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The role of quantitative pathology in clinical decision making for Barrett's oesophagus

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1. Clinical decision making in Barrett's oesophagus

Intestinal-type columnar epithelium in the (distal) oesophagus, known as Barrett's oesophagus (BO), is a well-defined premalignant condition [32]. The risk for the development of oesophageal adenocarcinoma in a patient with BO is at least 30 times higher as compared to the general population [9]. Invasive cancer in BO, so called Barrett cancer, is preceded by stages of progressively severe dysplastic changes [24]. For a symptomatic Barrett cancer, long-term survival rates are low. Therefore, attention should be focused on early detection of neoplastic changes, preferably in a preinvasive phase, i.e. dysplasia. An accurate and reproducible diagnosis of dysplasia in BO might ultimately lead to targeted therapeutic interventions or cancer prevention in the future.

At present, dysplasia in BO is the only clinically accepted marker of neoplastic potential. Strategies for endoscopic surveillance of BO are dictated by the grade of dysplasia on endoscopic biopsy [17,23]. When a diagnosis of low-grade dysplasia is made, surveillance should be intensified by shortening the time intervals between consecutive endoscopies and by applying more aggressive biopsy sampling [35,36]. High-grade dysplasia may indicate imminent progression into invasive carcinoma or even its occult presence [1,5,10,18,27,30]. When the diagnosis of (persistent) high-grade dysplasia is confirmed independently by two expert pathologists in a patient fit for major surgery, a 'prophylactic' oesophagectomy should be considered in an institution with low postoperative mortality. Alternatively, local endoscopic ablation with careful endoscopic follow-up could be applied, using endoscopic mucosal resection (EMR) and/or photodynamic therapy (PDT), although these promising new endoscopic techniques should still be considered experimental. The effectiveness of such surveillance strategy is hampered by substantial diagnostic variability in grading of dysplasia in BO biopsy samples (37–46%) [25,31,37].

2. Grading of dysplasia in Barrett's oesophagus

Dysplasia is usually defined as a process of unequivocal neoplastic proliferation, with loss of differentiation and/or maturation gradient [11,28]. The diagnosis of high-grade dysplasia or intramucosal carcinoma can be made with a high inter-observer reproducibility between expert pathologists (85–87%), especially if they come from the same institution [31]. However, diagnostic variability is a problem in lower grades of dysplasia [25]. It can be difficult to distinguish reactive inflammatory changes from genuine dysplasia. This can be partially overcome by microscopic re-assessment after optimal short-term medical anti-reflux therapy. However, we do not deal with distinct categories, but with a continuous spectrum from metaplasia (BO), through low-, (intermediate-), and high-grade dysplasia to invasive carcinoma. Because of lack of definitive

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histologic criteria and their subjective interpretation, both intra- and inter-observer variability are inherent to this scoring system.

3. Computerized immunoquantitation and morphometry of features associated with proliferation and differentiation

Computerized quantitative pathology appears a promising tool to decrease observer variability in grading of BO dysplasia. Dysplasia, by definition, represents loss of a differentiation/maturation gradient. Mucin histochemistry (like alcian blue pH 2.5/PAS) allows for demonstration of maturation loss in BO [16]. The features of differentiation and/or maturation can be objectively evaluated by means of morphometry. Morphometry enables to measure geometric features of tissue components, like stratification of nuclei within the epithelium and nuclear size related features (nuclear area and nuclear volume) [2]. It has been shown that, using morphometry, incomplete intestinal metaplasia of the gastric mucosa should be classified as low-grade dysplasia [34]. If the same holds true for BO, then morphometry may be useful as an additional diagnostic tool in grading of dysplasia.

Another feature of dysplasia is increased proliferation. This can be assessed by counting the mitotic figures on H&E sections, or by using special stains which enable to demonstrate cells that have entered the cell cycle (Nucleolar Organizer Regions Antigen – AgNORs; Proliferating Cell Nuclear Antigen – PCNA; Ki67; MIB1). Flow cytometry for this purpose is discouraged because the histological context is lost [6]. With the use of proliferative markers it is possible to localise rapidly dividing cells within dysplastic epithelium. In BO epithelium, the proliferative compartment is normally restricted to the bottom of the crypts (progenitor stem cells) [4]. An upward shift of proliferating cells towards the luminal surface is a characteristic feature of neoplastic progression [38]. The assessment of proliferative activity has been well documented in the dysplasia–carcinoma sequence of BO [8,12,14,15,26,39]. The most widely accepted proliferative parameter is the labelling index, defined as the proportion of positively labelled cells to the total number of cells (within the proliferative compartment). However, the assessment of the labelling index is based on counting a large number of cells under the microscope. This procedure needs standardised training in scoring and its reproducibility is limited [29]. Using computerized immunoquantitation of Ki67 by stereology with systematic random sampling, area percentage of positive nuclei (Ki67 area%) is an attractive alternative to labelling index [20].

Proliferation is partly controlled by tumour suppressor genes. Malignant progression in BO is the result of a stepwise accumulation of genetic alterations, in which p53 is thought to be a key factor [7]. The p53 protein accumulation is present in nearly two thirds of oesophageal adenocarcinomas arising in BO, and with similar frequency in areas of high-grade dysplasia [21]. In dysplastic areas adjacent to Barrett's adenocarcinomas, a positive correlation between p53 protein dysfunction and increased proliferative activity (as assessed with Ki67) was found [22]. The use of a proliferative marker (Ki67) in combination with the assessment of the p53 protein status might be of help to discriminate between different grades of dysplasia in BO. This combination may be of special interest, since Ki67 assessment may to some extent have an additional value to p53 immunohistochemistry (IHC). There is now increasing evidence that p53 IHC can give false positive results, and therefore it is not the optimal way to assess the p53 gene status [3,32].

4. Quantitative pathology as adjunct tool for grading of dysplasia

Alternatively, objective quantitative analysis of p53 IHC (measurement of molecular cell features = cytometry) could possibly be an adjunct to the conventional grading of dysplasia in BO. When the pathologist is not sure about the presence of dysplasia (often due to inflammatory and/or reactive changes), the diagnosis of 'indefinite for dysplasia' is made. There is evidence that p53 immunoquantitation might be of help to distinguish between indefinite for dysplasia and 'genuine' dysplasia [22]. Moreover, assessment of p53 area% may add to subjective biopsy examination in differentiating non-dysplastic from dysplastic BO [37].

Objective diagnostic information in BO can be obtained by computerized quantitation of features associated with differentiation (stratification, nuclear size related features) and proliferation (Ki67, p53) with various combinations [2]. Moreover, most of the cases with disagreement on subjective BO dysplasia grading can be classified uniquely according to the values emerging from the discriminant analyses of quantitative pathological parameters [19,37]. Since grading of dysplasia in BO carries important clinical consequences for

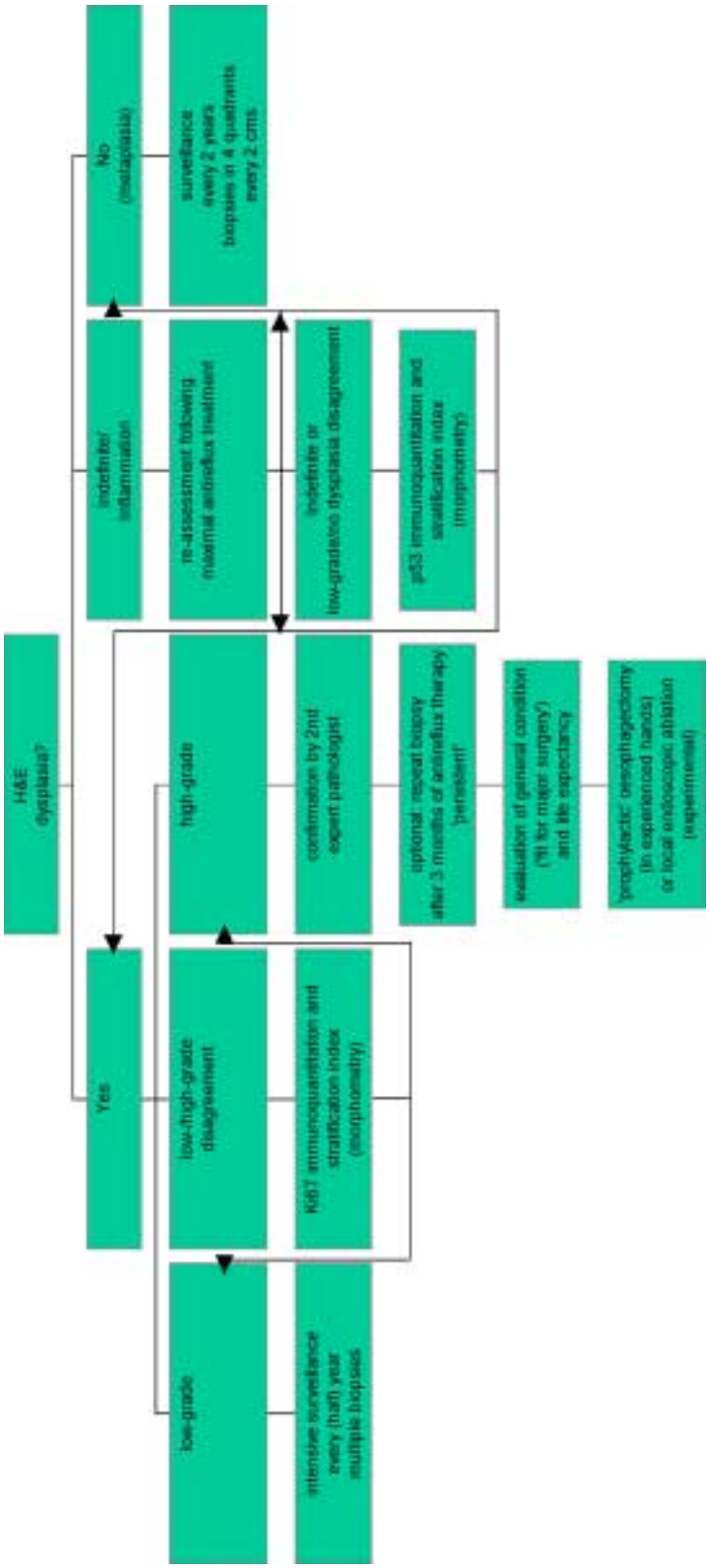

Fig. 1. Potential application of additional diagnostic tools of quantitative pathology for grading of dysplasia in patients with Barrett's oesophagus.

the individual patient (intensification of endoscopic surveillance, local endoscopic ablation, 'prophylactic' oesophagectomy), clinical application of the quantitative classification method might dramatically influence the relatively poor effectiveness of BO surveillance programmes. It has been shown that p53 area% and Ki67 area%, and stratification index are the most powerful parameters for discrimination between different grades of dysplasia in surgical resection specimens with BO [19]. Analysis of the same set of quantitative pathological parameters has been also feasible on BO surveillance biopsies provided that well-defined biopsy criteria are used [37]. Importantly, the quantitative pathological analysis may assist in reducing diagnostic variability in the grading of dysplasia during surveillance of patients with BO. Although quantitative pathology on BO is a powerful research tool, its clinical use should be limited to expert centres. This reasoning can be explained by stable quality of IHC, availability of high-technology computerized equipment (QPRODIT system with systematic random sampling, automated scanning stage), gaining much experience with the course of time, and finally accessibility of expertise pathologists. Although nearly all biopsies are usually judged by two pathologists (at least in-training pathologist and senior-pathologist, or two expert pathologists) in these centres, so-called 'quantitative pathological grade' may be also obtained [19]. The agreement cases between subjective grading by two expert pathologists and quantitative pathological grading serve as constantly growing database for discriminant analysis. Most of the disagreement cases on subjective grading can be classified uniquely according to the values emerging from the discriminant analysis [37]. Such experience may be used in referral centres offering revision of pathology in BO. Initial results indicate that reassessment of biopsies, including additional quantitative pathology, led to adjustment of the grading of dysplasia in 50% of cases referred as high-grade, and 90% as low-grade dysplasia [13].

An algorithm for the potential application of quantitative pathology in grading of dysplasia in BO has been proposed [21]. Graphs showing decision thresholds based on both, surgical resection material and endoscopic biopsy material, have been published previously [19,38]. A modified algorithm includes the recently published results of quantitative pathology on BO biopsies, and is presented in Fig. 1. The clinical value of this algorithm should be tested in a prospective clinical study with long-term follow-up. The potential of quantitative pathological features to discriminate between different grades of dysplasia in BO could be used for the future refinement of histological criteria in grading of dysplasia.

References

[1] N.K. Altorki, M. Sunagawa, A.G. Little and D.B. Skinner, High-grade dysplasia in the columnar-lined esophagus, *American Journal of Surgery* **161**(1) (1991), 97–99.

[2] J.P.A. Baak, J. Oort, J.C. Fleege, P.J. van Diest and J.S. Ploem, Techniques in quantitative pathology, in: *Manual of Quantitative Pathology in Cancer Diagnosis and Prognosis*, J.P.A. Baak, ed., Springer-Verlag, Berlin, 1991, pp. 45–56.

[3] I.O. Baas, F.M. van den Berg, J.W. Mulder, M.J. Clement, R.J. Slebos, S.R. Hamilton and G.J.A. Offerhaus, Potential false-positive results with antigen enhancement for immunohistochemistry of the p53 gene product in colorectal neoplasms, *Journal of Pathology* **178**(3) (1996), 264–267.

[4] G.L. Eastwood, A review of gastrointestinal epithelial renewal and its relevance to the development of adenocarcinomas of the gastrointestinal tract, *Journal of Clinical Gastroenterology* **21** (Suppl. 1) (1995), S1–11.

[5] M.J. Edwards, D.R. Gable, A.B. Lentsch and J.D. Richardson, The rationale for esophagectomy as the optimal therapy for Barrett's esophagus with high-grade dysplasia, *Annals of Surgery* **223**(5) (1996), 585–589.

[6] M.B. Fennerty, R.E. Sampliner, D. Way, R. Riddell, K.X. Steinbronn and H.S.Garewal, Discordance between flow cytometric abnormalities and dysplasia in Barrett's esophagus, *Gastroenterology* **97**(4) (1989), 815–820.

[7] P.C. Galipeau, D.S. Cowan, C.A. Sanchez, M.T. Barrett, M.J. Emond, D.S. Levine, P.S. Rabinovitch and B.J. Reid, 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus, *Proceedings of National Academy of Science USA* **93**(14) (1996), 7081–7084.

[8] P. Gillen, M. McDermott, D. Grehan, D.O. Hourihane and T.P. Hennessy, Proliferating cell nuclear antigen in the assessment of Barrett's mucosa, *British Journal of Surgery* **81**(12) (1994), 1766–1768.

[9] R.C. Haggitt, Barrett's esophagus, dysplasia, and adenocarcinoma, *Human Pathology* **25**(10) (1994), 982–993.

[10] R.F. Heitmiller, M. Redmond and S.R. Hamilton, Barrett's esophagus with high-grade dysplasia. An indication for prophylactic esophagectomy, *Annals of Surgery* **224**(1) (1996), 66–71.

[11] P. Hermanek, Dysplasia in the gastrointestinal tract: definition and clinical significance, *Surgical Endoscopy* **1** (1987), 5–10.

[12] M.K. Hong, W.B. Laskin, B.E. Herman, M.H. Johnston, J.J. Vargo, S.M. Steinberg, C.J. Allegra and P.G. Johnston, Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus, *Cancer* **75**(2) (1995), 423–429.

[13] J.B. Hulscher, J. Haringsma, J. Benraadt, G.J. Offerhaus, F.J. ten Kate, J.P. Baak, G.N. Tytgat, J.J. van Lanschot, Barrett Advisory Committee, Comprehensive Cancer Centre Amsterdam Barrett Advisory Committee: first results, *Netherlands Journal of Medicine* **58**(1) (2001), 3–8.

[14] S.Y. Iftikhar, R.J. Steele, S. Watson, P.D. James, K. Dilks and J.D. Hardcastle, Assessment of proliferation of squamous, Barrett's and gastric mucosa in patients with columnar lined Barrett's oesophagus, *Gut* **33**(6) (1992), 733–737.

[15] J. Jankowski, R. McMenemin, C. Yu, D. Hopwood and K.G. Wormsley, Proliferating cell nuclear antigen in oesophageal diseases; correlation with transforming growth factor alpha expression, *Gut* **33**(5) (1992), 587–591.

[16] G. Lapertosa, P. Baracchini and E. Fulcheri, Mucin histochemical analysis in the interpretation of Barrett's esophagus. Results of a multicenter study. The Operative Group for the Study of Esophageal Precancer, *American Journal of Clinical Pathology* **98**(1) (1992), 61–66.

[17] M. Miros, P. Kerlin and N. Walker, Only patients with dysplasia progress to adenocarcinoma in Barrett's oesophagus, *Gut* **32**(12) (1991), 1441–1446.

[18] M. Pera, V.F. Trastek, H.A. Carpenter, M.S. Allen, C. Deschamps and P.C. Pairolero, Barrett's esophagus with high-grade dysplasia: an indication for esophagectomy?, *Annals of Thoracic Surgery* **54**(2) (1992), 199–204.

[19] W. Polkowski, J.P.A. Baak, J.J.B. van Lanschot, G.A. Meijer, L. Schuurmans, F.J.W. ten Kate, H. Obertop and G.J.A. Offerhaus, Clinical decision making in Barrett's oesophagus can be supported by computerized immunoquantitation and morphometry of features associated with proliferation and differentiation, *Journal of Pathology* **184** (1998), 161–168.

[20] W. Polkowski, G.A. Meijer, J.P. Baak, F.J. ten Kate, H. Obertop, G.J. Offerhaus and J.J. van Lanschot, Reproducibility of p53 and Ki-67 immunoquantitation in Barrett's esophagus, *Analytical Quantitative Cytology and Histology* **19**(3) (1997), 246–254.

[21] W. Polkowski, J.J.B. van Lanschot and G.J.A. Offerhaus, Barrett esophagus and cancer: pathogenesis, carcinogenesis, and diagnostic dilemmas. (Invited Review), *Histology and Histopathology* **14** (1999), 927–944.

[22] W. Polkowski, J.J. van Lanschot, F.J. ten Kate, J.P. Baak, G.N. Tytgat, H. Obertop, W.J. Voorn and G.J. Offerhaus, The value of p53 and Ki67 as markers for tumour progression in the Barrett's dysplasia–carcinoma sequence, *Surgical Oncology* **4**(3) (1995), 163–171.

[23] D. Provenzale, J.A. Kemp, S. Arora and J.B. Wong, A guide for surveillance of patients with Barrett's esophagus, *American Journal of Gastroenterology* **89**(5) (1994), 670–680.

[24] B.J. Reid, P.L. Blount, C.E. Rubin, D.S. Levine, R.C. Haggitt and P.S. Rabinovitch, Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort, *Gastroenterology* **102** (1992), 1212–1219.

[25] B.J. Reid, R.C. Haggitt, C.E. Rubin, G. Roth, C.M. Surawicz, G. van Belle, K. Lewin, W.M. Weinstein, D.A. Antonioli, H. Goldman, W. MacDonald and D. Owen, Observer variation in the diagnosis of dysplasia in Barrett's esophagus, *Human Pathology* **19** (1988), 166–178.

[26] B.J. Reid, C.A. Sanchez, P.L. Blount and D.S. Levine, Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression, *Gastroenterology* **105**(1) (1993), 119–129.

[27] T.W. Rice, G.W. Falk, E. Achkar and R.E. Petras, Surgical management of high-grade dysplasia in Barrett's esophagus, *American Journal of Gastroenterology* **88**(11) (1993), 1832–1836.

[28] R.H. Riddell, H. Goldman, D.F. Ransohoff, H.D. Appelmann, C.M. Fenoglio, R.C. Haggitt, C. Ahren, P. Correa, S.R. Hamilton, B.C. Morson, S.C. Sommers and J.H. Yardley, Dysplasia in inflammatory bowel disease: standarized classification with provisional clinical applications, *Human Pathology* **14** (1983), 931–968.

[29] D.J. Roe, D.S. Alberts, M.J. Wargovich, R.M. Bostick, H.S. Garewal, J. Einspahr, L. Fosdick, L. Ramsey, C. Woods and D.L. McGee, Reproducibility of the measurement of colonic proliferation using bromodeoxyuridine across laboratories, *Cancer Epidemiology, Biomarkers and Prevention* **5**(5) (1996), 349–353.

[30] V.W. Rusch, D.S. Levine, R. Haggitt and B.J. Reid, The management of high grade dysplasia and early cancer in Barrett's esophagus. A multidisciplinary problem, *Cancer* **74**(4) (1994), 1225–1229.

[31] C. Sagan, J.F. Flejou, M.D. Diebold, F. Potet and M.F. Le Bodic, Reproducibilité des critères histologiques de dysplasie sur muqueuse de Barrett, *Gastroenterologie Clinique et Biologique* **18** (1994), D31–D34.

[32] R. Soong, P.D. Robbins, B.R. Dix, F. Grieu, B. Lim, S. Knowles, K.E. Williams, G.R. Turbett, A.K. House and B.J. Iacopetta, Concordance between p53 protein overexpression and gene mutation in a large series of common human carcinomas, *Human Pathology* **27**(10) (1996), 1050–1055.

[33] S.J. Spechler and R.K. Goyal, The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett, *Gastroenterology* **110**(2) (1996), 614–621.

[34] P. Tosi, M.I. Filipe, P. Luzi, C. Miracco, R. Santopietro, R. Lio, V. Sforza and P. Barbini, Gastric intestinal metaplasia type III cases are classified as low-grade dysplasia on the basis of morphometry, *Journal of Pathology* **169** (1993), 73–78.

[35] G.N.J. Tytgat, Does endoscopic surveillance in esophageal columnar metaplasia (Barrett's esophagus) have any real value?, *Endoscopy* **27**(1) (1995), 19–26.

[36] G.N.J. Tytgat and W. Hameeteman, The neoplastic potential of columnar-lined (Barrett's) esophagus, *World Journal of Surgery* **16**(2) (1992), 308–312.

[37] J.W. van Sandick, J.P.A. Baak, J.J.B. van Lanschot, W. Polkowski, F.J.W. ten Kate, H. Obertop and G.J.A. Offerhaus, Computerized quantitative pathology for the grading of dysplasia in surveillance biopsies of Barrett's oesophagus, *Journal of Pathology* **190** (2000), 177–183.

[38] I.C.E. Wesdorp, J.F. Bartelsman, M.E.I. Schipper, G.J. Offerhaus and G.N.J. Tytgat, Malignancy and premalignancy in Barrett's esophagus: a clinical-, endoscopical-, and histological study, *Acta Endoscopica* **11** (1981), 317–326.

[39] L. Yacoub, H. Goldman and R.D. Odze, Transforming growth factor-alpha, epidermal growth factor receptor, and MiB-1 expression in Barrett's-associated neoplasia: correlation with prognosis, *Modern Pathology* **10**(2) (1997), 105–112.