have limited practical value. The nuclear presence of abnormal proteins such as mutant p53 can be identified using immunohistochemistry and appropriate antibodies and can be useful. A negative finding does not exclude the presence of dysplasia however. Flow cytometry can identify aneu-

ploid cell populations and Fluorescent In Situ Hybridization (FISH) can identify chromosomal gains and losses. These techniques provide valuable additional information, but as they do not identify the same phenomenon which is responsible for the development of dysplasia, they do not replace the morphologic diagnosis.

Development of Dysplasia

Cancer development is associated with defects in genes controlling cell proliferation and cell death and with defects of genes controlling cell structure. Aberrant expression of proteins and abnormalities in genes coding for proteins involved in the preservation of cell shape and cohesion such as cytokeratins, CD44 and e-cadherin have been reported in epithelial dysplasia and in many cancers. Cytokeratins are members of a large family of proteins that compose the intermediate filaments in the cytoplasm of most, if not all, epithelial cells. The intermediate filament system and its associated proteins represent a chain of molecular connecting links between the nucleus and the cell surface. They are important for tissue structure and integrity. E-cadherins are adhesion molecules that play a key role in the organization and maintenance of the epithelial structure. They mediate cell-cell adhesion. Abnormalities in the structure or loss of these proteins, due to genetic defects, will result in changes in cellular shape and size (cytology) and in defects in cell cohesion (architecture) and polarity. Loss of polarity is one of the features used to diagnose dysplasia.

The microscopic features upon which a routine diagnosis of dysplasia is based are most probably related to genetic defects controlling proteins involved in the maintenance of cell shape and cohesion and to genetic defects controlling cell proliferation and death. Dysplasia is thus the result of multiple events. Its development may further be influenced by growth factors such as Epidermal Growth Factor and Epidermal Growth Factor Receptor.

In Barrett's dysplasia, cells have indeed proliferative controls that are uncoupled from the appropriate regulatory systems. An altered DNA content (aneuploidy) can be demonstrated by flow cytometry in Barrett's metaplasia. Several chromosomal abnormalities including Y chromosome losses, trisomies, and translocations involving chromosomes 7 and 11, have been documented in Barrett's adenocarcinoma and adjacent metaplastic epithelium. This may result of altered expression of cytokines and growth factors and of the acquisition of genomic alterations of cell cycle associated genes (5,15). The cell cycle genes include increased cyclin D1 expression (chromosome 11q13) and mobilization of cells from G0 to G1. Furthermore, p53 mutations are acquired progressively being present in 65% of cases with low-grade dysplasia and in 75% of cases with high-grade dysplasia. High-grade dysplasia is associated with decreased apoptosis and transformation towards carcinoma is associated with decreased cell adhesion.

Natural History of Dysplasia

These different genetic events occur in a certain order accompanying the morphologic transition from metaplasia towards low- and high-grade dysplasia and invasive adenocarcinoma and the etiology of these events is not known. This explains why it is difficult to predict the natural history of dysplasia. Low-grade dysplasia may persist for long periods. A direct progression towards carcinoma has been noted although more often an increase in the severity of the dysplasia, before the development of carcinoma, is seen during the observation period. High-grade dysplasia can also persist for many months, sometimes even years without obvious evolution but it can also progress rapidly to carcinoma. In a Dutch study high-grade dysplasia persisted for 36 and 44 months respectively in two patients, without evidence of further neoplastic progression while in 5 patients the time lag between the finding of low-grade dysplasia and cancer varied from 1.5 to 4 years (27). In a study by Reid et al., four patients with high-grade dysplasia remained histologically stable after a mean follow-up time of 14 months (range : 11-20 months). Progression to adenocarcinoma occurred in an average period of 14 months (range : 5-21 months) (28). In a prospective study of 81 patients, three patients developed adenocarcinoma. In two of these, high-grade dypslasia persisted for a period of 2.6-4.5 years before the discovery of adenocarcinoma on biopsy. The third patient had documented low-grade dysplasia during 4.3 years before the development of the adenocarcinoma (29). High-grade dysplasia, persistent over a period as long as 5 years was noted in one patient in a series of 34. In the same series another patient developed invasive cancer in a four year period (30). In some studies a transition from high-grade dysplasia towards invasive cancer is noted to occur in less than one year (31). In these patients it is however difficult to rule out the possibility that the cancer existed already at the initial endoscopy because of the low number of biopsies taken. In a study of 70 patients undergoing regular endoscopic biopsy procedures as part of their prospective surveillance, 12 were found to have early esophageal carcinoma on prompt follow-up endoscopy (mean, 2 months). Fifteen patients progressed to carcinoma over a period of 27 months (range 12-72) while 43 patients (74%) remained stable or regressed to less severe histologic diagnoses during a mean follow-up of 30 months (32).

Several studies report regression of dysplasia, even high-grade dysplasia in Barrett's esophagus, either spontaneously or after surgical or medical therapy but this remains highly controversial. Since neoplastic progression is associated with genetic abnormalities, spontaneous regression of dysplasia (defined as unequivocally neoplastic) is highly unlikely. A regression

towards a less severe stage has been observed but such a regression or an absence of dysplasia on follow-up biopsies is more likely explained either by sampling error or by inter- or intraobserver error (33,34).

Conclusion

Barrett's mucosa is a special type of metaplasia because it is usually composed of a mixture of different types of epithelium and it is also usually inflamed. It is a associated with an increased risk for cancer. The development of an invasive adenocarcinoma follows a multistep process from metaplasia, through different stages of dysplasia : the metaplasia - dysplasia - adenocarcinoma sequence. This progression is characterized by the occurrence of multiple genetic events. These induce defects in genes controlling cell proliferation and cell death and of genes controlling cell and tissue structure. The genetic events follow a certain order. Dysplasia can be identified by morphological techniques. Additional techniques identifying the genetic defects or their consequences are extremely useful but they do not identify the morphologic lesion of dysplasia, which itself is the result of a complex process involving different defects.

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