

## **Markers of malignant progression in Barrett's metaplasia (Barrett's esophagus)**

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### Abbreviations used:

EA: Esophageal adenocarcinoma; BM: Barrett's metaplasia; ND: Negative for dysplasia; IND: Indefinite for dysplasia; LGD: Low grade dysplasia; HGD: High grade dysplasia; IMC: Intramucosal carcinoma

## **INTRODUCTION:**

The incidence of esophageal adenocarcinoma (EA) has been rising steadily in the United States and western Europe since the mid 1970s. For yet unknown reasons, this increase is most profound in white males. However, the incidence of EA in white females has increased about five folds during the same time interval. Once rare, EA is now one of the 15 most common cancers in the U.S. and is associated with high mortality rate. The difference in 5-year survival between T1 tumors and T2 tumors, 85% vs. 40% respectively, is strikingly large and is the main reason why great emphasis is placed on early detection and treatment.

The precursor of EA is Barrett's esophagus/Barrett's metaplasia (BM).

Barrett's esophagus is defined as columnar epithelium-lined esophagus of any length (<3 cm : short segment; > 3 long segment). Goblet cells are ESSENTIAL for the diagnosis; they may be absent in dysplastic epithelium.

Previously three histologic patterns were called BM, one of which is the specialized columnar metaplasia in which goblet cells are present. Only this type of metaplasia is associated with malignant potential. For practical purposes, and because of the implications for clinical management, it is now widely accepted that the diagnosis of BM is reserved only to cases where goblet cells are present. This is what most (if not all) of your gastroenterologists will assume when you use the term "Barrett's metaplasia" or "Barrett's esophagus" in your diagnosis, so please be very careful with this distinction.

Goblet cells in BM resemble intestinal goblet cells. They are goblet-shaped cells with wider side to side diameter, slender elongated nucleus, and on H&E stain show bluish mucinous material filling almost all the cytoplasm with distinct coarse granularity. Alcian Blue stain should never be used for diagnosing BM. Positive staining with Alcian Blue will be seen in far more cases than those with true goblet cells, and will lead to misdiagnosis and unnecessary patient anxiety, almost life long follow-up with associated discomfort, cost, ..etc. If you are not sure that what you see on H&E are goblet cells, they are probably not goblet cells.

The dysplasia-carcinoma sequence in BM is well established. In almost all cases with well documented follow-up biopsies, BM negative for dysplasia (ND) progresses to EA through first transformation into low grade dysplasia (LGD) followed by high grade dysplasia (HGD), and finally EA. Not all cases in a lower grade progress to a higher grade. Because of this known sequence of events, histologic grading of dysplasia is the cornerstone for current surveillance programs aimed at early detection of EA in patients with BM. During a scheduled endoscopy procedure for a patient with BM, four quadrant biopsies are taken every 2 cms from the segment of esophagus showing endoscopic changes suggestive of BM, using either regular or jumbo forceps. The number and sampling may be increased in patients with dysplasia, and persons with higher grade of dysplasia are followed up at closer intervals. There are many variations in surveillance frequency among gastroenterologists (1). The action taken following a diagnosis of HGD is more controversial, but usually leads to esophagectomy in most institutions. With available new technology, some patients with HGD, or even IMC, are treated with ablative therapy, especially if they are not good surgical candidates.

### **MARKERS OF MALIGNANT POTENTIAL IN BM:**

In the past few years, several markers have been proposed. However, Dysplasia, p53 protein accumulation, and DNA ploidy are the most promising. Because their value have been demonstrated by several independent groups, and they have been tested on follow-up biopsies, they are either already in practice (dysplasia grading), very close to being adopted into practice (p53), or close (DNA ploidy).

#### **A. Dysplasia**

Morphological grading of dysplasia: REALLY WORKS. EA detected in patients with BM who are in surveillance programs are usually at a lower stage and are associated with significantly better prognosis than EA detected in patients who are not in surveillance programs (2). Moreover, it does not cost extra money.

However, several serious problems exist:

1. There is significant inter- and intra-observer variability in grading dysplasia even among experts (3).
2. Not all cases with dysplasia progress to higher grade or carcinoma (4, 5).
3. Sometimes it is difficult to distinguish high grade dysplasia from carcinoma in biopsy material, so a combined HGD/CA diagnosis has been recommended (6).
4. The difficulty in distinguishing LGD from IND lead some investigators to suggest combining them in one category IND/LGD (6), which dilutes the predictive value of LGD.
5. For a confident diagnosis of dysplasia, the characteristic morphologic changes should involve the surface epithelium (7), however:
  - a. If dysplastic changes involve glands but not the surface epithelium, in the absence of active inflammation, not even the experts can guarantee that it will not progress to HGD or CA.
  - b. In many patients with BM who are treated with proton pump inhibitors, with or without ablation therapy, there is full or partial re-epithelialization of the mucosal surface with squamous epithelium. Barrett's metaplasia has been detected underneath the squamous epithelium in 30% of the cases (8, 9). Since the potential of progression to HGD/CA remains despite successful treatment of acid reflux (10-14), and a confident diagnosis of LGD can not be made in cases showing all histologic criteria for a diagnosis of LGD but have squamous epithelium at the surface, this is a serious problem that is likely to get even worse.
6. Because of the relatively low sensitivity of dysplasia diagnosis, the cost of surveillance is high.

#### **B. p53 protein accumulation as detected by immunohistochemistry.**

We previously hypothesized that p53 protein accumulation may be an objective marker of malignant potential in BM (15). More importantly, we have later shown that p53 protein accumulation is a better marker of malignant potential in BM than a diagnosis of LGD, having a sensitivity of 100%, specificity of 93%, and a predictive value of a positive test of 0.56, compared to sensitivity of 100%, specificity of 64%, and predictive value of a positive test of 0.2 for a histologic diagnosis of LGD/IND (16).

Three subsequent independent studies have confirmed our findings (17-19).

Those who criticize the role of p53 immunostaining as a marker of malignant potential in BM usually cite two studies to support their argument (20) :

1. The study by Hamelin et al: They claim that this study show no correlation between p53 immunostaining and p53 gene mutation (21).
2. The study by Bani-Hani et al: They claim that using cancer as an end-point, there is only a non-significant trend for increased risk of progression in patients whose biopsies were p53 immunoreactive (22)

HOWEVER:

1. In the study by Hamelin et al.:
  - a. Positive p53 immunostaining correlated with p53 gene mutations in 13 of 14 (93%) samples of BM, dysplasia and carcinoma, which is not bad.
  - b. Negative p53 immunostaining was associated with p53 gene mutation in 6 of 17 (35%) such samples. This is the bad part, but
    - i) The apparently high false negative rate of p53 immunostaining may be significantly less than reported, because the authors considered immunostaining to be positive only when nuclear staining was present in the majority of epithelial cells, whereas the sensitive molecular techniques that they used “could detect p53 gene mutations even if present in a single cell in the same tissue”.
    - ii) They studied p53 mutation in the mutational “hot spot”, which is exons 5-8; some mutations may be present outside the hot spot.
    - iii) Certainly, some p53 mutations are not associated with protein accumulation, however, since it has not been shown that these cases progress to HGD/CA, the notion that p53 protein accumulation has a high false negative rate is meaningless.
2. In the study by Bani-Hani et al.
  - a. There was no uniform biopsy protocol: “during the period of this study, the number and site of biopsies were not standardized”.
  - b. Only 45% of the cancers were p53-positive (technical problems?)
  - c. In the discussion it was stated that “not all biopsy and tumor specimens were analyzed for p53 protein because the limited amount of specimen remaining on the histologic block after Cyclin-D1 analysis”
  - d. Histologic and Immunohistochemical Assessment: “p53 staining was graded as ..... 0 (no visible staining), 1+ (low-intensity staining in at least part of a section), or 2+ (high-intensity staining in at least part of the section). However, because most p53 staining was of low intensity and focal, the data was dichotomized to + (positive) and 0 (no visible

staining) for statistical analysis.”

- i. Again, the fact that in most cases p53 staining was weak and focal indicate some sort technical difficulty.

### **DNA Ploidy:**

Using image analysis of Fuelgen-stained tissue sections, James and Atkinson were the first to propose that DNA aneuploidy is a marker for malignant progression in BM(23). Later, Reid et. al, using flow cytometry, showed that not only aneuploidy, but increased G2M/tetraploidy is a significant predictor of malignant potential (6). We have recently confirmed these findings in a study using image analysis on tissue sections, and we suggested that during malignant progression in BM, increased G2M/tetraploidy precedes p53 protein accumulation, which is followed by aneuploidy, then HGD/CA (24).

The major criticism for DNA ploidy is purely technical.

#### 1. Flow cytometry:

- a. It is not possible to correlate DNA ploidy status with the grade of dysplasia in the same biopsy sample. Although the tissue adjacent to that submitted for flow cytometry is usually assessed morphologically for dysplasia, this may not necessarily represent the portion of the biopsy submitted for flow cytometry because dysplastic changes can be patchy and may be limited to few glands.
- b. A minimum of 10,000 events (not including debris or aggregates) is necessary for reproducible determination of S-phase ( and, similarly, G2M), and more than 10,000 events are needed when the proportion of DNA aneuploid events is small (25).

#### 2. Image analysis:

The advantage of this technique is that DNA ploidy can be assessed on the very same biopsy used for morphologic evaluation using adjacent step sections. Another advantage of image analysis over flow cytometry is that the former can be done on fewer cells. However, nuclear overlapping force the operator to exclude a few nuclei from the analysis, although this did not seem to have practical significance in the study by James and Atkinson or in our recent study.

### **Final note:**

A prospective study in which the combined use of p53 immunostaining, DNA ploidy determination by image analysis, and dysplasia grading, using both biopsy and cytology material, is currently in progress at our institution. The ultimate goal is to devise a more accurate and cost-effective surveillance program that can be used for early detection and to monitor patient response to chemoprevention and other non-surgical treatment modalities.

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