Barrett’s esophagus: The pathomorphological and molecular genetic keystones of neoplastic progression

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Abstract
Barrett’s esophagus is a widespread chronically progressing disease of heterogeneous nature. A life threatening complication of this condition is neoplastic transformation, which is often overlooked due to lack of standardized approaches in diagnosis, preventative measures and treatment. In this essay, we aim to stratify existing data to show specific associations between neoplastic transformation and the underlying processes which predate cancerous transition. We discuss pathomorphological, genetic, epigenetic, molecular and immunohistochemical methods related to neoplasia detection on the basis of Barrett’s esophagus. Our review sheds light on pathways of such neoplastic progression in the distal esophagus, providing valuable insight into progression assessment, preventative targets and treatment modalities. Our results suggest that molecular, genetic and epigenetic alterations in the esophagus arise earlier than cancerous transformation, meaning the discussed targets can help form preventative strategies in at-risk patient groups.

KEYWORDS
Barrett’s esophagus, epigenetic changes, esophageal cancer, molecular pathways, oncotransformation, preventative targets

1 | INTRODUCTION
Esophageal adenocarcinoma (EAC) is predominantly found in the distal third of the esophagus. Early diagnostics of EAC is challenging due to the lack of specific symptoms. More than 50% of EAC cases are diagnosed at stages III-IV, which explains the poor prognosis associated with this malignancy. The recently reported 5-year survival of patients with EAC is around 20.1%–23.4%.1,2 Risk factors of EAC development include male gender, gastro-esophageal reflux disease (GERD), Barrett’s esophagus and smoking.1,3–8 Barrett’s esophagus (BE) is a premalignant condition for EAC. Risk of developing EAC is significantly higher in patients with BE compared to the general population.6 Routine endoscopic surveillance with histopathological assessment in BE patients aims for early detection of neoplasia.9–12 Detection of dysplastic BE and T1a stage of EAC prompts endoscopic treatment which delivers high 5-year survival rates.13,14 Nonetheless, the role of BE and different types of metaplasia in the distal esophagus region in progression to EAC is under discussion15 and existing algorithms of endoscopic surveillance...
are suboptimal because most of patients diagnosed with EAC do not have any history of BE. Analysis of existing information on the different types of esophageal metaplasia pathways and their contribution to development of EAC will help delineate possible diagnostic and therapeutic targets. Our essay is focused on morphological diagnosis, immunohistochemical (IHC) examination and molecular-genetic methods for dysplasia detection and prediction of neoplastic progression.

All images presented in this study were obtained following approval by the ethics committee at the 31st State City Hospital of Moscow (№03-19 from 06.12.2019). All patients included in the pathomorphological study provided informed written consent.

2 | RISK OF EAC IN METAPLASTIC PROCESSES OF THE ESOPHAGUS

Long lasting reflux exposure in distal esophagus results in initiation of columnar-lined esophagus. Cardiac type metaplasia is the earliest morphologic finding, although multitude of gland structure phenotypic variants arises in segment of metaplasia in distal esophagus over time. Proportion of glands goes through enteralization which causes development of intestinal metaplasia (IM, or so called specialized metaplasia) with easily found hallmark goblet cells (GCs) that are inserted among foveolar cells. Enteralization is believed to start with expression of immunohistochemical markers of intestinal differentiation in columnar epithelium, such as CDX2, villin and Das-1, followed by MUC2 expression and development of GCs. Paneth cells are detected in some cases of specialized metaplasia. Segment of metaplasia may also contain different variants of gastric metaplasia: glands of cardiac, oxynto-cardiac and fundic type. Various phenotypes of metaplasia can be identified in biopsy pieces of distal esophagus separately or in combination.

There are two ultimately different approaches to BE diagnostics. British Society of Gastroenterology (BSJ) and international consensus BOB CAT define BE as any type of columnar metaplasia in distal esophagus. Meanwhile, American Gastroenterological Association (AGA) and Russian Society of Pathologists (RSP) require mandatory presence of IM for diagnosis of BE because IM is associated with increased risk of EAC development.

For a long time, it was accepted that more than 90% of all EAC arise at background of IM. In a large epidemiological study, Bhat S. et al. identified incidence of high-grade dysplasia (HGD)/EAC in patients with IM to be 0.58% a year, and only 0.07% a year in patients without IM (hazard ratio 3.54, 95% CI 2.09–6.00, p < 0.001), whereas in other research incidence of HGD/EAC did not differ in patients with IM and gastric metaplasia at initial biopsy. Tan M.C. et al. demonstrated in meta-analysis that BE (IM) is detected only in 56.6% patients (95% CI 48.5%–64.6%) at the time of EAC diagnosis. In addition, BE is more frequently identified in patients with early EAC: in studies, where early EAC was diagnosed in 100% of cases, BE was confirmed in 91.3% patients (95% CI 82.4%–97.6%). Sawas T. et al. observed IM only in 45.0%–49.9% patients with EAC and the frequency of BE detection in patients with different stages of EAC was nearly equal that contradicts overgrowth of IM by tumor. Sawas T. et al. identified two phenotypes of EAC with different prognosis based on the presence or absence of BE: EAC with BE at background was characterized by better prognosis than EAC without BE. The authors suppose ultra-short segment of IM to be the source of EAC without BE. Nevertheless, it is widely accepted that the chance of IM detection rises with increase in segment length. Considering that IM is rare in ultra-short segment (it is detected only in 14.8% patients) and in most cases it comprises cardiac and oxynto-cardiac metaplasia, it seems logical to assume the source of such EAC to be ultra-short and short segments of gastric type metaplasia (Figure 1).
This proposition is supported by results of several studies. Takubo K. et al.\textsuperscript{35} demonstrated that more than 70% cases of minute EAC arise at background of cardiac or fundic-type metaplasia surrounding the tumor. Performing IHC examination Watanabe G. et al.\textsuperscript{36} detected gastric phenotype (expression of gastric differentiation markers MUC5A and MUC6 with negative expression of intestinal markers) more frequently in minute tumors. Several phenotypes of dysplasia and EAC were identified based on IHC examination with gastric and intestinal markers that confirm presence of two distinct pathways in carcinogenesis: intestinal and foveolar,\textsuperscript{37,38} although genetic analysis showed that both metaplasia types harbor the same mutations.\textsuperscript{39} Using histological, IHC examination and genetic analysis, Lavry D.L. et al.\textsuperscript{40} revealed that even when IM is present EAC arises from gastric type metaplasia. On the other hand, high density of GCs in BE is associated with a decrease in the risk of EAC development and may represent a protective mechanism of adaptation.\textsuperscript{41-43} Inhibition of Notch-signaling causes proliferative cells in metaplastic glands to become terminally differentiated GC.\textsuperscript{44,45} Thus, induction of GC differentiation may represent a potential therapeutic strategy of EAC prevention in patients with BE.\textsuperscript{41,44}

3 | ENDOSCOPIC EVALUATION IN BE AND EAC: STANDARD PROCEDURE AND COMPUTER-AIDED DETECTION (CAD)

White light endoscopy (WLE) with four-quadrant biopsy each 2 cm plus biopsy from any suspicious visual lesions is recommended by most of the guidelines\textsuperscript{9,10,12} as an effective tool for dysplasia detection.\textsuperscript{46} Nevertheless, adherence to standard protocol is low, comprising between 24.1% and 82.7%\textsuperscript{47-49} and is even lower in long segment of dysplasia, where dysplasia is more likely to be found. Standard protocol is time and cost consuming, prone to sampling error and results in high load of pathologists with abundant biopsies.\textsuperscript{16} That is why a lot of different endoscopy modalities and techniques were tried for visualization of dysplasia and precise biopsy, among them narrow-band imaging (NBI),\textsuperscript{50-52} acetic acid chromoendoscopy (AAC),\textsuperscript{53-56} autofluorescence imaging (AFI),\textsuperscript{57,58} confocal laser endomicroscopy (CLE)\textsuperscript{59-63} and volumetric laser endomicroscopy (VLE).\textsuperscript{64-66} Although some studies demonstrated different imaging modalities to be efficient, in other studies the use of these techniques did not report benefits in dysplasia detection rate.\textsuperscript{67-70} Sensitivity of standard protocol with 4-quadrant biopsy ranged from 28% to 85% in different studies and specificity varied from 56% to 100%, this led American Society for Gastrointestinal Endoscopy to set thresholds for any Preservation and Incorporation of Valuable endoscopic Innovations (PIVI)\textsuperscript{71}: an imaging technology with targeted biopsies should have a per-patient sensitivity of 90% or greater, negative predictive value (NPV) of 98% or greater for detecting HGD or early EAC and specificity of at least 80% to allow a reduction in the number of biopsies compared to standard protocol.

However, recent research showed benefits of CAD using WLE images\textsuperscript{72-76} in dysplasia and early EAC detection. At first, F. van der Sommen et al.\textsuperscript{72} used 100 WLE images obtained from 44 patients with BE to develop CAD model based on machine learning algorithm that identified HGD and early EAC with sensitivity of 86% and specificity of 87% at the patient level. Next, Mendel R. et al.\textsuperscript{73} performed a convolutional neural networks (CNN) analysis of BE using 50 WLE images of EAC and 50 BE images from an open access database (Endoscopic Vision Challenge MICCAI 2015) and achieved sensitivity of 94% and specificity of 88%. Notably, A.J. de Groof et al.\textsuperscript{74} developed a hybrid ResNet-UNet model CAD system using 5 independent WLE endoscopy datasets. Pre-training was performed using large series of 494,364 labelled endoscopic images. Then 1247 images of early neoplasia and non-dysplastic BE (NDBE) were used in the second-step training and other 297 images (3rd step) – for internal validation. Two sets (4th and 5th step) each of which containing 40 neoplastic and 40 NDBE images served for external validation. At the 5th step, accuracy was 88%, sensitivity 93% and specificity 83% that outperformed results of general endoscopists (73%, 72% and 74%, respectively). The computational speed for classification and delineation of the endoscopic images in this study was compatible for use in real time during endoscopic surveillance. Hashimoto R. et al.\textsuperscript{75} also developed CNN algorithm for detection of dysplastic BE and NDBE with sensitivity of 96.4%, specificity of 94.2% and accuracy of 95.4%. This study also suggested possibility of real-time implementation. It was practically proved by Ebigbo A. et al.\textsuperscript{76} In this study, 129 endoscopic images were used for CAD system training and validation was performed in real-time assessing images from 14 patients with further histological confirmation. In this study, CAD sensitivity of 83.7%, specificity of 100.0% and overall accuracy of 89.9% were reached. Few studies also assessed CAD dysplasia detection using VLE.\textsuperscript{77-79} The data are summarized in Table 1.
Table 1: Standard WLE and CAD models in diagnostics of dysplasia and EAC

| Method                         | Advantages                                                                 | Disadvantages                                                                 | Articles                                      | Number of patients/images | Sensitivity | Specificity |
|--------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------|---------------------------|-------------|-------------|
| WLE with standard 4-quadrant biopsy | Is recommended by most of guidelines as effective                           | Poor adherence to protocol                                                    | ASGE PIVI (2012)71                          | —                         | 28%–85%    | 54%–100%   |
|                               |                                                                             | Prone to sampling error                                                        |                                              |                           |             |             |
|                               |                                                                             | High load of pathology department                                             |                                              |                           |             |             |
| WLE + CAD                     | Helps to avoid subjectivity in evaluation.                                  | Not currently used in general practice.                                       | van der Sommen F. et al. (2016)72           | 44 patients (100 images) | 86%         | 87%         |
|                               |                                                                             | Evaluation in real-time needs to be developed and validated.                  | Mendel R. et al. (2017)73                    | 100 images from MICCAI database | 94%         | 88%         |
|                               |                                                                             |                                                                             | de Groof A.J. (2020)74                      | Pre-training - 494,364 images               | 93%         | 83%         |
|                               |                                                                             |                                                                             |                                              | Training – 1247 images               |             |             |
|                               |                                                                             |                                                                             |                                              | Internal validation – 297 images         |             |             |
|                               |                                                                             |                                                                             |                                              | External validation – 2 sets of images (80 + 80) |             |             |
|                               |                                                                             |                                                                             | Hashimoto R. et al. (2020)75                | Training - 65 patients (1835 images)       | 96.4%       | 94.2%       |
|                               |                                                                             |                                                                             |                                              | Validation - 458 images                  |             |             |
|                               |                                                                             |                                                                             | Bigbo A. et al. (2020)76                    | Training - 129 images                   | 83.7%       | 100.0%      |
|                               |                                                                             |                                                                             |                                              | Validation - 14 patients (62 images)      |             |             |

Table 5: Pathomorphological features of dysplasia in Barrett’s esophagus

Neoplastic progression in BE goes through the following stages: nondysplastic BE (NDBE) → low-grade dysplasia (LGD) → high-grade dysplasia (HGD) → EAC (Figure 2). Morphological detection of the pits that may mimic dysplasia and invasion, but cannot substitute forces biopsy samples, because it does not access the architecture changes for diagnostics of dysplasia or different glands at the bases of the pits. The inter-observer agreement among pathologists for the diagnosis using WATS was better than for standard random 4-quadrant forceps biopsies (kappa 0.95–0.97). The kappa values for HGD/EAC, IND/LGD, and NDBE comprised 0.95 (95% CI 0.88–0.99), 0.74 (95% CI 0.61–0.85), and 0.98 (95% CI 0.83–0.94), respectively. WATS is an adjuvant for diagnosis of dysplasia. The rate of detection of dysplasia by WATS was 13.1% and increased to 13% increasing the yield of dysplasia detection from 0.68% to 2.33% in meta-analysis. WATS significantly improved the overall detection of dysplasia by 242% (95% CI 195%–315%). Rate of BE detection by forceps biopsy was 13.1% and WATS raised relative increase of 205 in the detection rate of esophageal dysplasia (95% CI 1.98–2.05, P < 0.0001). The detection of BE by 15% to 13% increasing the overall detection of BE by 205 in the detection rate of esophageal dysplasia (95% CI 1.98–2.05, P < 0.0001). The detection of BE by 15% to 13% increasing the overall detection of BE by 205 in the detection rate of esophageal dysplasia (95% CI 1.98–2.05, P < 0.0001).
of dysplasia in BE represents a clinically relevant factor for stratification of EAC development risk.\textsuperscript{94–100} The risk of EAC is 10-fold higher in LGD compared with NDBE.\textsuperscript{94} Gradation of neoplastic changes at pathological examination is held in accordance with Vienna classification\textsuperscript{101} or criteria proposed by Reid B.J. et al.\textsuperscript{102} (Table 3). Both diagnostic systems are consistent with current clinical practice.\textsuperscript{103}

Four morphological criteria were developed for dysplasia identification\textsuperscript{90,104}: (1) surface maturation versus epithelium in the glands, (2) architecture of glands, (3) cytological features of proliferation, and (4) presence of inflammation, ulcers or erosions.

NDBE specimens of esophageal mucosa are lined with columnar epithelium with round-shaped glands containing GCs, surface maturation is obvious, extent of mixed inflammatory infiltration in stroma varies greatly (Figure 3). GCs are necessary to distinguish with pseudogoblet cells (pseudo-GCs)—foveolar cells distended by mucus.\textsuperscript{105,106} In most cases, it can be done in specimens stained with hematoxylin and eosin. GCs are more round in shape, with clear to bluish cytoplasm and triangle nuclei, and they are scattered through epithelium, whereas pseudo-GCs are more elongated, with homogenous clear to pink cytoplasm and are organized in linear groups. In difficult cases, PAS/Alcian blue stain can be used to distinguish GCs and pseudo-GCs. PAS/Alcian blue stains blue cytoplasm of GCs, whereas cytoplasm of pseudo-GCs in most cases stains purple (Figure 4), although sometimes cytoplasm of pseudo-GCs stains blue by PAS/Alcian blue like cytoplasm of GCs. In such cases, IHC examination with MUC2—a highly specific marker of GCs—is of value\textsuperscript{107} (Figure 5). At the other hand, Srivastava et al.\textsuperscript{106} stated that ancillary stains are not necessary in diagnosis of BE, because they do not add accuracy in GCs detection.

There are two main types of dysplasia: more common adenomatous and rare foveolar.\textsuperscript{108–112} LGD shows weak or absent surface maturation. Inflammatory infiltration of stroma is scarce. Mild architecture distortion is typical: glands are slightly crowded, round and angulated, lined with columnar epithelium with nuclei located at the basal 1/2 of cells, and few nuclei may contain nucleoli. In adenomatous dysplasia (Figure 6), nuclei are mildly enlarged, slightly elongated, stratified and hyperchromatic, with few mitoses. In foveolar dysplasia, epithelial cells are cuboid with round to oval, and nuclei are slightly enlarged with hyperchromatosis (Figure 7).

HGD is characterized by prominent changes in architecture and/or pronounced features of cytological atypia as well as absent surface maturation. In adenomatous HGD (Figure 8) glands are crowded, with “back-to-back” appearance, and stroma between glands is scarce. Glands are of irregular shapes, some glands may be distended,
and few glands may represent micropapillary or cribriform pattern. Loss of cellular polarity and prominent nuclear stratification is identified. Nucleo-cytoplasmic ratio is highly increased, nuclei are elongated (pencil-like), hyperchromatic, nuclear membrane is irregular, nucleoli may be easily found. Mitoses, including atypical ones, are

Table 3: Comparison of two systems of dysplasia gradation in BE: proposed by Reid (1988) and the Vienna classification of gastrointestinal epithelial neoplasia (2000)

| The Vienna classification of gastrointestinal epithelial neoplasia, 2000<sup>101</sup> | Consensus for grading dysplasia in BE, 1988<sup>102</sup> |
|-----------------------------------------------|-----------------------------------------------|
| Negative for dysplasia/neoplasia              | Negative for dysplasia/neoplasia              |
| Indefinite for dysplasia/neoplasia            | Indefinite for dysplasia                      |
| Non-invasive low-grade neoplasia (low-grade adenoma/dysplasia) | Low-grade dysplasia                           |
| Non-invasive high-grade dysplasia              | High-grade dysplasia                          |
| High-grade dysplasia                           |                                               |
| Non-invasive adenocarcinoma (carcinoma in situ) |                                               |
| Suspicious for invasive carcinoma             |                                               |
| Invasive neoplasia                             | Adenocarcinoma                                |
| Intramucosal adenocarcinoma                    | Intramucosal adenocarcinoma                   |
| Submucosal adenocarcinoma or beyond            | Invasive adenocarcinoma                       |

Figure 2: Schematic illustration that demonstrates changing morphological features during neoplastic progression in BE and non-intestinal metaplasia of distal esophagus

Figure 3: Nondysplastic BE. Specimen of IM in distal esophagus with high density of goblet cells, stroma shows inflammatory infiltration and extravasation: (A) hematoxylin and eosin staining, (B) PAS/Alcian blue staining, magnification ×100
readily identified. Foveolar HGD (Figure 9) harbors less extensive architecture changes but severe enlargement of nuclei, hyperchromatosis and noticeable nucleoli.

LGD and HGD are distinguished based on severity of (1) architecture distortion and (2) cytological atypia. In subset of cases, prominent cytological

FIGURE 4 Gastric metaplasia with pseudo-GCs in distal esophagus. Specimen of metaplastic distal esophagus with distended foveolar cells, containing apical mucus at the surface. (A) hematoxylin and eosin staining, (B) PAS/Alcian blue staining: cytoplasm of epithelial cells stains purple, magnification ×100

FIGURE 5 Pseudogoblet cells in gastric metaplasia. Specimen of columnar-lined esophagus with elongated distended cells at the surface with apical mucus. (A) hematoxylin and eosin staining, (B) PAS/Alcian blue staining: cytoplasm of surface epithelium stains blue, (C) IHC evaluation with MUC2 shows negative expression, magnification ×200

FIGURE 6 Adenomatous low-grade dysplasia, hematoxylin and eosin staining: (A) magnification ×100, (B) magnification ×200. Specimen of columnar-lined esophagus with lack of surface maturation. Most of glands are simple, round or angulated, few of them are dilated. Nuclear stratification and enlarged nucleo-cytoplasmic ratio is obvious. Nuclei are pencillated, located in basal ½ of cells, mitoses are readily identified.
atypia with markedly enlarged, stratified, pleomorphic nuclei and a lot of mitoses is sufficient for diagnosis HGD even if changes in architecture are moderate. Prominent architecture distortion even accompanied with mild cytological atypia should be classified as HGD. In biopsy specimens with LGD count of GCs varies greatly—from few GCs to high density GCs. Although depletion of GCs is typical for dysplasia in general, Bansal et al.\textsuperscript{31} found association between LGD and high count of GCs. In HGD and EAC, count of GCs is usually decreased.

Intramucosal EAC is diagnosed when there is invasion through the basal membrane into the lamina propria but not deeper than muscularis mucosae and invasive carcinoma is characterized by deeper invasion. In intramucosal EAC glands acquire “back-to-back” appearance, syncytial growth pattern and single cells or small clusters

FIGURE 7 Foveolar low-grade dysplasia, hematoxylin and eosin staining: (A) magnification ×200, (B) magnification ×400. Surface maturation is absent. Glands are mainly round shape, lined with cuboid epithelium with increased nucleo-cytoplasmic ratio. Nuclei are round and hyperchromatic, with nucleoli. Few mitoses as well as apoptotic bodies are identified

FIGURE 8 Adenomatous high-grade dysplasia, hematoxylin and eosin staining, (A) magnification ×200, (B) magnification ×400. Specimen of columnar-lined esophagus with complex structure of glands, including dilated glands with micropapillae. Nuclei of epithelial cells are prominently enlarged, elongated and hyperchromatic. Mark nuclear stratification and loss of polarity are also features of HGD

FIGURE 9 Foveolar high-grade dysplasia, hematoxylin and eosin staining, (A) magnification ×200, (B) magnification ×400. Glands are predominantly round in shape, highly crowded, lined with columnar epithelium. Nuclei are round to oval, with severe enlargement, hyperchromatosis and a number of mitoses
within the lamina propria. At this stage desmoplasia is either absent or subtle. Obvious desmoplasia and infiltrative growth pattern appear in tumors with deeper invasion (Figure 10).\textsuperscript{113,114}

Differential diagnosis of HGD and EAC in biopsy specimens is problematic with intraobserver agreement at about 0.30–0.65.\textsuperscript{90,115–117} In early studies, when HGD was an indication to operative treatment, EAC was identified in 40%–70% esophagectomies after pre-operative diagnosis HGD.\textsuperscript{118–120} Several features when they are identified in HGD are suspicious of unsampled EAC, including extensive cribriforming, dilated glands filled with necrotic debris, ulceration, intraluminal neutrophils and pagetoid pattern of neoplastic cells extension into squamous epithelium.\textsuperscript{114}

In some observations it is troublesome to judge about dysplasia: morphological features are suspicious for dysplasia, but not sufficient to be definite.\textsuperscript{105,113,121} In these cases the appropriate diagnosis is indefinite for dysplasia—IND (Figure 11). Such situations derive from technical issues causing artificial changes, lack of surface epithelium or scarce biopsy pieces. Also IND may be diagnosed in specimens with abundant inflammation, ulcers or erosions resulting in reactive changes of epithelium that display focal weak surface maturation and cytological atypia (increased nucleo-cytoplasmic ratio, hyperchromatosis and mitoses).

Incidence of EAC in patients with NDBE is estimated as 0.12%–0.33% a year.\textsuperscript{26,122,123} Rate of EAC detection increases with duration of surveillance and represents 0.19% a year in first 5 years after BE was diagnosed and 0.63% a year after 20 years of surveillance.\textsuperscript{124} Incidence of EAC in patients with LGD varies from 0.76 to 28% a year.\textsuperscript{26,98,99} The main reason for such a variety involves low intra-observer agreement and poor reproducibility in diagnostics of presence and grade of dysplasia.\textsuperscript{90–92,99,100}

At least two pathologists should independently perform histological examination in each case to avoid subjectivity in dysplasia detection.\textsuperscript{9–12,120,125} In several studies, the number of pathologists that confirmed dysplasia was associated with rate of progression.\textsuperscript{92,93,98,120,126} Curvers W.L. et al.\textsuperscript{126} estimated incidence of HGD/EAC as 13.4% when initial diagnosis LGD was confirmed by expert pathologist and only as 0.49% in cases when expert pathologist down-graded the lesion to NDBE. In a prospective study of Duits L.C. et al.\textsuperscript{98} risk of progression to HGD/EAC increased 10-fold when one pathologist established LGD, 27-fold when two pathologists recognized dysplasia and 47-fold when all three pathologists confirmed LGD. Nevertheless LGD is overdiagnosed in 28%–85% of observations.\textsuperscript{98,99,126} and HGD—in 40% of cases,\textsuperscript{99,127} that leads to more aggressive treatment. IHC evaluation provides an opportunity not only to increase reproducibility of dysplasia diagnostics,
but also to identify patients who are at high risk of neoplastic progression.

6 | IMMUNOHISTOCHEMICAL MARKERS OF DYSPLASIA AND PROGRESSION PREDICTORS IN BE

IHC with p53. Inactivation of p53 is a key feature that occurs early in BE carcinogenesis,\textsuperscript{126–130} though it is not surprising that IHC evaluation with p53 is used for precise diagnostics of dysplasia. Two patterns of aberrant p53 expression are identified: more frequently detected p53 overexpression (Figure 12) is associated with missense mutation of TP53, whereas absent p53 expression is caused by deletion or truncating mutation of TP53.\textsuperscript{93}

Use of IHC evaluation with p53 improves reproducibility in morphological assessment of BE specimens and aids to avoid overdiagnosis of dysplasia.\textsuperscript{92,93,131–133}

Moreover, aberrant expression of p53 is associated with increased risk of progression to EAC.\textsuperscript{92,133–145} Murray L. et al.\textsuperscript{135} showed that diffuse expression of p53 is a predictor of progression to HGD/EAC (odds ratio – OR 8.42 [95% CI 2.37–30.0]), although p53 alone is not a reliable marker as in 2/3 of patients who progressed to HGD/EAC pattern of p53 expression was normal. In other studies OR of development HGD/EAC in aberrant p53 expression varied from 3.0 to 21.6.\textsuperscript{139,142} Kastelein F. et al.\textsuperscript{138} demonstrated that prognostic value to predict neoplastic progression increased from 15% for morphological diagnosis of LGD to 33% for LGD with aberrant expression of p53.

In a prospective study, Younes M. et al.\textsuperscript{141} detected progression to HGD/EAC in 31.25% patients with expression of p53 in aggregates of epithelial cells and in 75% patients with p53 expression in multifocal aggregates of epithelial cells at initial biopsy (Kaplan–Meier analysis, $p < 0.0001$). In this study progression to HGD/EAC was seen in 40% observations with overexpression of p53 and only 0.3% patients with negative expression of p53 (Kaplan–Meier analysis, $p < 0.0001$).

Different definitions of aberrant IHC staining with p53 were used in various studies that make them difficult to compare. Although relevant association of aberrant p53 expression with neoplastic progression in BE was proved in meta-analyses. Janmaat V.T. et al.\textsuperscript{143} estimated overall OR of progression to HGD/EAC in aberrant expression of p53 as 3.86 (95% CI 2.03–7.33), whereas in patients with NDBE with aberrant expression, overall OR comprised 6.12 (95% CI 2.99–12.52) and in patients with LGD it was as high as 8.64 (95% CI 3.62–20.62). More stringent criteria for aberrant staining definition resulted in higher overall OR.\textsuperscript{144} In other meta-analysis performed by Snyder P. et al.\textsuperscript{144} OR of neoplastic progression in patients with aberrant expression of p53 in case–control studies varied from 3.84 to 5.95, as well as hazard ratio in cohort studies was estimated as 14.25 and 17.31 in different statistical models.
Use of IHC examination with p53 in routine practice was recommended by BSG$^9$ and European Society of Gastrointestinal Endoscopy (ESGE).$^{134}$

**IHC with Ki67.** Level of Ki67 expression that characterizes proliferative activity of cells increases in line: NDBE—LGD—HGD—EAC.$^{136,137,142,146,147}$ Expansion of Ki67-positive epithelial cells from proliferative zone at the middle third of crypts to surface is observed during neoplastic progression (Figure 13).$^{148–152}$ Diffuse positive immunostaining of Ki67 at the surface is usually detected in HGD that helps us to distinguish HGD from LGD, where only minority of surface epithelium shows Ki67 expression.$^{136,149,150}$ Use of IHC with Ki67 improves reproducibility of dysplasia diagnosis in BE.$^{131,147,153}$ Extensive expression of Ki67 is also associated with progression to HGD/EAC.$^{136,137,145}$

**IHC with AMACR.** The most controversial results were obtained for use of AMACR. In several studies, staining of AMACR was either absent$^{154–156}$ or was detected in few cases of NDBE.$^{151}$ Frequency of detection and extension of AMACR expression rises in line LGD—HGD—EAC (Figure 14).

Shi X.Y. et al.$^{151}$ estimated sensitivity of AMACR expression for distinguishing between NDBE and dysplastic BE as 72.4% and specificity as 94.8%; staining of AMACR correlated with expression of p16, cyclin D1 and Ki67. Staining of AMACR was helpful to distinguish NDBE from IND/LGD and LGD from HGD. In other research, expression of AMACR did not differ between NDBE, IND and LGD, but was elevated in HGD.$^{157}$ Sensitivity of AMACR expression varied widely: from 38 to 91.3% for LGD, from 64 to 95.8% for HGD and from 72 to 96% for EAC and specificity comprised 100%.$^{154–156}$ Nevertheless, Strater J. et al.$^{158}$ showed weak expression of AMACR in 83% cases of NDBE, indicating low sensitivity of AMACR in BE-associated dysplasia detection.

In case–control study with large amount of samples (12,127 biopsies derived from 635 patients), Kastelein F. et al.$^{159}$ demonstrated that strong AMACR expression was associated with progression to HGD/EAC (relative risk 4.8, 95% CI 1.9–12.6), although positive predictive value of strong AMACR expression (22%) was too low to use AMACR as the only marker of progression.

To sum up, IHC examination with p53, Ki67 and AMACR aims for precise diagnostics of dysplasia in BE (Table 4). Moreover, expression of these IHC markers has some prognostic value (Table 5), although predictive value of any IHC marker alone is limited. New challenge is to develop a combination of IHC markers for precise diagnostics of dysplasia in BE and prediction of progression.

### MACHINE LEARNING ALGORITHMS IN DIGITAL PATHOLOGY

To overcome low inter-observer agreement on dysplasia diagnosis, attempts were made to develop machine learning approach applying to high-resolution digital images with evaluation of morphometric and immunoquantitative parameters to distinguish between NDBE, dysplastic

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**FIGURE 13** IHC evaluation with Ki67 in BE, magnification ×400: (A) nondysplastic BE: nuclear expression of Ki67 in the middle 1/3 of crypts, (B) BE with LGD: expression of Ki67 in the middle and the upper 1/3 of crypts, (C) BE with HGD: expression of Ki67 at the surface, (D) EAC: diffuse expression of Ki67
FIGURE 14  IHC with AMACR in BE, magnification ×400: (A) BE without dysplasia: weak attenuated expression in cytoplasm (background expression), (B) BE with LGD: granular expression of AMACR in proportion of epithelial cells, (C) BE with HGD: granular expression of AMACR in majority of epithelial cells, (D) EAC: granular expression of AMACR in proportion of epithelial cells

TABLE 4  Histological evaluation and immunohistochemical assay in diagnostics of BE

| Diagnostic method | Markers | Advantages | Disadvantages |
|-------------------|---------|------------|---------------|
| Histopathology assessment of forceps biopsy | Presence and grade of dysplasia | Standard diagnostic procedure, Routinely used, Cost-effective, Easy to perform, LGD histology is associated with progression | Poor inter-observer agreement, Low reproducibility, High rate of dysplasia overdiagnosis, Need for second opinion/evaluation by expert |
| IHC evaluation | p53 | Confirming presence or absence of dysplasia, Proved efficient in diagnostics, Low cost, Prognostic tool, Recommended as a routine method by BSG9 and ESGE125, Extensively studied marker | Lack of standardization in interpretation of staining: different definitions and cut-points are used in various studies,106 Although some studies demonstrate good inter-observer agreement,93,138 Positive staining is observed in up to 10% of NDBE105,106 Although aberrant expression is highly associated with progression, proportion of patients with scattered staining also develops EAC135 |
| | Ki67 | Additional tool to evaluate proliferative activity, Some data suggest association with progression, Is available in routine practice, Low cost | Nonspecific marker that stains both dysplasia and reactive epithelium, Low value as a predictive marker |
| | AMACR | Additional tool to assess dysplasia in BE, Has some prognostic value, Is available in routine practice, Low cost | Sensitivity and specificity varies greatly in different studies, Low value as a predictive marker |
BE and EAC (Table 6). The earliest work in this field was the study of Polkowski W. et al. (1998) which suggested that quantitative assessment of cytometric and morphometric features associated with proliferation and differentiation could help in interpretation of BE histology. Combination of stratification index (SI) and Ki67 quantitative analysis gave the best classification result, but quantitation of p53 area added no value. Van Sandick J.W. et al. also showed benefit of SI and Ki67 area combination for distinguishing between LGD and HGD (91% correct classification), although combination of SI and p53 area was superior for distinguishing between NDBE and LGD (89% correct classification). Importantly, Baak J.P. et al. reported only 35% agreement between pathologists and experts. Experts downgraded high proportion of lesions due to severe inflammation, reactive changes, ulcers, proximity to squamo-columnar junction and tangential cutting. In adequate sections morphometrical classification was closer to experts' grading (75% of agreement compared with 53% for pathologists). Sabo E. et al. developed neural network algorithm (NNET) for dysplasia grading using nuclear appearance (size, shape, chromatin texture, pleomorphism, symmetry and pseudostratification) that was able to correctly classify 89% of cases in distinguishing between NDBE and LGD and 87.5% of cases in differentiation between LGD and HGD. Moreover, in this study some of the variables were predictive for progression. Recently, Tomita N. et al. proposed new attention-based network model that classified NDBE, dysplastic BE and EAC with mean accuracy of 0.83.

TissueCypher. TissueCypher (Cernostics, Inc.) is a tissue system pathology assay using set of immunofluorescent markers (p16, AMACR, p53, CD68, COX-2, CD45RO, HIF1a, HER2/neu and K20). Quantitative integrated image analysis of expression and co-expression of these markers in combination with morphological changes in nuclei in biopsy specimens of distal esophagus was used to develop a risk assessment model based on 15 parameters that allows identifying patients with low, intermediate and high risk of neoplastic progression. TissueCypher result predicts progression independently of pathology analysis, segment length, age, sex or p53 overexpression. Use of TissueCypher in patients with NDBE is of great interest: rate of progression in high-risk patients established by TissueCypher is comparable to rate of progression in patients with LGD. These results allow us to choose personalized treatment for patients with BE. In a prospective study, TissueCypher result influenced management decisions for choosing surveillance interval or method of treatment (endoscopic eradication therapy) in 55% cases.

8 IMPORTANT MOLECULAR AND GENETIC EVENTS ASSOCIATED WITH NEOPLASTIC PROGRESSION IN BE

NDBE and especially EAC are marked by high mutational load, surpassed only by lung cancer and melanoma. Patients with NDBE who further progress to EAC (progressors) have initially higher mutational load than patients with NDBE who remain stable (non-progressors). Various genetic alterations were described in BE and EAC including point mutations, losses of heterozygosity (LOH), as well as large genomic rearrangements, namely, chromothripsis, kataegis and bridge-fusion-bridge (BFB) along with aneuploidy and tetraploidy. Some genetic alterations happen irrespective of carcinogenesis stage, but several genetic events tend to occur at a particular stage of neoplastic progression (Figure 15).

Loss of heterozygosity in BE and EAC. LOH is a chromosomal event that leads to deletion of the whole gene and adjacent area at one chromosome requiring transcription from other chromosome containing mutant or inactivated gene. The most common LOHs in BE and EAC include LOH in locus 9p21 (involved gene CDKN2A) and locus 17p13 (TP53). Majority of patients with HGD display mosaic of clones and subclones with different patterns of LOH. Inactivation on CDKN2A serves as the earliest, initiating event in pathogenesis of dysplasia and EAC. Although CDKN2A inactivation was identified both in patients with dysplasia/EAC and NDBE, selective sweep of lesions in CDKN2A caused by 9pLOH, promotor methylation or mutation followed by second event in CDKN2A or TP53 (17pLOH or mutation) is implemented during BE carcinogenesis. Generating of clones with TP53 mutations within segment of metaplasia in distal esophagus is key event of progression, leading to increment accumulation of mutations. Mutations in TP53 are identified in 72%–82.6% of EAC; they may arise long before morphological detection of dysplasia in progressors and are seen only in 5% of non-progressors.

Large genomic rearrangements. Maley C.C. et al. demonstrated that patients with more clonal diversity at segment of BE progress more frequently. Generally non-progressors display small localized deletions in fragile sites and 9pLOH without copy number alterations. In contrary, progressors at 24 months before diagnostics of EAC show huge clonal diversity at segment with large genomic rearrangements including multiple losses and gains as well as whole genome doubling (WGD). Stachler M.D. et al. revealed that TP53 mutations result in rapid WGD followed by genomic instability and oncogene amplification.
in tumor cells. It is worth mentioning that pathway of WGD is accomplished more often (in 62.5% of EAC) than classical pathway of gradual accumulation of mutations.

Among oncogene amplifications **SMAD4** is remarkable. **SMAD4** gene product forms complexes with other SMAD family proteins and regulates TGFβ-dependent...
transcription. New generation sequencing revealed that SMAD4 mutations are identified only in patients with EAC that may help to distinguish HGD from EAC.176

Third pathway of neoplastic progression in BE involves genomic catastrophes.177 Whole genome sequencing samples with EAC identified that large genomic

| RR     | OR     | Sens.   | Sp.     | PPV | NPV  |
|--------|--------|---------|---------|-----|------|
| 9.7; 95% CI 4.4–21.5 | 78%     | 80%     | 42%    | 95% |

(Fore consensus LGD)

| 4.2; 95% CI 2.4–7.3 | 44%     | 78%     | 15%   |

| 9.28; 95% CI 4.39–19.64 for persistent LGD |
| 7.5; 95% CI 1.7–32.8 |
| 4.18; 95% CI 1.03–17.1 for persistent LGD |

| 11.7; 95% CI 1.93–71.4 |

| 80%     | 68%     | 70%    | 78% |
|---------|---------|--------|-----|
| 49%     | 86%     |        |
| 63.6%   | 92.5%   | 53.8%  | 94.9% |
| Article                        | Number of patients | Tissue material                      | Staining | Number of images/areas | Agreement between pathologists |
|-------------------------------|--------------------|--------------------------------------|----------|------------------------|------------------------------|
| Polkowsky W. et al. (1998)    | 35                 | Resection specimens after esophagectomies | HE, Ki67, p53 | 73 areas (58 – training set, 9 – second set, 6 – couldn’t be assessed) | 79%                          |
| van Sandick J.W. et al. (2000)| 18                 | Biopsy specimens                      | HE, Ki67, p53 | 105 areas derived from 371 biopsies | 63%                          |
| Baak J.P. et al. (2002)       | —                  | Biopsy specimens                      | HE, Ki67  | 143 specimens          | 35% with experts             |
| Sabo E. et al. (2006)         | 152                | Biopsy specimens                      | HE       | Not mentioned          | Not mentioned                |
| Tomita N. et al. (2019)       | Not mentioned      | Biopsy specimens                      | HE       | 180 whole-slide images (116 images – training set, 64 – testing set) separated into 379 images | —                            |
| Critchley-Thorne R.J. et al.  | 366                | Biopsy specimens                      | HE, p16, AMACR, p53, CD68, COX-2, CD45RO, HIF1a, HER2/neu, K20 | — | — |
| Equipment | Classes | Parameters | Results |
|-----------|---------|------------|---------|
| QPRODIT1 version 6.1 (Leica Imaging Systems Ltd., Cambridge, UK) | NDBE | Mean nuclear area (MNA) | Combination of SI and Ki67 area was the most valuable to discriminate between NDBE and LGD and between LGD and HGD (both – 94% of correctly classified areas). Discrimination between HGD and ImCA was lower than 80% of correct classification with any parameters. |
| | LGD | Mean nuclear volume (MNV) | |
| | HGD | Mitotic activity index (MAI) | |
| | ImCA | MAI in the upper half of mucosa (MAI Up) | |
| | | Stratification index (SI) | |
| | | Ki67 area | |
| | | Ki67 area Up | |
| | | p53 area | |
| QPRODIT1 version 6.1 (Leica Imaging Systems Ltd., Cambridge, UK) | NDBE | MNA | Combination of SI and p53 area helped to distinguish between NDBE and LGD (89% of correctly classified areas). Combination of SI and Ki67 area allowed discriminating between LGD and HGD (91% of correctly classified areas). Combination of SI, Ki67 area and MNV gave advantage in discriminating LGD and HGD (94% of correctly classified areas). |
| | LGD | MNV | |
| | HGD | MAI | |
| | | SI | |
| | | Ki67 area | |
| | | p53 area | |
| — | NDBE | SI | Agreement between morphometric model and experts reached 75%. |
| | IND | MNA | |
| | LGD | Ki67 area | |
| | HGD | | |
| Image Pro Plus version 5.1 software (MediaCybernetics, MD, USA) | NDBE | Nuclear size | The neural network algorithm (NNET) correctly classified 86% of the cases in distinguishing between NDBE and LGD (70% of NDBE and 95% of LGD) and 87% of cases in distinguishing between the LGD and HGD groups in the training set. In testing set NNET differentiated NDBE from LGD in 89% of the cases (80% of NDBE and 91.7% of LGD) and to differentiate LGD from HGD in 85.7% of the cases (71.4% of LGD and 100% of HGD). |
| | IND | Nuclear shape | |
| | LGD | Nuclear chromatin texture | |
| | HGD | Nuclear pleomorphism | |
| | | Nuclear symmetry | |
| | | Nuclear pseudostratification | |
| convolutional neural network ResNEt-18 and a grid-based attention network ImageNet | Normal | Not mentioned | Classification accuracies of attention-based model were 0.85 (95% CI, 0.81–0.90) for the NDBE class, 0.89 (95% CI, 0.84–0.92) for dysplastic BE class, and 0.88 (95% CI, 0.84–0.92) for the EAC class. The proposed model achieved a mean accuracy of 0.83 (95% CI, 0.80–0.86) and outperformed the sliding window approach on the same testing set. |
| | NDBE | | |
| | Dysplastic BE | | |
| | EAC | | |
| TissueCypher Image Analysis Platform (Cernostics, Inc.) | Low, intermediate or high risk of progression | Expression and co-expression of markers | 15-feature classifier was developed to predict progression (AUROC 0.804). HRs were 2.45 (95% CI, 0.99–6.07) for the comparison of the intermediate-risk versus low-risk group and 9.42 (95% CI, 4.61–19.24), for high-risk versus low-risk. NPV 0.98, PPV 0.26. |
| | | | |
| | | | (Continues) |
rearrangements may result in oncogene amplification through chromothripsis with generation of double-minute chromosomes (MYC and MDM2), kataegis or BFB (KRAS, MDM2 and RFC3).178

**Chromothripsis.** Chromothripsis represents a catastrophic event during carcinogenesis with large-scale genomic rearrangements including chromosome shattering, gains and losses involving several genes at once and may lead to rapid oncogene activation and inactivation of tumor suppressor genes.179 Rausch T. et al.180 revealed that chromothripsis is associated with TP53 gene mutations in children with Sonic-Hedgehog medulloblastoma caused by Li-Fraumeni syndrome. Authors proposed three mechanisms contributing to chromothripsis in patients with TP53 mutations: (1) critical telomere shortening and chromosome end-to-end fusion, (2) premature condensation of chromatin due to alteration of cell cycle regulation (i.e., transition from G2 to M phase), and (3) impaired DNA reparation and apoptosis induction. High rate of TP53 mutations and telomere shortening in EAC elucidate chromothripsis being identified in 30%–32.5% cases.178,181 Chromothripsis quiet commonly coincide with kataegis.

**Kataegis** means hypermutation pattern of clustered C > T and C > G at TpC dinucleotides, that was first described in breast cancer.182,183 Kataegis arises as a result of APOBEC protein activity that serves as catalytic component of an RNA editing complex. DNA mutator activity of APOBEC is due to C-to-U deamination.184 In cytoplasm...
APOBEC restricts replication of DNA-viruses, including HIV, and comprises a component of natural retroviral defense. APOBECs predominantly target single-stranded DNA, and can produce a cluster of strand coordinated mutations that affect cytosine bases in the same strand. Kataegis is detected at the breakpoints of chromothriptic rearrangements caused by telomere crisis. So the reason for kataegis is breakage of chromatin bridges of dicentric chromosome by 3’repair exonuclease 1 (TREX1) with generation of single strain DNA acting as a substrate for APOBEC deaminases. Kataegis is diagnosed in 31%–86% observations of EAC.

BFB (break-fusion-bridge) cycles are initiated by telomere loss followed by fusion of unprotected ends of chromosomes or sister chromatids. These chromosomes then rupture in anaphase. This process may repeat during several cell cycles resulting in inverted duplications with high copy number alterations. Tumor growth activation originates when these amplified areas involve oncogenes. BFB is detected in 27.3% of EAC and leads to amplification of potent oncogenes (RCF3, MDM2, VEGFA, BCAT1 ÷ KRAS) through double-minute chromosome generation. These data give evidence that genomic catastrophes are important in neoplastic transformation of BE and represent an alternative mechanism of malignization. Genomic catastrophes that are often seen in HGD and EAC may probably result in rapid progression.
Aneuploidy. Aneuploidy is defined as abnormal number of chromosomes in cell. Using flow cytometry, Rabinovitch P.S. et al.\textsuperscript{189} detected aneuploidy in tumor cells of EAC and in epithelial cells adjacent to tumor. Later Rabinovich P.S. et al.\textsuperscript{190} developed cut-points to assess aneuploidy (>2.7N) and tetraploidy (4N > 6%) in BE in order to predict progression to EAC. In a retrospective study of biopsy archives of patients with EAC for a 9-year period, it was shown that DNA ploidy anomalies were detected more often in more advanced lesions (NDBE—13%, LGD—60%, HGD—73%, EAC—100%).\textsuperscript{191} Reid B.J. et al.\textsuperscript{129} proposed that aneuploidy is a late event in EAC development that happens after 17pLOH or TP53 mutation. It was further proved that aneuploidy and/or tetraploidy in clones with 17pLOH is associated with progression to EAC.\textsuperscript{192}

In research of Sikkema M. et al.,\textsuperscript{137} univariate analysis showed that aneuploidy, strong Ki67 overexpression and moderate p53 overexpression were all associated with increased risk of progression to HGD/EAC. Although multivariable analysis revealed that in the presence of LGD, p53 overexpression, and to a lesser extent, Ki67 overexpression remained important risk factors for neoplastic progression, whereas aneuploidy was no longer predictive. Nevertheless, detection of aneuploidy in patients with NDBE long time before progression makes it a plausible biomarker for identifying patients at-risk of progression.\textsuperscript{193} Thus, Killcoyne S. et al.\textsuperscript{194} demonstrated that genomic copy number abnormalities may appear 10 years before dysplasia detection in BE and are strong predictors of neoplastic transformation. Recently, Douville C. et al.\textsuperscript{195} proposed a method of assessment of aneuploidy in esophageal brushings that identifies early and late chromosomal lesions specific for neoplastic progression in BE.

9 | EPIGENETIC MARKERS OF BE NEOPLASIA AND PREDICTORS OF PROGRESSION

Epigenetic changes begin at early stages of neoplastic transformation and are regarded as potential predictive markers of progression. Several epigenetic changes are implemented during carcinogenesis\textsuperscript{196,197}: (1) DNA methylation, (2) posttranslational modifications of histones, (3) specific miRNAs and (4) nucleosome positioning. In our review, we will mostly focus on DNA methylation and miRNA expression, as these processes were extensively studied in BE and EAC.

**Methylation of DNA.** DNA methylation is performed by DNA methyltransferases (DNMTs) at 5-position of cytosine, usually dinucleotide sequence CpG serves as a substrate for DNMTs. Most of CpG in mammalian cells are methylated except for CpG islands enriched by CpG sequenced, which are located in promotor regions of 60%–70% genes. Aberrant methylation of CpG islands in carcinogenesis usually results in silencing of gene expression, whereas methylation of CpG sequences outside of promotor regions (gene body methylation), in contract, leads to transcriptional activation of corresponding genes.\textsuperscript{197} DNA methylation is the most studied epigenetic feature associated with neoplastic progression in BE. Not only hypermethylation of CpG islands,\textsuperscript{198—200} but also hypomethylation outside of
them serves as epigenetic hallmark of progression. Thus, Alvarez H. et al. showed significant genomewide hypomethylation in NDBE compared to squamous epithelium; second shift toward hypomethylation was seen in HGD and EAC. Widespread hypomethylation was associated with transcriptional activation of XCL1, XCL3, GATA6 and DMBT1. In accordance, Xu E. et al. demonstrated decreased DNA methylation level outside of CpG islands and increased methylation in CpG islands in patients with BE and EAC compared to squamous epithelium. These coexisting epigenetic phenomena cause global changes of transcriptome that are involved in EAC development and appear early in carcinogenesis. Hypermethylation of genes SFRP1, GBX2, ADAM12, PTGDR, DMRT1, PTPRT, SH3GL3, LAMA1, COL5A1 and AJAP1, that were identified in cancers of other locations, was seen in BE as well as in EAC. In retrospective study hypermethylation of CDKN2A, RUNX3 and HPP1 was identified in patients with BE 2 years before EAC diagnostics and was associated with increased risk of progression. Based on methylation index of these genes, pathomorphological features and segment length authors developed three-tiered risk stratification model to predict progression in BE.

Alvi et al. studied methylation of imprinted genes and genes located on X chromosome in patients with BE and EAC. They detected 4 genes (SLC22A18, PIGR, GIA12 and RIN2) differently methylated in NDBE, dysplastic BE and EAC (AUC = 0.988). In a prospective cohort of patients, methylation of less than 2 genes was seen in patients with low risk of progression to EAC, and methylation of 2 genes was associated with intermediate risk and >2 genes – with high risk of EAC development.

Kaz A.M. et al. identified 4 unique methylation profiles in BE and EAC: BE with low and high methylation epiphenotype and EAC with low and high methylation epiphenotype. Authors also found 17 differently methylated sites of CpG (differently methylated positions [DMPs]) that may distinguish BE and EAC and 3 DMPs for NDBE and HGD. Yu M. et al. showed that high methylation is associated with mutations or amplification of ERBB2, and also harbors higher mutational load. Moreover, authors revealed that cell lines with different DNA methylation level are characterized by different sensitivity to drugs (SN-38, topotecan and palbociclib). Therefore, assessment of DNA methylation level is useful for indication of target treatment. Jammula S. et al. also defined 4 subtypes of patients with BE and EAC based on DNA methylation intensity. Patients with 1 subtype showed DNA hypermethylation with high mutational load and mutations in cell cycle controlling genes (CCND1, CCNE1, MYC, CDK6) and receptor tyrosine signaling pathways (GATA4, ERBB2, KRAS). Subtype 2 consisted predominantly of patients with BE with upregulation of transcriptional factors HNF4A/G, FOXA1/2/3, GATA6 and CDX2, as well as high expression of genes associated with ATP synthesis and fatty acid oxidation. Patients of subtype 3 did not show changes in methylation pattern, compared with control tissue, but displayed heavy inflammatory infiltration enriched with cytotoxic cells, B-cells, mast cells and neutrophils along with cancer associated fibroblasts and reduced levels of T-helper cells. Subtype 3 was associated with the lowest survival, whereas the highest survival was expectedly found in subtype 2. At last, patients with subtype 4 showed hypomethylation accompanied with large-scale genomic rearrangements, copy number alterations and amplification of CCNE1 and ERBB2.

Number of DMPs varied in squamous epithelium and BE as well as in BE and EAC is tremendous. Li D. et al. identified 12 from 458 DMPs that are valuable in distinguishing of squamous epithelium, BE, EAC and esophageal squamous carcinoma and found 3 CpG sites in EAC and 2 CpG sites in esophageal squamous cell carcinoma (ESSC), methylation of which was prognostic (associated with survival). After detection of 257 DMPs, specific for EAC, Peng W. et al. developed a model for early diagnostics of EAC based on 4 DMPs (cg07589773, cg10474350, cg13011388 and cg15208375, localized in IKZF1, HOXA7, EFS and TSHZ3, AUC = 0.903).

In all aforementioned studies, DNA was derived from biopsy samples of distal esophagus, although several noninvasive methods were proposed for detection of TFF1, VIM, CCNA1 и VIM methylation for BE diagnostics. Posttranslational modifications of histones. Histone modifications regulate gene transcription as well as replication and DNA repair. Among posttranscriptional modifications, imbalance between acetylation and deacetylation of histones was shown to be implicated in cancer development and particularly in esophageal carcinogenesis. Acetylation of lysine residues’ by histone acetyltransferases (HATs) results in the relaxation of DNA structures and facilitates gene transcription, whereas hypoacetylation of histones is a hallmark of inactive heterochromatin. Cancer cells are characterized with impaired balance between HATs and histone deacetylases (HDACs) which severely alters chromatin structure and, as a consequence, alter gene expression, including genes, involved in the cell cycle regulation, differentiation and apoptosis. For example, HDACs repression causes hyperacetylation of histones which increases transcriptional activity, including rise in expression of potent onco genes, initiating carcinogenesis. On the other hand, HDACs overexpression leads to histone hypoacetylation and impaired cell cycle (increase in cyclin dependent kinases 2 and 4 and abundant phosphorylation of retinoblastoma protein) that results in augmented cellular proliferation. HDACs inhibitors are valuable novel
anti-cancer drugs that arrest tumor growth, promote apoptosis, help us to overcome chemotherapy resistance and increase reactive oxygen species, causing DNA and membrane damage in cancer cells. Moreover, HDACs inhibitors impair miRNA expression showing huge interaction between different epigenetic modifications.

Like posttranslational modifications of histones, nucleosome positioning modulates accessibility of regulatory DNA sequences for transcriptional factors. Specific information about nucleosome positioning and its close interaction with DNA methylation is provided in several papers.

miRNA. miRNAs are small noncoding sequences of 20–25 nucleotides that maintain posttranscriptional regulation of target genes. MiRNAs express tissue-specific way and control wide spectrum of biological processes, including proliferation, apoptosis and differentiation. Numerous data comparing miRNA expression profiles in tumors and corresponding normal tissues demonstrate widespread changes in miRNAs expression during carcinogenesis. MiRNAs function either as tumor suppressors or as oncogenes, depending on target genes.

Maru D.M. et al. showed that increased level of miRNA-196a in biopsy samples of distal esophagus is a potential biomarker of progression from NDBE to EAC, therein expression of target genes (SPRR2C, S100A9 and KRT5) falls rapidly through neoplastic transformation. Fassan M. et al. revealed different miRNA expression profiles of esophageal squamous epithelium, IM without dysplasia, LGD, HGD and EAC. Authors detected increase in miR-215 and miR-192 accompanied by decrease in miR-205, miR-203 and let-7c levels during carcinogenesis. In prospective research Revilla-Nuin B. et al. identified, that elevated levels of 4 miRNAs (miR-192, 194, 196a and 196b) are associated with progression to EAC. Many other miRNAs involved in neoplastic progression in BE were identified. In meta-analysis miR-192, miR-194, miR-203, miR-205 and miR-215 were found to be perspective tissue biomarkers for BE diagnosis.

MiRNAs are also used in non-invasive diagnostics of BE, e.g., using Cytosponge (combination of miR192, miR196a, miR199a and TFF3). Circulating miRNAs of plasma may also serve as a diagnostic sample. For example, Bus P. et al. validated combination of circulating miRNA for differential diagnostics of BE and EAC. In addition, level of miR130a increased gradually in line NDBE—LGD—HGD—EAC stage I, II—AKI stage III, IV.

Value of miRNAs in diagnostics is obvious (Table 7), besides levels of specific miRNAs may serve as prognostic markers and are also applicable for assessment of treatment efficacy and as therapeutic targets.

Epigenetic changes are the earliest in pathogenesis of BE, anticipating any genetic or molecular alterations during Barrett’s carcinogenesis. Several epigenetic changes serve as stage-specific markers of neoplastic transformation which is important for precise diagnosis. DNA methylation and miRNA profiles are promising tools for non-invasive diagnostics of BE and EAC. Moreover, epigenetic alterations provide new targets for treatment.

### 10 | Microenvironment Markers in Progression to Barrett’s Adenocarcinoma

Microenvironment during carcinogenesis can be divided into 3 dynamic stages: tumor precursor microenvironment, tumor microenvironment (TME) and pre-metastatic

| Advantages | Disadvantages | Markers, elevated with progression | Markers, decreased with progression |
|---|---|---|---|
| Personized diagnostics | Ongoing search for clinically relevant and cost-effective markers of progression. Need for validation of novel markers in clinical trials. | ↓miR-21, ↓miR-25, ↓miR-92a-3p, ↓miR130a, ↓miR-136-5p, ↓miR-192, ↓miR-194, ↓miR196a, ↓miR-196b, ↓miR-199a, ↓miR215, ↓miR-223, ↓miR-301b, ↓miR-382-5p, ↓miR-618, ↓miR-17-92 cluster | ↓let-7c, ↓miR-23b, ↓miRNA-133a-3p, ↓miR-199a-3p, ↓miR-203, ↓miR-205, ↓miR-320c, ↓miR-375, ↓miR-378 |
| Capability to use different specimens (biopsy pieces, Cytosponge brushing, plasma, serum) | Potential tool for prognosis and assessment of treatment efficacy. | Markers, elevated with progression | Markers, decreased with progression |
| Potential tool for prognosis and assessment of treatment efficacy. | May represent a therapeutic target. | Markers, elevated with progression | Markers, decreased with progression |

| TABLE 7 Overview of miRNA, associated with neoplastic progression in BE | Markers, elevated with progression | Markers, decreased with progression |
|---|---|---|---|
| Personized diagnostics | Ongoing search for clinically relevant and cost-effective markers of progression. Need for validation of novel markers in clinical trials. | ↓miR-21, ↓miR-25, ↓miR-92a-3p, ↓miR130a, ↓miR-136-5p, ↓miR-192, ↓miR-194, ↓miR196a, ↓miR-196b, ↓miR-199a, ↓miR215, ↓miR-223, ↓miR-301b, ↓miR-382-5p, ↓miR-618, ↓miR-17-92 cluster | ↓let-7c, ↓miR-23b, ↓miRNA-133a-3p, ↓miR-199a-3p, ↓miR-203, ↓miR-205, ↓miR-320c, ↓miR-375, ↓miR-378 |
niches.\textsuperscript{235} TME consists of adaptive and innate immune cells, fibroblasts, adipocytes, endothelial cells and extra-cellular matrix (ECM) components.

Chronic inflammation, caused by gastric and bile acid reflux, results in recruiting of immune cells and releasing a variety of mediators (e.g. IL-1β, IL-8 and IL-6), which together establish BE microenvironment that favors dysplasia initiation and further development of EAC.\textsuperscript{235–238} Numerous immune changes in BE were associated with progression to EAC. Flow based single cell analysis showed that B cell rich microenvironment in normal esophagus changes into predominantly T cell rich landscape in BE.\textsuperscript{239} Using IHC evaluation, Porter et al.\textsuperscript{240} revealed that NDBE is associated not only with reduced lymphocytic infiltration of CD20+ B-cells, but also with lower level of CD4+ T-cell and CD8+ T-cell infiltration compared with squamous epithelium of esophagus. In this study dysplastic BE demonstrated an increase of CD20+ B-cells, CD8+ T-cells and Foxp3+ Tregs compared with NDBE. Importantly, individuals with dysplasia also showed increased CD20 + B-cells in background NDBE compared with nonprogressors, and patients with EAC displayed increased CD20+, CD4+ and CD8+ lymphocytes in the background NDBE compared with nonprogressors. In rat model Miyashita T. et al.\textsuperscript{241} showed that M2 phenotype CD163+ macrophages (tumor-associated macrophages, TAMs) infiltration contributes to tumor development along with Foxp3+ Tregs via Stat3-pathway.

Kavanagh ME et al.\textsuperscript{242} demonstrated Th2 phenotype in BE, characterized by elevated levels of IL-4 producing CD4+ T-cells and secreted levels of IL-6, and immuno-compromised T-cells infiltrating EAC with low expression of CD45RO and CD69 that facilitate tumor progression and may represent a target for immune therapy. The same researchers identified that circulating T cells in EAC patients exhibited impaired migratory capacity with decreased frequencies of Th1-associated CXCR3+ and Th17-associated CCR6+ cells.\textsuperscript{243} Interestingly, neutrophil-lymphocyte ratio (NLR) in blood gradually increased from NDBE to EAC. NLR >2.27 was able to diagnose EAC with 80% sensitivity and 71% specificity (area under the curve = 0.8).\textsuperscript{244}

RNA-Seq and the genomic cellular analysis tool xCell revealed a linear increase in Th1, Th2, Treg, and pro-B cell populations in EAC compared with precancerous lesions (dysplastic BE and NDBE) as well as a linear increase in M1 and M2 macrophages between HGD and EAC.\textsuperscript{245} Although multiplex IHC showed that immune cell populations tended to increase in a stepwise fashion from BE to LGD to HGD, followed by a decline in all evaluated immune cell populations in EAC tissues that coincided with increased PD-L1 expression.\textsuperscript{245} PD-L1 has been shown to cause T cell apoptosis and suppress antitumor immunity.\textsuperscript{246,247} PD-L1 expression in subset of EAC patients means that these individuals may benefit from immunomodulatory therapy, such as anti–PD-1, anti–PD-L1 or anti-CTLA4 therapy.

Changes of the ECM in the BE microenvironment also are important in carcinogenesis. Matrix metalloproteinases (MMPs) are components of ECM involved in inflammation and tumor metastasis. IHC showed that MMP-7 was weakly expressed in squamous epithelium adjacent to EAC but increased progressively in epithelial cells in NDBE, LGD, HGD and EAC, particularly at the invasive front.\textsuperscript{248} Moreover, MMP-7 was weakly expressed in the stroma myofibroblasts of dysplastic BE and EAC, especially at the invasive front. Authors supposed that MMP-7 in BE epithelial cells was regulated by PI3-K kinases and could stimulate stromal cell migration, invasion and remodeling of the microenvironment.\textsuperscript{248} MMP9 and MMP13 are also up-regulated in BE.\textsuperscript{249} Expression of MMP13 was higher in NDBE, whereas expression of MMP-9 was higher in EAC. Herszenyi L et al.\textsuperscript{250} demonstrated that MMP9 expression level gradually increased from NDBE to EAC making MMP9 a prognostic biomarker. Wang Z et al.\textsuperscript{251} demonstrated that expression levels of COL1A2 (encoding α2 chain of collagen I) and related genes (COL1A1, COL3A1, ZNF469, and POSTN) were positively correlated with the infiltration levels of macrophages and dendritic cells, and the expression levels of ZNF469 was also positively correlated with the infiltration levels of CD4+ T cells in both EAC and ESCC. These results indicated these genes might be the candidate genes for assessing the immune infiltration levels in esophageal cancer. COL1A2 is known to play a role in the invasion and metastasis of ovarian cancer.\textsuperscript{252} COL1A2 also up-regulates proliferation, migration and invasion of ESCC\textit{ in vitro}.\textsuperscript{253}

Changes in different immune cell populations as well as components of ECM are elucidated across the progression from BE to EAC. Some of immune changes are of value because they represent targets for immunomodulatory treatment. A lot of novel markers associated with BE progression to EAC are identified in scientific studies and need to be evaluated in clinical trials before becoming part of the routing diagnostics.

\section*{11 \ | CONCLUSIONS AND OUTLOOK}

Endoscopic examination with morphologically confirmed IM is a standard of BE diagnostics. Morphological verification of dysplasia is challenging and provides great variability in diagnosis. In difficult cases IHC evaluation is reasonable. IHC examination with p53, Ki67 and AMACR not only allows identifying presence and grade of
dysplasia, but also has implication in determining prognosis. TissueCypher technology provides quantitative analysis of epithelial and stromal immunofluorescent markers expression (p16, AMACR, p53, CD68, COX-2, CD45RO, HIF1a, HER2/neu and K20) in biopsy specimens with BE. TissueCypher results are interpreted in terms of low, intermediate or high risk of progression to EAC.

Population of patients with BE is heterogeneous: although some patients are stable with NDBE, others may rapidly evolve to dysplasia and EAC. Analysis of genetic and epigenetic alterations in BE and EAC sheds light on pathways of neoplastic progression in distal esophagus and gives a key to stratification of progression risk in each individual patient, meaning that molecular and genetic alterations arise earlier than morphologically identifiable dysplasia. Noninvasive detection of epigenetic markers of BE and EAC or detection of markers in plasma or serum of patients is a promising alternative to EGS with biopsy and is valuable for diagnosis, progression and survival prognosis and assessment of therapy efficacy.

CONFLICT OF INTERESTS
The authors declare no conflicts of interest in the writing and preparation of this article.

AUTHOR CONTRIBUTIONS
K.M., A.D., D.A., M.S., L.M. developed the methodology and developed the goals and main criterion for the project. K.M., A.K. and M.S. gathered the methodology, gathered literature, and prepared primary drafts. M.S., K.M., and L.M. analyzed the primary results and wrote the primary article. K.M., A.D., D.A. and M.S. prepared the manuscript. All authors reviewed the final manuscript.

DATA AVAILABILITY STATEMENT
All associated data are available from the corresponding author upon reasonable request.

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REFERENCES
1. Then EO, Lopez M, Saleem S, et al. Esophageal cancer: an updated surveillance epidemiology and end results database analysis. World J Oncol. 2020;11(2):55-64.
2. Haiyu Z, Xiaofeng P, Xiangqiong M, et al. Incidence and survival changes in patients with esophageal adenocarcinoma during 1984–2013. Biomed Res Int. 2019;12(2019):7431850.
3. Peters Y, Al-Kaabi A, Shaheen NJ, et al. Barrett oesophagus. Nat Rev Dis Primers. 2019;5(1):35.
4. Thrumurthy SG, Chaudry MA, Thrumurthy SSD, Mughal M. Oesophageal cancer: risks, prevention, and diagnosis. BMJ. 2019;366:l4373. Erratum in: BMJ. 2019 Sep 6;366:l3591.
5. Coleman HG, Xie SH, Lagergren J. The epidemiology of esophageal adenocarcinoma. Gastroenterol. 2018;154(2):390-405.
6. Thrift AP. Barrett’s esophagus and esophageal adenocarcinoma: how common are they really? Dig Dis Sci. 2018;63(8):1958-1996.
7. Runge TM, Abrams JA, Shaheen NJ. Epidemiology of Barrett’s esophagus and esophageal adenocarcinoma. Gastroenterol Clin North Am. 2015;44(2):203-231.
8. Lepage C, Drouillard A, Jouve JL, Faivre J. Epidemiology and risk factors for oesophageal adenocarcinoma. Dig Liver Dis. 2013;45(8):625-629.
9. Fitzgerald RC, di Pietro M, Raganath K, et al; British Society of Gastroenterology. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett’s oesophagus. Gut. 2014;63(1):7-42.
10. Bennett C, Moayyedi P, Corley DA, et al; BOB CAT Consortium. BOB CAT: a large-scale review and Delphi consensus for management of Barrett’s esophagus with no dysplasia, indefinite for, or low-grade dysplasia. Am J Gastroenterol. 2015;110(5):662-682. quiz 683.
11. Shaheen NJ, Falk GW, Iyer PG, Gerson LB, American College of Gastroenterology. ACG Clinical guideline: diagnosis and management of Barrett’s esophagus. Am J Gastroenterol. 2016;111(1):30-50. quiz 51.
12. Barrett’s Esophagus pathology. Clinical recommendations. Russian Society of Pathology. 2016, http://www.patolog.ru/news/utverzhdennyе-rop-klinicheskie-rekomendaciipomorfológicheskoy-diagnostikze-zabolvaneyi
13. Qumseya BJ, Panossian AM, Rizk C, et al. Survival in esophageal high-grade dysplasia/adenocarcinoma post endoscopic resection. Dig Liver Dis. 2013;45(12):1028-1033.
14. Pech O, May A, Manner H, et al. Long-term efficacy and safety of endoscopic resection for patients with mucosal adenocarcinoma of the esophagus. Gastroenterol. 2014;146(3):652-660.e1.
15. Zhang L, Sun B, Zhou X, et al. Barrett’s esophagus and intestinal metaplasia. Front Oncol. 2021;17(11):630837.
16. Patel A, Gyawali CP. Screening for Barrett’s esophagus: balancing clinical value and cost-effectiveness. J Neurogastroenterol Motil. 2019;25(2):181-188.
17. Tan MC, Mansour N, White DL, Sisson A, El-Serag HB, Thrift AP. Systematic review with meta-analysis: prevalence of prior and concurrent Barrett’s oesophagus in oesophageal adenocarcinoma patients. Aliment Pharmacol Ther. 2020;52(1):20-36.
18. Verbeek RE, Leenders M, Ten Kate FJ, et al. Surveillance of Barrett’s esophagus and mortality from esophageal adenocarcinoma: a population-based cohort study. Am J Gastroenterol. 2014;109(8):1215-1222.
19. Biswas S, Quante M, Leedham S, Jansen M. The metastatic mosaic of Barrett’s oesophagus. Virchows Arch. 2018;472(1):43-54.
20. McDonald SA, Graham TA, Lavery DL, Wright NA, Jansen M. The Barrett’s gland in phenotype space. Cell Mol Gastroenterol Hepatol. 2014;1(1):41-54.
21. McDonald SA, Lavery D, Wright NA, Jansen M. Barrett oesophagus: lessons on its origins from the lesion itself. Nat Rev Gastroenterol Hepatol. 2015;12(1):50-60.
22. Hahn HP, Blount PL, Ayub K, et al. Intestinal differentiation in metaplastic, non-goblet columnar epithelium in the esophagus. *Am J Surg Pathol*. 2009;33(7):1006-1015.

23. Srivastava S, Liew MS, McKeon F, et al. Immunohistochemical analysis of metaplastic non-goblet columnar lined oesophagus shows phenotypic similarities to Barrett’s oesophagus: a study in an Asian population. *Dig Liver Dis*. 2014;46(2):170-175.

24. Mikhailova LM, Volykivskaya KS, Fedorov ED, et al. Columnar metaplasia and Barrett’s esophagus: morphological heterogeneity and immunohistochemical phenotype. *Bulletin of RSMU*. 2019;6:77-83.

25. Salimian KJ, Waters KM, Eze O, et al. Definition of Barrett esophagus in the United States: support for retention of a requirement for goblet cells. *Am J Surg Pathol*. 2018;42(2):264-268.

26. Bhat S, Coleman HG, Yousef F, et al. Risk of malignant progression in Barrett’s esophagus patients: results from a large population-based study. *J Natl Cancer Inst*. 2011;103(13):1049-1057.

27. Gatenby PA, Ramus JR, Caygill CP, Shepherd NA, Watson A. Relevance of the detection of intestinal metaplasia in non-dysplastic columnar-lined oesophagus. *Scand J Gastroenterol*. 2008;43(5):524-530.

28. Kelty CJ, Gough MD, Van Wyk Q, Stephenson TJ, Ackroyd R. Barrett’s oesophagus: intestinal metaplasia is not essential for cancer risk. *Scand J Gastroenterol*. 2007;42(11):1271-1274.

29. Sawas T, Killcoyne S, Iyer PG, et al; OCCAMS Consortium. Identification of prognostic phenotypes of esophageal adenocarcinoma in 2 independent cohorts. *Gastroenterol*. 2018;155(6):1720-1728.e4.

30. Sawas T, Azad N, Killcoyne S, et al. Comparison of phenotypes and risk factors for esophageal adenocarcinoma at present vs prior decades. *Clin Gastroenterol Hepatol*. 2020;18(12):2710-2716.e1.

31. Bansal A, McGregor DH, Anand O, et al. Presence or absence of intestinal metaplasia but not its burden is associated with prevalent high-grade dysplasia and cancer in Barrett’s esophagus. *Dis Esophagus*. 2014;27(8):751-756.

32. Harrison R, Perry I, Haddadin W, et al. Detection of intestinal metaplasia in Barrett’s esophagus: an observational comparator study suggests the need for a minimum of eight biopsies. *Am J Gastroenterol*. 2007;102(6):1154-1161.

33. Chandrasoma PT, Der R, Ma Y, Peters J, Demeester T. Histologic classification of patients based on mapping biopsies of the gastroesophageal junction. *Am J Surg Pathol*. 2003;27(7):929-936.

34. Oberg S, Johansson J, Wenner J, et al. Endoscopic surveillance of columnar-lined esophagus: frequency of intestinal metaplasia detection and impact of antireflux surgery. *Ann Surg*. 2001;234(5):619-626.

35. Takubo K, Aida J, Naomoto Y, et al. Cardiac rather than intestinal-type background in endoscopic resection specimens of minute Barrett adenocarcinoma. *Hum Pathol*. 2009;40(1):65-74.

36. Watanabe G, Ajioka Y, Takeuchi M, et al. Intestinal metaplasia in Barrett’s oesophagus may be an epiphenomenon rather than a preneoplastic condition, and CDX2-positive cardiac-type epithelium is associated with minute Barrett’s tumour. *Histopathol*. 2015;66(2):201-214.

37. Demicco EG, Farris AB 3rd, Baba Y, et al. The dichotomy in carcinogenesis of the distal esophagus and esophagogastric junction: intestinal-type vs cardiac-type mucosa-associated adenocarcinoma. *Mod Pathol*. 2011;24(9):1177-1190.

38. Khor TS, Alfaro EE, Ooi EM, et al. Divergent expression of MUC5AC, MUC6, MUC2, CD10, and CDX2 in dysplasia and intramusosal adenocarcinomas with intestinal and foveolar morphology: is this evidence of distinct gastric and intestinal pathways to carcinogenesis in Barrett Esophagus? *Am J Surg Pathol*. 2012;36(3):331-342.

39. Liu W, Hahn H, Odze RD, Goyal RK. Metaplastic esophageal columnar epithelium without goblet cells shows DNA content abnormalities similar to goblet cell-containing epithelium. *Am J Gastroenterol*. 2009;104(4):816-824.

40. Lavery DL, Martinez P, Gay LJ, et al. Evolution of oesophageal adenocarcinoma from metaplastic columnar epithelium without goblet cells in Barrett’s oesophagus. *Gut*. 2016;65(6):907-913.

41. Kunze B, Wein F, Fang HY, et al. Notch signaling mediates differentiation in Barrett’s esophagus and promotes progression to adenocarcinoma. *Gastroenterol*. 2020;159(2):575-590.

42. Schellnegger R, Quante A, Rospieszcz S, et al. Goblet cell ratio in combination with differentiation and stem cell markers in Barrett esophagus allow distinction of patients with and without esophageal adenocarcinoma. *Cancer Prev Res (Phila)*. 2017;10(1):55-66.

43. Srivastava A, Golden KL, Sanchez CA, et al. High goblet cell count is inversely associated with ploidy abnormalities and risk of adenocarcinoma in Barrett’s esophagus. *PLoS One*. 2015;10(7):e0133403.

44. Menke V, van Es JH, de Lau W, et al. Conversion of metaplastic Barrett’s epithelium into post-mitotic goblet cells by gamma-secretase inhibition. *Dis Model Mech*. 2010;3(1–2):104-110.

45. van Es JH, van Gijn ME, Riccio O, et al. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature*. 2005;435(7044):959-963.

46. Abela JE, Going JJ, Mackenzie JF, McKernan M, O’Mahoney S, Stuart RC. Systematic four-quadrant biopsy detects Barrett’s dysplasia in more patients than nonsystematic biopsy. *Am J Gastroenterol*. 2008;103(4):850-855.

47. Abrams JA, Kapel RC, Lindberg GM, et al. Adherence to biopsy guidelines for Barrett’s esophagus surveillance in the community setting in the United States. *Clin Gastroenterol Hepatol*. 2009;7(7):736-742. quiz 710.

48. Westerveld D, Khullar V, Mramba L, et al. Adherence to quality indicators and surveillance guidelines in the management of Barrett’s esophagus: a retrospective analysis. *Endosc Int Open*. 2018;6(3):E300-E307.

49. Wani S, Williams JL, Komanduri S, Muthusamy VR, Shaheen NJ. Endoscopists systematically undersample patients with long-segment Barrett’s esophagus: an analysis of biopsy sampling practices from a quality improvement registry. *Gastrointest Endosc*. 2019;90(5):732-741.e3.

50. Sharma P, Hawes RH, Bansal A, et al. Standard endoscopy with random biopsies versus narrow band imaging targeted biopsies in Barrett’s oesophagus: a prospective, international, randomised controlled trial. *Gut*. 2013;62(1):15-21.

51. Sharma P, Bergman JJ, Goda K, et al. Development and validation of a classification system to identify high-grade dysplasia and esophageal adenocarcinoma in Barrett’s esophagus using narrow-band imaging. *Gastroenterol*. 2016;150(3):591-598.

52. Nogales O, Caballero-Marcos A, Clemente-Sánchez A, et al. Usefulness of non-magnifying narrow band imaging in EVIS EXERA III video systems and high-definition endoscopes to diagnose dysplasia in Barrett’s esophagus using the Barrett...
International NBI Group (BING) classification. Dig Dis Sci. 2017;62(10):2840-2846.

53. Tholoor S, Bhattacharyya R, Tsagkournis O, Longcroft-Wheaton G, Bhandari P. Acetic acid chromoendoscopy in Barrett's esophagus surveillance is superior to the standardized random biopsy protocol: results from a large cohort study (with video). Gastrointest Endosc. 2014;80(3):417-424.

54. Coletta M, Sami SS, Nachiappan A, Fraquelli M, Casazza G, Ragunath K. Acetic acid chromoendoscopy for the diagnosis of early neoplasia and specialized intestinal metaplasia in Barrett’s esophagus: a meta-analysis. Gastrointest Endosc. 2016;83(1):57-67.e1.

55. Chedgy FI, Subramaniam S, Kandiah K, Thayalasekaran S, Bhandari P. Acetic acid chromoendoscopy: improving neoplasia detection in Barrett’s esophagus. World J Gastroenterol. 2016;22(25):5753-5760.

56. Kandiah K, Chedgy FJQ, Subramaniam S, et al. International development and validation of a classification system for the identification of Barrett’s neoplasia using acetic acid chromoendoscopy: the Portsmouth acetic acid classification (PREDICT). Gut. 2018;67(12):2085-2091.

57. Kara MA, Peters FP, Fockens P, ten Kate FJ, Bergman JJ. Endoscopic video-autofluorescence imaging followed by narrow band imaging for detecting early neoplasia in Barrett’s esophagus. Gastrointest Endosc. 2006;64(4):176-185.

58. Borovicka J, Fischer J, Neuweiler J, et al. Autofluorescence endoscopy in surveillance of Barrett’s esophagus: a multicenter randomized trial on diagnostic efficacy. Endoscopy. 2006;38(9):867-872.

59. Bajbouj M, Vieth M, Rösch T, et al. Probe-based confocal laser endomicroscopy compared with standard four-quadrant biopsy for evaluation of neoplasia in Barrett’s esophagus. Endoscopy. 2010;42(6):435-440.

60. Bertani H, Frazzoni M, Dabizzi E, et al. Improved detection of incident dysplasia by probe-based confocal laser endomicroscopy in a Barrett’s esophagus surveillance program. Dig Dis Sci. 2013;58(1):188-193.

61. Sharma P, Meining AR, Coron E, et al. Real-time increased detection of neoplastic tissue in Barrett’s esophagus with probe-based confocal laser endomicroscopy: final results of an international multicenter, prospective, randomized, controlled trial. Gastrointest Endosc. 2011;74(3):465-472.

62. Canto MI, Anandasabapathy S, Brugge W, et al; Confocal Endomicroscopy for Barrett’s esophagus or Confocal Endomicroscopy for Barrett’s Esophagus (CEBE) Trial Group. In vivo endomicroscopy improves detection of Barrett’s esophagus-related neoplasia: a multicenter international randomized controlled trial (with video). Gastrointest Endosc. 2014;79(2):211-221.

63. Vranic L, Nadarević T, Stićmac D. Probe-based confocal laser endomicroscopy and Barrett’s esophagus - just a scientific toy or significant improvement in diagnosis? Dig Dis. 2021. doi: 10.1159/000516257. Epub ahead of print. PMID: 33794523.

64. Smith MS, Cash B, Konda V, et al. Volumetric laser endomicroscopy and its application to Barrett’s esophagus: results from a 1,000 patient registry. Dis Esophagus. 2019;32(9):doz029.

65. Jain D, Fatima S, Jain S, Singhal S. Volumetric laser endomicroscopy for Barrett’s esophagus - looking at the fine print. J Gastrointestin Liver Dis. 2017;26(3):291-297.

66. Suter MJ, Gora MJ, Lauwers GY, et al. Esophageal-guided biopsy with volumetric laser endomicroscopy and laser cautery marking: a pilot clinical study. Gastrointest Endosc. 2014;79(6):886-896.

67. Beg S, Mensa M, Fullard M, Finerty E, Richman P, Leahy A. Impact of advanced endoscopic imaging on Barrett’s esophagus in daily clinical practice. Gastrointest Endosc. 2018;87(5):1189-1194.

68. Boerwinkel DF, Swager A, Curvers WL, Bergman JJ. The clinical consequences of advanced imaging techniques in Barrett’s esophagus. Gastroenterol. 2014;146(3):622-629.e4.

69. Giacchino M, Bansal A, Kim RE, et al. Clinical utility and interobserver agreement of autofluorescence imaging and magnification narrow-band imaging for the evaluation of Barrett’s esophagus: a prospective tandem study. Gastrointest Endosc. 2013;77(3):711-718.

70. Egger K, Werner M, Meining A, et al. Biopsy surveillance is still necessary in patients with Barrett’s oesophagus despite new endoscopic imaging techniques. Gut. 2003;52(1):18-23.

71. Sharma P, Savides TJ, Canto MI, et al; ASGE Technology and Standards of Practice Committee. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on imaging in Barrett’s esophagus. Gastrointest Endosc. 2012;76(2):252-254.

72. van der Sommen F, Zinger S, Curvers WL, et al. Computer-aided detection of early neoplastic lesions in Barrett’s esophagus. Endoscopy. 2016;48(7):617-624.

73. Mendel R, Ebigbo A, Probst A, Messmann H, Palm C. Barrett’s esophagus analysis using convolutional neural networks. In: Maier-Hein KH, Deserno TM, Handels H, Tołdorff T, eds. Bildverarbeitung für die Medizin. Springer; 2017:80-85. Informatik Aktuell.

74. de Groof AJ, Struyvenberg MR, van der Putten J, et al. Deep-learning system detects neoplasia in patients with Barrett’s esophagus with higher accuracy than endoscopists in a multistep training and validation study with benchmarking. Gastroenterol. 2020;158(4):915-929.e4.

75. Hashimoto R, Requa J, Dao T, et al. Artificial intelligence using convolutional neural networks for real-time detection of early esophageal neoplasia in Barrett’s esophagus (with video). Gastrointest Endosc. 2020;91(6):1264-1271.e1.

76. Ebigbo A, Mendel R, Probst A, et al. Real-time use of artificial intelligence in the evaluation of cancer in Barrett’s oesophagus. Gut. 2020;69(4):615-616.

77. Swager AF, van der Sommen F, Klomp SR, et al. Computer-aided detection of early Barrett’s neoplasia using volumetric laser endomicroscopy. Gastrointest Endosc. 2017;86(5):839-846.

78. Trindade AJ, McKinley MJ, Fan C, Leggett CL, Kahn A, Pleskow DK. Endoscopic surveillance of Barrett’s esophagus using volumetric laser endomicroscopy with artificial intelligence image enhancement. Gastroenterol. 2019;157(2):303-305.

79. Struyvenberg MR, van der Sommen F, Swager AF, et al. Improved Barrett’s neoplasia detection using computer-assisted multiframe analysis of volumetric laser endomicroscopy. Dis Esophagus. 2020;33(2):doz065.

80. Smith MS, Ikonomi E, Bhuta R, et al; US Collaborative WATS Study Group. Wide-area transepithelial sampling with computer-assisted 3-dimensional analysis (WATS) markedly improves detection of esophageal dysplasia and Barrett’s
esophagus: analysis from a prospective multicenter community-based study. *Dis Esophagus.* 2019;32(3):doy099.

81. Srinivasan S, Agha YH, Hyder J, et al. 361 WATS3D vs. traditional forceps biopsy in screening of Barrett’s esophagus: a community hospital experience. *Am J Gastroenterol.* 2019;114:S212-S213.

82. Vennalaganti PR, Kaul V, Wang KK, et al. Increased detection of Barrett’s esophagus-associated neoplasia using wide-area trans-epithelial sampling: a multicenter, prospective, randomized trial. *Gastrointest Endosc.* 2018;87(2):348-355.

83. Gross SA, Smith MS, Kaul V, US Collaborative WATS3D Study Group. Increased detection of Barrett’s esophagus and esophageal dysplasia with adjunctive use of wide-area transepithelial sample with three-dimensional computer-assisted analysis (WATS). *United European Gastroenterol J.* 2018;6(4):529-535.

84. Agha YH, Srinivasan S, Hyder J, et al. WATS3D versus forceps biopsy in screening for Barrett’s esophagus: experience in community endoscopy centers. *Am Gastroenterol.* 2021;34(2):164-168.

85. Suresh Kumar VC, Harne P, Patthipati VS, et al. Wide-area transepithelial sampling in adjunct to forceps biopsy increases the absolute detection rates of Barrett’s oesophagus and oesophageal dysplasia: a meta-analysis and systematic review. *BMJ Open Gastroenterol.* 2020;7(1):e000494.

86. Singer ME, Smith MS. Wide Area Transepithelial Sampling with Computer-Assisted Analysis (WATS3D) is cost-effective in Barrett’s esophagus screening. *Dig Dis Sci.* 2021;66(5):1572-1579.

87. Vennalaganti PR, Naag Kanakadandi V, Gross SA, et al. Inter-Observable agreement among pathologists using wide-area transepithelial sampling with computer-assisted analysis in patients with Barrett’s esophagus. *Am J Gastroenterol.* 2015;110(9):1257-1260.

88. Canto MI, Montgomery E. Wide-area transepithelial sampling with 3-dimensional cytology: does it detect more dysplasia or yield more hype? *Gastrointest Endosc.* 2018;87(2):356-359.

89. Tschanz ER. Do 40% of patients resected for Barrett esophagus with high-grade dysplasia have unsuspected adenocarcinoma? *Arch Pathol Lab Med.* 2005;129(2):177-180. doi:10.1043/2005-129-177-DOPRFB. PMID:15679415.

90. Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol.* 2001;32(4):368-378.

91. Kerkhof M, van Dekken H, Steyerberg EW, et al; CYBAR study group. Grading of dysplasia in Barrett’s oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. *Histopathol.* 2007;50(7):920-927.

92. Kaye PV, Haider SA, Ilyas M, et al. Barrett’s dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. *Histopathol.* 2009;54(6):699-712.

93. Kaye PV, Ilyas M, Soomro I, et al. Dysplasia in Barrett’s oesophagus: p53 immunostaining is more reproducible than haematoxylin and eosin diagnosis and improves overall reliability, while grading is poorly reproducible. *Histopathol.* 2016;69(3):431-440.

94. Moyes LH, Oien KA, Foulis AK, Fullarton GM, Going JJ. Prevalent low-grade dysplasia: the strongest predictor of malignant progression in Barrett’s columnar-lined oesophagus. *Gut.* 2016;65(2):360-361.

95. Sikkema M, Looman CW, Steyerberg EW, et al. Predictors for neoplastic progression in patients with Barrett’s esophagus: a prospective cohort study. *Am J Gastroenterol.* 2011;106(7):1231-1238.

96. Song KY, Henn AJ, Gravely AA, et al. Persistent confirmed low-grade dysplasia in Barrett’s esophagus is a risk factor for progression to high-grade dysplasia and adenocarcinoma in a US Veterans cohort. *Dis Esophagus.* 2020;33(2):doz061.

97. Thota PN, Lee HJ, Goldblum JR, et al. Risk stratification of patients with Barrett’s esophagus and low-grade dysplasia or indefinite for dysplasia. *Clin Gastroenterol Hepatol.* 2015;13(3):459-465.e1.

98. Duits LC, van der Wel MJ, Cotton CC, et al. Patients With Barrett’s esophagus and confirmed persistent low-grade dysplasia are at increased risk for progression to neoplasia. *Gastroenterol.* 2017;152(5):993-1001.e1.

99. Duits LC, Phoa KN, Curvers WL, et al. Barrett’s oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut.* 2015;64(5):700-706.

100. Montgomery E, Goldblum JR, Greenson JK, et al. 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(4):379-388.

101. Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut.* 2000;47(2):251-255.

102. Reid BJ, Haggitt RC, Rubin CE, et al. Observer variation in the diagnosis of dysplasia in Barrett’s esophagus. *Hum Pathol.* 1988;19(2):166-178.

103. WHO Classification of Tumours. *Digestive System Tumours.* 5th ed. Edited by the WHO Classification of Tumours Editorial Board. Lyon, IARC; 2019.

104. Montgomery E. Update on grading dysplasia in Barrett’s esophagus. *Pathol Case Rev.* 2002;7(1):35-42.

105. Naini BV, Souza RF, Odze RD. Barrett’s esophagus: a comprehensive and contemporary review for pathologists. *Am J Surg Pathol.* 2016;40(5):e45-e66.

106. Srivastava A, Appelman H, Goldsmith JD, Davison JM, Hart J, Krasinskas AM. The use of ancillary stains in the diagnosis of Barrett esophagus and Barrett esophagus-associated dysplasia: recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. *Am J Surg Pathol.* 2017;41(5):e8-e21.

107. McNtiere MG, Soucy G, Vaughan TL, Shahsafaei A, Odze RD. MUC2 is a highly specific marker of goblet cell metaplasia in the distal esophagus and gastroesophageal junction. *Am J Surg Pathol.* 2011;35(7):1007-1013.

108. Brown IS, Whiteman DC, Lauwers GY. Foveolar type dysplasia in Barrett esophagus. *Mod Pathol.* 2010;23(6):834-843.

109. Mahajan D, Bennett AE, Liu X, Bena J, Bronner MP. Grading of gastric foveolar-type dysplasia in Barrett’s esophagus. *Mod Pathol.* 2010;23(1):1-11.

110. Rucker-Schmidt RL, Sanchez CA, Blount PL, et al. Nonadenomatous dysplasia in Barrett esophagus: a clinical, pathologic, and DNA content flow cytometric study. *Am J Surg Pathol.* 2009;33(6):886-893.

111. Serra S, Ali R, Bateman AC, et al. Gastric foveolar dysplasia: a survey of reporting habits and diagnostic criteria. *Pathol.* 2017;49(4):391-396.

112. Karamchandani DM, Zhang Q, Liao XY, Xu JH, Liu XL. Inflammatory bowel disease- and Barrett’s esophagus-associated neoplasia: the old, the new, and the persistent struggles. *Gastroenterol Rep (Oxf).* 2019;7(6):379-395.
113. Odze RD. Diagnosis and grading of dysplasia in Barrett's oesophagus. *J Clin Pathol.* 2006;59(10):1029-1038.

114. Montgomery E, Voltaggio L. Biopsy Interpretation of The gastrointestinal Tract Mucosa, 3rd ed, vol. 2. Wolters Kluwer; 2018:12-34.

115. Downs-Kelly E, Mendelin JE, Bennett AE, et al. Poor interobserver agreement in the distinction of high-grade dysplasia and adenocarcinoma in pretreatment Barrett's oesophagus biopsies. *Am J Gastroenterol.* 2008;103(9):2333-2340. quiz 2341.

116. Hornick JL, Odze RD. Neoplastic precursor lesions in Barrett's oesophagus. *Gastroenterol Clin North Am.* 2007;36(4):775-796.

117. Ormsby AH, Petras RE, Henricks WH, et al. Observer variation in the diagnosis of superficial oesophageal adenocarcinoma. *Gut.* 2002;51(5):671-676.

118. Dar MS, Goldblum JR, Rice TW, Falk GW. Can extent of high grade dysplasia in Barrett's oesophagus predict the presence of adenocarcinoma at oesophagectomy? *Gut.* 2003;52(4):486-489.

119. Konda VJ, Ross AS, Ferguson MK, et al. Is the risk of concomitant invasive esophageal cancer in high-grade dysplasia in Barrett's esophagus overestimated? *Clin Gastroenterol Hepatol.* 2008;6(2):159-164.

120. Skacel M, Petras RE, Henricks WH, et al. Observer variation in the superficial oesophageal adenocarcinoma. *Gut.* 2002;51(5):671-676.

121. Grin A, Streitker CJ. Histopathology in Barrett esophagus and Barrett esophagus-related dysplasia. *Clin Endosc.* 2014;47(1):31-39.

122. Desai TK, Krishnan K, Samala N, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut.* 2012;61(7):970-976.

123. Hvid-Jensen F, Pedersen L, Drewes AM, Sørensen HT, Punch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med.* 2011;365(15):1375-1383.

124. Kroep S, Lansdorp-Vogelaar I, Rubenstein JH, et al. An accurate cancer incidence in Barrett's esophagus: a best estimate using published data and modeling. *Gastroenterol.* 2015;149(3):577-585.e4, quiz e14-5.

125. Weusten B, Bisschops R, Coron E, et al. Endoscopic management of Barrett's esophagus: European Society of Gastrointestinal Endoscopy (ESGE) position statement. *Endoscopy.* 2017;49(2):191-198.

126. Curvers WL, ten Kate FJ, Krishnadath KK, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *Am J Gastroenterol.* 2010;105(7):1523-1530.

127. Sangle NA, Taylor SL, Emond MJ, Depot M, Overholt BF, Bronner MP. Overdiagnosis of high-grade dysplasia in Barrett's esophagus: a multicenter, international study. *Mod Pathol.* 2015;28(6):758-765.

128. Caspa Gokulan R, Garcia-Buitrago MT, Zaika AI. From genetics to signaling pathways: molecular pathogenesis of esophageal adenocarcinoma. *Biochim Biophys Acta Rev Cancer.* 2019;1872(1):37-48.

129. Reid BJ, Barrett MT, Galipeau PC, et al. Barrett's esophagus: ordering the events that lead to cancer. *Eur J Cancer Prev.* 1996;5(Suppl 2):57-65.

130. Stachler MD, Camarda ND, Deitrick C, et al. Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. *Gastroenterol.* 2018;155(1):156-167.

131. Lörinc E, Jakobsson B, Landberg G, Veress B, Ki67 and p53 immunohistochemistry reduces interobserver variation in assessment of Barrett's oesophagus. *Histopathol.* 2005;46(6):642-648.

132. van der Wel MJ, Coleman HG, Bergman JG, Jansen M, Meijer SL, BOLERO working group. Histopathologist features predictive of diagnostic concordance at expert level among a large international sample of pathologists diagnosing Barrett's dysplasia using digital pathology. *Gut.* 2020;69(5):811-822.

133. Skacel M, Petras RE, Rybicki LA, et al. p53 expression in low grade dysplasia in Barrett's oesophagus: correlation with interobserver agreement and disease progression. *Am J Gastroenterol.* 2002;97(10):2508-2513.

134. Weston AP, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's oesophagus: immunohistochemical marker predictive of progression. *Am J Gastroenterol.* 2001;96(5):1355-1362.

135. Murray L, Sudo A, Scott M, et al. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut.* 2006;55(10):1390-1397.

136. Kerkhof M, Steyerberg EW, Kusters JG, et al. Aneuploidy and high expression of p53 and Ki67 is associated with neoplastic progression in Barrett esophagus. *Cancer Biomark.* 2008;4(1):1-10.

137. Sikkema M, Kerkhof M, Steyerberg EW, et al. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's oesophagus: a case-control study. *Am J Gastroenterol.* 2009;104(11):2673-2680.

138. Kastelein F, Biermann K, Steyerberg EW, et al; ProBar-study group. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. *Gut.* 2013;62(12):1676-1683.

139. Davelaar AL, Calpe S, Lau L, et al. Aberrant TP53 detected by combining immunohistochemistry and DNA-FISH improves Barrett's esophagus progression prediction: a prospective follow-up study. *Genes Chromosomes Cancer.* 2015;54(2):82-90.

140. Horvath B, Singh P, Xie H, Thota PN, Sun X, Liu X. Expression of p53 predicts risk of prevalent and incident advanced neoplasia in patients with Barrett's esophagus and epithelial changes indefinite for dysplasia. *Gastroenterol Rep (Oxf).* 2016;4(4):304-309.

141. Younes M, Brown K, Lauwers GY, et al. p53 protein accumulation predicts malignant progression in Barrett's metaplasia: a prospective study of 275 patients. *Histopathol.* 2017;71(1):27-33.

142. Duits LC, Lao-Sirieix P, Wolf WA, et al. A biomarker panel predicts progression of Barrett's esophagus to esophageal adenocarcinoma. *Dis Esopha.* 2019;32(1):doy102.

143. Jannaat VT, van Olphen SH, Biermann KE, Looijenga LHI, Bruno MB, Spaander MCW. Use of immunohistochemical biomarkers as independent predictor of neoplastic progression in Barrett's oesophagus surveillance: a systematic review and meta-analysis. *PLoS One.* 2017;12(10):e0186305.

144. Snyder P, Dunbar K, Ciper DJ, Souza RF, Spechler SJ, Konda VJA. Aberrant p53 immunostaining in Barrett's esophagus predicts neoplastic progression: systematic review and meta-analyses. *Dig Dis Sci.* 2019;64(5):1089-1097.

145. Altaf K, Xiong JJ, la Iglesia D, Hickey L, Kaul A. Meta-analysis of biomarkers predicting risk of malignant progression in Barrett's oesophagus. *Br J Surg.* 2017;104(5):493-502.

146. Baak JP, ten Kate FJ, Offerhaus GJ, van Lanschot JJ, Meijer GA. Routine morphometrical analysis can improve reproducibility of dysplasia grade in Barrett's oesophagus surveillance biopsies. *J Clin Pathol.* 2002;55(12):910-916.
147. Feith M, Stein HJ, Mueller J, Siewert JR. Malignant degeneration of Barrett's esophagus: the role of the Ki-67 proliferation fraction, expression of E-cadherin and p53. *Dis Esophagus*. 2004;17(4):322-327.

148. Hong MK, Laskin WB, Herman BE, et al. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. *Cancer*. 1995;75(2):423-429.

149. Olvera M, Wickramasinghe K, Brynes R, Bu X, Ma Y, Chandrasoma P. Ki67 expression in different epithelial types in columnar lined esophagus indicates varying levels of expanded and aberrant proliferative patterns. *Histopathol.* 2005;47(2):132-140.

150. Polkowski W, van Lanschot JJ, Ten Kate FJ, et al. The value of Shi XY, Bhagwandeen B, Leong AS, p16, cyclin D1, Ki-67, and AMACR as markers for tumour progression in the Barrett's dysplasia-carcinoma sequence. *Surg Oncol.* 1995;4(3):163-171.

151. Shi XY, Bhagwandeen B, Leong AS. p16, cyclin D1, Ki-67, and AMACR as markers for dysplasia in Barrett's esophagus. *Appl Immunohistol Mol Morphol.* 2008;16(5):447-452.

152. Yacoub L, Goldman H, Odze RD. Transforming growth factor-alpha, epidermal growth factor receptor, and MiB-1 expression in Barrett's-associated neoplasm: correlation with-progression. *Mod Pathol.* 1997;10(2):105-112.

153. Sabour S, Yousaft H, Hayat U, et al. Surface Ki-67 expression improves reproducibility of dysplasia diagnosis in Barrett's esophagus: methodologic issues to avoid mismanagement. *Am J Clin Pathol.* 2020;154(3):426-427.

154. Dorer R, Odze RD. AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, ulcerative colitis, and Crohn's disease. *Am J Surg Pathol.* 2006;30(7):871-877.

155. Lisovsky M, Falkowski O, Bhuiya T. Expression of alpha-methylacyl-coenzyme A racemase in dysplastic Barrett's epithelium. *Hum Pathol.* 2006;37(12):1601-1606.

156. 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(8):961-967.

157. Kinra P, Gahlot GPS, Yadav R, et al. Histological assessment & use of immunohistochemical markers for detection of dysplasia in Barrett's esophageal mucosa. *Pathol Res Pract.* 2018;214(7):993-999.

158. 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. *Histopathol.* 2008;52(3):399-402.

159. Kastelein F, Biermann K, Steyerberg EW, et al; ProBar study group. Value of α-methylacyl-CoA racemase immunohistochemistry for predicting neoplastic progression in Barrett's oesophagus. *Histopathol.* 2013;63(5):630-639.

160. Polkowski W, Baak JP, van Lanschot JJ, et al. Clinical decision making in Barrett's oesophagus can be supported by computerized immunounquantitation and morphometry of features associated with proliferation and differentiation. *J Pathol.* 1998;184(2):161-168.

161. van Sandick JW, Baak JP, van Lanschot JJ, et al. Computerized quantitative pathology for the grading of dysplasia in surveillance biopsies of Barrett's oesophagus. *J Pathol.* 2000;190(2):177-183.

162. Sabo E, Beck AH, Montgomery EA, et al. Computerized morphometry as an aid in determining the grade of dysplasia and progression to adenocarcinoma in Barrett's esophagus. *Lab Invest.* 2006;86(12):1261-1271.

163. Tomita N, Abdollahi B, Wei J, Ren B, Suriawinata A, Hassanpour S. Attention-based deep neural networks for detection of cancerous and precancerous esophageal tissue on histopathological slides. *JAMA Netw Open*. 2019;2(11):e1914645.

164. Kobayashi S, Saltz JH, Yang VW. State of machine and learning in histopathological applications in digestive diseases. *World J Gastroenterol.* 2021;27(20):2545-2575.

165. Critchley-Thorne RJ, Duits LC, Prichard JW, et al. A tissue system pathology assay for high-risk Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev.* 2016;25(6):958-968.

166. Frei NF, Konte K, Bossart EA, et al. Independent validation of a tissue systems pathology assay to predict future progression in nondysplastic Barrett's esophagus: a spatial-temporal analysis. *Clin Transl Gastroenterol.* 2020;11(10):e00244.

167. Davison JM, Goldblum J, Grewal US, et al. Independent blinded validation of a tissue systems pathology test to predict progression in patients with Barrett's esophagus. *Am J Gastroenterol.* 2020;115(6):843-852.

168. Diehl DL, Khara HS, Akhtar N, Critchley-Thorne RJ. TissueCypher Barrett's esophagus assay impacts clinical decisions in the management of patients with Barrett's esophagus. *Endosc Int Open.* 2021;9(3):E348-E355.

169. Stachler MD, Taylor-Weiner A, Peng S, et al. Paired exome analysis of Barrett's esophagus and adenocarcinoma. *Nat Genet.* 2015;47(9):1047-1055.

170. Dulak AM, Stojanov P, Peng S, et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet.* 2013;45(5):478-486.

171. Ross-Innes CS, Becq J, Warren A, et al. Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. *Nat Genet.* 2015;47(9):1038-1046. doi: 10.1038/ng.3357. Epub 2015 Jul 20. PMID: 26192915; PMCID: PMC4556068.

172. Galipeau PC, Prevo LJ, Sanchez CA, Longton GM, Reid BJ. Clonal expansion and loss of heterozygosity at chromosomes 9p and 17p in premalignant esophageal (Barrett's) tissue. *J Natl Cancer Inst.* 1999;91(24):2087-2095.

173. Maley CC, Galipeau PC, Li X, Sanchez CA, Paulson TG, Reid BJ. Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res.* 2004;64(10):3414-3427.

174. Maley CC, Galipeau PC, Finley JC, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nat Genet.* 2006;38(4):468-473.

175. Li X, Galipeau PC, Paulson TG, et al. Temporal and spatial evolution of somatic chromosomal alterations: a case-cohort study of Barrett's esophagus. *Cancer Prev Res (Phila).* 2014;7(1):114-127.

176. Weaver JMJ, Ross-Innes CS, Shannon N, et al. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. *Nat Genet.* 2014;46(8):837-843.

177. Contino G, Vaughan TL, Whiteman D, Fitzgerald RC. The evolving genomic landscape of Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterol.* 2017;153(3):657-673.e1.

178. Nones K, Waddell N, Wayte N, et al. Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. *Nat Commun.* 2014;29(5):5224.

179. Stephens PJ, Greenman CD, Fu B, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell.* 2011;144(1):27-40.
180. Rausch T, Jones DT, Zapatka M, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell. 2012;148(1–2):59-71.

181. Secrèr M, Li X, de Silva N, et al; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium. Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance. Nat Genet. 2016;48(10):1131-1141. Erratum in: Nat Genet. 2017 Jan 31;49(2):317.

182. Nik-Zainal S, Alexandrov LB, Wedge DC, et al; Breast Cancer Working Group of the International Cancer Genome Consortium. Mutational processes molding the genomes of 21 breast cancers. Cell. 2012;149(5):979-993.

183. Maciejowski J, de Lange T. Telomeres in cancer: tumour suppression and genome instability. Nat Rev Mol Cell Biol. 2017;18(3):175-186. doi:10.1038/nrm.2016.171. Epub 2017 Jan 18. Erratum in: Nat Rev Mol Cell Biol. 2019 Apr;20(4):259.

184. Harris RS, Petersen-Mahrt SK, Neuberger MS. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. Mol Cell. 2002;10(5):1247-1253.

185. Harris RS, Dudley JP. APOBECs and virus restriction. Virol. 2015;479-480:131-145.

186. Maciejowski J, Li Y, Bosco N, Campbell PJ, de Lange T. Chromothripsis and kataegis induced by telomere crisis. Cell. 2015;163(7):1641-1654.

187. Murnane JP. Telomeres and chromosome instability. DNA Repair (Amst). 2006;5(9–10):1082-1092.

188. Newell F, Patel K, Gartside M, et al. Complex structural rearrangements are present in high-grade dysplastic Barrett’s oesophagus samples. BMC Med Genomics. 2019;12(1):31.

189. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett’s esophagus is associated with genomic instability. Lab Invest. 1989;60(1):65-71.

190. Rabinovitch PS, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett’s esophagus III: baseline flow cytometric variables. Am J Gastroenterol. 2001;96(11):3071-3083.

191. Fang M, Lew E, Klein M, Sebo T, Su Y, Goyal R. DNA abnormalities as marker of risk for progression of Barrett’s esophagus to adenocarcinoma: image cytometric DNA analysis in formalin-fixed tissues. Am J Gastroenterol. 2004;99(10):1887-1894.

192. Maley CC, Galipeau PC, Li X, et al. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Res. 2004;64(20):7629-7633.

193. Qureshi AP, Stachler MD, Haque O, Odze RD. Biomarkers for Barrett’s esophagus - a contemporary review. Expert Rev Mol Diagn. 2018;18(11):939-946.

194. Killcoyne S, Gregson E, Wedge DC, et al. Genomic copy number predicts esophageal cancer years before transformation. Nat Med. 2020;26(11):1726-1732.

195. Douville C, Moinova HR, Thota PN, et al. Massively parallel sequencing of esophageal brushings enables an aneuploidy-based classification of patients with Barrett’s esophagus. Gastroenterol. 2021;160(6):2043-2054.e2.

196. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31(1):27-36.

197. Kaz AM, Grady WM, Stachler MD, Bass AJ. Genetic and epigenetic alterations in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterol Clin North Am. 2015;44(2):473-489.

198. Schulmann K, Sterian A, Berki A, et al. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett’s-associated neoplastic progression and predicts progression risk. Oncogene. 2005;24(25):4138-4148.

199. Alvi MA, Liu X, O’Donovan M, et al. DNA methylation as an adjunct to histopathology to detect prevalent, inconspicuous dysplasia and early-stage neoplasia in Barrett’s esophagus. Clin Cancer Res. 2013;19(4):878-888.

200. Xu E, Gu J, Hawk ET, et al. Genome-wide methylation analysis shows similar patterns in Barrett’s esophagus and esophageal adenocarcinoma. Carcinogenesis. 2013;34(12):2750-2756.

201. Alvarez H, Opalinska J, Zhou L, et al. Widespread hypomethylation occurs early and synergizes with gene amplification during esophageal carcinogenesis. PLoS Genet. 2011;7(3):e1001356. Erratum in: PLoS Genet. 2011 May;7(5).

202. Sato F, Jin Z, Schulmann K, et al. Three-tiered risk stratification model to predict progression in Barrett’s esophagus using epigenetic and clinical features. PLoS One. 2008;3(4):e1890.

203. Kaz AM, Wong CJ, Luo Y, et al. DNA methylation profiling in Barrett’s esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics. 2011;6(12):1403-1412.

204. Yu M, Maden SK, Stachler M, et al. Subtypes of Barrett’s oesophagus and oesophageal adenocarcinoma based on genome-wide methylation analysis. Gut. 2019;68(3):389-399.

205. Jammula S, Katz-Summerscorn AC, Li X, et al; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) consortium, Fitzgerald RC. Identification of subtypes of Barrett’s esophagus and esophageal adenocarcinoma based on DNA methylation profiles and integration of transcriptome and genome data. Gastroenterol. 2020;158(6):1682-1697.e1.

206. Li D, Zhang L, Liu Y, et al. Specific DNA methylation markers in the diagnosis and prognosis of esophageal cancer. Aging (Albany NY). 2019;11(23):11640-11658.

207. Peng W, Tu G, Zhao Z, et al. DNA methylome and transcriptome analysis established a model of four differentially methylated positions (DMPs) as a diagnostic marker in esophageal adenocarcinoma early detection. PeerJ. 2021;7(9):e11355.

208. Chettouh H, Mowforth O, Galeano-Dalmau N, et al. Methylation panel is a diagnostic biomarker for Barrett’s oesophagus in endoscopic biopsies and non-endoscopic cytology specimens. Gut. 2018;67(11):1942-1949.

209. Moinova H, Leidner RS, Ravi L, et al. Aberrant vimentin methylation is characteristic of upper gastrointestinal pathologies. Cancer Epidemiol Biomarkers Prev. 2012;21(4):594-600.

210. Moinova HR, Laframboise T, Loomba T, et al. Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett’s esophagus. Sci Transl Med. 2018;10(424):eaao5848.

211. Damaskos C, Garmpis N, Valsami S, et al. Histone deacetylase inhibitors: an attractive therapeutic strategy against breast cancer. Anticancer Res. 2017;37(1):35-46.

212. Schizas D, Mastoraki A, Naar L, et al. Concept of histone deacetylases in cancer: reflections on esophageal carcinogenesis and treatment. World J Gastroenterol. 2018;24(41):4635-4642.

213. Ali SR, Humphreys KJ, McKinnon RA, Michael MZ. Impact of histone deacetylase inhibitors on microRNA expression and cancer therapy: a review. Drug Dev Res. 2015;76(6):296-317.

214. Jiang C, Pugh BF. Nucleosome positioning and gene regulation: advances through genomics. Nat Rev Genet. 2009;10(3):161-172.

215. Kelly TK, Liu Y, La FD, Liang G, Berman BP, Jones PA. Genome-wide mapping of nucleosome positioning and DNA
methylation within individual DNA molecules. Genome Res. 2012;22(12):2497-2506.

216. Collings CK, Anderson JN. Links between DNA methylation and nucleosome occupancy in the human genome. Epigenetics Chromatin. 2017;11(10):18.

217. Baldi S. Nucleosome positioning and spacing: from genome-wide maps to single arrays. Essays Biochem. 2019;63(1):5-14.

218. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as onco- genes and tumor suppressors. Dev Biol. 2007;302(1):1-12.

219. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834-838.

220. Maru DM, Singh RR, Hannah C, et al. MicroRNA-196a is a potential marker of progression during Barrett’s metaplasia-dysplasia-invasive adenocarcinoma sequence in esophagus. Am J Pathol. 2009;174(5):1940-1948.

221. Fassan M, Volinia S, Palatini J, et al. MicroRNA expression profiling in human Barrett’s carcinogenesis. Int J Cancer. 2011;129(7):1661-1670.

222. Revilla-Nuñ B, Parrilla P, Lozano JJ, et al. Predictive value of MicroRNAs in the progression of Barrett esophagus to ad- enocarcinoma in a long-term follow-up study. Ann Surg. 2013;257(5):886-893.

223. Wu X, Ajani JA, Gu J, et al. MicroRNA expression signatures during malignant progression from Barrett’s esophagus to esophageal adenocarcinoma. Cancer Prev Res (Phila). 2013;6(3):196-205.

224. Slaby O, Srnová J, Radova L, et al. Dynamic changes in microRNA expression profiles reflect progression of Barrett’s esophagus to esophageal adenocarcinoma. Carcinogenesis. 2015;36(5):521-527.

225. Craig MP, Rajakurana S, Pally O, et al. Differential MicroRNA signatures in the pathogenesis of Barrett’s esophagus. Clin Transl Gastroenterol. 2020;11(1):e00125.

226. Mallick R, Patnaik SK, Wani S, Bansal A. A systematic review of esophageal microRNA markers for diagnosis and monitoring of Barrett’s esophagus. Dig Dis Sci. 2016;61(4):1039-1050.

227. Li X, Kleeman S, Coburn SB, et al. Selection and application of tissue microRNAs for nonendoscopic diagnosis of Barrett’s esophagus. Gastroenterol. 2018;155(3):771-783.e3.

228. Bus P, Kestens C, Ten Kate FJ, et al. Profiling of circulating mi- croRNAs in patients with Barrett’s esophagus and esophageal adenocarcinoma. J Gastroenterol. 2016;51(6):560-570.

229. Pavlov K, Kluiver J, Meijer C, et al. Circulating miRNAs in patients with Barrett’s esophagus, high-grade dyspla- sia and esophageal adenocarcinoma. J Gastrointest Oncol. 2018;9(6):1150-1156.

230. Fassan M, Realdon S, Cascone L, et al. Circulating microRNA expression profiling revealed miR-92a-3p as a novel biomarker of Barrett’s carcinogenesis. Pathol Res Pract. 2020;216(3):152907.

231. Wang L, Ji F, Liu G, et al. Upregulation of circulating miR-130a is correlated with development of Barrett’s esophagus and esoph- ageal adenocarcinoma. Onco Targets Ther. 2018;17(12):1-7.

232. Zarrilli G, Galuppi F, Angerilli V, et al. miRNAs involved in esophageal carcinogenesis and miRNA-related therapeutic perspectives in esophageal carcinoma. Int J Mol Sci. 2021;22(7):3640.

233. Lv J, Zhao HP, Dai K, Cheng Y, Zhang J, Guo L. Circulating exosomal miRNAs as potential biomarkers for Barrett’s esoph- agus and esophageal adenocarcinoma. World J Gastroenterol. 2020;26(22):2889-2901.
early events in esophageal carcinogenesis. *World J Gastroenterol*. 2007;13(5):676-682.

251. Wang Z, Chen M, Qiu Y, et al. Identification of potential biomarkers associated with immune infiltration in the esophageal carcinoma tumor microenvironment. *Biosci Rep*. 2021;41(2):BSR20202439.

252. Fishman DA, Kearns A, Chilukuri K, et al. Metastatic dissemination of human ovarian epithelial carcinoma is promoted by alpha2beta1-integrin-mediated interaction with type I collagen. *Invasion Metastasis*. 1998;18:15-26. doi:10.1159/000024495

253. Li G, Jiang W, Kang Y, Yu X, Zhang C, Feng Y. High expression of collagen 1A2 promotes the proliferation and metastasis of esophageal cancer cells. *Ann Transl Med*. 2020;8(24):1672.

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