Barrett's Esophagus and Cancer Risk: How Research Advances Can Impact Clinical Practice

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Barrett's esophagus (BE) is the only known precursor to esophageal adenocarcinoma (EAC), whose incidence has increased sharply in the last 4 decades. The annual conversion rate of BE to cancer is significant, but small. The identification of patients at a higher risk of cancer therefore poses a clinical conundrum. Currently, endoscopic surveillance is recommended in BE patients, with the aim of diagnosing either dysplasia or cancer at early stages, both of which are curable with minimally invasive endoscopic techniques. There is a large variation in clinical practice for endoscopic surveillance, and dysplasia as a marker of increased risk is affected by sampling error and high interobserver variability. Screening programs have not yet been formally accepted, mainly due to the economic burden that would be generated by upper gastrointestinal endoscopy. Screening programs have not yet been formally accepted, mainly due to the economic burden that would be generated by widespread indication to upper gastrointestinal endoscopy. In fact, it is currently difficult to formulate an accurate algorithm to confidently target the population at risk, based on the known clinical risk factors for BE and EAC. This review will focus on the clinical and molecular factors that are involved in the development of BE and its conversion to cancer and on how increased knowledge in these areas can improve the clinical management of the disease. **(Gut Liver 2014;8:356-370)**

Key Words: Barrett esophagus; Dysplasia; Cancer; Screening

INTRODUCTION

Barrett's esophagus (BE) is an acquired condition in which a metaplastic columnar lining with intestinal differentiation replaces the stratified squamous epithelium in the distal esophagus. The metaplastic epithelium comprises three different cell types: atrophic gastric-fundic-type epithelium containing parietal and chief cells; a transitional-type epithelium with cardiac mucous-secreting glands; and specialized columnar epithelium with intestinal-type goblet cells.[1] While American gastroenterological societies consider the specialized epithelium with goblet cells a requirement for the diagnosis of BE,[2] British guidelines consider the possibility of including BE with gastric metaplasia only.[3]

The true prevalence of BE is still unclear. In recent years Italian and Swedish researchers were able to show a prevalence of 1.3% and 1.6%, respectively, although in both studies a selection bias may have led to an over-estimate.[4,5] BE generally develops in the context of chronic gastroesophageal reflux disease (GERD) and it is about 10 times more frequent in individuals who complain of reflux symptoms.[5-7] BE is the only known precursor to esophageal adenocarcinoma (EAC), with an annual conversion rate of approximately 0.3%.[8-10] In recent U.K. statistics, the esophagus was rated as the 7th most common cancer site among males and 14th among females. However esophageal malignancy was the fourth most common cause of cancer-related death in men and sixth in women in this geographical area. Although these data related to both of the most common histologic types, adenocarcinoma and squamous cell carcinoma (SCC), it is known that the overall prognosis of these two types of cancer is similar.[11] The discrepancy between incidence and mortality rates stems from the fact that esophageal cancer is aggressive in nature and relatively asymptomatic at early stages leading to a low overall 5-year survival rate (<15%).[12,13] There is a large geographical variation in the incidence of esophageal cancer (Fig. 1A),[14] with a higher incidence of SCC in African and Asian countries. Notably, the incidence of esophageal adenocarcinoma has been worryingly increasing over the last 3 to 4
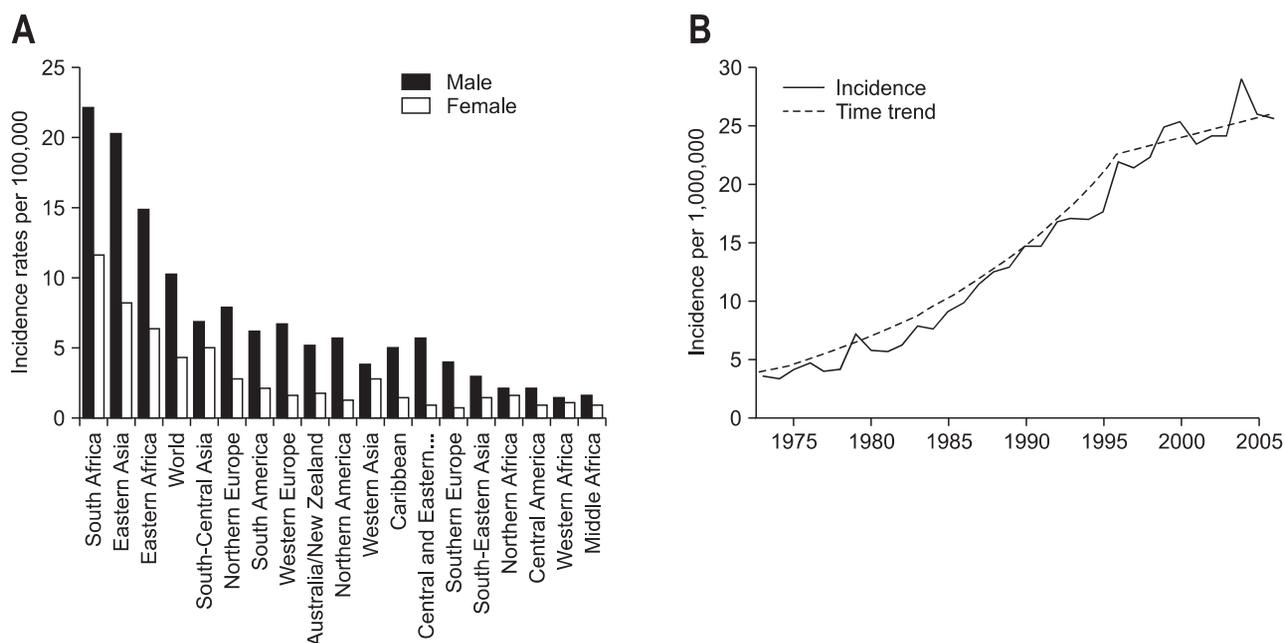
A

B

Fig. 1. (A) World age-standardized incidence rates of esophageal cancer per 100,000 population. Estimates derived from Cancer Research UK statistics (Ferlay J, *et al*. GLOBOCAN 2008 v1.2, cancer incidence and mortality worldwide).[14] (B) Relative change in the incidence of esophageal adenocarcinoma (1973 to 2006). With permission from Pohl H, *et al*. Cancer Epidemiol Biomarkers Prev 2010;19:1468-1470.[15]

decades in the Western world (Fig. 1B),[15,16] where it has become the most common esophageal malignancy.[17,18] In keeping with this, GERD is also increasing in incidence in the Western population[19,20] and has been found to be the most common gatrointestinal (GI) diagnosis in an outpatient setting in the United States.[21] This epidemiological picture has led to the question of whether screening programs for BE are justified.[22] Since the gold standard for a diagnosis of BE is endoscopy with biopsies, this screening method would be too costly and invasive to be applied to the general population. All of the most recently published guidelines do not recommend screening of the unselected population, but do suggest to target the population at higher risk of BE.[2,3] Here we review the current knowledge on clinical and molecular factors associated to the risk of BE and EAC and analyse how an improved understanding of this condition can influence clinical algorithms for the management of this disease.

RISK FACTORS FOR BE

There are numerous risk factors for BE and they are generally shared with EAC. Gastroesophageal acid reflux is considered the most important factor. In a population-based case-control study, gastroesophageal reflux was associated with BE and EAC, with an odd ratio (OR) of 12.0 (95% confidence interval [CI], 7.64 to 18.7) and 3.48 (95% CI, 2.25 to 5.41), respectively.[23] A recent meta-analysis showed that GERD symptoms increased the odds of long segment BE by fivefold.[24] The prevalence of BE in patients with GERD varies between 3% and 15% depending on the

study.[6,7,22,23] This large range mostly relates to the stringency of criteria used for the selection of patients with reflux disease.

Obesity is the second strongest risk factor for the development of BE and EAC.[23,25] Obesity and GERD have synergistic effects according to a population-based case-control study, which demonstrated that obese individuals with symptoms of acid reflux had markedly higher risks of BE (OR, 34.4; 95% CI, 6.3 to 188) than people with reflux alone (OR, 9.3; 95% CI, 1.4 to 62.2) or obesity alone (OR, 0.7; 95% CI, 0.2 to 2.4).[26] The distribution of fat also has a role in determining the risk in that large amount of visceral abdominal fat relative to subcutaneous fat is associated with a significant increase in the risk of BE.[27,28]

Smokers and ex-smokers are also at increased risk of EAC.[23] A meta-analysis demonstrated a strong association between cigarette smoking and EAC with a dose-response relation to disease outcome. In addition longer smoking cessation was associated with a decreased risk of adenocarcinoma.[29] However, the association of smoking with BE remains controversial according to different studies.[30,31]

Other risk factors include male sex, white race, low vegetables intake and high red meat consumption, whereas data have showed an inverse correlation with *Helicobacter pylori* infection.[8,16,32-35]

BE has also been shown to occur in familial clusters. Studies in different populations of patients with BE and EAC confirmed that about 7% of cases are familial.[36,37] Juhasz and collaborators[38] studied 47 first degree relatives of patients with EAC and BE-related high-grade dysplasia from 23 families and confirmed BE in 13 relatives (27.7%).

A genetic background to this disease is supported by recent genome-wide association studies (GWAS). A first GWAS report demonstrated that variants at two loci were associated with disease risk; chromosome 6p21 (OR, 1.21; 95% CI, 1.13 to 1.28), within the major histocompatibility complex locus, and chromosome 16q24 (OR, 1.14; 95% CI, 1.10 to 1.19), in close proximity to *FOXF1* gene, which is implicated in esophageal development and structure.[39] In a second GWAS study Levine and coworkers[40] compared EAC cases (n=2,390) and individuals with BE (n=3,175) with 10,120 controls. Three new association loci were identified; 19p13 within *CRTC1*, whose activation has been associated with oncogenic activity, 9q22 within *BARX1*, which encodes a transcription factor involved in esophageal specification and 3p14 near the transcription factor *FOXP1*, which regulates esophageal development.

MOLECULAR PATHWAYS RELATED TO BARRETT'S DEVELOPMENT AND PROGRESSION TO CANCER

The cell of origin of BE within the esophagus remains a controversial issue. Recent evidence in mice-models showed that BE may originate from progenitor cells present within the gastric cardia in close proximity with the gastroesophageal junction. Two models have been proposed to recapitulate the origin of BE. In *p63*-deficient mice, it was shown that the normal squamous re-epithelisation of the esophagus during embryogenesis is impaired and this gives rise to upward migration of embryonic columnar remnant cells located at the level of the squamocolumnar junction (SCJ), generating a columnar epithelium reminiscent of BE.[41] In a different study, Quante and coworkers[42] were able to show that mice overexpressing interleukin-1β have an inflammatory response at the SCJ, which leads to a columnar lined esophagus that is molecularly similar to BE. In these mice, increased esophageal exposure to bile and acid triggered a sustained inflammatory response that reinforces Barrett's like carcinogenesis in a Notch-dependent fashion. Overall, these mouse models provide support to the theory that BE may originates from progenitor cells located at the SCJ and would explain why BE is generally in anatomical continuity with the cardia epithelium. However, the different anatomy of the murine esophagus warrant further studies to translate these models into the human pathology. An alternative theory is that BE may originate through a process of transdifferentiation of squamous cells or reprogramming of esophageal stem cell towards a different phenotype. This would likely involve epigenetic reprogramming of esophageal cells. In support of this theory is the evidence that genes normally involved in differentiation and gut axial specification are modulated in BE. Increased expression of the caudal-related gene CDX2 and CDX1, which are normally highly expressed in colon, has been shown in BE and related to the acquisition of the intestinal phenotype.[43] This gene regulation has recently been linked to change in the methylation status of the promoter[44] and associated to the acid/bile induced inflammation through the activation of nuclear factor κB, a crucial transcription factor in the inflammatory response.[45] In addition, acquired deregulation of HOX genes during adulthood has been linked to carcinogenesis. We have recently showed that three HOXB genes (*HOXB5*, *HOXB6*, and *HOXB7*) are activated in BE through an epigenetic mechanism involving histone posttranslational modifications. Alterations to the HOX gene expression in esophageal cells was associated with the induction of genes linked to an intestinal-phenotype.[46] The cell target of the epigenetic reprogramming of differentiation genes remain to be established, especially after the recent evidence of lack of *bona fide* stem cells in the human esophagus.[47]

Chronic reflux of acid and bile into the esophagus normally results in an acute and chronic inflammatory process. *In vivo* and *ex vivo* exposure of esophageal cells to acid and bile salts can induce the production of reactive oxygen species and nitric oxide,[48,49] which are related to oxidative DNA damage and double-strand breaks.[50,51] These events have been linked in general to carcinogenesis and more recently to the metaplasia, dysplasia to cancer sequence in BE.[50] In addition, oxidative DNA damage in BE causes telomerase activation and telomere instability, which are known to result in mutation of cancer-related genes and promotion of cancer.[52]

Inflammation is also related to recruitment of immune cells. Naive T cells, macrophages and dendritic cells are enriched in both nondysplastic and dysplastic BE, as well as in EAC.[53-55] These cells could contribute to tumorigenesis through production of cytokines, chemokines and growth factors, which are released as part of the inflammatory response and can promote proliferation and angiogenesis.[56]

Exposure to acid and bile salts has also been related to deregulation of microRNAs (miRNA),[57,58] a class of short noncoding RNA involved in a variety of cellular processes. In particular miRNA-145 was linked to the activation of BMP4 pathway,[59] which has been previously implicated in the development of BE through the activation of the Hedgehog pathway.[60] BE and EAC present a distinct miRNA expression profile,[61,62] which could be potentially useful for diagnostic purposes due to the fact that miRNAs are stable and detectable in blood.[63]

Another class of noncoding RNA, long noncoding RNA (lncRNA), which have diverse cellular properties including gene regulation and control of cell growth and migration,[64] has recently also been implicated in Barrett's carcinogenesis. Wu and collaborators[65] showed that the lncRNA AFAP1-AS1 is hypomethylated and overexpressed in BE and EAC and its silencing *in vitro* inhibited invasion and promoted apoptosis.

CLINICAL PREDICTORS OF CANCER RISK

Until recently the only clinical factor with practical implications in the management of BE was the histological diagnosis

of dysplasia. The two largest population studies in the Northern Irish and Danish cohorts confirmed that the cancer risk in patients with low grade dysplasia (LGD) is approximately 5 times higher than nondysplastic patients.[8,10] It is standard practice to monitor patient with LGD at closer intervals. Unfortunately a histopathological diagnosis of dysplasia is often associated to a high degree of interobserver variability even among expert GI pathologists, hence doubts have been shed on the exact clinical usefulness of this marker for patient stratification.[66,67] There are additional clinical factors that have been shown to influence the risk of progression of BE to cancer. These clinical elements have the potential to inform the physician about the surveillance and management of patients with BE. Several studies have shown that increasing BE length is associated with higher risk of progression to high grade dysplasia (HGD) and malignancy.[8,9,68-70] The most common cutoff used in the literature for the definition of long segment of BE is 3 cm or more; however there is high variability in the literature in the cutoffs used. Overall it is justified to consider long segment of BE at higher risk. The 2013 British Society of Gastroenterology (BSG) guidelines for the management of BE recommend to tailor surveillance interval on basis of the length of the BE.[3]

The large Northern Irish population study has also found that the presence of intestinal metaplasia (IM) was associated with a hazard ratio for progression to cancer of 3.54 (95% CI, 2.09 to 6.00).[8] However, the issue of whether IM confers increased cancer risk conceptually applies only to countries, such as United Kingdom, where IM is not required for a diagnosis of BE.[2,3]

Visible endoscopic lesions including ulcers are also associated with a high risk of HGD and early cancer and warrant close monitoring,[71] but it must be recognized that the absence of dysplasia in the presence of visible lesions is often due to sampling error. Overall, it is clear that there is a paucity of clinical factors which can inform the physician about individual cancer risk and those that are currently used are affected by a significant degree of subjectivity either in the diagnosis, i.e., dysplasia, or in the definition, i.e., length. Hence there is the need for more objective risk stratification tools to inform patient management.

MOLECULAR BIOMARKERS

Molecular biomarkers have been investigated over the last 20 years in the field of BE with the aim of providing the physician with predictors of disease behaviour and hence aiding clinical management. The advantage of biomarkers over the current standard, i.e., dysplasia, relies on the possibility to provide an objective measure of the molecular changes in tissue, which are known to correlate with progression of disease. In addition, since molecular abnormalities can extend within the BE over larger epithelial surface than cellular dysplasia, they could be
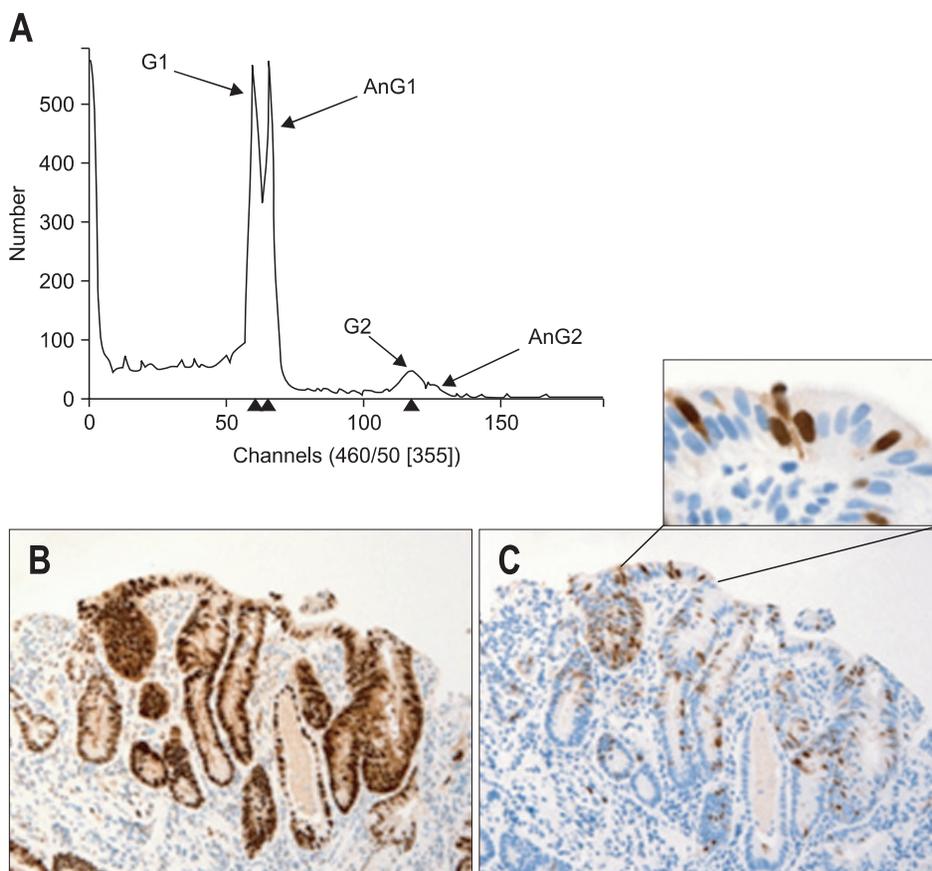
Fig. 2. Patient with Barrett's esophagus, with positivity at three different biomarkers. (A) Flow-cytometric analysis of nuclear DNA content. The aneuploidy peaks (AnG1 and AnG2) can be clearly identified as separate from the normal G1 and G2 peaks. (B) Overexpression of p53 detected by immunohistochemistry (×10). (C) Immunohistochemistry staining for cyclin A shows positive cells on the surface of the epithelium (insets, ×40). Positive cells in deep glands are considered within the normal limit.

less subject to sampling error.[72]

Gain or more rarely loss of individual chromosomes (aneuploidy) or duplication of the entire genome (tetraploidy) are common events in EAC and can precede the development of cancer or even dysplasia (Fig. 2A).[73] Gross abnormalities in the DNA content are tumorigenic since these can lead to altered expression of cancer-related genes. In particular loss of heterozygosity at tumor suppressor genes, such as p16 and p53, have been linked to acquisition of dysplasia in BE.[74,75] Reid and collaborators[76] have contributed significantly to the understanding of the timing and distribution of these molecular changes and have conducted large retrospective studies on prospectively collected samples to evaluate the usefulness of these biomarkers as cancer predictors. For example they have showed that among patients with nondysplastic BE or at most LGD, those without aneuploidy had a 0% 5-year cumulative cancer incidence compared with 28% for those with aneuploidy. In another study, the prevalence of 17p (p53) loss of heterozygosity (LOH) at baseline increased from 6% in nondysplastic patients to 57% in patients with HGD. Using baseline 17p (p53) LOH as a predictor of progression in 325 patients with BE, those with this marker had increased risk of HGD and cancer with a relative risk (RR) of 3.6 (95% CI, 1.3 to 10) and 16 (95% CI, 6.2 to 39), respectively.[77] In a follow-up study three biomarkers (abnormal DNA content, p53 LOH, and p16 LOH) were evaluated as a panel in a cohort of 243 patients, and a step-wise increase in the cancer progression risk was found with increasing number of positive biomarkers. This showed a RR for cancer of 38.7 (95% CI, 10.8 to 138.5) at 10 years of follow up when all three biomarkers were positive.[78] The main limitation of these studies was that assessment of aneuploidy was performed with a complex methodology involving flow-cytometric analysis on snap-frozen biopsies. However, it is now possible to assess aneuploidy with alternative techniques, which are potentially more applicable to clinical setting. One of them is image cytometry (IC), which can be performed on thick sections from paraffin-embedded specimens. IC was showed to be comparable to flow-cytometry for the assessment of aneuploidy in BE tissue.[79] A retrospective case-control study confirmed that a panel consisting of LGD and two molecular biomarkers (aneuploidy by IC and immunohistochemistry [IHC] for *Aspergillus oryzae* lectin) effectively separated progressors from nonprogressors.[80] Each individual positive marker was associated with an OR of 3.74 (95% CI, 2.43 to 5.79) for progression to HGD/EAC. An alternative method for assessment of aneuploidy is fluorescent *in situ* hybridization (FISH), which employs fluorescent probes to target specific DNA sequences. FISH has been studied in BE in combination with cytological brushings, which has the advantage over biopsies to sample larger epithelial areas. In particular it was found that FISH for chromosome 7 and 17 was more accurate that IC for detection of aneuploidy on cytological preparations and could detect HGD/EAC with a sensitivity and a specificity of 85% and 84%, respectively.[81]

The same group used FISH to detect copy changes of cancer-related genes, such as c-myc, EGFR, and 20q13 locus, which were found to be amplified in up to 14% and 50% of cases with HGD and EAC, respectively.[82] Similarly, a different group of authors found that FISH for four cancer-related loci (c-myc, HER2, 20q13, and p16) on brushing samples had better accuracy than conventional cytology or IC on brushings for the diagnosis of dysplasia.[83] A case-control study with FISH markers is currently being undertaken to predict disease progression in a Dutch cohort of patients with BE.

Mutation in the tumor suppressor gene *p53* is the most recurrent genetic hit in EAC.[84] p53 function is associated with G1 arrest during cell cycle and apoptosis; as a result, mutation of the *p53* gene will adversely affect control of cell proliferation and impair activation of apoptosis, promoting carcinogenesis.[52,85] Mutation of *p53* leads to either stabilization of an inactive product or complete absence of the protein. Both events can be efficiently detected by IHC, which is a cost-effective test applicable to clinical setting (Fig. 2B).[86] A case-control study by Murray and coworkers[87] found that abnormal p53 protein expression was associated with progression to EAC at follow-up, with an OR of 11.7 (95% CI, 1.93 to 71.7). It was proposed that p53 expression can be used as biomarker of malignant expression in BE, however due to the low sensitivity it was also suggested that additional biomarkers would have needed as adjunct. These results have been confirmed in a more recent and larger case controlled study on 720 patients with BE, where p53 protein expression was associated with an increased risk of neoplastic progression (RR, 5.6; 95% CI, 3.1 to 10.3) and proved to be a more powerful predictor of neoplastic progression than histological diagnosis of LGD.[88] p53 IHC has also been shown to be a useful adjunct to the histopathological diagnosis of dysplasia, assisting the pathologist in interpreting less straightforward pathological patterns.[89] In keeping with this, the 2013 BSG guidelines recommend the use of p53 IHC as adjunct to conventional histopathology.[3]

Promoter hypermethylation can lead to silencing of gene expression and cancer and has been shown to be associated with widespread epigenetic changes involving global DNA hypomethylation and targeted hypermethylation of tumor suppressor genes.[90] Kaz and collaborators[91] used a microarray-based approach on 96 esophageal samples to determine the methylation profiles of normal esophagus, nondysplastic BE, BE with HGD and EAC, and they found increasing methylation levels at gene promoters along the pathological progression. Hence, similarly to p53, methylation markers could represent a useful adjunct to histopathology. In a different study, a four-gene (SLC22A18, PIGR, GJA12, and RIN2) methylation panel was found to stratify patients with different stages of BE into three risk groups based on the number of genes methylated, with potential clinical utility (low risk: <2 genes, intermediate: 2, and high: >2).[92]

Hypermethylation of p16 and APC was also found to associ-

ate with dysplasia at a biopsy level and correlate with cancer risk at a patient level, with an OR for combined HGD/EAC of 14.97 (95% CI, 1.7-inf) when both genes were methylated.[93] In a different study methylation of 10 genes (HPP1, RUNX3, RIZ1, CRBP1, 3-OST-2, APC, TIMP3, p16, MGMT, p14) were analysed in a large cohort of EAC cases (n=77), BE (n=93), and normal esophageal specimens (n=64). Three of them, p16, RUNX3, and HPP1, showed the most significant hypermethylation levels in cancer and in a case control cohort were associated with the risk of histological progression of BE to cancer at 2-year follow-up with an OR of 1.74 (95% CI, 1.33 to 2.2), 1.8 (95% CI, 1.08 to 2.81), and 1.77 (95% CI, 1.06 to 2.81), respectively.[94]

Cyclin A is a protein that is involved in the regulation of progression through the cell cycle. In normal columnar gastrointestinal tissue, including nondysplastic BE, the expression of cyclin A is confined to the base of the crypts. With increasing grades of dysplasia, the expression of cyclin A moves towards the upper third of the crypts and the surface epithelium (Fig. 2C). In a study including 16 cases of BE that progressed to cancer and twice as many nonprogressor controls, surface expression of cyclin A correlated with the risk of progression with an OR for cancer of 7.5 (95% CI, 1.8 to 30.7).[95]

Despite the large number of molecular biomarkers studied, there is generally a lack of large prospective studies that have validated these and this has made introduction into clinical practice problematic. The biomarker with the largest data available is p53 IHC, which, due to the ample validation in independent cohorts and simplicity of the methodology, is likely closer than other biomarkers to clinical application. Aneuploidy is also very promising, but validation with the use of cost-effective techniques is needed to make it compatible with a clinical setting.

GUIDELINES

There are recent guidelines on screening and management of patients with BE. This review will focus on those published in the last 3 years, as these have taken into account the most recent data on epidemiological aspects of BE.[2,3,96] Recent data have not provided strong evidence to support screening programs. The American Society of Gastrointestinal Endoscopy (ASGE) guidelines concluded that endoscopic screening for BE is controversial due to lack of randomized controlled trials (RCT), hence it cannot be recommended.[96] On the other hand, the American Gastroenterology Association (AGA) states that the practice of screening in the United States remains widespread among physicians. The current AGA guidelines suggest that patients with multiple risk factors associated with BE and EAC should be screened. Risk factors were defined as age 50 years or older, male sex, white race, chronic GERD, hiatus hernia, elevated body mass index, and intra-abdominal distribution of body fat, but the threshold of risk factors that should trigger interven-

tion remained undefined.[2] This recommendation is in agreement with that issued by the BSG, which however is more practical with concern to the definition of the population at risk when considering multiple risk factors. These guidelines state that endoscopic screening should be taken into account in a selected population with gastroesophageal reflux symptoms and multiple risk factors (at least three of age 50 years or older, white race, male sex, obesity).[3] It is also advised that for individuals with a positive family history of BE and EAC the threshold for screening should be lowered. The issue of whether screening should focus on individuals with reflux symptoms remains unresolved. The AGA working group decided that screening should not be directed only to individuals with reflux, as this is extremely common in the general population,[21] yet approximately 40% of patients with EAC do not report a symptomatic history of gastroesophageal reflux.[97] On the other hand, GERD is the strongest risk factor for BE and EAC, and included as generic risk factor among other may result in justifying screening in a large population of individual (e.g., every white male over 50 years of age), with significant burden on the health care system. Clearly there is a need to tailor recommendations for screening interventions in order to target the largest proportion of patients with prevalent disease, without exposing an unjustified number of individuals to procedures which may generate psychological morbidity, reduce the quality of life and increase insurance premiums in places where health provision is mainly insurance based. In addition, screening performed with conventional endoscopy and tissue biopsies is expensive and would have significant bearing on the health care budget. Hence there is a need for less invasive and cost-effective devices for BE screening, ideally applicable to primary care. Non-endoscopic cell collection devices like the Cytosponge™, office-based transnasal esophagoscopy and tethered or untethered capsule endoscopy are the most promising tools but more studies are required to make conclusions regarding their diagnostic accuracy and feasibility on a larger scale.[22]

Surveillance in BE is also a controversial issue. While it is generally accepted that patients with BE should be monitored over time, definitive evidence that systematic endoscopic surveillance improves survival is still lacking. Several retrospective studies have showed that EAC and junctional adenocarcinomas diagnosed within a previous background of known BE have an earlier stage and improved survival compared to cancers presenting *de novo*.[98-100] However these studies are limited by lead time bias. By contrast, a more recent case-control study from Corley and collaborators[101] has suggested that previous endoscopic surveillance has no significant impact on mortality from EAC. The authors, however, found an unusually high prevalence of advanced stage cancers in patients undergoing surveillance, suggesting that in this cohort of patients endoscopic surveillance did not efficiently achieve the expected goal of detecting early disease. Also in this study, there was a higher proportion

of dysplasia in previous biopsies of cases that died of EAC compared to controls that did not die of this disease. Hence, there may be methodological problems with surveillance protocols in routine practice outside of specialist centers.

Nevertheless the practice of surveillance is generally accepted and recommended by all gastroenterology societies; the AGA working group indeed commented on the fact that it remains unclear whether endoscopic surveillance is beneficial, hence it was not possible to make meaningful recommendations regarding the optimal intervals between endoscopic procedures.[102]

The surveillance programs recommended by the BSG, the ASGE, and the AGA are summarized in Table 1. Overall, while we wait for convincing evidence that endoscopic surveillance is beneficial, in view of the well-established association between BE and EAC and the very poor outcomes from this cancer, it seems clinically sensible to survey BE patients over time. A multicenter U.K. based RCT (BOSS trial) is currently being undertaken to address the long-term clinical impact of endoscopic surveillance.[103] In this study, patients with BE without dysplasia are being randomized into surveillance versus no surveillance (with OGD on demand if needed). This will hopefully provide scientific evidence to support the practice of endoscopic surveillance.

One of the main implications of widespread surveillance is that the current gold standard is endoscopy with biopsies, which is invasive and expensive. Research is focusing currently on two directions to improve cost-effectiveness of surveillance. As discussed above, one is the development of biomarkers to risk stratify patients into low and high risk individuals. The rationale is to provide a more objective assessment of the individual cancer risk to overcome the shortfalls of a pathological assessment of dysplasia. This would allow stretching out intervals for surveillance in low risk patients with the potential to discharge them and on the other hand anticipate ablation treatment in high risk patients. The second research goal is to devise a less invasive and more cost-effective technologies for surveillance. Differently from screening devices, those applicable to surveillance setting would need some form of tissue collection either for pathological analysis or biomarker assessment.

Currently little progress has been made with regards to chemoprevention, and this remains a key area for investigation. There are retrospective data that suggest that proton pump inhibitors (PPI) correlate with decreased risk of HGD and EAC,[104] but definitive proof is lacking due to difficulties in designing RCTs with a placebo arm. The only drug that has made its way to an RCT is aspirin (AspECT study). Aspirin inhibits cyclo-oxygenase 1 and 2 (COX-1 and COX-2), regulator enzymes of prostaglandin E_2 production, which has been shown to be involved in angiogenesis and invasiveness in EAC and other GI malignancy.[105-107] The results of the AspECT study are awaited

Table 1. Comparison of Surveillance Recommendations in Recently Published Guidelines

	BSG (2013)		ASGE (2012)	AGA (2011)
Nondysplastic BE				
Length of BE taken into consideration	Yes		No	No
Gastric metaplasia compatible with BE diagnosis	Yes		No	No
Repeat OGD in	<3 cm	≥3 cm	3–5 yr	3–5 yr
	3–5 yr*	2–3 yr		
Indefinite for dysplasia				
Acid suppression advised	Yes		Yes	No recommendation made
Repeat OGD advised	Yes		Yes	
	In 6 mo†		No specific time frame	
Low grade dysplasia				
Initially repeat OGD in	6 mo		6 mo	6–12 mo
Surveillance OGD every	6 mo		12 mo	6–12 mo
High grade dysplasia				
Plan	MDT discussion with the view to perform endoscopic therapy with RFA+/- EMR‡		Endoscopic therapy with RFA+/- EMR to be preferred to surgery and endoscopic surveillance‡	Endoscopic therapy with RFA+/- EMR Surgery and 3-monthly surveillance in alternative‡

BSG, British Society of Gastroenterology; ASGE, American Society for Gastrointestinal Endoscopy; AGA, American Gastroenterological Association; BE, Barrett's esophagus; OGD, osophagogastroduodenoscopy; MDT, multi-disciplinary team; RFA, radiofrequency ablation; EMR, endoscopic mucosal resection.

*Discharge recommended in case of short segment of BE (<3 cm) without intestinal metaplasia; †If no definite dysplasia found in 6 months, patient should be regarded as nondysplastic; ‡RFA seems the ablative technique with the best safety and efficacy profile.

to conclude whether Aspirin in combination to PPI can be part of the management algorithm of patients with BE. Since this trial is also randomizing patients between two different doses of esomeprazole, some information on the chemopreventive effect of PPI will transpire.

ADVANCED ENDOSCOPIC IMAGING TO IDENTIFY HIGH RISK PATIENTS

There has been a great deal of research over the last years in an attempt to develop novel endoscopic techniques to enhance detection of inconspicuous dysplasia (Table 2). This would have the potential advantage to enable biopsies to be targeted towards areas containing histological dysplasia and eliminate the need of multiple random sampling. The benefit would be twofold: 1) better cost-effectiveness due to shorter endoscopies and reduced work-load for the pathologist; and 2) improved patient tolerance. Three main fields have been explored so far; i.e., dye chromoendoscopy, light filtering, and electronic image reprocessing.

Chromoendoscopy is a technique by which a chemical agent is sprayed on the Barrett's mucosa in an attempt to enhance the detection of dysplasia. Several different agents have been studied including methylene blue (MB), Lugol's solution, indigo carmine (IC), and acetic acid (AA). MB is a vital agent that is avidly incorporated by cells with intestinal differentiation and has been the first dye investigated in the field of BE. There are conflicting results on the utility of MB in dysplasia detection. A recent meta-analysis by Ngamruengphong et al. concluded

that MB does not provide a clinical advantage compared to the Seattle protocol (random quadrantic biopsies every 2 cm).[108]

IC is a contrast agent which helps highlight areas of subtle mucosal irregularity which are otherwise very difficult to identify on conventional white light endoscopy. IC has been studied by Kara and collaborators[109] in a small randomized crossover study, which compared high resolution endoscopy (HRE), IC chromoendoscopy and narrow band imaging (NBI). In this study, HRE had equal yield of dysplasia compared to advanced imaging techniques.

AA at the concentration of 2% to 3% is an inexpensive and safe imaging adjunct that when in contact with surface epithelium causes protein denaturation and induces a typical whitening effect on BE mucosa. Increased vascularisation of areas of early neoplasia results in enhanced and rapid loss of acetowhitening, which appears as area of redness on a white background. Despite two early randomized studies which failed to show increased detection rate of dysplasia by AA chromoendoscopy,[110,111] a more recent large single-center retrospective study has found a higher histological yield in patients which received AA enhanced chromoendoscopy.[112] More studies are needed to ascertain whether AA is a useful adjunct for dysplasia detection.

NBI is based on optical filters controlled by a button switch, which allows one to isolate narrow wave-lengths corresponding to the green and blue spectra of light. In the blue-green range light has reduced penetration into tissues and therefore this helps visualization of superficial vessels and mucosal pits.[113] NBI can be less time consuming and easier to perform in comparison to white light endoscopy, but it is still subject to interob-

Table 2. Comparison of Imaging Techniques Investigated to Increase Detection Rate of Dysplasia in Barrett's Esophagus

Technique	Advantages	Disadvantages
Methylene blue chromoendoscopy	Cheap	Conflicting data
	Widely available	Concerns about DNA toxicity
Indigo carmine chromoendoscopy	Cheap	Comparable to high resolution endoscopy
	Widely available	
Acetic acid chromoendoscopy	Cheap	Conflicting data
	Widely available	Validation required
Narrow band imaging	Widely available	Conflicting data
	Endoscope integrated	Narrow field if combined to magnification
Autofluorescence imaging	Endoscope integrated	Conflicting data
	Easy read out	High false positive rate
	Wide field of view	Not widely available
Confocal laser endomicroscopy	Real time histology	Narrow field of view
	Compatible with other red flag techniques	Costs
		Intravenous dye required
Optical coherence tomography	Real time readout of histological patterns	Preliminary data only
	Wide field of view	Complex readout of imaging patterns
		Costs

server variability. In a prospective study with a tandem design, Wolfsen and collaborators[114] found that NBI was superior to standard-resolution white light endoscopy with random biopsies for the detection of higher grades of dysplasia. A more recent multicenter randomized crossover study which compared NBI with high-resolution white light endoscopy only found a higher histological yield on the per-location analysis but not in the per-patient analysis, suggesting that the clinical overall value of NBI may be limited.[115] NBI however required fewer biopsies per patient compared with the standard approach, which may lead to cost savings.

A meta-analysis by Mannath et al.[116] included 446 patients with 2,194 lesions and they reported that NBI with magnification shows high diagnostic precision in detecting high-grade dysplasia, with a sensitivity of 96% and specificity 94%.

Autofluorescence imaging (AFI) utilizes high frequency blue light, which has the property to excite endogenous fluorophores to emit green fluorescence. In the presence of BE with early neoplasia, architectural and molecular changes in the columnar mucosa lead to reduction of green fluorescence. Dysplastic lesions therefore can be flagged-up as purple-red areas on a green background. Despite early enthusiasm for the utility of AFI in dysplasia detection,[117-119] two crossover studies and a recent analysis of available clinical trials have showed a very limited diagnostic value in this technology for BE endoscopic surveillance.[120,121] This is partly due to the high false positive rate of AFI, which in some studies has reached 80%. The significance of this false positivity is not yet clear. A multicenter study has been conducted by our institution with European collaborators, where biopsies directed by AFI were processed for a large panel of molecular biomarkers and the outcome of the biomarker analysis was compared with that of the Seattle protocol. This study found that AFI positivity correlated with molecular abnormalities of the Barrett's tissue and even if that area was not dysplastic on a focal biopsy there was a very high correlation between the molecular read-out from these areas and the overall dysplasia status of the patient.[122] In the per-patient analysis, a small panel of three biomarkers (p53 IHC, cyclin A, and aneuploidy) assessed on AFI positive areas had equal diagnostic accuracy to the Seattle protocol. AFI could therefore be a useful tool to direct biopsies for the detection of biomarkers and hence more objectively determine the risk status of the patient. In the future the combination of advanced imaging and molecular biomarkers could represent an improved strategy for improved stratification of BE patients.[123]

Other imaging technologies include confocal laser endomicroscopy, optical coherence tomography, diffuse reflectance spectroscopy and light scattering spectroscopy.

Confocal laser endomicroscopy (CLE) allows for high resolution assessment of the mucosa using endoscopically delivered laser light with magnification beyond ×1,000 allowing for imaging of cellular and subcellular structures and capillaries.[124] An international multicenter, prospective, randomized, controlled trial by Sharma et al.[125] showed that probe-based CLE used as part of a multimodal imaging approach in combination with high-definition white-light endoscopy (HD-WLE) and NBI improved the sensitivity for dysplasia detection compared with HD-WLE alone. Another RCT on 192 patients compared HD-WLE with Seattle protocol versus HD-WLE plus endoscope-integrated CLE (eCLE) and targeted biopsies.[126] This study found that the addition of eCLE increased the diagnostic yield for neoplasia from 6% to 22%, with a 4.8-fold reduction in the number of total biopsies required. However, the main issue of CLE is the narrow field of view and the best flagging technique to direct the operator as to which regions to analyse with the CLE probe remains to be established.

Optical coherence tomography (OCT) relies on the backscattering of light to obtain cross-sectional images of the tissue. It enhances the endoscopic image of the superficial layers of the esophagus. The technique is similar to endosonography, but the image formation in OCT depends on variations in the reflectance of light from different tissue layers. OCT imaging has demonstrated anatomic structures such as crypts and glands that could potentially permit endoscopists' to diagnose mucosal abnormalities such as BE, including dysplastic changes.[127,128]

Intrinsic fluorescence, reflectance, and light-scattering spectroscopy provide complementary data on biochemical and morphologic changes that occur during the development of dysplasia.[129,130] However convincing data are still lacking on the clinical applicability of these techniques, neither as single modality or in combination.[131]

In conclusion, currently there is insufficient evidence to recommend advanced imaging modalities for routine Barrett's surveillance. High-resolution endoscopy should be the minimum standard and the addition of more complex imaging modalities should be reserved to tertiary referral centers with a high volume of dysplastic cases. In the future multi-modal imaging, in combination with molecular information has the potential to overcome many of the limitations of the current clinical standard.

CONCLUSIONS AND FUTURE PERSPECTIVE

It is now increasingly clear that BE is a multifactorial disease, where a genetic predisposition interacts with the environment. Only very recently GWAS studies have started to provide the first insights into the genetic variants that predispose to the development of BE and EAC, but we are still far from being able to draw a risk profile based on the inherited genetic factors. Since there are multiple risk loci, each conferring a low increased risk, it may be difficult to make a clinical-risk tool from this information. In the absence of practical ways to identify individuals at high risk based on their genetic profile, for the time being it seems logical to look for clinical risk factor. Presently,

clinical factors, such as reflux symptoms, age >50 years, white race, male sex and obesity, are the key elements that trigger referral for endoscopic screening. However, there is uncertainty about how many factors should be present to define a high risk population. For example, reflux is regarded as the strongest risk factor for BE; however, more than 1/3 of patients with EAC deny previous history of heartburn and the prevalence of BE among reflux sufferers is only about 10%. Hence, the population that needs to be screened to diagnose enough cases of BE or cancer to impact on the overall mortality, is very large. As a consequence, it is mandatory to identify a minimally invasive screening test, with low cost and wide applicability to primary care. This is a very relevant area for future research.

The current surveillance algorithm heavily relies on the histological assessment of dysplasia based on random biopsies. It is still debated whether endoscopic surveillance is an effective measure to improve survival in patients with BE, due to controversial published data. This likely depends on the fact that dysplasia is difficult to detect endoscopically, as well as the fact that endoscopists adhere poorly to recommended protocols and pathologist struggle to agree on the diagnosis of dysplasia.[66,67,132] Flagging endoscopic techniques have been investigated to inform biopsy sampling, however up to now single modalities have not been proven to be superior to the current gold standard. A multimodal approach might represent an attractive possibility which has not been intensively studied so far. Meanwhile, the minimum standard seems to be high-resolution endoscopy, allowing for sufficient time for careful inspection and targeted biopsies on suspicious mucosal areas.[3,133]

Controversial data have been published on the cancer risk associated with a diagnosis of dysplasia, likely due to high interobserver variability and possibly also a different threshold used for the diagnosis of dysplasia in different countries or practices.[66,67] More objective measures of cancer risk are needed to inform clinical decisions. Biomarkers are natural candidates as molecular changes not only correlate with dysplasia, but can precede it and are often more objective. Even though several biomarkers have been showed to correlate with prevalent dysplasia and cancer risk, it is clear that panels of biomarkers provide the most accurate measure.[78,80] Biomarkers need to be cheap, easy to interpret and applicable to the clinical setting. p53 IHC is an example of such biomarker, which couple low costs with good clinical performance. More studies within prospective case-control cohort are needed to validate existing and novel biomarkers. The emerging sequencing technology needs also to be explored as it is becoming increasingly affordable and can provide large scale information potentially able to uncover unexplored areas of the genome associated with cancer risk. Multicenter studies are the ideal setting to test biomarkers in order to provide large enough cohorts of patients to achieve meaningful conclusions.

In the future, it is possible to envisage a scenario where in-expensive and minimally invasive screening techniques will help diagnose a large proportion of unknown BE. Coupled with the objective assessment of an individual's risk for cancer, this will allow tailoring patient management with choosing between early ablation in high risk BE (nondysplastic with aberrant molecular profile as well as frankly dysplastic cases) and prolonged endoscopic surveillance intervals or monitoring with minimally invasive devices in patients with low risk BE.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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