What makes an expert Barrett's pathologist?

Concordance and pathologist expertise within a digital review panel

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CHAPTER 1

ROLE OF PATHOLOGIC CONFIRMATION FOR BARRETT'S ESOPHAGUS AND DYSPLASIA

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ABSTRACT

Barrett's esophagus (BE) is a premalignant condition defined by the replacement of squamous epithelium by columnar epithelium in the distal part of the esophagus. Patients with BE have an increased risk of progression to esophageal adenocarcinoma (EAC). Advanced EAC has a poor 5-year survival rate. However, if EAC is diagnosed at an early stage, endoscopic treatment has proven to be a safe and effective treatment, with excellent long-term survival rates. Currently it is not possible to accurately predict which patients with BE will develop EAC. Despite promising developments in genetic and molecular biomarker research, grade of dysplasia is still the best predictor for progression to EAC. Present guidelines advise surveillance endoscopies with biopsies for BE patients to detect early neoplasia at a treatable stage. Surveillance intervals are determined by length of the BE segment and on the histopathologic diagnosis of the biopsies. Accurate histopathologic assessment of biopsies to define surveillance intervals or to decide on a treatment strategy, is therefore of the utmost importance.

INTRODUCTION

Barrett's esophagus (BE) is a premalignant condition defined by the replacement of squamous epithelium by columnar epithelium in the distal part of the esophagus. Patients with BE have an increased risk of malignant progression, thought to develop through a gradual process from nondysplastic BE (NDBE) to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and eventually esophageal adenocarcinoma (EAC). [1] Advanced EAC has a poor 5-year survival rate. However, if EAC is diagnosed at an early stage, endoscopic treatment has proven to be a safe and effective treatment, with excellent long-term survival rates. [2 3]

Currently it is not possible to accurately predict which patients with BE will develop EAC. Despite promising developments in genetic and molecular biomarker research, these techniques are not ready yet to be integrated into clinical practice. Grade of dysplasia is therefore still the best predictor for progression to EAC. [4]

Present guidelines advise surveillance endoscopies with biopsies for BE patients to detect early neoplasia at a treatable stage. Next to the use of high-definition white light endoscopy, random 4-quadrant biopsies should be taken every 1-2 cm of Barrett's mucosa according to the Seattle protocol, after obtaining target biopsies from visible lesions. Most guidelines advise surveillance intervals based on the length of the BE segment and on the histopathologic diagnosis of the biopsies, with shorter surveillance intervals in patients with a diagnosis of LGD. In the absence of visible lesions, the presence of LGD or HGD in a Barrett's segment may lead to the decision to treat a patient with endoscopic ablation therapy. [4-10] A histological diagnosis of cancer without visible lesions, requires a dedicated repeat endoscopy, to inspect if any lesions that require endoscopic resection were missed. [11] Next to dedicated endoscopic inspection, accurate histopathological assessment of biopsies to define surveillance intervals or to decide on a treatment strategy, is therefore of the utmost importance in the management of BE patients.

HISTOPATHOLOGICAL DIAGNOSIS OF BE

The definition of BE has varied in time and around the world since the original description of the 'lower esophagus lined by columnar epithelium' by dr. Norman

Barrett in 1957. [12] Endoscopic confirmation of columnar mucosa in the distal esophagus is an indisputable criterion, but the histological counterpart remains a topic of controversy. In 1976 Paull et al. [13] described three types of metaplastic columnar epithelium; fundic-cardiac type comprising parietal and chief cells, cardiac (junctional) type with cardiac mucous glands and intestinal (distinctive specialized) type mucosa (intestinal metaplasia, IM) containing goblet cells. Pathological studies showed that IM in particular is associated with an increased risk of malignant progression, leading to the inclusion of IM in the diagnosis of BE in most guidelines. [14 15] Bhat et al. [16] confirmed in a population-based study that the risk of cancer was significantly higher in patients with IM than in patients whose biopsies did not show IM at first biopsy (0.38% vs 0.07% per year, HR=3.53). [16] Some studies suggest that the cardiac type epithelium also predisposes to the development of esophageal cancer. [17 18] In a series of 141 small esophageal adenocarcinomas, Takubo et al. [19] found that 71% were adjacent to cardiac or fundic-type metaplasia rather than IM, and no IM was observed in 56.6% of the endoscopic mucosal resection specimens. [19] However, they omitted to report the location of the tumors and if IM was ever demonstrated in prior biopsies. If IM was previously detected the removal of IM from the definition of BE as suggested by the authors does not seem justified. [20] Since most studies describing the risk of malignant progression mainly or exclusively included BE with IM, the progression risk of cardiac and fundic-type columnar epithelium remains unclear.

Different views exist about the influence of the progression risk on the definition of BE. The American Gastroenterological Association (AGA) states that the definition of a medical condition depends on the clinical importance, which is predisposition to cancer when defining BE. Any form of metaplastic columnar epithelium that predisposes to cancer should therefore be included in the definition. Since IM is the only type of columnar epithelium that evidently predisposes to malignancy, presence of IM is required for the diagnosis of BE in the AGA guidelines. [9] The AGA is uniform with the American College of Gastroenterology (ACG), the American Society for Gastrointestinal Endoscopy (ASGE), and the European Society of Gastrointestinal Endoscopy (ESGE), who include columnar-lined epithelium containing IM in its definition of BE. [7-9] The European Society of Gastrointestinal Endoscopy (ESGE) shares this view. On the contrary, the United Kingdom and Japan do not require histological evidence of IM for the diagnosis of BE. According to the British Society of Gastroenterology (BSG) the definition should not depend on malignant potential,

but should be descriptive of the metaplastic state. However, the BSG takes presence of IM into account when deciding on the clinical management, since it has proven to increase risk of malignant progression. [4] In the Asia-Pacific consensus of 2008 presence of IM was required, but the update of 2016 excluded IM from the definition based on the possibility of missing IM by sampling bias and the suggestion of neoplastic potential by nongoblet columnar metaplasia. [21]

HISTOPATHOLOGIC ASSESSMENT OF DYSPLASIA

Dysplasia in BE is assessed according to the revised Vienna criteria, which are based on the dysplasia classification in inflammatory bowel disease. [22 23] Evaluation of cytological and architectural severity and invasion status leads to assignment in one of the following categories: negative for dysplasia, indefinite for dysplasia (IND), LGD, noninvasive HGD, and invasive neoplasia. Key characteristics used to assess the dysplasia grade in BE are surface maturation, glandular architecture, and cytonuclear changes.

Histologic assessment of BE is not without pitfalls and complicated by the inflammatory nature of the disorder. Regeneration can cause a wide variety of changes in the epithelium, depending on the presence of active inflammation or ulceration, thereby resembling dysplasia. In addition, the grades of dysplasia are not sharply delineated categories, but follow a gradually changing continuous spectrum.

BE graded "negative for dysplasia" usually consists of columnar epithelium containing goblet cells (i.e., IM), with surface maturation and glandular architecture generally intact (**Figure 1**). A key feature of dysplasia is the presence of a so-called clonal step, in which morphological changes are sharply demarcated compared to the surrounding tissue. Moreover, dysplastic samples often show loss of surface maturation, with stratified epithelium at the mucosal surface that contains an increased number of (hyper- chromatic) cells. In HGD, this nuclear stratification is usually seen in the surface epithelium as well as in the glands below, while in LGD the stratified epithelium is usually limited to the mucosal surface. In addition, in both LGD and HGD the number of goblet cells can be decreased or they can even be absent. In LGD, cytological changes include mildly enlarged nuclei that still have regular contours, but sometimes have increased chromatin. The glandular architecture is still relatively preserved, with

glandular crowding but typically no back-to-back formation. In HGD, the cytological and architectural changes become more advanced. The nuclei can exhibit even more marked changes in size and shape, mitoses become atypical and increased and cells will start to show loss of polarity. Glandular architecture becomes complex and can exhibit changes like back-to-back formation, cribriform growth, branching, or budding.

Sometimes it is not possible to make a proper diagnosis, because of inflammation or poor quality of the biopsy material. In these cases, the category IND is used. In dysplasia abnormal architectural arrangement is seen, but inflammation and regeneration cause a change in glandular shape and size as well. Furthermore, some cytonuclear abnormalities caused by inflammation could imitate both LGD and HGD (**Figure 2**). The differentiation between NDBE and LGD appears the most problematic, but IND can also be diagnosed when the distinction between other categories, for example reactive changes and HGD cannot be made. [23-25]

INTEROBSERVER VARIABILITY

Although the Vienna classification has helped resolve discrepancies between Western and Eastern pathologists in the diagnosis of dysplasia and early carcinoma, above mentioned challenges regarding pathologic evaluation remain, resulting in considerable inter-observer variability. [26] The distinction between LGD and reactive changes seems to be the most challenging and may explain discrepancy in reported progression rates.

Studies have shown a poor to fair interobserver agreement (with κ- values ranging from 0.14-0.32) for diagnosing LGD in BE, although higher interobserver agreements were seen in studies with expert gastrointestinal (GI) pathologists (κ = 0.48-0.50). [27-32] Furthermore studies with expert GI pathologists showed that LGD is widely over-diagnosed in general practice. Curvers et al. showed that an expert panel of GI pathologists downstaged 85% of the LGD diagnosis by community pathologists to NDBE or to IND. Duits et al. showed similar results with 73% of the LGD patients downstaged. [30 31]

Follow-up of patients with confirmed LGD by an expert panel showed an increased risk for neoplastic progression of 9.1-13.4% per patient year. The patients with a

downstaged diagnosis showed a similar risk of neoplastic progression as NDBE patients of 0-0.9% per patient year. A recent meta-analysis by Qumseya et al showed that confirmation of LGD by an expert pathologist or panel was associated with a significantly higher incidence rate of progression to HGD or EAC compared with studies without expert pathology review (3.1% vs 1.2%, $P < 0.001$), The reported progression rate is lower than the abovementioned studies describe. This might be due to the fact that progression in the first year of follow-up after LGD diagnosis was not included in this analysis as these cases may represent prevalent HGD/EAC. Nonetheless, these data confirm the need for confirmation of LGD by an expert GI pathologist or panel. Interobserver agreement for the combined category of HGD/EAC is considerably better with substantial agreement (κ 0.64-0.72). [27 33 34]

HISTOLOGICAL DIAGNOSIS AND CLINICAL SIGNIFICANCE

Histologic assessment of biopsies in BE is important since both surveillance intervals and treatment decisions are influenced by the histologic diagnosis. As there are different opinions about the definition of BE, there are also some dissimilarities between guidelines on the management of NDBE, IND, LGD, and HGD. A summary of guideline recommendations for BE can be found in **Table 1**.

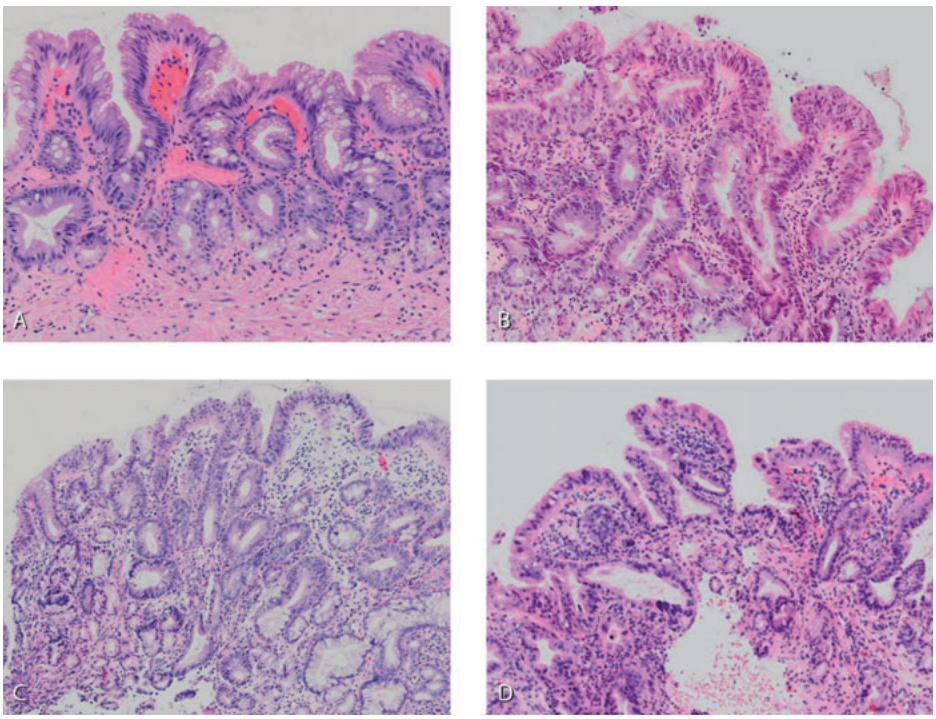
No intestinal metaplasia

In patients with a columnar lined distal esophagus, without IM in biopsies, the BSG recommends a repeat endoscopy in 3-5 years for patients with a short (< 3cm) segment of columnar epithelium without IM to account for sampling error. If absence of IM is confirmed discharge of surveillance is encouraged. [4] The ACG recommends with very low level of evidence to consider a repeat endoscopy in 1-2 years in patients with suspected BE and lack of IM on histology. [8] As mentioned above, other guidelines do not consider columnar epithelium without IM to be BE and do not mention any advice on surveillance for this category. [6 7 9]

Non-dysplastic BE (NDBE)

If no dysplasia is found in the random biopsies and no visible lesions are detected, surveillance intervals depend on the length of the BE segment according to the most recent ESGE (2017) and BSG guidelines. ESGE guidelines advise no surveillance for an irregular *Z*-line or columnar-lined esophagus o1 cm. For segment lengths of 1-3 cm the ESGE recommends a 5-year surveillance interval, while the BSG proposes a range of 3-5 years taking individual cancer risk into account. [47] For BE of ≥3 to ≤10cm, the ESGE and BSG recommend surveillance every 3 years and 2-3 years. Both societies advise referral to a BE expert center in the case of a segment longer than 10 cm. Other guidelines don't take length of BE segment into account and advise a surveillance interval of 3-5 years.

Figure 1: Examples of non-dysplastic and dysplastic Barrett's esophagus



A) Non-dysplastic Barrett's esophagus. B) Indefinite for dysplasia. C) Low-grade dysplasia. D) High-grade dysplasia.

Indefinite for dysplasia

The ACG, ASGE, and ESGE require confirmation by a second expert GI pathologist for a diagnosis of IND. [6-8] A repeat gastroscopy with biopsies after 3-6 months of optimal acid suppression therapy is advised. The ACG advises surveillance after 12 months for BE with confirmed IND. Other guidelines state that if no definite dysplasia is found at repeat endoscopy after optimal acid suppression therapy, the surveillance recommendations for NDBE should be followed.

Low-grade dysplasia (LGD)

The interobserver variability and the difference in reported progression rates, makes the management of LGD a much-debated issue. All guidelines state that the diagnosis of LGD should be confirmed by a second expert pathologist. The AGA and ESGE recommend that patients with confirmed LGD should be referred to an endoscopist with expertise in BE. [7 10] According to the AGA a repeat endoscopy should be performed after 8-12 weeks with maximal acid suppression, others recommend repeat endoscopy after 6 months. [5-7 10] If LGD persists on a second endoscopy the management options of endoscopic eradication therapy vs ongoing surveillance should be discussed. A randomized clinical trial comparing radiofrequency ablation (RFA) with endoscopic surveillance in 136 patients with confirmed LGD showed a reduced risk of neoplastic progression over 3 years of follow-up in the ablation group (1.5% vs 26.5%, $P < 0.001$). These results led to premature closure of the study. In addition, sustained complete eradication of dysplasia and IM was seen in the majority of treated patients (92.6% and 88.2%) [35]. A recent meta-analysis by Qumseya et al [36] comparing RFA with surveillance in BE patients with LGD included 19 studies. This study showed a relative risk of disease progression of 0.14% (95% CI: 0.04-0.45, P 0.001) in the RFA group compared with surveillance, with an absolute risk reduction of 10.9%. Correspondingly, the progression rate was significantly higher among patients with surveillance compared with those treated with RFA (0.022 vs 0.005, P 0.001). These results confirm that RFA should be considered as a treatment option for BE patients with confirmed LGD. If surveillance is the management of choice, the ACG and ASGE recommend surveillance esophagogastroduodenoscopy every 12 months. The AGA proposes surveillance every 6 months for 1 year, then annually unless there is reversion to NDBE. According to the ESGE, surveillance should be performed every 6 months, and if no dysplasia is found again after 1 year. If 2 subsequent endoscopies were negative for dysplasia the recommendation for NDBE should be followed.

High grade dysplasia without a visible lesion

Confirmation by a second expert GI pathologist is required in case of a diagnosis of HGD. The BSG and ESGE recommend referral to a BE expert center where a high definition endoscopy should be repeated. A Dutch study showed that in 79 patients referred with a biopsy diagnosis of HGD or cancer without a visible lesion, 76% of these patients did have a macroscopic abnormality requiring ER, after expert assessment. [11]

If no abnormalities are detected after expert endoscopic assessment and the worst histologic diagnosis remains HGD, RFA treatment should be scheduled. In addition, the BSG recommends referral to a multidisciplinary team, including an endoscopist, upper GI cancer surgeon, radiologist, and GI pathologist to discuss management when therapy is considered.

The ASGE proposes endoscopic treatment and to only consider surveillance in patients unfit or unwilling to undergo therapy. [6] The ACG recommends endoscopic therapy unless life limiting comorbidities exist, but does not mention an alternative surveillance interval. [8]

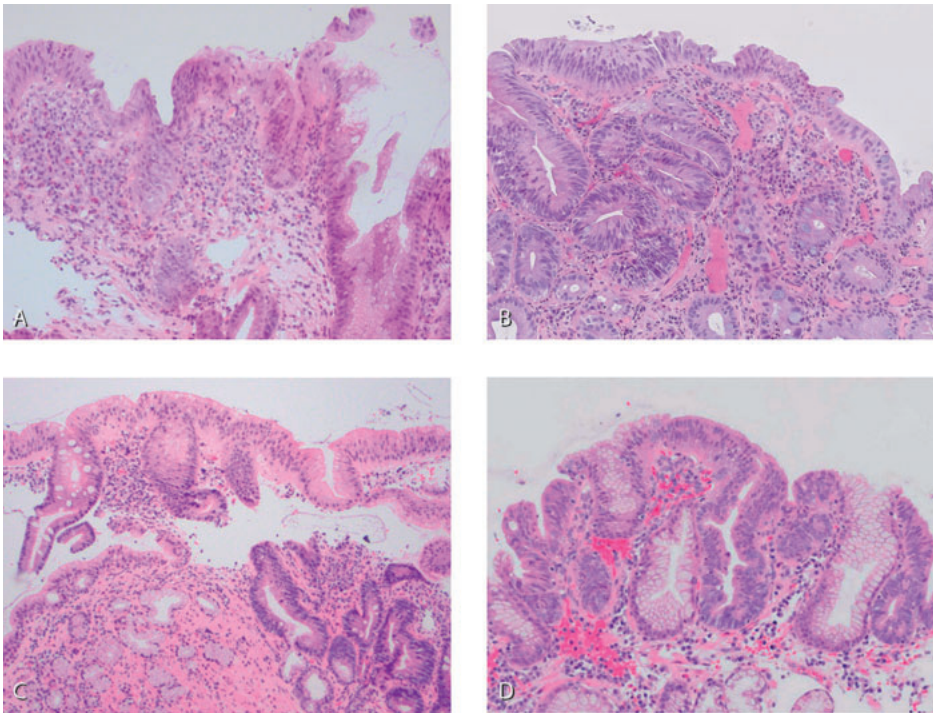
Cancer

A biopsy diagnosis of cancer requires expert endoscopist inspection to identify macroscopic abnormalities that should then be removed by ER. The ER specimen allows for accurate histologic diagnosis of the tumor, including infiltration depth, differentiation grade, presence of lymphovascular invasion, and radicality of the resection.

Importance of pathologic confirmation of dysplasia in case of visible lesions

In case of endoscopically visible lesions, histopathologic diagnosis of the lesion by biopsies is of less importance. Any visible abnormality should be removed by ER, both for therapy as for staging purposes. Pathologic assessment of the ER specimen is important for treatment decisions. ER leads to better histopathologic grading compared to biopsies and studies showed a change in diagnosis in 30%-49%. [37-39] Furthermore, ER improves the interobserver agreement. This is easily explained by larger tissue specimens compared with biopsy material and the ability to evaluate the presence of mucosal and submucosal invasion. [40 41]

Figure 2: Examples of discrepancies between reactive and dysplastic changes



A) Non-dysplastic BE with active inflammation. B) Low-grade dysplasia with active inflammation. C) Non-dysplastic BE with pseudo-stratification. D) Low-grade dysplasia with stratification.

IMPROVING HISTOLOGICAL ASSESSMENT

Improving and centralizing expertise

There are a number of ways to improve interobserver agreement in the diagnostic work-up of BE. First, the expertise of expert BE pathologists can be employed. The gold standard for BE diagnostics still consists of morphology assessment by the human eye, i.e., a pathologist. Studies suggest that expert BE pathologists reach a high level of agreement and can adequately predict progression. [27 30 31 33 42-45]

A study by Montgomery et al. employed 12 GI pathologists that scored a total of 250 slides of BE biopsies. Intra-observer agreement was substantial and inter-observer agreement was moderate when the diagnoses were divided in 4 diagnostic categories (ND, IND and LGD, HGD, carcinoma). [27] One recent study showed that progression

risk of patients with low-grade dysplasia could be related to the number of expert pathologists agreeing on the diagnosis. [30] When 3 pathologists confirmed the diagnosis of low-grade dysplasia, the odds of progression increased 47 times. When 2 pathologists confirmed the diagnosis, the odds of progression were still 27-fold increased. While this study shows that the pathologists participating in this study are experts in separating progressors from non-progressors, it has not yet been defined what actually makes them an expert.

Second, when group discussions are employed, experienced BE pathologists appear to be even more consistent with themselves and among each other, i.e. have a higher intra- and interobserver agreement. [33 42 45] One study from our group (yet unpublished), conducted at our center, employed 5 expert BE pathologists with experience in BE diagnostics ranging from 5-30 years. After assessing and discussing discrepancies of a case set consisting of single-slide BE biopsy cases enriched for dysplasia, they twice assessed a case set of 60 whole endoscopy cases, also enriched for dysplastic cases. Their mean weighted intraobserver agreement improved to 0.84 in 3 diagnostic categories (NDBE, IND, and LGD HGD) These findings are strengthened by the study by Montgomery et al that was mentioned earlier. In this study a group discussion was employed after scoring the first 125 slides. After scoring the second 125 slides, the intraobserver and interobserver agreement increased for all diagnostic category cutoffs, except for the one with 4 categories that required distinction of IND and LGD (4 diagnostic categories: ND, IND, LGD, HGD, or more). [27]

So, in order to structurally employ the expertise of pathologists, a platform should be provided in which they can assess the cases as a group and hold group discussions when they do not reach consensus. In the Netherlands, expert BE pathologists and gastro- enterologists are clustered in BE expert centers. Together they review all dysplastic cases of BE and treat them if the dysplasia is confirmed. Diagnostics of BE is considered a joint effort of the pathologists and gastroenterologists working in these centers (www.barrett.nl).

Table 1: A summary of guideline recommendations based on histological diagnosis of BE and dysplasia

	Definition of BE	Management of NDBE	Management of LGD	Management of Indefinite for dysplasia	Management of HGD
AGA (2011, 2016) [9 10]	Any extent of metaplastic columnar epithelium in the distal esophagus with IM	Surveillance EGD every 3-5 years, length not taken into account	Confirm with expert GI pathologist If confirmed: - Refer to expert center - Repeat EGD in 8-12 weeks under maximal acid suppression. - Consider endoscopic eradication with confirmed and persistent LGD Or - Surveillance EGD every 6 months x2, then annually unless there is reversion to NDBE	Not mentioned	Confirmation by expert GI pathologist If confirmed: - Endoscopic eradication with ablation or EMR Or - Surveillance EGD every 3 months Alternative: - Esophagectomy
ACG (2016) [8]	Salmon-colored mucosa extending ≥ 1cm proximal to GEJ with IM	Surveillance EGD every 3-5 years, length not taken into account	If confirmed & without life-limiting comorbidity: -Endoscopic therapy Or -Surveillance EGD every year	Repeat EGD under maximal acid suppression in 3-6 months . If IND confirmed: -Surveillance interval of 12 months	If confirmed: -Endoscopic eradication therapy (with ER for visible nodularity followed by ablation)

Table 1: Continued

	Definition of BE	Management of NDBE	Management of LGD	Management of Indefinite for dysplasia	Management of HGD
ASGE (2012) [6]	Presence of IM of the tubular esophagus	Surveillance EGD every 3-5 years Consider endoscopic ablation / no surveillance in select cases	Confirm with expert GI pathologist. - Repeat EGD in 6 months - Surveillance EGD every year - Consider endoscopic resection or ablation	Clarify presence and grade of dysplasia with expert GI pathologist. -Increase antisecretory therapy -Repeat EGD and biopsy	Confirm with expert GI pathologist. - RA or RFA ablation. Or - Surveillance EGD every 3 months in select patients - Consider EUS and surgical consultation

Table 1 (cont'd)

	Definition of BE	Management of NDBE	Management of LGD	Management of Indefinite for dysplasia	Management of HGD
ESGE (2017) [7]	Distal esophagus with columnar epithelium ≥1 cm (tongues or circular) containing IM	<1 cm or limited life expectancy / advanced age (75yrs): no surveillance ≥1 cm & ≤3cm: EGD every 5 years ≥3 cm & ≤10cm: -EGD every 3 years ≥10 cm: -Referral BE expert center	If confirmed by 2nd expert GI pathologist: - Referral to BE expert center - Repeat EGD in 6 months If no dysplasia: - EGD after 1 year. After 2 subsequent negative endoscopies: -Follow NDBE recommendation If confirmed LGD in subsequent endoscopies: - Offer ablation	If confirmed by 2nd expert GI pathologist: - Repeat EGD under maximal acid suppression in 6 months. If no definite in subsequent biopsies: - Follow NDBE recommendation	If confirmed: - Referral to BE expert center. - Repeat EGD If NDBE: - Repeat EGD every 3 months If confirmed: endoscopic ablation (preferably RFA)

Table 1: Continued

	Definition of BE	Management of NDBE	Management of LGD	Management of Indefinite for dysplasia	Management of HGD
BSG (2014, 2017) [4 5]	Endoscopically visible and histopathologically confirmed metaplastic columnar epithelium (≥1cm) above GEJ	<3cm (without IM or dysplasia): repeat EGD, if no IM: discharge from surveillance <3cm (with IM): surveillance EGD 3-5 years ≥3cm: surveillance EGD every 2-3 years ≥10 cm: consider referral BE expert center	Repeat EGD with maximal acid suppression in 6 months. If confirmed LGD in ≥2 sets of biopsies: - Offer endoscopic ablation therapy, preferably with RFA, after review by MDT Or - Surveillance EGD every 6 months	Repeat EGD with maximal acid suppression in 6 months. If no definite dysplasia: - Follow NDBE recommendation	MDT referral If confirmed: -EGD in tertiary center Visible lesions: - ER No visible lesions: - Ablation Endoscopic therapy is preferred over oesophagectomy or endoscopic surveillance

Abbreviations: EGD, esophagogastroduodenoscopy; ER, endoscopic resection; GI, gastrointestinal; RFA, radiofrequency ablation; PDT, photodynamic therapy.

Use of immunohistochemistry (p53)

Additional immunohistochemical staining for p53 can improve agreement between pathologists for the diagnosis of dysplasia. A mutation in the p53 tumor suppressor gene pathway can lead to an overexpression pattern or a complete absence of p53 labeling. The BSG and ESGE agree on the useful role of p53 immunostaining and state that it should be considered as adjunct diagnostic tool. The AGA finds that adding p53 to histopathologic assessment of LGD in routine clinical practice needs further clarification since the largest study had only 71% sensitivity and 68% specificity. [46] The ACG acknowledges that an absent or increased expression by immunohistochemistry could be associated with an increased risk of progression but considers no biomarkers or panels of bio- markers ready for routine clinical practice at this point.

Optimizing biopsy sampling

Third, an important prerequisite for an optimal histologic diagnosis is the availability of optimally sampled biopsy material. It is therefore important to have expert endoscopists as well as expert pathologists. From clinical practice, we know that expert endoscopists are able to pick out subtle visible lesions within a Barrett's segment that might be missed by others that are less experienced. [11] Discovery and concurrent sampling of these subtle visible lesions gives the pathologists a better chance at diagnosing dysplasia that might be present within the segment.

When no visible abnormalities are seen, obtaining an adequate number of random biopsies of the entire Barrett segment according to the Seattle protocol is very important. Nonadherence to the biopsy protocol is associated with reduced detection of dysplasia. [47-49] It is important to realize that surveillance with biopsies, even when adhering to the Seattle protocol, is subject to sampling error since dysplasia may be present only focally. Therefore, one should bear in mind that regression of a diagnosis of dysplasia during follow-up endoscopy, may reflect sampling error. A promising technique that may overcome the problem of biopsy sampling error will be discussed in the future paragraph section.

IMPLEMENTATIONOF THESE IMPROVEMENTS

Improving and centralizing expertise

In 2015 set up a national digital review panel for dysplastic Barrett's esophagus was set up in the Netherlands. This panel is formed by a core of 5 expert BE pathologists that have been working in the field of BE for many years and have proven their expertise in many earlier studies. [45] It has been extended to include pathologists from every Barrett expert center in the Netherlands, bringing the number of pathologists in this panel to 14. This Dutch national digital review panel functions as follows: dysplastic cases are sent from a referring hospital to our central laboratory by a gastroenterologist or pathologist. All cases will be stained for p53. Cases are then scanned with a digital slide scanner and made available online in a slide viewer. The pathologists are invited by email to view the case and are provided with the referral pathology report. After they have independently diagnosed the case, their answers are integrated and the cases without a majority diagnosis are discussed in a monthly, online group discussion, in which the slides are also reviewed. The outcome is then sent back to the referring doctor, who can then decide on further management. The outcome of all these common efforts is the improved stratification of patients with dysplastic BE so that patients can be treated when they are found to have confirmed low-grade dysplasia.

Optimizing biopsy sampling

To improve endoscopic detection of early Barrett's neoplasia training of the endoscopist is essential. Recently, an online interactive tool "The BORN project" has become available. This training tool consists of a teaching module with high-quality videos of BE with neoplasia. This module can help train endoscopists in recognition and delineation of lesions, and will help to target biopsies from suspicious areas most likely to harbor dysplasia (www.BEST-Academia.eu).

FUTURE PERSPECTIVES

In the future, hopefully extension of the Dutch national digital review panel to more pathologists can be realized and eventually European coverage can be provided. At the moment all 14 pathologists are assessing all cases. An algorithm is being designed to calculate how many pathologists should view a case in order to yield a reliable diagnosis. Furthermore, the slide sets that have been used to evaluate the

intraobserver and interobserver agreement between the pathologists participating in the Dutch national digital review panel are an excellent source of training material. An online training tool using the digitally available slide sets with annotations and strong consensus diagnosis, will facilitate training of pathologists who would like to specialize themselves further in the field of Barrett's histology.

A promising research effort concerns the field of automated histologic image analysis where fluorescence biomarker labeling is combined with digital imaging and image analysis. Current limitations of histopathologic assessment by pathologists like interobserver and intraobserver variability, subjectivity, and logistic issues can be bypassed by this approach. Another advantage is that this technique does not require the tissue to be digested, as is the case with technologies that measure changes in gene expression or mutations. This technique objectively quantifies multiple epithelial, stromal and morphologic biomarkers, and multiple pathways associated with malignant progression are assessed. The molecular and cellular changes associated with these pathways can precede the morphologic changes that pathologists can evaluate by histology. Formalin-fixed, paraffin-embedded slides are colored by multiple fluorescence bio- markers, imaged by whole slide scanning and analyzed by an image analysis platform using algorithms. [50] This quantitative and objective approach has proven to be able to differentiate between low and high risk on malignant progression within 5 years in BE patients, [51] and might be a promising future technique.

Another novel appealing concept is wide-area transepithelial sampling (WATS) with computer-assisted analysis. With the use of an abrasive brush deep transepithelial tissue from a wide area of the BE segment can be sampled. Then a computer identifies the 200 most suspicious areas based on cellular morphology and molecular diagnostics, after which a trained pathologist evaluates the sample on a high-resolution video monitor. WATS has proven to have a high interobserver agreement for NDBE, LGD, and HGD or cancer (κ 0.88, 0.74 and 0.95, respectively) in a study where 149 slides were assessed. [52] A large prospective trial has shown that the addition of WATS to 4-quadrant biopsies increased the overall detection of IM by 39.8% in a population screened for BE and dysplasia. [53] A smaller study in BE surveillance patients resulted in increased yield of dysplasia detection. [54] In a high-risk BE surveillance population WATS in combination with 4-quadrant biopsy sampling increased the yield of esophageal

dysplasia detection by 42% compared with biopsy sampling alone. [55] WATS may overcome the problem of sampling error that is inherent to biopsy sampling, even when adhering to the Seattle protocol.

CONCLUSION

Histopathologic assessment of BE and dysplasia is essential in choosing the right treatment strategy or surveillance interval. The continuous spectrum of dysplasia and the inflammatory nature of BE that can mimic dysplasia complicate making the correct histologic diagnosis. To improve histologic assessment, the use of a panel of expert pathologists, immunohistochemistry, and new techniques like automated histologic image analysis should be implemented and developed further.

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