# Biomarkers for Predicting Neoplastic Progression in Barrett's Esophagus

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**Abstract**

Barrett's esophagus (BE), a complication of chronic gastroesophageal reflux disease (GORD), represents the strongest risk of esophageal adenocarcinoma (EAC). The low risk of progression together with the economic costs for surveillance argue for biomarkers predicting the likelihood of BE progression. In the last decades several promising biomarkers have been developed to estimate the risk of malignant transformation. In this review we summarize the current knowledge regarding these biomarkers for an individualized risk prediction and therapeutic outcome.

## Abbreviations

| | |
|---|---|
| APC | Adenomatous polyposis coli |
| BE | Barrett's esophagus |
| COX | Cyclooxygenase |
| DNA | Deoxyribonucleic acid |
| EAC | Esophageal adenocarcinoma |
| EGF | Epidermal growth factor |
| EGFR | Epidermal growth factor receptor |
| FISH | DNA fluorescent in situ hybridization |
| GERD | Gastroesophageal reflux |
| HGD | High-grade dysplasia |
| LGD | Low-grade dysplasia |
| LOH | Loss of heterozygosity |
| Mcm protein | Minichromosome maintenance protein |
| miRNA | MicroRNA |
| NBI | Narrowband imaging |
| ND | Nondysplastic |
| NF-kB | Nuclear factor "kappa-light-chain-enhancer" of activated B cells |
| PCNA | Proliferating cell nuclear antigen |
| TFF3 | Trefoil factor |
| TGF-α | Transforming growth factor alpha |

## 16.1 Introduction: Need for Biomarkers

The importance of Barrett's esophagus (BE) lies in its increasing prevalence and strong association to esophageal adenocarcinoma (EAC) [1]. While the risk and incidence of distal gastric cancer are decreasing worldwide, EAC has the most rapidly rising incidence in the Western world [2–4]. BE is characterized by the replacement of the normal stratified squamous epithelium of the distal esophagus by columnar epithelium with specialized intestinal metaplasia (IM) containing goblet cells [5] (Fig. 16.1). It is a premalignant condition, and patients with BE have a 30–60 times greater risk of developing adenocarcinoma of the esophagus than the general population [3, 5, 6]. The risk of developing cancer is higher among men, older patients, and patients with long segments of Barrett's mucosa or dysplasia [7].

Despite the increased risk of cancer development, the natural history of BE is incompletely understood [1]. The progression of BE from a columnar-lined esophagus to EAC is an established, gradual process from nondysplastic (ND) BE to low-grade dysplasia (LGD) and high-grade dysplasia (HGD) before the development of invasive cancer [8]. However, the individual risk of cancer progression is difficult to ascertain as only a small number of patients with BE will progress to EAC [2, 9]. Approximately 0.2–0.5% of patients with ND BE will develop EAC annually [9, 10], and only 5% of patients with EAC are known with a prior diagnosis of BE [5, 11]. Besides, some patients with dysplastic BE will also regress, with no further dysplasia detectable [12]. At present, there are no clinical or histological features to stratify the risk of progression or regression of patients with BE [8], and these patients are evaluated by the histological grade of dysplasia [5]. Based on this finding, the interval of endoscopic surveillance is determined individually [5, 12, 13]. Furthermore, despite advanced techniques, including narrowband imaging (NBI) and chromoendoscopy, endoscopic detection of BE is difficult [4, 10], and dysplastic areas in BE can be missed because of biopsy sampling errors [9]. Dysplasia is often patchy in extension and severity, and several biopsies are necessary to detect BE reliably [14]. Besides, histological diagnosis and grading of dysplasia are also potential limitations [9]. Interobserver variability

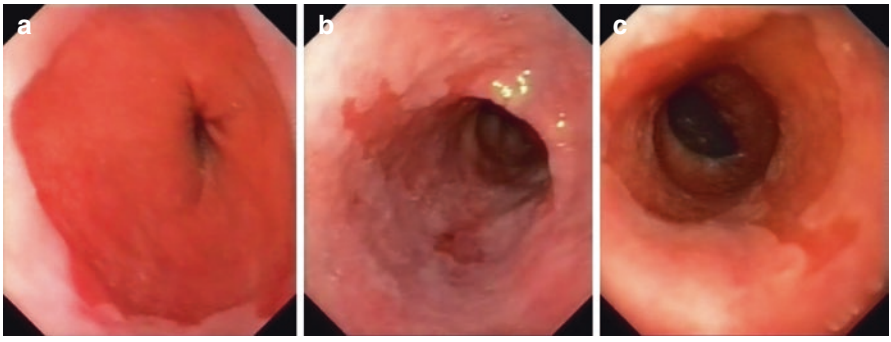


**Fig. 16.1** Endoscopic findings of (**a**) short-segment Barrett's esophagus and (**b**, **c**) long-segment Barrett's esophagus

is a known problem especially for discrimination between ND and LGD. Most studies comparing diagnosis of dysplasia among different pathologist have concluded that there is a significant intra- and interobserver variability [15, 16]. Consequently, recent international guidelines for the management of LGD in BE recommend that the diagnosis of LGD should be confirmed by a second pathologist with specialized expertise in gastrointestinal (GI) pathology [13]. Furthermore, the difficulty to discriminate inflammatory and reactive changes from true dysplasia complicates the diagnosis of dysplasia [17].

These limitations, the low risk of progression, together with the economic costs for surveillance, argue for biomarkers predicting the likelihood of BE progression [14] and allowing targeting of screening for those most at risk [8, 9, 12]. The increasing number of publications, seen in the past few years, reflects the ongoing research for effective biomarkers, as well as the lack of clinically validated prognostic tools [9]. Several clinical, endoscopic, and genetic markers have been studied to risk stratify patients with BE in terms of their risk of progression [18].

## 16.2 Biomarkers

To validate and integrate biomarkers for the early detection of cancer and for clinical use, the National Cancer Institute Early Detection Research Network (EDRN) has proposed five phases, which are analogue to the process in therapeutic drug studies [4, 19]. Phase I consists of preclinical exploratory studies to identify potential biomarkers; phase II comprises clinical assay developments to determine sensitivity and specificity of markers in patients with the disease compared to healthy controls; phase III is composed of retrospective studies on specimens from subjects prior to their diagnosis; phase IV consists of prospective screening studies; phase V constitutes cancer control studies to detect whether screening with biomarkers reduces cancer incidence [4]. In BE, the majority of biomarkers have never been studied beyond phase I or II, and most studies used complex technologies not useful for clinical practice [4, 12, 14, 20].

Similar to other malignant tumors, carcinogenesis of EAC is characterized by several genetic and epigenetic aberrations [4, 9]. At least 5–10 genetic alterations are necessary to generate a malignant phenotype [3]. In the last few decades, multiple genes have been identified which seem to be involved in the development of neoplastic lesions in BE [4]. These markers are proliferation/cell cycle proteins, tumor suppressor genes, adhesion molecules, DNA content, and inflammation-associated markers [5, 12]. Some of these changes are early events in the development of cancer and might serve as biomarkers for risk stratification [9].

## 16.3 Proliferation/Cell Cycle Proteins

Hyperproliferation of endothelial cells is detectable in BE with an increase during progression from metaplasia to dysplasia [4]. In general, proliferative stimuli to cells in chronic GERD and BE are gastric acid and bile acids [21]. Some studies have demonstrated that pulsatile exposure to low pH leads to hyperproliferation of endothelial cells compared to growth at neutral pH [22]. To replace injured tissue after gastroesophageal

reflux, cells need to progress from the G1 to the S phase in cell cycle [5], which is controlled by several key proteins. Mutations of any of these proteins regulating cell cycle may result in BE progression and may be useful to predict progression.

## 16.4 Ki67

The proliferation marker Ki67 (usually stained with the monoclonal antibody MIBI), which is upregulated in all active phase of cell cycle, may be a reliable biomarker [2]. The determination of KI67 expression has become routine in various malignant tumors, for example, it is a major biomarker for treatment decisions in breast cancer [23]. Ki67 expression in BE showed a stepwise increase with neoplastic progression [5, 24] and differences in expression levels between ND, LGD, and HGD BE [25]. The number and localization of Ki67-positive nuclei were significantly altered between ND, LGD, or HGD BE and EAC [26–28].

## 16.5 PCNA

The proliferation marker PCNA (proliferating cell nuclear antigen) is an indicator of cell cycle progression at the G1/S transition [2]. Studies have shown an increased proportion of cells stained with this antibody parallel to progression from metaplasia to dysplasia [2]. PCNA immunostaining was mainly seen in the basal cells of the epithelial compartment of glands in ND BE [20]. However, in mucosa of HGD BE, the proliferative compartment extended upward into the superficial layers of glands [28, 29].

## 16.6 Cyclins

Cyclins are potential biomarkers to predict BE progression. These proteins play a key role in cell cycle regulation [30]. Cyclin D1 is a proto-oncogene controlling the G1-S transition [12]. Studies have postulated that BE showing cyclin D1 overexpression is 6–7 times more likely to develop EAC [31]. However, other studies were not able to confirm this finding [5, 32–34]. At present, abnormalities of cyclin D1 expression cannot be used as routine biomarkers to predict progression risk [12]. The proto-oncogene cyclin A is expressed in 76% of patients with BE in the proliferative compartment [12]. With increasing grades of dysplasia, cyclin A expression shifts toward the mucosal surface [12]. In ND BE, 24% of patients express cyclin A at the surface epithelium compared with 59% of patients with LGD, 87% of patients with HDG, and 100% of patients with EAC [35].

## 16.7 Mcm

Mcm proteins (minichromosome maintenance proteins) are essential for DNA replication [4] and are expressed in all proliferating cells throughout the cell cycle [4]. Overexpression of the minichromosome maintenance deficient 2 (Mcm2) in BE

biopsies was significantly associated with higher risk of EAC [9, 36]. BE biopsies of patients who progressed to EAC had Mcm2 overexpression in 28.4% of the luminal cells compared with 3.4% in nonprogressors [36].

In summary, abnormalities of proteins controlling cell cycle may be biomarkers to predict neoplastic progression [5]. However, further larger prospective studies with standardized techniques and definitions to measure proliferation are needed [18].

## 16.8 Tumor Suppressor Genes

Tumor suppressor genes regulate cell proliferation, apoptosis, cell adhesion, and gene expression [3]. Various studies have evaluated their ability to predict progression in BE.

## 16.9 P53

P53 is expressed by the TP53 gene (chromosome 17p) and is one of the most commonly mutated tumor suppressor genes in human cancers [37]. P53 is responsible for the activation of DNA repair mechanisms, activates cell cycle arrest at the G1/S cell cycle checkpoint, and initiates apoptosis if DNA damage cannot be repaired [8, 38, 39]. Alterations of p53 in EAC and its precursor lesions have been detected in several studies [4, 12].

The p53 protein has a short half-life and is, in general, not detectable immunohistochemically at all or only at low levels [12, 40]. In dysplastic BE, p53 function is often lost due to point mutations in the DNA binding domain of the gene [8]. This results in an increased half-life of p53 protein, and its accumulation in the cell nucleus generates levels that can be detected by immunohistochemistry [5, 8]. A stepwise overexpression of p53 with increasing grades of dysplasia in BE has been shown in several studies [24, 41–43]: immunohistochemical analysis has shown a low percentage of p53 overexpression in ND BE (5%), increasing to 10–20% in LGD and to more than 60% in HGD [44, 45]. Patients with LGD show an increased risk of progression to HGD and cancer in case of p53 overexpression [46]. Besides, TP53 point mutations in EAC can be detected in up to 70% [7, 47, 48] and are associated with poor tumor differentiation, as well as reduced overall survival following surgical resection [20].

Next to point mutations, loss of heterozygosity (LOH) is a frequent alteration of p53 in BE. LOH refers to the loss of the normal, functional allele at a heterozygous locus in which the other allele has already been inactivated [9, 49]. Studies have revealed that LOH of p53 (17pOH) could be a biomarker to predict cancer progression in BE. 17pOH has been shown to occur in 0–6% of BE without dysplasia, in 20–27% with LGD, in 57% with HGD, and in 54–92% with EAC [5]. In BE biopsies containing different grades of dysplasia, the 3-year cumulative incidence of cancer was 38% (95% CI, 26.0–54.0) in those with proven 17pLOH compared to

3.3% (95% CI, 1.4–8.0) in biopsy samples without 17pLOH [50]. Reid et al. reported that 17pLOH is associated with a 16-fold increased risk of progression to cancer [50]. In that study, 17pLOH was a significant predictor of progression to HGD in patients with initial ND, indefinite dysplasia, or LGD [50].

In conclusion, p53 gene alterations (mutations and LOH) are early and frequent events in EAC and seem to be associated with malignant transformation of BE [3]. However, the sensitivity of this marker alone to predict cancer risk seems to be of limited value [5]. Immunohistochemistry of the mutated p53 was shown to be 88–100% sensitive and 75–93% specific for predicting progression from LGD BE to HGD [46, 48, 51] but only 32% sensitive to predict progression from ND BE to HGD [34]. Besides, some mutations result in a truncated p53 protein, which is undetectable by immunohistochemistry [40]. There was no detectable accumulation by immunohistochemistry in 31% of patients with proved p53 mutation [52]. In addition, not all p53 protein accumulations are caused by mutations, as inflammation or cellular stress can upregulate p53, too [5, 18, 40, 53].

Consequently, 17pLOH and p53 immunostaining seem to represent useful biomarkers to predict BE progression, especially in combination with other high-risk markers [3, 4]. However, they have to be proved in large-scale, multicenter trials [18], and newer genotyping technologies may overcome some of the current limitations surrounding p53 [12].

## 16.10 P16

The tumor suppressor gene p16 is located at chromosome 9p21 and encodes a cell cycle regulator protein. Its inactivation results in uncontrolled cell proliferation [5]. Acid and bile exposure of the esophageal mucosa may mediate inactivation of p16, resulting in BE progression to dysplasia and EAC [54]. Alterations of p16 can be detected in all grades of dysplasia [12] and in up to 85% of EAC [9]. It occurs as a result of hypermethylation, mutation, LOH, or methylation of the promotor regions [55].

Hypermethylation of the p16 promoter is a common mechanism of p16 inactivation during neoplastic progression in BE and is already present in ND premalignant BE [56]. In a retrospective study of 53 patients, it was associated with an increased risk of progression from ND to HGD BE or invasive cancer (OR 1.74: 95% CI 1.33–2.20) [57]. Another genetic event leading to loss of p16 is LOH, detectable in approximately 75% of EAC tissue samples [58]. P16 LOH seems to be associated with subsequent clonal expression along the Barrett segment, favoring further mutations and disease progression [59]. The combination of p16 mutations and LOH in 9p21 seems to occur early, prior to the development of aneuploidy or cancer [5, 58], and may be a predictive biomarker panel. Furthermore, allelic loss of p16 seems to predict lack of response to photodynamic therapy in patients with HGD BE and cancer [9, 18]. However, larger studies evaluating the efficiency of p16 as biomarker for tumor progression have to be performed.

## 16.11 Further Promising Tumor Suppressor Genes/Proto-oncogenes to Predict BE Progression

The tumor suppressor p27 inhibits cyclin E/Cdk2 complexes, preventing cells from entering cell cycle into S phase [12]. P27 knockout mice showed an increased risk of EAC development compared to wild-type mice [60]. In BE and EAC, loss of p27 expression is associated with malignant transformation and a poorer prognosis [12, 60].

The tumor suppressor gene adenomatous polyposis coli (APC), a regulator of the WNT pathway, seems to be altered in BE by methylation and LOH [61, 62]. However, further studies have to determine its predictive ability [18].

A strong association has been found between 17p13 LOH and an abnormal flow cytometric DNA content in BE [63]. Reid et al. showed that 37% of patients with LOH at 17p13 progressed from ND BE to EAC, compared to 3% of patients without LOH at this allele [64].

The bcl-2 proto-oncogene, which blocks apoptosis, seems to be overexpressed early in the dysplasia-to-carcinoma sequence of BE [3] and may be a potential biomarker for predicting progression.

However, all these genes have to be evaluated in further studies to assess their role in predicting BE progression to EAC.

## 16.12 Chromosomal Abnormalities

A further possibility of predicting BE progression to EAC lies in chromosomal abnormalities. DNA content abnormalities refer to numerical and structural changes in chromosomes, including aneuploidy and tetraploidy [9]. Aneuploidy is the presence of an abnormal number of chromosomes in a cell, unlike the normal content of 46 chromosomes [18]. Tetraploidy refers to the instance when the chromosomal number of a cell is twice as high as that of normal cells [18].

Abnormalities in DNA ploidy correlate well with conventional histologic diagnoses of dysplasia and carcinoma, and several studies suggest that this marker might represent a valuable adjunctive tool in the evaluation of patients with Barrett's esophagus [49, 55]. In biopsies with ND or LGD BE without aneuploidy or increased tetraploidy, the 5-year cancer incidence was found to be 0% [49]. However, with biopsies containing the same grades of dysplasia demonstrating either aneuploidy or increased tetraploidy, the 5-year risk of cancer progression was 28% [49, 64, 65]. Over 90% of HGD BE and EAC show DNA aneuploidy, and there is a significant relation between the presence of DNA aneuploid population and the progression form ND BE to dysplasia and EAC [2, 66].

In summary, DNA content abnormalities seem to be an accurate marker of progression in subjects with BE, but have not been widely used due to technical challenges with flow cytometry [18].

## 16.13 FISH

DNA fluorescent in situ hybridization (FISH) is a technique in which small fluorescently labeled DNA probes are used for detection of chromosomal and gene aberrations [9]. This method can detect various types of cytogenetic alterations, including aneusomy, duplication, amplification, deletion, and translocation [18]. In the past, several studies used FISH probes directed against different tumor suppressor or proto-oncogenes like p53 (17q13.1), p16 (9p21), or HER-2/neu (17q11.2) to find biomarkers predicting progression of BE [67–70]. Amplification of at least one of these loci occurred in 14% of HGD and increased to 50% in EAC [71]. A prospective follow-up study showed promising results in identifying high-risk BE patients with a FISH assay, including the tumor suppressor genes p53 and p16 and centromeric probes of chromosomes 7 and 17 to detect aneuploidy [9]. Aberrations of chromosomes 7 and 17 were detected in 13% of ND BE, increased with dysplastic stage, and detected HGD/EAC with a sensitivity and specificity of 85% and 84% [9, 72]. Besides, a multicolored FISH assay has been developed for detection of dysplasia in BE [73]. This probe set showed a sensitivity of 84–93% and specificity of 93% to identify HGD and EAC [18, 73]. Furthermore, FISH-based biomarkers may also be used to predict response to ablation therapy and help to guide therapy decisions [74]. In summary, genetic abnormalities detected by FISH appear to be a promising method for BE progression. However, further validation in larger studies is needed [18].

## 16.14 Methylation

DNA hypermethylation is an early event in tumorigenesis and causes inactivation of tumor suppressor genes, as well as chromosomal instability [4]. Methylation-induced inactivation of genes, which is involved in cell cycle and cell differentiation during BE pathogenesis, was shown in several studies [4], and patients with a dense methylation pattern in EAC showed a worse survival after surgery [57].

Methylation of the p16 tumor suppressor gene is a common genetic abnormality found in BE [18] and can be detected in 34–66% [57, 75, 76]. Methylation of the tumor suppressor gene CDKN2A, which inhibits cell cycle progression and abrogates expression of p16, seems to be associated with the progression from BE to EAC [8]. It occurs early in the metaplasia-dysplasia-carcinoma sequence [77] and can be detected in 3–77% of BE patients [77]. Besides, it was shown to be related to 17pLOH and chromosomal abnormalities like tetraploidy and aneuploidy [8]. Based on the methylation of some genes (p16, HPP1, RUNX3) and clinical parameters (gender, BE segment length, and histopathology), a model was developed to stratify patients with BE into low-, intermediate- and high-risk groups [78]. This may represent a useful biomarker panel to predict BE progression. Hypermethylation of other genes like APC and T1MP1

has been detected in patients with BE [18]. However, convincing studies on their predictive ability are lacking [18]. Moreover, DNA methylation is a reversible event [4]. Consequently, modulation of the epigenetically involved pathways by using small molecules might become a therapeutically option for patients with BE [4].

## 16.15 Biomarker Panels

Combinations of biomarkers in panels may be better in predicting the risk of neoplastic progression in patients with BE than individual biomarkers alone [68, 69, 79]. Biopsies demonstrating high diversity seem to be more likely to progress to EAC [8, 70]. Due to technical progression, several molecular aberrations can be analyzed simultaneously with the aid of panels of biomarkers [8]. Using aneuploidy/increased tetraploidy, 17pLOH, and 9pLOH in combination, the presence of all three abnormalities predicted an 80% risk of cancer progression in BE at 6 years [9, 79, 80]. Moreover, a study demonstrated that the combination of LGD, abnormal DNA ploidy, and *Aspergillus oryzae* lectin can predict progression from BE to HGD and EAC [81]. Besides, multicolored FISH might be an option to analyze several biomarkers in a single assay [69].

## 16.16 Further Potential Biomarkers

### 16.16.1 HER2/neu

The proto-oncogene HER2/neu (c-erbB2) encodes a transmembrane glycoprotein with intrinsic tyrosine kinase activity [3]. Alteration of HER2/neu can be detected in approximately 10–70% of EAC [3, 82]. HER2/neu overexpression in EAC correlates significantly with tumor invasion, distant metastasis, lymph node involvement, and status of residual tumor after resection [3, 83], but it offers therapeutic options in the combination of chemotherapy and trastuzumab [84]. As HER2/neu overexpression is not detectable in dysplastic BE, it seems to be a late event in the dysplasia to carcinoma sequence [85]. Further studies evaluating the potential of HER2/neu to predict BE progression are necessary.

## 16.17 Several Growth Factors

Epidermal growth factor (EGF), epidermal growth factor receptor (EGFR), and transforming growth factor alpha (TGF-α) are important members of the family of growth factors [3]. Some studies show the correlation of EGF, EGFR, and TGF-α overexpression with the degree of mucosal dysplasia and the occurrence of EAC [3, 82, 86–88].

Neovascularization seems to be an early event in the pathogenesis of BE [4]. An increased number of small vessels can be detected in dysplastic BE, and an increasing microvessel density can be seen from BE to HGD or intramucosal carcinoma [4, 89]. Overexpression of VEGF and VEGFR can be detected in dysplastic BE and EAC [90]. Furthermore, COX-2 expression is associated with neovascularization, suggesting that bile and gastric acid may induce angiogenesis via COX-2 expression [89]. Besides, in other tissues, COX-2 inhibitors can suppress vessel growth [89]. However, trails of a selective COX-2 inhibitor, celecoxib, did not show any protective effect against BE progression to EAC [91].

## 16.18 NF-kB

The transcription factor NF-kB (nuclear factor "kappa-light-chain-enhancer" of activated B cells) regulates proinflammatory genes, differentiation, and growth [12]. Cytokines, free radicals, and acid stimulate translocation of NF-kB to the nucleus, where it binds specific DNA sites and upregulates the expression of genes involved in inflammatory process [92]. NF-kB expression is stepwise increased in patients with BE adjacent to EAC [93, 94]. In patients with ND BE, NF-kB overexpression was detected in 50%, with LGD BE in 63% and with HGD BE in 100% [93]. NF-kB can be activated by deoxycholic acid, a bile acid and a common component of reflux, or acid pH [89]. However, further studies are needed to determine the role of this molecule in the metaplasia-carcinoma sequence [12].

## 16.19 Cyclooxygenase 2 (COX-2)

Cyclooxygenase (COX) catalyzes the rate-limiting step in prostaglandin synthesis [3]. COX-1 is constitutively expressed, whereas COX-2 is undetectable in most cells. However, it can be activated by cytokines, gastric acid, and bile acids. Studies revealed that COX-2 is involved in cell proliferation, reducing apoptosis and promoting angiogenesis [12, 95]. Unconjugated bile acids, one of the major components of gastroesophageal reflux, can stimulate COX-2 expression through a reactive oxygen species-mediated signaling pathway [96]. COX-2 expression cannot be measured in normal esophageal mucosa [4], but a progressive increase of COX-2 expression along the metaplasia-dysplasia sequence was described [97]. Additionally, COX-2 is expressed in 70–80% of patients with EAC [3]. Besides, nonsteroidal anti-inflammatory drug (NSAID) intake was shown to have a protective effect and reduces the risk of EAC, especially in patients with several molecular high-risk abnormalities [80]. However, at the moment, there are not enough data that support the role of COX-2 as a useful biomarker [12].

## 16.20 MicroRNAs

MicroRNAs (miRNAs) are small segments of noncoding RNA of 20–24 nucleotides regulating the translation of mRNA. They play a role in cell proliferation and function as tumor suppressor genes or oncogenes [4]. MicroRNAs may be useful biomarkers, as they are present in circulating blood plasma in a highly stable, cell-free form included in lipid or lipoprotein complexes [98]. Several studies have examined the role of miRNAs in progression from BE to EAC [98–100] and detected alterations in miRNA expression profiles between ND BE, HGD BE, and EAC [98]. Alterations in miR-25, miR-93, and miR-106b have been reported in BE and EAC compared to normal esophageal tissue [100]. Furthermore, in samples of EAC, an upregulation of mi-21 and mi-192 has been detected [101].

There are several miRNAs that have been found to be up- or downregulated in different stages in the progression from BE to EAC [98]. Identifying specific miRNA patterns in BE might help to detect dysplasia with more progressive potential and might help to distinguish low-risk from high-risk patients [4]. Further work is required in order to use miRNAs for risk stratification in the progression from BE to EAC [98].

## 16.21 Endoscopic Measurements

Next to reliable biomarkers, methods to detect areas of concern for biopsies are needed. The direct application of molecular markers during endoscopy to allow visualization of dysplasia without the need for histopathology is a further promising field of BE research [8]. The use of fluorescent probes to molecules involved in the dysplasia sequence of BE may allow for targeting areas of concern [8]. The majority of these studies rely on the use of confocal imaging [8]. The development of a polyclonal antiperiostin antibody against periostin, which is expressed differentially in ND and dysplastic BE, is an example for this new method [102]. However, periostin is also expressed in inflamed tissue [8] limiting the sensitivity and specificity of this marker. The peptide probe sequence ASYNYDA has been fluorescently labeled to be visible in vivo by using fluorescence microscopy [8]. The sensitivity and specificity for the detection of dysplastic BE was 82% and 85%, respectively [8]. However, at present, confocal imaging is not a standard endoscopic technique, and a more clinically applicable fluorescence dye visible with a standard endoscope is needed [8].

## 16.22 Non-endoscopic Methods to Detect Dysplasia

The costs, as well as discomfort of the numerous surveillance endoscopies of patients with BE, have argued for non-endoscopic alternatives to detect BE. The Cytosponge is a capsule on a string that is swallowed by the patient. When the

capsule reaches the stomach, the capsule dissolves and releases a spherical sponge which is retrieved [8]. During the retrieval through the esophagus, cells adhere to the sponge and can be immunohistochemical analyzed for the presence of TFF3 (trefoil factor) [8]. TFF3 is a marker of columnar epithelium and is expressed in a variety of tissues, including goblet cells of the intestines and colon. It promotes mucosal healing and epithelial restitutions in vivo in the gastrointestinal mucosa. Detecting TFF3-positive glandular cells in the Cytosponge indicates the presence of BE. In a study with 500 patients, the sensitivity and specificity of this method for detection of BE were 73 and 93% for short-segment BE and increased to 90 and 93% for long-segment BE [103]. Furthermore, in 19 of 22 sponge samples taken from patients with known high-grade dysplasia, mutations in the TP53 gene could be detected. By contrast, no TP53 mutations were found in the sponge samples of healthy controls or patients with BE without dysplasia [47]. However, due to false positivity, the clinical utility of TFF3 may be limited in the cardia [104].

Serum biomarkers for the detection of patients at an increased risk of EAC are under intensive investigation [8]. Telomere length in blood samples of patients with BE without dysplasia was assessed and followed for 5.8 years. Patients with shorter telomere length at baseline were at increased risk of developing EAC [105].

## Conclusion

The major risk of patients with BE of developing EAC has generated interest in defining subgroups of high-risk patients who can be surveilled effectively [106]. However, the natural history of BE is still very difficult to predict for one individual patient [106]. Several promising candidate biomarkers and biomarker panels have been described: proliferation markers, chromosomal abnormalities, tumor suppressor genes, DNA hypermethylation, as well as FISH or microRNAs might be able to predict Barrett's progression. The development of a Barrett's risk score incorporating clinical variables and biomarker panels may be an option to stratify patients into low-risk and high-risk subsets [18]. However, there are several problems to translate the use of these biomarkers into practice like the need for special media for biopsies, interlaboratory variation in methodology, and lack of standardization [18]. The majority of these studies were performed retrospectively and included only a small number of patients [106]. Consequently, the majority of these markers need to be evaluated in large-scale prospective clinical trials. Prolonged follow-up of patients ranging between 5 and 10 years is required leading to logistical problems [18]. Besides, in order to develop useful biomarkers, we need to further understand molecular and genetic abnormalities associated with BE [12], and it still needs to be proven that these biomarkers will reduce cancer incidence [106].

In the next years, we can expect more studies attempting to find new methods that effectively predict BE progression.

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