Usefulness of histopathological markers in diagnosing Barrett's intraepithelial neoplasia (dysplasia)

A. Jouret-Mourin[1], C. Sempoux[1], K. Ho Minh Duc[1], K. Geboes[2]

(1) Dept of Pathology, UCL, Brussels ; (2) Dept of Pathology, KUL, Leuven.

Abstract

The incidence of oesophageal adenocarcinoma has significantly increased in Europe over the last 30 years. The progression from normal mucosa to adenocarcinoma has been associated with genetic and morphological traits regrouped under the term "intraepithelial neoplasia" (IEN) according to the Vienna classification. The early detection of such lesions represents the first step in the identification of high-risk patients. The morphological criteria of IEN are the gold standard to identify such patients.

Firstly described by Riddell et al in 1983, IEN is based on morphological criteria including both cytological and architectural alterations and is classified into different stages of severity. However, large studies have clearly demonstrated the lack of reproducibility, with large inter-individual discrepancies for both discrete and severe lesions.

Discrepancies between high grade IEN and adenocarcinoma can be minimized by using the Vienna classification, which groups both of these lesions under the "stage IV".

Discrepancies between low-grade IEN and uncertain lesions remain too important. Erroneous and overstated diagnosis of low grade IEN induces an unnecessary follow-up of patients with obvious psychological and economic consequences. Recent studies have demonstrated that the reading of the slides by 2 to 3 gastrointestinal (GI) pathologists significantly decreases interpretation mistakes.

Because of these interpretation problems, scientists have looked for non-morphological criteria to confirm the pre-cancerous state. The current PubMed literature proposes many putative biomarkers. However, none of these has been correctly validated in large prospective case-control studies, which hampers their use in clinical routine.

DNA quantification by flux cytometry and morphometry represent alternative methods of documenting IEN but these techniques are complex and expensive. The use of the proliferation marker Ki67 needs deep sampling with correct orientation and standardized cell counting. P504 S has been studied in Barrett's disease and might be a novel tool. The only promising tool thus far is the overexpression of p53 as shown in prospective studies demonstrating a nice correlation with clinical evolution and is easy to use in clinical routine. (Acta gastroenterol. belg., **2009**, 72, **425-432**).

Key words : Intraepithelial neoplasia, dysplasia, Barrett, p53, Ki67, AMACR.

The incidence of oesophageal adenocarcinoma has steadily risen in the Western World during the last 30 years. It varies from one in 52 to one in 441 cases per patient year, thereby exceeding, in white males, that of tumours of the colo-rectum, lung, prostate, and skin (1,2). Barrett's oesophagus (BO) nowadays represents the most common cause of oesophageal adenocarcinoma. The progression from normal mucosa to adenocarcinoma in the oesophagus has been associated with genetic and morphological traits regrouped under the term "dysplasia" which, for the digestive system, is synonymous to intraepithelial neoplasia (IEN). The risk of developing cancer increases with the degree of severity of IEN. In comparison to controls, patients with Barrett's IEN have a 30-to 100 fold higher risk of developing oesophageal adenocarcinoma. Therefore, the early detection of any dysplastic changes represents the first step in the identification of high-risk patients and the rapid initiation of endoscopic therapy or curative surgical resection.

Morphological criteria of dysplasia

Proposed by Riddell *et al.* in 1983, the term "dysplasia" firstly was defined as an unequivocal non invasive neoplastic transformation of the epithelium excluding all reactive changes (3). Following the Vienna Classification in 2000 (4) the idiom "intraepithelial neoplasia", which is more descriptive and recommended in BO by the World Health Organisation and other experts' consensus reports, progressively replaced the expression "dysplasia" (4,5,6).

The Vienna Classification includes a grading score based on the cytological aspect and the criteria of invasion in order to resolve nomenclature discrepancies between Western and Japanese pathologists. Indeed, the "Western diagnosis" of carcinoma requires an invasion, which is defined by a tumoral involvement of the lamina propria, whereas the "Japanese criteria" of carcinoma are exclusively based on cytological and architectural changes. Consequently, Japanese pathologists regularly use the expression "mucosal carcinoma", without stating whether or not there is invasion into the lamina propria.

Definition of IEN refers to morphological criteria including both cytological and architectural alterations established by an international consensus (2-10) (Table 1). The architectural abnormalities include structural distorsion, elongation with budding, branching, dilatation and intraluminal folding, irregularly shaped

Correspondence to : Prof. A. Jouret-Mourin, Dept of Pathology, Cliniques Universitaires St Luc, Avenue Hippocrate 10, 1200 Bruxelles. E-mail : anne.mourin@uclouvain.be

Submission date : 03/08/2009
Acceptance date : 04/11/2009

Table 1. — **Morphological criteria for intraepithelial neoplasia**

Low-grade intraepithelial neoplasia
– Preserved crypt architecture or mild architectural alterations
– Stratified nuclei without loss of cellular polarity
– Enlarged hyperchromatic nuclei
– Presence of mitotic figures in the upper portion of crypts
– Surface maturation similar to deeper glands
High-grade intraepithelial neoplasia
– Distortion of crypt architecture with branching, lateral budding or cribriform pattern
– Extension of the abnormalities to the mucosal surface
– Pronounced nuclear stratifications
– Loss of nuclear polarity
– Nuclear pleomorphism
– Mitotic activity and abnormal mitosis

glands and bridging. Regarding the cytological abnormalities, the glands are lined by numerous tall cells with abnormal cellular differentiation and loss of mucus production, as well as stratified cells with hyperchromatic nuclei which are variable in size and shape. Mitoses are more frequent than usual, and can be found in surface epithelium. Cytologically, the nuclei are large and irregular, and may contain nucleoli. The nuclear to cytoplasmic ratio is increased, with a loss of nuclear polarity.

Although neoplastic transformation is a continuum, IEN has been conventionally classified into different stages. The grading criteria rely on the severity of both cytological and architectural abnormalities, and the classification of IEN in BO is an adaptation of that originally used for inflammatory bowel diseases, which distinguishes three categories, i.e. negative, indefinite and positive for IEN (3).

"Indefinite for intraepithelial neoplasia" (ID) refers to cases in which the cellular changes are not sufficient for the diagnostic of IEN, but are too important for being neglected. Practically, it consists of light distorted architecture with loss of mucus production and a surface maturation associated with increased basophilia, sometimes in a context of inflammation. Nuclear abnormalities can be observed, but are less severe than those seen in typical dysplasia. Mitoses are increased in deeper glands, with a normal aspect.

The "positive for intraepithelial neoplasia" (PD) category is subdivided into low- and high-grade upon the extent of cytological and architectural changes.

In low-grade intraepithelial neoplasia (LGD), crypt architecture shows mild alterations, with glandular crowding. The surface maturation is similar to deeper glands. Of note, preserved crypt architecture can also be found, with no distinction from non dysplastic epithelium. However, dysplastic nuclei are enlarged, elongated, hyperchromatic and stratified without loss of cellular polarity (Fig. 1a,b). Mitotic figures may be present in the upper portion of crypts. The presence of an abrupt transition between non dysplastic and dysplastic epithelium, with uniform nuclear changes extending evenly from the crypt base to the mucosal surface, is helpful for diagnosing LGD (8). Indeed, the presence of surface maturation in an atypical crypt is a feature that would usually help pathologists exclude the diagnosis of IEN in favour of crypt regeneration. However, Lomo *et al.* have described dysplastic-like atypia limited to the bases of the crypts without involvement of the surface epithelium. Such lesions were previously considered as "ID" by most authors, but may represent a proper subtype of IEN (11,12).

In contrast to LGD, high-grade intraepithelial neoplasia (HGD) is characterized by severe architecture abnormalities, i.e. distortion of crypts, branching, crowding, lateral budding or cribriform pattern, which usually extend to the mucosal surface (Fig. 2a,b). In addition, severe cytologic changes, like pronounced nuclear stratifications, loss of nuclear polarity, pleomorphism, nucleoli, and mitotic activity, are observed.

Inter-observer variability in intraepithelial neoplasia assessment

Inter-observer variability in diagnosing IEN is unavoidable, and mainly due to the subdivision of the continuous spectrum of tumour development into distinctive categories and the wide spectrum of features. Large studies have clearly demonstrated the lack of reproducibility of IEN categories, with large intra and inter-individual discrepancies for both discrete and severe lesions (7-10,13-14).

Discrepancies between high-grade IEN and adenocarcinoma (AD) can be minimized by using the 2000 Vienna Classification (4,15) (Table 2). Indeed, the category 4 includes all lesions with morphological features more severe than those seen in LGD, without unequivocal invasion. These patients with such severe lesions need an endoscopic mucosal resection.

There remains a considerable inter-observer variability among pathologists concerning the diagnosis of ID and LGD. These categories show the lowest k values, and thus the highest variability (1,8,13,16-17). Regenerating epithelium can be difficult to distinguish from true IEN, particularly in case of significant inflammation and/or ulceration in the biopsy specimen. Differentiating reactive changes from LGD is a challenge for most pathologists. An erroneous and overstated diagnosis of LGD is associated with unnecessary follow-up of patients, with direct psychological and economic consequences.

Inter-observer discrepancies occur not only between general pathologists and pathologists with specific expertise in gastro-intestinal (GI) diseases, but also between GI experts. In 2007, Kerkhof *et al.* demonstrated such inter-observer variability in the assessment of IEN grade in a large prospective multicentric study. The inter-observer reproducibility between the initial diagnosis of general pathologists and the final diagnosis of a panel of experts for the grade of dysplasia was fair

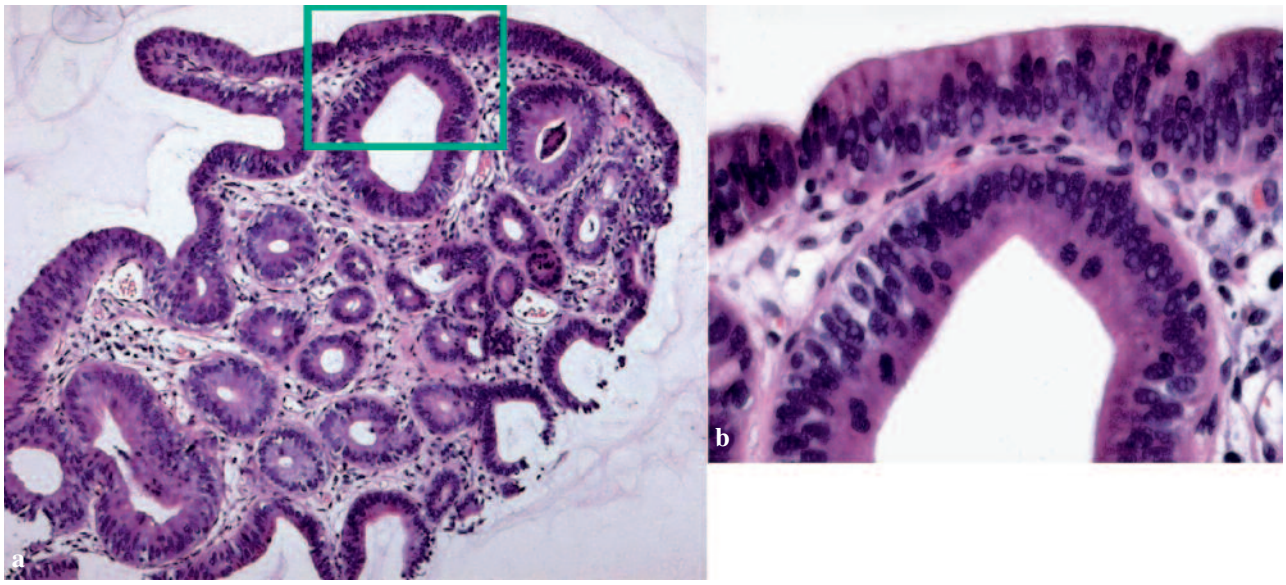

Fig. 1a,b. — Low grade Intraepithelial Neoplasia. HE. ×10 (a), ×40 (b)

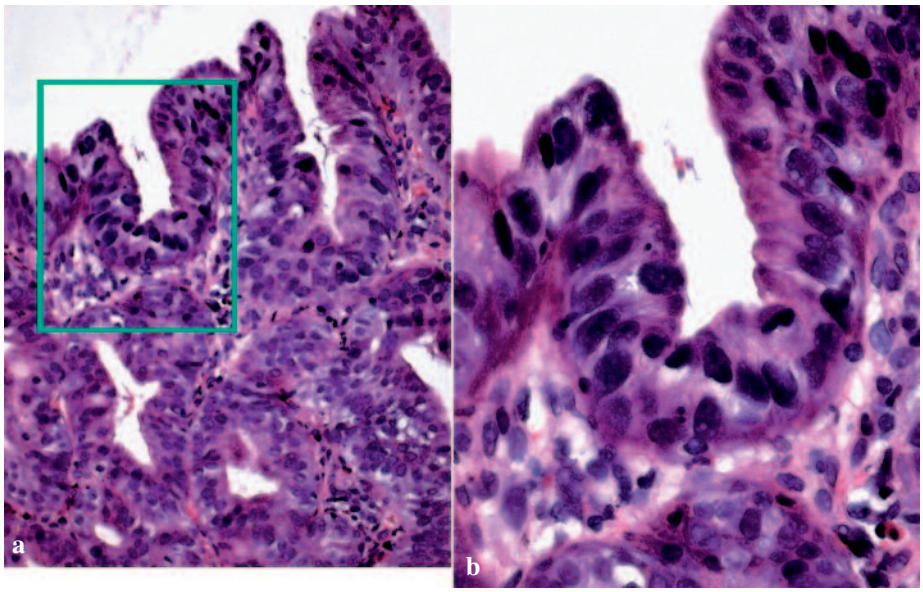

Fig. 2a,b. — High Grade Intraepithelial Neoplasia. HE. ×20 (a), ×40 (b)

(k = 0.25). However, the evaluation of inter-observer variability between GI pathologists highlights a poor agreement between two expert panels (14). Pech *et al.* analysed the divergence between the diagnoses of general and specialized GI pathologists, and further questioned the validity of LGD diagnosis. Among 50 cases regarded as LGD by general pathologists, only half of them was confirmed by GI pathologists. 21 patients presented Barrett's metaplasia without IEN, and 4 patients were categorized as HGD (16). Skacel *et al.* reviewed a cohort of 25 patients with an original diagnosis of LGD, and calculated a fair (k = 0.28) to poor (k = 0.20) agreement between GI pathologists. These authors further showed that, when a consensus diagnosis of LGD came out among GI pathologists, an increased risk of progression from low-grade to high-grade IEN was observed (17). In the study of Reid et al, experienced GI pathologists only showed 60% agreement in the comparison of biopsies that where negative for IEN to those considered as ID or LGD (9). Similarly, Montgomery *et al.* have published a substantial agreement among 12 GI pathologists for the diagnosis of HGD (k = 0.65), but only fair (k = 0.32) and slight (k = 0.15) agreement for LGD and ID, respectively. The percentage of cases agreed by experienced GI pathologists was much lower for low-grade histological spectrum in comparison to HGD (13).

Table 2. — **Vienna Classification (4)**

Classification of gastrointestinal epithelial neoplasia
CATEGORY 1 Negative for neoplasia/dysplasia
CATEGORY 2 Indefinite for neoplasia/dysplasia
CATEGORY 3 Non-invasive neoplasia low grade (low grade adenoma/dysplasia)
CATEGORY 4 Non-invasive neoplasia high grade 4.1. High grade adenoma/dysplasia 4.2. Non-invasive carcinoma (carcinoma in situ)[a] 4.3. Suspicious for invasive carcinoma
CATEGORY 5 Invasive neoplasia 5.1. Intramucosal carinoma[b] 5.2. Submucosal carcinoma or beyond

[a] *"Non-invasive" stands for absence of evident invasion.*
[b] *"Intramucosal" stands for invasion into the lamina propria or muscularis mucosae.*

In summary, these studies firstly emphasize the need for a second opinion from a GI pathologist before establishing the diagnosis of IEN and its major therapeutic decisions and consequences for the patient. In addition, the important discrepancy regarding IEN grading, even between expert GI pathologists, requires the identification and the validation of non-morphological criteria to help physicians identifying pre-cancerous states.

Non-morphological markers of intraepithelial neoplasia

To improve the diagnostic accuracy of IEN, the scientific community has recently focused on the identification and validation of non-morphological criteria. More than 60 potential biomarkers have been proposed, but few if any have confirmed their initial promises (1,18,19). Morphometry and DNA content detected by flow cytometry have been assessed (20-24). In addiction, distinct types of tissue biomarkers, such as p53 and Ki67, have been evaluated by immunohistochemistry (25-36). Recently, a methylacyl-coA racemase has been described as a novel putative useful biomarker (37-42).

Morphometry

Morphometry which is a quantitative measurement of morphological characteristics requires dedicated and expensive equipment, as well as an expertise which is not universally available for daily routine (21). Furthermore, morphometry provides no solution for technical issues like tangential cutting or severe inflammation, as recently reviewed and described by Baak *et al.* (22).

Flowcytometric analysis of DNA content

Flowcytometric analysis of DNA content has been reported to be promising in determining the risk of progression to malignancy. Reid et al have demonstrated in a series of 322 BO patients that patients with ND, IND or LGD in oesophagus biopsies and an aneuploid or tetraploid nuclear DNA content had a 28% 5-year-cumulative risk of developing oesophageal adenocarcinoma in comparison to 0% for patients without DNA content abnormalities in baseline biopsies (23). Unfortunately, flowcytometric abnormalities can also be found in a subset of patients without IEN. Conversely, some patients with HGD can only show diploid cell population (2,23). Flowcytometric analysis of DNA content is currently assessed as a routine biomarker in phase IV development studies in some selected specialized centres. However, technical difficulties and expensive costs still represent significant barriers for its use in clinical practice (1,18).

Ki67

Ki67 protein is present inside the nucleus in all active phases of the cell cycle and is a well-known proliferation marker. Ki67-positive immunoreactivity is defined as a strong and complete nuclear staining undoubtedly recognizable at ×10 magnification (Fig. 3). The distribution of Ki67-positive proliferative cells in the normal gastric mucosa is restricted to the deep third of foveolar pits, with no significant staining in surface epithelium or in the superficial part of the foveolar pits. Therefore, some studies have postulated that aberrant Ki67 staining may represent a useful marker for IEN (25-31). Original data showed that Ki67-positive fraction significantly increases from columnar metaplasia to IEN and invasive carcinoma, with a marked expansion of the proliferative component (25-27). However, Olvera *et al.* demonstrated in 2005 that Ki67 staining has no value in differentiating LGD from reactive changes (26). Indeed, regenerating epithelium also displays increased cell proliferation, which in some instances approaches that seen in LGD (27).

Thus, Ki67 expression cannot be regarded as sufficiently reliable to confirm IEN. Moreover, Ki67 assessment is technically limited by the need for well-oriented sections in order to correctly determine the border between the upper and lower halves of the crypts, as well as for a standardisation of cell counting (28). Elaborate methods have been described and characterized in the scientific literature, which are excellent in the research field but unsuited for routine clinical practice (29-31).

p53

The p53 gene encodes for a protein implicated in the regulation of the entry of proliferating cells into the S phase of the cell cycle. Mutations of *p53* gene resulting in the overexpression of altered p53 protein have been

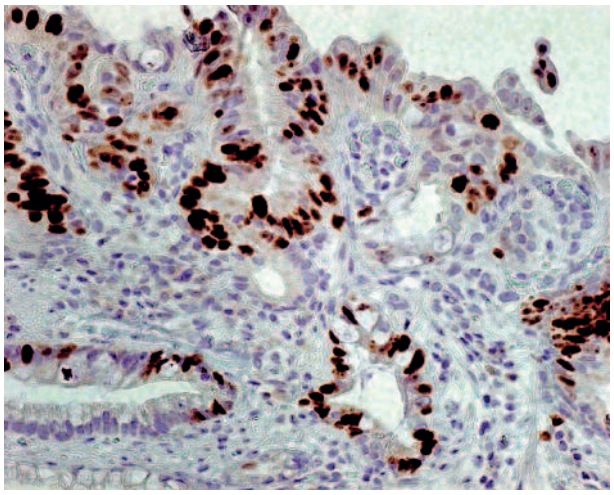

Fig. 3. — KI 67 immunostaining : Aberrant proliferation in IEN : strong nuclear staining. ×20.

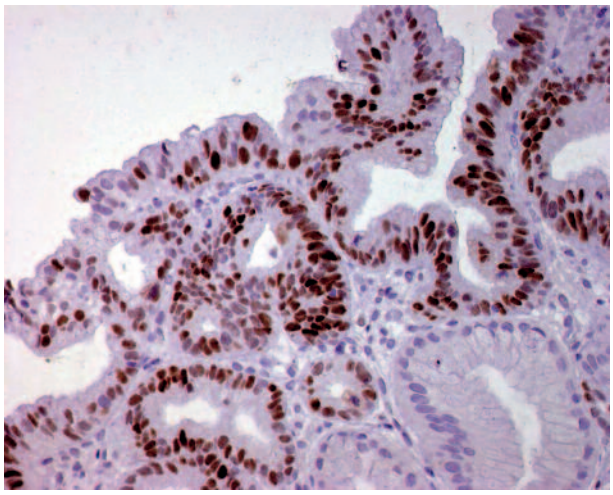

Fig. 4. — p53 immunostaining. Low grade IEN. strong nuclear staining. ×20.

observed with a high frequency in BO-related HGD and adenocarcinoma. Such p53 overexpression is characterized by a strong nuclear staining by immunohistochemistry (Fig. 4). Several studies have supported a place of p53 immunohistochemical analysis in the selection of patients with LGD who will progress to HGD or adenocarcinoma (32-36). The accumulation of p53 in biopsies with LGD is associated with an increased predictive value for the development of HGD (34). Moreover, p53 positivity in non-dysplastic lesions may indicate a first and early step in the progression towards neoplasia (15,35,36), suggesting that changes in p53 expression or mutation may antedate histological IEN. However, not all p53 gene mutations result in p53 overexpression. In addition, p53 overexpression can occur in the absence of gene mutation and may be detected in up to 10% of biopsies that are histologically negative for IEN. Thus, most authors state that the overexpression of p53 has a low sensitivity and cannot be advocated as a routine marker for diagnostic use.

Although negative findings do not rule out the existence of IEN, a positive finding of nuclear p53 expression detected by immunohistochemistry facilitates the interpretation of the histological lesion. It may indeed help confirm a suspected diagnosis of IEN and assist with the distinction between low- and high-grade IEN (2,15,32,33,35). Skacel *et al.* have reviewed biopsies of 16 patients with LGD in BO, and showed that p53 immunoreactivity was correlated with the clinical progression (32). Similarly, Weston *et al.* have followed 48 BO patients with LGD, and suggested that p53 detection in LGD specimens represents a significant risk factor for LGD progression (33). However, a case-control study has showed that p53 staining was significantly associated with the risk of malignant progression, only if positive in the initial biopsies (35). The impact of adding p53 immunohistochemistry on reproducibility and prediction of outcome has been recently further assessed. Diagnostic agreement (k values) improved with positive p53 immunostaining. However, p53 negativity should not rule out IEN diagnosis in a histologically equivocal case (15). Indeed, the absence of p53 immunoreactivity may correspond to those cases which do not harbour mutant p53 or in which the mutation is associated with the loss of p53 expression.

AMACR

A methylacyl-CoA racemase (AMACR, also known as p504s) is an enzyme that catalyses the racemisation of A methyl branched carboxylic coenzyme A thioesters. AMACR is overexpressed in a variety of neoplasms, such as prostate and colon cancer. Immunohistochemistry positivity is a faint and granular cytoplasmic staining (Fig. 5). Recent studies have evaluated AMACR expression in the metaplasia-dysplasia- carcinoma sequence in BO (Table 3). Dorer *et al.* and Livovsky *et al.* have shown that AMACR is expressed in dysplastic epithelium in BO with a reasonably high degree of sensitivity and absolute specificity (37,38). For Dorer *et al.*, AMACR staining was negative in all cases of BO considered as negative for IEN whereas 20% of ID, 38% of LGD, 81% of HGD, and 72% of adenocarcinoma cases were positive (37). Similar results were observed in Lisovsky's series. AMACR immunostaining could not be detected in negative or indefinite IEN cases whereas 11% of LGD, 64% of HGD and 75% of adenocarcinoma were positive (38). In 2008, Scheil Bertram *et al.* published a retrospective study of early Barrett's adenocarcinoma treated by surgery. They analysed the AMACR expression in 127 different specimens (multi-tissue array with reactive and neoplastic samples from each patient). Barrett's epithelium without IEN did not disclose AMACR immunoreactivity. Conversely, AMACR immunoreactivity was found in 27% of ID, 91% with LGD and 96% with HGD or early cancer (39).

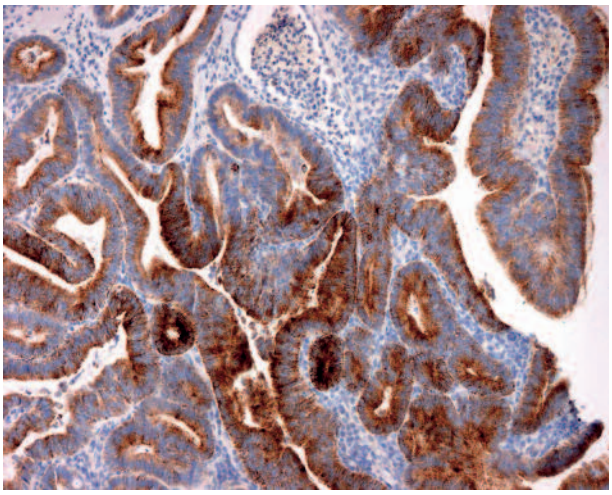

Fig. 5. — AMACR immunostaining. High grade IEN. Faint, granular cytoplasmic staining. ×20.

Theses studies have outlined the high degree of specificity of AMACR for dysplasia/carcinoma, and supported that it may be useful to detect neoplastic epithelium. However, Strater *et al.* have recently shown that a weak AMACR expression could be detected in 83% of BO cases without IEN, and moderate to strong expression of AMACR was restricted to neoplastic lesions (40). In a study of 101 cases, Shi *et al.* have reported similar observations, with 12% of AMACR positivity for BO without IEN, 47% in cases with ID, 44% in LGD and 93% in HGD (41). Our group has further confirmed these findings with a study of 50 BO cases (27 biopsies and 23 endoscopic mucosal resection) reviewed by a resident and two GI pathologists according to the Vienna Classification. Seventeen cases were negative of ID, 10 cases showed LGD, 19 cases HGD, and 2 corresponded to adenocarcinoma. Immunostaining for p504S (racemase- Dako, clone 13H4) was performed, and immunoreactivity pattern was semi-quantitatively analyzed by two independent examinators (AJM and CS) according to a three-point scale : negative / weak (+) / strong (++). No inter-observer variations were observed. AMACR was weakly positive in 4/17 cases without IEN ; 4/10 with LGD and 4/19 with HGD. No strong signal could be found in Barrett's without IEN. Samples with LGD and HGD showed strong immunoreactivity in 1/10 and 10/19, respectively. A racemase positivity either weak or strong was observed only in 50% of LGD cases and 74% of HGD lesions. These results further emphasize the poor sensitivity and specificity of racemase detection in the diagnosis of IEN in Barrett's oesophagus (42).

In conclusion, the risk of cancer development increases with the degree of IEN. The early detection of such lesions therefore represents the first step in the identification of high-risk patients. However, the identification and assessment of early dysplastic stages remain difficult in most cases. In order to improve the scoring accuracy based on morphological criteria, a second opinion of GI expert pathologists is required and is characterized by a lower inter-observer variation, a higher specificity and predictive values, even for LGD lesions. In addition, the Vienna Classification increases the degree of reproducibility and decreases disagreement for high-grade lesions. Besides morphological criteria, no biomarker has yet emerged as reliable in the identification and scoring of IEN. The development of dysplastic lesions is a complex process involving multiple abnormalities. The currently available ancillary techniques usually focus on

Table 3. — **AMACR Expression in Barrett's oesophagus (Imunostaining : Weak : + Strong : ++)**

	Dorer (37)	Lisowsky (38)	Scheil Bertram (39)	Strater (40)	Shi (41)	Ho Minh Duc (42)
No patient	134	96	127	31	101	50
Neg	0/36 0%	0/23 0%	0/30 0%	5+ 5/6 83%	2+/1++ 3/25 12%	4+ 4/17 23%
Ind	1+/2++ 3/14 21%	0/16 0%	8+ 8/30 27%	/	6+/2++ 8/17 47%	/
LG	2+/4++ 6/16 38%	1+/1++ 2/19 10%	10+/8++ 18/20 90%	5+/1++ 6/8 80%	5+/3++ 8/18 44%	4+/1++ 5/10 50%
HG	9+/17++ 26/32 81%	7+/7++ 14/22 64%	3+/19++ 22/23 96%	4+/1++ 5/8 60%	3+/11++ 14/15 93%	4+/10++ 14/19 74%
AD	5+/21++ 26/36 72%	6+/6++ 12/16 75%	3+/20++ 23/24 96%	1+/6++ 7/9 75%	5+/20++ 25/26 96.2%	2++ 2/4 50%

one single event of this continuous spectrum, which might explain why they have not been able, thus far, to replace the morphological evaluation.

In summary, the histological assessment of IEN remains the gold standard routine method for assessing the risk of malignant change. The p53 immunohistochemistry may be of help in difficult cases by identifying ID cases with the highest risk of progression. Although negative findings do not rule out the existence of IEN, a positive nuclear p53 expression by immunohistochemistry facilitates the interpretation of the histological lesion. The wide variation in reported rates of progression of IEN to malignancy, as well as problems of reproducibility, are strong arguments to go on with the identification and validation of IEN biomarkers.

References

1. ODZE R.D. Update on the diagnosis and treatment of Barrett esophagus and related neoplastic precursor lesions. *Arch. Pathol. Lab. Med.*, 2008, **132** : 1577-1585.
2. GEBOES K., VAN EYKEN P. The diagnosis of dysplasia and malignancy in Barrett's oesophagus. *Histopathology*, 2000, **37** : 99-107.
3. RIDDELL R.H., GOLDMAN H., RANSOHOFF D.F., APPELMAN H.D., HAGGITT R.C., AHREN C., CORREA P., HAMILTON R.C., MORSON B.C. Dysplasia in IBD : standardized classification with provisional clinical applications. *Hum. Pathol.*, 1983, **14** : 931-968.
4. SCHLEMPER R.J., RIDDELL R.H., KATO Y., BORCHARD F., COOPER H.S., DAWSEY S.M., DIXON M.F., FENOGLIO-PREISER C.M., FLEJOU J.F., GEBOES K., HATTORI T., HIROTA T., ITABASHI M., IWAFUCHI M., IWASHITA A., KIM Y.I., KIRCHNER T., KLIMPFINGER M., KOIKE M., LAUWERS G.Y., LEWIN K.J., OBERHUBER G., OFFNER F., PRICE A.B., RUBIO C.A., SHIMIZU M., SHIMODA T., SIPPONEN P., SOLCIA E., STOLTE M., WATANABE H., YAMABE H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut*, 2000, **47** : 251-255.
5. OFFERHAUS G.J.A., CORREA P., VAN EEDEN S.., GEBOES K., DRILLENBURG P., VIETH M., VAN VELTHUYSEN M.L., WATANABE H., SIPPONEN P., TEN KATE F.J., BOSMAN F.T., BOSMA A., RISTIMAKI A., VAN DEKKEN H., RIDDELL R., TYTGAT G.N. Report of an Amsterdam Working group on Barrett esophagus. *Virchows Arch.*, 2003, **443** : 601-607.
6. WERNER M., FLEJOU J.F., HAINAUT P. *In* : Pathology and Genetics of the Digestive System. IARC Press Lyon, 2000, 20-26.
7. MONTGOMERY E., GOLDBLUM J.R., GREENSON J.K., HABER M.M., LAMPS L.W ., LAUWERS G.Y., LAZENBY A.J., LEWIN D.N., ROBERT M.E., WASHINGTON K., ZAHURAK M.L., HART J. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus : a follow-up study based on 138 cases from a diagnostic variability study. *Hum. Pathol.*, 2001, **32** : 379-388.
8. HORNICK J.L., ODZE R.D. Neoplastic precursor lesions in Barrett's esophagus. *Gastroenterol. Clin. N. Am.*, 2007, **36** : 775-796.
9. REID B.J., HAGGITT R.C., RUBIN C.E., ROTH G., SURAWICZ C.M., VAN BELLE G., LEWIN K., WEINSTEIN W.M., ANTONIOLI D.A., GOLDMAN H. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum. Pathol.*, 1988, **19** : 166-178.
10. LAUWERS G., RIDDELL B. Gastric epithelial dysplasia. *Gut*, 1999, **45** : 784-790.
11. LOMO L.C., BLOUNT P.L., SANCHEZ C.A., LI X., GALIPEAU P.C., COWAN D.S., AYUB K., RABINOVITCH P.S., REID B.J., ODZE R.D. Crypt dysplasia with surface maturation : a clinical, pathological and molecular study of a Barrett's esophagus cohort. *Am. J. Surg. Pathol.*, 2006, **30** : 423-435.
12. FLEJOU J.F. Histological assessment of oesophageal columnar mucosa. Best Pract. Res. Clin.Gastroenterol., 2008, 22 : 671-686.
13. MONTGOMERY E., BRONNER M.P., GOLDBLUM J.R., GREENSON J.K., HABER M.M., HART J., LAMPS L.W., LAUWERS G., LAZENBY A.J., LEWIN D.N., ROBERT M.E., TOLEDANO A.Y., SHYR Y., WASHINGTON K. Reproducibility of the diagnosis of dysplasia in Barrett oesophagus : a reaffirmation. *Hum. Pathol.*, 2001, 32 : 368-378.
14. KERKHOF M., VAN DEKKEN H., STEYERBERG E.W., MEIJER G.A., MULDER A.H., DE BRUINE A., DRIESSEN A., TEN KATE F.J., KUSTERS J.G., KUIPERS E.J., SIERSEMA P.D. Grading of dysplasia in Barrett's oesophagus : substantial interobserver variation between general and GI pathologists. *Histopathology*, 2007, **50** : 920-927.
15. KAYE P.V., HAIDER S.A., ILYAS M., JAMES P.D., SOOMRO I., FAISAL W., CATTON J., PARSONS S.L., RAGUNATH K. Barrett's dysplasia and the Vienna classification : reproductibility prediction of progression and impact of consensus reporting and p53 immunochemistry. *Histopathology*, 2009, **54** : 699-712.
16. PECH O., VIETH M., SCHMITZ D., GOSSNER L., MAY A., SEITZ G., STOLTE M., ELL C. Conclusions from histological diagnosis of low grade intraepithelial neoplasia in Barrett's oesophagus. *Scand. J. Gastroenterol.*, 2007, **42** : 682-688.
17. SKACEL M., PETRAS R.E., GRAMLICH T.L., SIGEL J.E., RICHTER J.E., GOLDBLUM J.R. The diagnosis of low grade dysplasia in Barrett's esophagus and its implications for disease progression. *Am. J. Gastroenterol.*, 2000, **95** : 3383-3387.
18. FLEJOU J.F., SVRCEK M. Barrett's oesophagus- a pathologist's view. *Histopathology*, 2007, **50** : 3-14.
19. PRESTON S.L., JANKOWSKI J.A. Drinking from the fountain of promise : biomarkers in the surveillance of Barrett's esophagus – the glass is half full ! *Gut*, 2006, **55** : 1377-1379.
20. REID B.J., BLOUNT P.L., RABINOVITCH P.S. Biomarkers in Barrett's oesophagus. *Gastrointest. Endosc. Clin. N. Am.*, 2003, **13** : 369-397.
21. POLKOWSKI W., BAAK J.P., VAN LANSCHOT J.J. Clinical decision making in Barrett's oesophagus can be supported by computerized immunoquantification and morphometry of features associated with proliferation and differentiation. *J. Pathol.*, 1998, **184** : 161-168.
22. BAAK J.P., TEN KATE F.J., OFFERHAUS G.J., VAN LANSCHOT J.J., MEIJER G.A. Routine morphological analysis can improve reproductibility of dysplasia grade in Barrett's oesophagus surveillance biopsies. *J. Clin. Pathol.*, 2002, **55** : 910-916.
23. REID B.J., LEVINE D.S., LONGTON G., BLOUNT P.L., RABINOVITCH P.S. Predictors of progression to cancer in Barrett's esophagus baseline histology and flow cytometry indentify low and high risk patients subsets. *Am. J. Gastroenterol.*, 2000, **95** : 1669-1676.
24. RABINOVITCH G., LONGTON G., BLOUNT P.L., RABINOVITCH P.S., LONGTON G., BLOUNT P.L., LEVINE D.S., REID B.J. Predictors of progression in Barrett's esophagus baseline flow cytometric variables. *Am. J. Gastroenterol.*, 2001, **96** : 3071-3083.
25. FEITH M., STEIN H.J., MUELLER J., SIEWERT J.R. Malignant degeneration of Barrett's esophagus : the role of the Ki67 proliferation fraction, expression of E cadherin and p53 . *Dis. Esophagus*, 2004, **17** : 322-327.
26. OLVERA M., WICKRAMASINGHE K., BRYNES R., BU X., MA Y., CHANDRASOMA P. KI67 expression in different epithelial types in columnar lined oesophagus indicates varying levels of expanded and aberrant proliferative patterns. *Histopathology*, 2005, **47** : 132-140.
27. HONG M.K., LASKIN W.B., HERMAN B.E., JOHNSTON M.H., VARGO J.J., STEINBERG., ALLEGRA C.J., JOHNSTON P.G. Expansion of the KI67 proliferative compartment correlates with degree of dysplasia in Brrett's oesophagus. *Cancer*, 1995, **75** : 423-429.
28. LÖRING E., JAKOBSSON B., LANDBERG G., VERESS B. KI67 and p53 immunohistochemistry reduces interobserver variation in assessment of Barrett's oesophagus. *Histopathology*, 2005, **46** : 642-648.
29. WHITTLES C.E., BIDDLESTONE L.R., BURTON A., BARR H., JANKOWSKI J.A., WARNER P.J., SHEPERD N.A. Apoptotic and proliferative activity in the neoplastic progression of Barrett's oesophagus : a comparative study. *J. Pathol.*, 1999, **187** : 535-540.
30. VAN SANDICK J.W., BAAK J.P., VAN LANSCHOT J.J., POLKOWSKI W., TEN KATE F.J., OBERTOP H., OFFERHAUS G.J. Computerized quantitative pathology for the grading of dysplasia in surveillance biopsies of Barrett's oesophagus. *J. Pathol.*, 2000, **190** : 177-183
31. HALM U., TANNAPFEL A., BREITUNG B., BREIDERT M., WITTEKIND C.W., MÖSSNER J. Apoptosis and cell proliferation in the metaplasia-dysplasia-carcinoma sequence of Barrett's oesophagus *Hepatogastroenterol.*, 2000, **47** : 962-966.
32. SKACEL M., PETRAS R., RYBICKI L., GRAMLICH T.L., RICHTER J.E., FALK G.W., GOLDBLUM J.R. p53 expression in Low grade dysplasia in Barret's esophagus. Correlation with interobserver agreement and disease progression. *Am. J. Gastroenterol.*, 2002, **97** : 2508-2513.
33. WESTON A.P., BANERJEE S.K., SHARMA P., TRANG M., TRAN M., RICHARDS R., CHERIAN R. p53 protein overexpression in LGD in Barrett's esophagus : immunhistochemical marker predictive of progression. *Am. J. Gastroenterol.*, 2001, **96** : 1355-1362.
34. YOUNES M., ERTAN A., LECHAGO LV., SOMOANO J.R., LECHAGO J. p53 protein accumulation is a specific marker of malignant potential in Barrett's metaplasia. *Dig. Dis. Sci.*, 1997, **42** : 697-701.

35. MURRAY L., SEDO A., SCOTT M., Mc MANUS D., SLOAN J.M., HARDIE L.J., FORMAN D., WILD C.P. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut*, 2006, 55 : 1390-1397.
36. KERKHOF M., STEVERBERG E.W., KUSTERS J.G., VAN DEKKEN H., VAN VUUREN A.J., KUIPERS E.J., SIERSEMA P.D. Aneuploidy and high expression of p53 and Ki67 is associated with neoplastic progression in Barrett esophagus. *Cancer Biomarkers*, 2008, **4** : 1-10.
37. DORER R., ODZE R.D. AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, Ulcerative colitis and Crohn's disease. *Am. J. Surg. Pathol.*, 2006, **30** : 871-877.
38. LISOVSKY M., FALKOWSKI O., BHUIYA T. Expression of alpha methylacyl coenzyme A racemase in dysplastic Barrett's epithelium. *Hum. Pathol.*, 2006, **37** : 1601-1606.
39. SCHEIL-BERTRAM S., LORENZ D., ELL C., SHEREMET E., FISSELER-ECKHOFF A. Expression of alpha methylacyl coenzyme A racemase in the dysplasia carcinoma sequence associated with Barrett's esophagus. *Mod. Pathol.*, 2008, **21** : 961-967.
40. STRÄTER J., WIESMÜLLER C., PERNER S., KUEFER R., MÖLLER P. Alpha methylacyl coA racemase (AMACR) immunohistochemistry in Barrett's and colorectal mucosa : only significant overexpression favours a diagnosis of intraepithelial neoplasia. *Histopathology*, 2008, **52** : 399-402.
41. SHI X.Y., BHAGWANDEEN B., LEONG A. p16, Cyclin D1, KI67 and AMACR as markers for dysplasia in Barrett esophagus. *Appl. Immunohistochem. Mol. Morph.*, 2008, 16 : 447-452.
42. HO MINH DUC K., SEMPOUX C., PIESSEVAUX H., DEPREZ P., JOURET-MOURIN A. Usefulness of p53 and p504S (racemase) in diagnosing Barrett's intraepithelial neoplasia. *Acta Gastroenterol. Belg.*, 2009, **72** : P 08.