**Name of journal: World Journal of Gastrointestinal Pathophysiology**

**ESPS Manuscript NO: 8511**

**Columns: TOPIC HIGHLIGHT**

WJGP 5th Anniversary Special Issues (4): Barrett’s

Biomarkers of Barrett’s esophagus

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

Yasser M Fouad, Ibrahim Mostafa, Reem Yehia, Hisham El-Khayat

**Yasser M Fouad, Reem yehia,** Gastroenterology and Hepatology Unit, Tropical Medicine Department, Minia University, Minia 11432, Egypt

**Ibrahim Mostafa, Hisham El-Khayat,** Gastroenterology and Hepatology Department, Theodore Research Institute, Cairo 11435, Egypt

**Author contributions:** All authors contributed to the manuscript and wrote a part and revise the other part; Fouad YM and Yehia R collected the data; Mostafa I and El- Khayat H revised the whole manuscript.

**Correspondence to:** **Yasser Mahrous Fouad, MD, Professor** of Gastroenterology and Hepatology, Tropical Medicine Departement, Minia University, Main Road, Minia 11432, Egypt. [yasserfouad10@yahoo.com](mailto:Yasserfouad10@yahoo.com)

**Telephone:** +20-1-114721500

**Received:** December 28, 2013  **Revised:** July 2, 2014

**Accepted:** July 17, 2014

**Published online:**

**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.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**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.

Fouad YM, Mostafa I, Yehia R, El-Khayat H. Biomarkers of Barrett’s esophagus. *World J Gastrointest Pathophysiol* 2014; In press

**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.

**REFERENCES**

1 **Sharma P**. Clinical practice. Barrett's esophagus. *N Engl J Med* 2009; **361**: 2548-2556 [PMID: 20032324 DOI: 10.1056/NEJMcp0902173]

2 **Pohl H**, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005; **97**: 142-146 [PMID: 15657344]

3 **Holmes RS**, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. *Semin Radiat Oncol* 2007; **17**: 2-9 [PMID: 17185192 DOI: 10.1016/j.semradonc.2006.09.003]

4 **Brown LM**, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002; **11**: 235-256 [PMID: 12424848 DOI: 10.1016/S1055-3207(02)00002-9]

5 **Kerkhof M**, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruïne A, Driessen A, ten Kate FJ, Kusters JG, Kuipers EJ, Siersema PD. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. *Histopathology* 2007; **50**: 920-927 [PMID: 17543082 DOI: 10.1111/j.1365-2559.2007.02706.x]

6 **Illig R**, Klieser E, Kiesslich T, Neureiter D. GERD-Barrett-Adenocarcinoma: Do We Have Suitable Prognostic and Predictive Molecular Markers? *Gastroenterol Res Pract* 2013; **2013**: 643084 [PMID: 23573078 DOI: 10.1155/2013/643084]

7 National cancer institute. http: //www.cancer.gov/dictionary?CdrID=45618)

8 **Jankowski JA**, Odze RD. Biomarkers in gastroenterology: between hope and hype comes histopathology. *Am J Gastroenterol* 2009; **104**: 1093-1096 [PMID: 19417749 DOI: 10.1038/ajg.2008.172]

9 **Paulson TG**, Maley CC, Li X, Li H, Sanchez CA, Chao DL, Odze RD, Vaughan TL, Blount PL, Reid BJ. Chromosomal instability and copy number alterations in Barrett's esophagus and esophageal adenocarcinoma. *Clin Cancer Res* 2009; **15**: 3305-3314 [PMID: 19417022 DOI: 10.1158/1078-0432.CCR-08-2494]

10 **Li X**, Galipeau PC, Sanchez CA, Blount PL, Maley CC, Arnaudo J, Peiffer DA, Pokholok D, Gunderson KL, Reid BJ. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev Res (Phila)* 2008; **1**: 413-423 [PMID: 19138988 DOI: 10.1158/1940-6207.CAPR-08-0121]

11 **Selaru FM**, Zou T, Xu Y, Shustova V, Yin J, Mori Y, Sato F, Wang S, Olaru A, Shibata D, Greenwald BD, Krasna MJ, Abraham JM, Meltzer SJ. Global gene expression profiling in Barrett's esophagus and esophageal cancer: a comparative analysis using cDNA microarrays. *Oncogene* 2002; **21**: 475-478 [PMID: 11821959 DOI: 10.1038/sj.onc.1205111]

12 **Reid BJ**, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000; **95**: 1669-1676 [PMID: 10925966 DOI: 10.1111/j.1572-0241.2000.02196.x]

13 **Rabinovitch PS**, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001; **96**: 3071-3083 [PMID: 11721752 DOI: 10.1111/j.1572-0241.2001.05261.x]

14 **Galipeau PC**, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007; **4**: e67 [PMID: 17326708 DOI: 10.1371/journal.pmed.0040067]

15 **Reid BJ**, Prevo LJ, Galipeau PC, Sanchez CA, Longton G, Levine DS, Blount PL, Rabinovitch PS. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001; **96**: 2839-2848 [PMID: 11693316 DOI: 10.1111/j.1572-0241.2001.04236.x]

16 **Murray L**, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, Forman D, Wild CP. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut* 2006; **55**: 1390-1397 [PMID: 16682429 DOI: 10.1136/gut.2005.083295]

17 **Bian YS**, Osterheld MC, Fontolliet C, Bosman FT, Benhattar J. p16 inactivation by methylation of the CDKN2A promoter occurs early during neoplastic progression in Barrett's esophagus. *Gastroenterology* 2002; **122**: 1113-1121 [PMID: 11910361 DOI: 10.1053/gast.2002.32370]

18 **Zhuang Z**, Vortmeyer AO, Mark EJ, Odze R, Emmert-Buck MR, Merino MJ, Moon H, Liotta LA, Duray PH. Barrett's esophagus: metaplastic cells with loss of heterozygosity at the APC gene locus are clonal precursors to invasive adenocarcinoma. *Cancer Res* 1996; **56**: 1961-1964 [PMID: 8616831]

19 **Schulmann K**, Sterian A, Berki A, Yin J, Sato F, Xu Y, Olaru A, Wang S, Mori Y, Deacu E, Hamilton J, Kan T, Krasna MJ, Beer DG, Pepe MS, Abraham JM, Feng Z, Schmiegel W, Greenwald BD, Meltzer SJ. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 2005; **24**: 4138-4148 [PMID: 15824739 DOI: 10.1038/sj.onc.1208598]

20 **Mokrowiecka A**, Wierzchniewska-Ławska A, Smolarz B, Romanowicz-Makowska H, Małecka-Panas E. p16 gene mutations in Barrett's esophagus in gastric metaplasia - intestinal metaplasia - dysplasia - adenocarcinoma sequence. *Adv Med Sci* 2012; **57**: 71-76 [PMID: 22440936 DOI: 10.2478/v10039-012-0003-0]

21 **Maley CC**, Galipeau PC, Li X, Sanchez CA, Paulson TG, Blount PL, Reid BJ. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. *Cancer Res* 2004; **64**: 7629-7633 [PMID: 15492292 DOI: 10.1158/0008-5472.CAN-04-1738]

22 **Jin Z**, Cheng Y, Gu W, Zheng Y, Sato F, Mori Y, Olaru AV, Paun BC, Yang J, Kan T, Ito T, Hamilton JP, Selaru FM, Agarwal R, David S, Abraham JM, Wolfsen HC, Wallace MB, Shaheen NJ, Washington K, Wang J, Canto MI, Bhattacharyya A, Nelson MA, Wagner PD, Romero Y, Wang KK, Feng Z, Sampliner RE, Meltzer SJ. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res* 2009; **69**: 4112-4115 [PMID: 19435894 DOI: 10.1158/0008-5472.CAN-09-0028]

23 **Shi XY**, Bhagwandeen B, Leong AS. p16, cyclin D1, Ki-67, and AMACR as markers for dysplasia in Barrett esophagus. *Appl Immunohistochem Mol Morphol* 2008; **16**: 447-452 [PMID: 18665038 DOI: 10.1097/PAI.0b013e318168598b]

24 **van Dekken H**, Hop WC, Tilanus HW, Haringsma J, van der Valk H, Wink JC, Vissers KJ. Immunohistochemical evaluation of a panel of tumor cell markers during malignant progression in Barrett esophagus. *Am J Clin Pathol* 2008; **130**: 745-753 [PMID: 18854267 DOI: 10.1309/AJCPO31THGVEUIDH]

25 **Bani-Hani K**, Martin IG, Hardie LJ, Mapstone N, Briggs JA, Forman D, Wild CP. Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1316-1321 [PMID: 10944553 DOI: 10.1093/jnci/92.16.1316]

26 **Lao-Sirieix P**, Brais R, Lovat L, Coleman N, Fitzgerald RC. Cell cycle phase abnormalities do not account for disordered proliferation in Barrett's carcinogenesis. *Neoplasia* 2008; **6**: 751-760 [PMID: 15720801 DOI: 10.1593/neo.04280]

27 **Going JJ**, Keith WN, Neilson L, Stoeber K, Stuart RC, Williams GH. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. *Gut* 2002; **50**: 373-377 [PMID: 11839717 DOI: 10.1136/gut.50.3.373]

28 **Hong MK**, Laskin WB, Herman BE, Johnston MH, Vargo JJ, Steinberg SM, Allegra CJ, Johnston PG. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. *Cancer* 1995; **75**: 423-429 [PMID: 7812911]

29 **Pellish LJ**, Hermos JA, Eastwood GL. Cell proliferation in three types of Barrett's epithelium. *Gut* 1980; **21**: 26-31 [PMID: 7364315 DOI: 10.1136/gut.21.1.26]

30 **Reid BJ**, Sanchez CA, Blount PL, Levine DS. Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression. *Gastroenterology* 1993; **105**: 119-129 [PMID: 8514029]

31 **Chao DL**, Sanchez CA, Galipeau PC, Blount PL, Paulson TG, Cowan DS, Ayub K, Odze RD, Rabinovitch PS, Reid BJ. Cell proliferation, cell cycle abnormalities, and cancer outcome in patients with Barrett's esophagus: a long-term prospective study. *Clin Cancer Res* 2008; **14**: 6988-6995 [PMID: 18980994 DOI: 10.1158/1078-0432.CCR-07-5063]

32 **Merlo LM**, Shah NA, Li X, Blount PL, Vaughan TL, Reid BJ, Maley CC. A comprehensive survey of clonal diversity measures in Barrett's esophagus as biomarkers of progression to esophageal adenocarcinoma. *Cancer Prev Res (Phila)* 2010; **3**: 1388-1397 [PMID: 20947487 DOI: 10.1158/1940-6207.CAPR-10-0108]

33 **Ishikawa K**, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 2008; **320**: 661-664 [PMID: 18388260 DOI: 10.1126/science.1156906]

34 **Lee S**, Han MJ, Lee KS, Back SC, Hwang D, Kim HY, Shin JH, Suh SP, Ryang DW, Kim HR, Shin MG. Frequent occurrence of mitochondrial DNA mutations in Barrett's metaplasia without the presence of dysplasia. *PLoS One* 2012; **7**: e37571 [PMID: 22629421 DOI: 10.1371/journal.pone.0037571]

35 **Tan BH**, Skipworth RJ, Stephens NA, Wheelhouse NM, Gilmour H, de Beaux AC, Paterson-Brown S, Fearon KC, Ross JA. Frequency of the mitochondrial DNA 4977bp deletion in oesophageal mucosa during the progression of Barrett's oesophagus. *Eur J Cancer* 2009; **45**: 736-740 [PMID: 19211242 DOI: 10.1016/j.ejca.2009.01.013]

36 **Rossi E**, Grisanti S, Villanacci V, Della Casa D, Cengia P, Missale G, Minelli L, Buglione M, Cestari R, Bassotti G. HER-2 overexpression/amplification in Barrett's oesophagus predicts early transition from dysplasia to adenocarcinoma: a clinico-pathologic study. *J Cell Mol Med* 2009; **13**: 3826-3833 [PMID: 19292734 DOI: 10.1111/j.1582-4934.2008.00517]

37 **Rygiel AM**, Milano F, Ten Kate FJ, Schaap A, Wang KK, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Gains and amplifications of c-myc, EGFR, and 20.q13 loci in the no dysplasia-dysplasia-adenocarcinoma sequence of Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1380-1385 [PMID: 18559552 DOI: 10.1158/1055-9965.EPI-07-2734]

38 **Falk GW**, Skacel M, Gramlich TL, Casey G, Goldblum JR, Tubbs RR. Fluorescence in situ hybridization of cytologic specimens from Barrett's esophagus: a pilot feasibility study. *Gastrointest Endosc* 2004; **60**: 280-284 [PMID: 15278064 DOI: 10.1016/S0016-5107(04)01687-6]

39 **Brankley SM**, Wang KK, Harwood AR, Miller DV, Legator MS, Lutzke LS, Kipp BR, Morrison LE, Halling KC. The development of a fluorescence in situ hybridization assay for the detection of dysplasia and adenocarcinoma in Barrett's esophagus. *J Mol Diagn* 2006; **8**: 260-267 [PMID: 16645214 DOI: 10.2353/jmoldx.2006.050118]

40 **Wang KK**, Barr Fritcher E, Halling KC. The use of FISH in a multicenter blinded study to predict development of neoplasia in Barrett's esophagus. *Gastroenterology* 2009; **136:** A 157.

41 **Ong CA**, Lao-Sirieix P, Fitzgerald RC. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. *World J Gastroenterol* 2010; **16**: 5669-5681 [PMID: 21128316 DOI: 10.3748/wjg.v16.i45.5669]

42 **Fang D**, Das KM, Cao W, Malhotra U, Triadafilopoulos G, Najarian RM, Hardie LJ, Lightdale CJ, Beales IL, Felix VN, Schneider PM, Bellizzi AM. Barrett's esophagus: progression to adenocarcinoma and markers. *Ann N Y Acad Sci* 2011; **1232**: 210-229 [PMID: 21950815 DOI: 10.1111/j.1749-6632.2011.06053.x]

43 **Huang Q**, Hardie LJ. Biomarkers in Barrett's oesophagus. *Biochem Soc Trans* 2010; **38**: 343-347 [PMID: 20298180 DOI: 10.1042/BST0380343]

44 **Lao-Sirieix P**, Boussioutas A, Kadri SR, O'Donovan M, Debiram I, Das M, Harihar L, Fitzgerald RC. Non-endoscopic screening biomarkers for Barrett's oesophagus: from microarray analysis to the clinic. *Gut* 2009; **58**: 1451-1459 [PMID: 19651633 DOI: 10.1136/gut.2009.180281]

45 **Kadri SR**, Lao-Sirieix P, O'Donovan M, Debiram I, Das M, Blazeby JM, Emery J, Boussioutas A, Morris H, Walter FM, Pharoah P, Hardwick RH, Fitzgerald RC. Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study. *BMJ* 2010; **341**: c4372 [PMID: 20833740]

46 **Reid BJ**, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988; **19**: 166-178 [PMID: 3343032 DOI: 10.1016/S0046-8177(88)80344-7]

47 **Montgomery E**, Bronner MP, Goldblum JR, Greenson JK, Haber MM, Hart J, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Toledano AY, Shyr Y, Washington K. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001; **32**: 368-378 [PMID: 11331953]

48 **Reid BJ**, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101 [PMID: 20094044]

49 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 1995-2004 [PMID: 18923165 DOI: 10.1056/NEJMoa0804525]

50 **Wilson JF**. The rocky road to useful cancer biomarkers. *Ann Intern Med* 2006; **144**: 945-948 [PMID: 16785487 DOI: 10.7326/0003-4819-144-12-200606200-00022]

51 **Michiels S**, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 2005; **365**: 488-492 [PMID: 15705458 DOI: 10.1016/S0140-6736(05)17866-0]

52 **Rygiel AM**, Milano F, Ten Kate FJ, de Groot JG, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Assessment of chromosomal gains as compared to DNA content changes is more useful to detect dysplasia in Barrett's esophagus brush cytology specimens. *Genes Chromosomes Cancer* 2008; **47**: 396-404 [PMID: 18265409 DOI: 10.1002/gcc.20543]

53 **Fritcher EG**, Brankley SM, Kipp BR, Voss JS, Campion MB, Morrison LE, Legator MS, Lutzke LS, Wang KK, Sebo TJ, Halling KC. A comparison of conventional cytology, DNA ploidy analysis, and fluorescence in situ hybridization for the detection of dysplasia and adenocarcinoma in patients with Barrett's esophagus. *Hum Pathol* 2008; **39**: 1128-1135 [PMID: 18602665 DOI: 10.1016/j.humpath.2008.02.003]

54 **Dahlberg PS**, Jacobson BA, Dahal G, Fink JM, Kratzke RA, Maddaus MA, Ferrin LJ. ERBB2 amplifications in esophageal adenocarcinoma. *Ann Thorac Surg* 2004; **78**: 1790-1800 [PMID: 15511476]

55 **Hammoud ZT**, Dobrolecki L, Kesler KA, Rahmani E, Rieger K, Malkas LH, Hickey RJ. Diagnosis of esophageal adenocarcinoma by serum proteomic pattern. *Ann Thorac Surg* 2007; **84**: 384-92; discussion 392 [PMID: 17643604]

56 **Prasad GA**, Wang KK, Halling KC, Buttar NS, Wongkeesong LM, Zinsmeister AR, Brankley SM, Westra WM, Lutzke LS, Borkenhagen LS, Dunagan K. Correlation of histology with biomarker status after photodynamic therapy in Barrett esophagus. *Cancer* 2008; **113**: 470-476 [PMID: 18553366]

57 **Dunn JM**, Mackenzie GD, Oukrif D, Mosse CA, Banks MR, Thorpe S, Sasieni P, Bown SG, Novelli MR, Rabinovitch PS, Lovat LB. Image cytometry accurately detects DNA ploidy abnormalities and predicts late relapse to high-grade dysplasia and adenocarcinoma in Barrett's oesophagus following photodynamic therapy. *Br J Cancer* 2010; **102**: 1608-1617 [PMID: 20461081 DOI: 10.1038/sj.bjc.6605688]

58 **Langer R**, Ott K, Specht K, Becker K, Lordick F, Burian M, Herrmann K, Schrattenholz A, Cahill MA, Schwaiger M, Hofler H, Wester HJ. Protein expression profiling in esophageal adenocarcinoma patients indicates association of heat-shock protein 27 expression and chemotherapy response. *Clin Cancer Res* 2008; **14**: 8279-8287 [PMID: 19088045 DOI: 10.1158/1078-0432.CCR-08-0679]

59 **Wu X**, Gu J, Wu TT, Swisher SG, Liao Z, Correa AM, Liu J, Etzel CJ, Amos CI, Huang M, Chiang SS, Milas L, Hittelman WN, Ajani JA. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol* 2006; **24**: 3789-3798 [PMID: 16785472 DOI: 10.1200/JCO.2005.03.6640]

60 **Heeren PA**, Kloppenberg FW, Hollema H, Mulder NH, Nap RE, Plukker JT. Predictive effect of p53 and p21 alteration on chemotherapy response and survival in locally advanced adenocarcinoma of the esophagus. *Anticancer Res* 2004; **24**: 2579-2583 [PMID: 15330218]

61 **Nakashima S**, Natsugoe S, Matsumoto M, Kijima F, Takebayashi Y, Okumura H, Shimada M, Nakano S, Kusano C, Baba M, Takao S, Aikou T. Expression of p53 and p21 is useful for the prediction of preoperative chemotherapeutic effects in esophageal carcinoma. *Anticancer Res* 2000; **20**: 1933-1937 [PMID: 10928129]

62 **Kim MK**, Cho KJ, Kwon GY, Park SI, Kim YH, Kim JH, Song HY, Shin JH, Jung HY, Lee GH, Choi KD, Kim SB. ERCC1 predicting chemoradiation resistance and poor outcome in oesophageal cancer. *Eur J Cancer* 2008; **44**: 54-60 [PMID: 17976974 DOI: 10.1016/j.ejca.2007.09.006]

63 **Weston AP**, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's esophagus: immunohistochemical marker predictive of progression. *Am J Gastroenterol* 2001; **96**: 1355-1362 [PMID: 11374668 DOI: 10.1111/j.1572-0241.2001.03851.x]

64 **Cronin J**, McAdam E, Danikas A, Tselepis C, Griffiths P, Baxter J, Thomas L, Manson J, Jenkins G. Epidermal growth factor receptor (EGFR) is overexpressed in high-grade dysplasia and adenocarcinoma of the esophagus and may represent a biomarker of histological progression in Barrett's esophagus (BE). *Am J Gastroenterol* 2011; **106**: 46-56 [PMID: 21157443 DOI: 10.1038/ajg.2010.433]

65 **Lao-Sirieix P**, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. *Clin Cancer Res* 2007; **13**: 659-665 [PMID: 17255290 DOI: 10.1158/1078-0432.CCR-06-1385]

66 **Breton J**, Gage MC, Hay AW, Keen JN, Wild CP, Donnellan C, Findlay JB, Hardie LJ. Proteomic screening of a cell line model of esophageal carcinogenesis identifies cathepsin D and aldo-keto reductase 1C2 and 1B10 dysregulation in Barrett's esophagus and esophageal adenocarcinoma. *J Proteome Res* 2008; **7**: 1953-1962 [PMID: 18396902 DOI: 10.1021/pr7007835]

67 **Peters CJ**, Rees JR, Hardwick RH, Hardwick JS, Vowler SL, Ong CA, Zhang C, Save V, O'Donovan M, Rassl D, Alderson D, Caldas C, Fitzgerald RC. A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. *Gastroenterology* 2010; **139**: 1995-2004.e15 [PMID: 20621683]

68 **Lagarde SM**, Ver Loren van Themaat PE, Moerland PD, Gilhuijs-Pederson LA, Ten Kate FJ, Reitsma PH, van Kampen AH, Zwinderman AH, Baas F, van Lanschot JJ. Analysis of gene expression identifies differentially expressed genes and pathways associated with lymphatic dissemination in patients with adenocarcinoma of the esophagus. *Ann Surg Oncol* 2008; **15**: 3459-3470 [PMID: 18825457 DOI: 10.1245/s10434-008-0165-y]

69 **Mathé EA**, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaihara N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM, Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009; **15**: 6192-6200 [PMID: 19789312 DOI: 10.1158/1078-0432.CCR-09-1467]

70 **Izzo JG**, Wu TT, Wu X, Ensor J, Luthra R, Pan J, Correa A, Swisher SG, Chao CK, Hittelman WN, Ajani JA. Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. *J Clin Oncol* 2007; **25**: 698-707 [PMID: 17308274 DOI: 10.1200/JCO.2006.08.0283]

71 **Langer R**, Von Rahden BH, Nahrig J, Von Weyhern C, Reiter R, Feith M, Stein HJ, Siewert JR, Höfler H, Sarbia M. Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study. *J Clin Pathol* 2006; **59**: 631-634 [PMID: 16731604 DOI: 10.1136/jcp.2005.034298]

72 **D'Errico A**, Barozzi C, Fiorentino M, Carella R, Di Simone M, Ferruzzi L, Mattioli S, Grigioni WF. Role and new perspectives of transforming growth factor-alpha (TGF-alpha) in adenocarcinoma of the gastro-oesophageal junction. *Br J Cancer* 2000; **82**: 865-870 [PMID: 10732760 DOI: 10.1054/bjoc.1999.1013]

73 **von Rahden BH**, Stein HJ, Feith M, Pühringer F, Theisen J, Siewert JR, Sarbia M. Overexpression of TGF-beta1 in esophageal (Barrett's) adenocarcinoma is associated with advanced stage of disease and poor prognosis. *Mol Carcinog* 2006; **45**: 786-794 [PMID: 16921482 DOI: 10.1002/mc.20259]

74 **Kawakami K**, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, Demeester TR, Eads C, Laird PW, Ilson DH, Kelsen DP, Harpole D, Moore MB, Danenberg KD, Danenberg PV, Meltzer SJ. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 2000; **92**: 1805-1811 [PMID: 11078757 DOI: 10.1093/jnci/92.22.1805]

75 **Buskens CJ**, Van Rees BP, Sivula A, Reitsma JB, Haglund C, Bosma PJ, Offerhaus GJ, Van Lanschot JJ, Ristimäki A. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. *Gastroenterology* 2002; **122**: 1800-1807 [PMID: 12055587 DOI: 10.1053/gast.2002.33580]

76 **Gertler R**, Doll D, Maak M, Feith M, Rosenberg R. Telomere length and telomerase subunits as diagnostic and prognostic biomarkers in Barrett carcinoma. *Cancer* 2008; **112**: 2173-2180 [PMID: 18348304 DOI: 10.1002/cncr.23419]

77 **Saad RS**, El-Gohary Y, Memari E, Liu YL, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. *Hum Pathol* 2005; **36**: 955-961 [PMID: 16153457 DOI: 10.1016/j.humpath.2005.06.019]

78 **Falkenback D**, Nilbert M, Oberg S, Johansson J. Prognostic value of cell adhesion in esophageal adenocarcinomas. *Dis Esophagus* 2008; **21**: 97-102 [PMID: 18269642 DOI: 10.1111/j.1442-2050.2007.00749.x]

79 **Darnton SJ**, Hardie LJ, Muc RS, Wild CP, Casson AG. Tissue inhibitor of metalloproteinase-3 (TIMP-3) gene is methylated in the development of esophageal adenocarcinoma: loss of expression correlates with poor prognosis. *Int J Cancer* 2005; **115**: 351-358 [PMID: 15688381 DOI: 10.1002/ijc.20830]

**P-Reviewer:** Hillman LC **S-Editor:** Wen LL **L-Editor: E-Editor:**

**Table 1 Phases of biomarker production**

|  |
| --- |
| **Phases of biomarker validation and development**  **Phase 1** : Biomarkers of promise are identified based on application in  other cancers and elucidation of novel pathways  **Phase 2** :Cross sectional studies validate the biomarker of interest  to be sufficiently discriminatory and biomarker assays are  standardized  **Phase 3** :Case–control studies confirm the biomarker to be expressed  before the development of cancer with a retrospective but  longitudinal design  **Phase 4** :Prospective longitudinal studies avoid biases associated with  Case–control studies  **Phase 5**: Population-based studies show impact of biomarker detection  on 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] |
|  |  |  |  |  |
| Predictive  Progression markers  Prognostic biomarkers | P16 allelic loss  DNA ploidy abnormalities  HSP27  Ephrin B receptor  Genetic polymorphism  P21  P53  ERCC1  P53  DNA abnormalities  LOH of 157p and 9p  EGFR  Cyclin A  Cyclin D1  Hypermethylation of p16, RUNX2,HPP1  8 gene methylation panel  Catherpsin D,AKR1D10,AKR1C2 mRNA levels  DCK, PAPSS2, SIRT,TRIM44  P16 loss, C-MYC gain  ASS expression  MicroRNA expression profile  Cyclin D1  EGFR  TGF-α  TGF-β1  APC  COX-2  Telomerase  VEGF  Cadherin  TIMP | FISH  ICDA    IHC  Microarray  qRT-PCR    IHC  IHC  IHC  IHC  Flow cytometry  Flow cytometry  IHC  IHC  IHC  RT-PCR  RT-PCR  Western blot,qRt-PCR  RT-PCR, IHC  FISH  Microarray  Microarray, RT-PCR  IHC, FISH  IHC  IHC, ISH  RT-PCR, ELISA  PCR  IHC  Southern-blot and PCR  IHC  IHC  IHC, PCR | Response to therapy  Covariate value for recurrence  No response to therapy  Response to therapy in EAC  Associated with clinical outcome  Correlated with better CTX response  Correlated with better CTX response  Predicts CTX resistance  Limited efficacy as a progression marker  High risk for progression to EAC  Predict progression to EAC  Overexpression in HGD and EAC  Predicts progression to dysplasia  Risk of Progression to EAC  Risk of progression to EAC/HGD  Predicts progression to EAC/HGD  Dysregulation predicts progression to EAC/HGD  4 gene signature in EAC , predict 5 year survival  Associated with therapy response  Low expression associated with metastases  Low level associated with worse prognosis in EAC  Decreased survival  Decreased expression associated with decreased survival  High level indicates progression and metastases  High expression associated with decreased survival  High level associated with decreased survival  Associated with metastases and recurrence  Associated with decreased survival  Associated with metastases and decreased survival  Decreased level associated with decreased survival  Decreased 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.