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Biomarkers of Barrett’s esophagus

Fouad YM *et al*. Biomarkers of Barrett’s esophagus

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**Abstract**

Barrett’s esophagus (BE) is the strongest risk for esophageal adenocarcinoma (EAC). Metaplasia in patients with BE may progress to dysplasia and then invasive carcinoma. Well-defined diagnostic, progressive, predictive, and prognostic biomarkers are needed to identify the presence of the disease, estimate the risk of malignant transformation, predict the therapeutic outcome and survival for EAC patients. There are many predictive and prognostic markers that lack substantial proofs, and do not allow stratification of patients with gastroesophageal reflux disease in clinical practice for outcome and effectiveness of therapy. In this short review we discuss, in summary, the current knowledge regarding possible biomarkers focussing on the pathophysiologic mechanisms to improve prognostic and therapeutic approaches.

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**Key words**: Barrett’s esophagus; Esophageal adenocarcinoma; Biomarkers

**Core** **tip:** The importance of biomarkers of Barrett's eosophegus is to provide identification of the disease, estimate the risk of malignant transformation, predict the response to therapy, and indicate the overall survival-prognosis for esophageal adenocarcinoma patients. Proposed predictive and prognostic markers do not allow stratification of gastroesophageal reflux disease patients for progression, outcome, and effectiveness of therapy in clinical practice. The aim of this short review is to discuss the current knowledge regarding proposed biomarkers to improve prognostic and predictive therapeutic approaches focussing on the pathophysiologic mechanisms.

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**INTRODUCTION**

Barrett's esophegus (BE) is characterized by the replacement of squamous epithelium in the esophagus by metaplastic columnar epithelium with goblet cells[1]**.** BE is a well-known risk factor for esophageal adenocarcinoma (EAC), a malignancy with the most rapid increase in incidence (approximately 500%) over the past 3 decades in the western world and with persistently poor outcomes when diagnosed after the onset of symptoms (survival less than 20% at 5 years)[2].An important problem in treating the patients with BE is the absence of satisfactory surveillance programs inspite of the known stages of carcinogenesis from Barrett’s esophagus to adenocarcinoma. Over the past two decades, there have been a lot of trials to identify patients with BE and predict patients with a high risk of progression to adenocarcinoma[3-6]

In this review, definition, mechanisms of production and types of biomarker in patients with BE will be summarized.

**DEFINITION OF BIOMARKERS**

***The biomarker***

A biological molecule presenting a sign of condition or disease, normal or abnormal process. It is found in the blood, body fluids, and tissues. Moreover, the biomarker may be used for assessment of the response of the body to treatment of a disease or condition[7].

***Phases of biomarker Identification and validation***

Biomarker discovery has to pass through 5 to 6 phases before clinical application (Table 1). Phases 4, 5 and 6 present a significant challenge of the needed large samples sizes, long follow up and high costs[8].

**TYPES OF BIOMARKERS IN PATIENTS WITH BE**

***Genomic instability***

The similarity of genetic pattern between BE and EAC demonstrated by DNA microarray studies supported the hypothesis that BE is a step preceding EAC. The genomic instability has been shown to be a poor prognostic marker in BE patients. Chromosomal alterations , deletions, point mutations, methylation abnormalities, and loss of heterozygosity (LOH) are the main reflections of genomic instability in patients with BE[9-11].

***DNA abnormalities***

DNA abnormalities (*e.g*., aneuploidy or tetraploidy) assessed by flow cytometry, can be used as predictive markers in patients with BE with no or low grade dysplasia[12, 13]. Loss of heterozygosity (LOH) represents the loss of normal function of one allele of a gene in which the other allele was already inactivated. In a long-term follow-up study of BE patients, a panel combining 9p LOH, 17p LOH in addition to aneuploidy and tetraploidy was a strong predictor of EAC[14].

***Abnormalities of tumour Loci***

An important predictor of risk of dysplasia and EAC in patients with BE is LOH for p53. LOH for p53 was shown to be associated with a 16-fold increase in the risk of progression to cancer[15]. However, in another study, in patients with non-dysplastic BE, only 32.4% of patients with progression showed overexpression of p53 in their initial biopsy[16].Furthermore, alteration of APC, a regulator of the WNT pathway, by methylation[17] and LOH[18] was shown in patients with BE with nuclear predictive value.

***Epigenetics***

The post-transcriptional silencing of specific genes without a change in the DNA sequence. Variety of mechanisms are involved in epigenetics including methylation and acetylation. It has been shown that Hypermethylation and loss of p16, were independently associated with an increased risk of progression from intestinal metaplasia (IM) to HGD[19,20].

The p16 methylation was shown to be highly prevalent in patients with BE (34%–66%)[17,19,21]. Moreover , in a multicentre study, a panel of 8 genes (p16, RUNX3, HPP1, NELL1, TAC1, SST, AKAP12, and CDH13), was used to predict the risk of progression in patients with BE. In this study, 195 patients were included and sensitivities of prediction of progression approached 50%[22].

 ***Cell cycle predictors***

Dysregulated cell cycle may lead to accumulation of genetic aberrations in most cancer cells . Cyclins are cell cycle regulator proteins, potentially useful biomarkers for progression. In patients with BE, Cyclin D1 overexpression, was shown to be associated with progression to EAC[23-26].Further research in a large groups of patients is needed to confirm the predictive values of cyclins.

***Proliferation abnormalities***

The association between increasing proliferation and worsening of dysplasia In BE was shown in many studies[26-28], while other studies found no association[29,30]. Researchers explained the discrepancies between there results by the use different techniques, the different histological pattern between columnar and squamous epithelium and the use of different proliferative indices. One of the important markers of cellular proliferation is Ki67. However, In a long time follow up study, Ki67-positive proliferative fractions were not associated with risk of progression[31]. Further larger studies with standardized techniques are needed to measure proliferation.

***Clonal diversity in BE***

Genetic instabilities may lead to multiple distinct clones. The coexistence of multiple distinct clones is called clonal diversity. In patients with BE, clonal diversity measures were strong predictors of progression[32]. However, complicated methodology limited the use of clonal diversity as a predictive marker.

***Mitochondrial DNA***

The mitochondrial DNA (mtDNA) have been implicated in the process of carcinogenesis[33]. The mtDNA mutations were found in 53% of patients with BE without dysplasia[32]. In patients with BE, deletion of the mitochondrial genome (4977bp) was found in 15.4% in IM, 40% in LGD, 69.2% in HGD, and 90% in paratumoral tissue[34].

**FLUORESCENCE IN-SITU HYBRIDIZATION**

Fluorescence in-situ hybridization (FISH) is a technique which detects DNA content and loci abnormalities in the cells by fluorescent-tagged DNA probes. FISH can detect aneusomy (abnormalities of chromosome copy number), deletion, duplication, amplification and translocation at tumor suppressor loci and protooncogene loci.

In patients with BE, FISH was used to detect genetic abnormalities by investigators in different studies from multiple centres[35-39]. Detection of dysplasia in BE and identification of HGD and EAC using FISH four robe set has been shown with reasonable sensitivity (84%–93%) and specificity (93%)[39]. In another multicentre study, polysomy detected by FISH has been shown predicting risk of progression to HGD/EAC[40].

**CLASSIFICATION OF BIOMARKERS OF BE**

biomarkers of BE can be classified into 4 groups: (1) diagnostic biomarkers; (2) progression biomarkers; (3) predictive biomarkers; and (4)prognostic biomarkers. This classification is based on the previous intensive research, and review articles[6,41-43] (Table2).

***Diagnostic biomarkers***

Indicate the presence of disease. The histochemical analysis of biopsies of the gastro-esophageal junction remains the conventional approach for detection and diagnosis of BE. In patients with asymptomatic BE, the trefoil factor 3 (TFF3) combined with a noninvasive diagnostic technique has been investigated with promising results in screening of these patients[44,4 5].Further validation and assessment are needed to confirm the results of these studies.

***Progression biomarkers***

 The degree of dysplasia in obtained biopsies is the main marker of progression of BE although there is a lot of intra- and inter-observer errors[46-48].The most promising markers are MCM2 expression pattern and LOH on distinct gene loci, especially at 17p. The cost and time intensive experimental work limit the use of these markers in clinical practice.

***Predictive biomarkers***

Predict the response to therapy. Limited number of predictive biomarkers are available (Table 2) and this category is in need for further intensified research.

***Prognostic biomarkers***

Indicates overall survival-prognostic in EAC. The majority of biomarkers are present in this category. Prognostic biomarkers include growth signals, insensitivity to growth inhibitory signals, markers of evasion of programmed cell death, limitless replicative potential (Telomerase), markers of sustained angiogenesis, markers of invasion and metastasis, marker of tumor differentiation, and cancer-related inflammation (Table 2).

***Biomarkers in clinical field: problems and obstacles***

A lot of work is needed to put the biomarkers into clinical trials requiring the cooperation between clinical researchers and experts in molecular techniques. Moreover, the validation of a biomarker passes through 5 phases and requires multicentre studies, prohibitive costs and long term follow up.

The method of specimen collection is another challenge. While microarray studies require special equipment and may not be easily to access by clinical scientist, molecular profiling using formalin-fixed paraffin-embedded (FFPE) specimens is interesting to researchers because of easy availability. In patients with hepatocellular carcinoma, the use of a large scale (> 6000) gene profiling resulted in high quality data even from specimens archived for as long as 24 years[49].

The lack of prospective controlled trials is another important problem attributed to the high costs and the need for large sample sizes. Moreover, the lack of reproducibility of assays between laboratories represent another obstacle for identification of clinically useful cancer biomarkers[50]. The DNA microarray studies reanalysis showed that the selection of patients had an impact on the predictor role of genes in prognosis[51]. Careful interpretation of biomarker studies is needed by using large data sets such as DNA microarray repositories.

**CONCLUSION**

A biomarker in BE should help in population screening, improve the surveillance of patients with BE, and identify the prognostic groups and best therapy once EAC develop. Many biomarkers have been intensively studied to accurately predict the progress of BE to EAC. The MCM2 expression pattern, LOH on distinct gene loci, especially at 17p, hypermethylation of p16 and the expression pattern of P53 are promising markers especially for progression of disease. Important prognostic biomarkers include Cyclin D1, Ki-67, TGF-𝛼, APC, COX-2, telomerase and VEGF. Till now, no biomarker has been able to replace the current gold standard dysplasia in routine clinical practice. Panels of biomarkers seem to be better in predicting progression in accurate manner. The issue of costs and practicality of biomarkers should be considered before research. A model incorporating clinical data and biomarkers will be promising and can accurately predict the risk of progression, prognosis or response to therapy. Similar models have been used in other cancers and diseases such as the Nottingham prognostic index for breast cancer and MELD score for liver disease. After generation and validation of such model, it should then be rigorously validated in a large cohort of patients in a prospective fashion.

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**Table 1 Phases of biomarker production**

|  |
| --- |
| **Phases of biomarker validation and development** **Phase 1** : Biomarkers of promise are identified based on application inother cancers and elucidation of novel pathways**Phase 2** :Cross sectional studies validate the biomarker of interestto be sufficiently discriminatory and biomarker assays arestandardized**Phase 3** :Case–control studies confirm the biomarker to be expressedbefore the development of cancer with a retrospective butlongitudinal design**Phase 4** :Prospective longitudinal studies avoid biases associated withCase–control studies**Phase 5**: Population-based studies show impact of biomarker detectionon disease burden and cancer control |

**Table 2 Types of biomarkers in barrett’s esophagus**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biomarker | Method | Remarks | Ref. |
| Diagnostic  | TFF3 | IHC | To screen asymptomatic patients for BE | [49,50] |
|  | Chromosome 7 and 17 changes | IDKA and FISH | Early stages of BE | [52] |
|  | 8q24 (*C-MYC*), 17q12(*HER2*), and 20q13 changes | FISH | Early stages of BE | [53] |
|  | 17q11.2 (*ERBB2*) | Microarray analysis | EAC | [54] |
|  | Serum proteomic analysis | Mass spectrometry | EAC | [55] |
|  |  |  |  |  |
| PredictiveProgression markersPrognostic biomarkers | P16 allelic loss DNA ploidy abnormalities HSP27 Ephrin B receptorGenetic polymorphism P21P53ERCC1 P53 DNA abnormalitiesLOH of 157p and 9pEGFR Cyclin ACyclin D1Hypermethylation of p16, RUNX2,HPP18 gene methylation panelCatherpsin D,AKR1D10,AKR1C2 mRNA levels DCK, PAPSS2, SIRT,TRIM44P16 loss, C-MYC gainASS expressionMicroRNA expression profileCyclin D1EGFRTGF-αTGF-β1APC COX-2TelomeraseVEGFCadherinTIMP | FISH ICDA IHC MicroarrayqRT-PCR  IHCIHCIHCIHC Flow cytometryFlow cytometryIHCIHCIHCRT-PCRRT-PCRWestern blot,qRt-PCRRT-PCR, IHCFISHMicroarrayMicroarray, RT-PCRIHC, FISHIHCIHC, ISHRT-PCR, ELISAPCRIHC Southern-blot and PCRIHCIHCIHC, PCR | Response to therapyCovariate value for recurrenceNo response to therapyResponse to therapy in EACAssociated with clinical outcomeCorrelated with better CTX responseCorrelated with better CTX responsePredicts CTX resistanceLimited efficacy as a progression markerHigh risk for progression to EACPredict progression to EACOverexpression in HGD and EACPredicts progression to dysplasiaRisk of Progression to EACRisk of progression to EAC/HGDPredicts progression to EAC/HGDDysregulation predicts progression to EAC/HGD4 gene signature in EAC , predict 5 year survivalAssociated with therapy responseLow expression associated with metastasesLow level associated with worse prognosis in EACDecreased survivalDecreased expression associated with decreased survivalHigh level indicates progression and metastasesHigh expression associated with decreased survivalHigh level associated with decreased survivalAssociated with metastases and recurrenceAssociated with decreased survivalAssociated with metastases and decreased survival Decreased level associated with decreased survivalDecreased level associated with decreased survival | [56][57] [58][59][60] [61][62][16][13, 63][13] [14][64] [65] [19][22][66][67] [56] [68] [69] [70][71][72][73][73] [74][75][76][77][78][79] |

ACIS: Automated cellular imaging system; ASS: Argininosuccinate synthase; APC: Adenomatous polyposis coli; BE: Barrett’s esophagus; COX: Cyclooxygenase; DCK: Deoxycytidine kinase; DICM: Digital image cytometry; EAC: Esophageal adenocarcinoma; EGFR: Epidermal growth factor receptor; ELISA: Enzymelinked immunosorbent assay; FISH: Fluorescence *in-situ*-hybridization; ICDA: Image cytometric DNA analysis; HSP27: Heat-shock protein 27; IHC: Immunohistochemistry; LOH: Loss of heterozygosity; PAPSS2: 3󸀠-phosphoadenosine 5󸀠-phosphosulfate synthase 2; PCR: Polymerase chain reaction; qRT: Quantitative reverse transcriptase; MLPA: Multiplex ligation dependent probe amplification; NF-𝜅B: Nuclear factor kappa B; SIRT2: Sirtuin 2; SNP: Single nucleotide polymorphism; TFF3: Trefoil factor 3; TGF: Transforming growth factor; TIMP: Tissue inhibitors of metalloproteinases; TRIM44: Tripartite motifcontaining 44; uPA: Urokinase-type plasminogen activator; VEGF: Vascular endothelial growth factor.