REVIEW

Molecular changes in the progression of Barrett's oesophagus

Edyta Zagorowicz, Janusz Jankowski

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Barrett's oesophagus is a frequent complication of gastro-oesophageal reflux disease predicting oesophageal adenocarcinoma. The majority of Barrett's patients will not develop cancer, so that specific methods of identification of those at risk are required. Recent molecular studies have identified a selection of candidate biomarkers that need validation in prospective studies. They reflect various changes in cell behaviour during neoplastic progression. The ASPECT trial in the UK aims to establish whether chemoprevention with aspirin and a proton pump inhibitor will reduce adenocarcinoma development and mortality in patients with Barrett's oesophagus. It will also validate biomarkers for progression and clinical response and further study disease pathogenesis.

recent developments in molecular techniques offer new approaches to analyse and select tissue biomarkers that could undergo further assessment.

BIOMARKERS IN CANCER DEVELOPMENT

A biomarker is an indicator of a pathological process present in blood, tumour tissue, urine or faecal samples that may be measured or used to monitor the response to therapeutic interventions. Biomarkers, detectable before invasive tumour development, may replace rare or distant end points and be used as surrogate end points in cancer chemoprevention trials in order to decrease the sample size and duration of the trial.¹² An ideal biomarker is a substance secreted by tumour tissue and not secreted by non-tumour tissue that is easily and cheaply detectable. A model of formal biomarker development has been recently proposed,¹³ analogous to the process used in therapeutic drug evaluation and intervention studies. The five consecutive phases are as follows:

- (1) Preclinical exploratory studies to identify potentially useful biomarkers, including pre-clinical studies comparing tumour or precancerous tissue with tissue that is not affected. In addition to immunochemistry and western blots used for this purpose, gene-expression profiles based on microarrays that provide information on expression of thousands of genes or protein expression are increasingly used.
- (2) Clinical assay development to estimate the sensitivity and specificity of the biomarker in subjects with established disease compared to normal control subjects with sufficient sample sizes.
- (3) Retrospective longitudinal studies on clinical specimens collected from affected subjects before their clinical diagnosis and compared with those from control subjects to test the capacity of the biomarker to detect preclinical disease.
- (4) Prospective screening studies to determine whether tumours can be detected early before

Ten per cent to 20% of the Western population report regular gastro-oesophageal reflux symptoms.¹ Oesophageal squamous epithelium damaged by gastro-oesophageal reflux disease (GORD) may be replaced by Barrett's mucosa, a mosaic of metaplastic epithelium of gastric, intestinal, colonic or pancreatic types. It is the most common premalignant lesion affecting about 1.5% of the adult population.² The annual conversion to oesophageal adenocarcinoma is approximately 0.5% per year.³ The annual conversion rate from Barrett's to cancer is twice that elsewhere (1%/yr),⁴ while the 5 year survival of patients with oesophageal adenocarcinoma remains around 10%.⁵

Established demographic and clinical factors that correlate with the higher cancer incidence include white race, male sex, obesity, smoking, duration, frequency and severity of reflux, length of Barrett's oesophagus, presence of high grade dysplasia (HGD), a positive family history of Barrett's oesophagus or oesophageal adenocarcinoma, possibly the absence of *Helicobacter pylori* infection and the hiatal hernia size.^{6–8} Non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and perhaps proton pump inhibitor use show a negative correlation with the occurrence of oesophageal adenocarcinoma.^{9–11} The increasing burden of this disease and poor prognosis has stimulated research in the area but molecular and cellular mechanisms responsible for the development of Barrett's oesophagus and its conversion to neoplasia in a subgroup of patients are not fully understood. Predictive factors need to be identified for timing of preventive interventions and for targeted designs for future studies. The oesophagus is easily accessible to endoscopic biopsy and

See end of article for authors' affiliations

Correspondence to: Professor Janusz Jankowski, Department of Clinical Pharmacology, Radcliffe Infirmary, University of Oxford OX2 6HE, UK; janusz.jankowski@clinpharm.ox.ac.uk

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Abbreviations: APC, adenomatous polyposis coli; COX-2, cyclooxygenase-2; ECGF1, endothelial growth factor 1; GORD, gastro-oesophageal reflux disease; HGD, high grade dysplasia; hTERT, telomerase reverse transcriptase catalytic sub-unit; hTR, telomerase RNA; LGD, low grade dysplasia; LOH, loss of heterozygosity; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton pump inhibitor; PCNA, proliferating cell nuclear antigen; PGE2, prostaglandin E2; SFRP, secreted frizzled-related proteins; SPARC, secreted protein, acidic, cysteine-rich; TRAP, telomerase repeat assay protocol; TSPAN, tetraspanin

clinical diagnosis with the biomarker—that is, detection rate and false referral rate.

- (5) Cancer control studies that address whether screening with the biomarker reduces the burden of cancer on the population.

In addition to the above a sixth phase verification of these markers in multiple centres using the same protocols seems necessary so as to avoid issues peculiar to one centre's individual practice which might make generalisation of the method difficult.

Such recommendations with formulated criteria for when a biomarker can progress from one phase of development to the next will facilitate study designs and distribution of resources. For clinicians interpreting research literature, such a systematic approach gives valuable insight into how advanced the development of a new biomarker is, and its yield in specific clinical settings. Most publications on Barrett's oesophagus report studies in phases 1 and 2. There are few if any reports on the later phases 3–5, and we have yet to see a clinically useful test to manage cancer risk in Barrett's oesophagus.

MOLECULAR ALTERATIONS AND BIOMARKERS IN BARRETT'S METAPLASTIC TISSUE

Earliest abnormalities

Recent research has provided some insight into the earliest phases of Barrett's metaplasia development. Barrett's epithelium is characterised by the presence of goblet cells, and expression of intestinal markers such as MUC2, alkaline phosphatase, villin and sucrase isomaltase. It has been suggested that transcription factors that play an important role in normal intestinal differentiation may also be important in the development of metaplastic epithelium in the oesophagus. CDX1 and CDX2 are homeobox proteins that play major roles in the development of intestinal epithelium in utero. The expression of these proteins in the early phase of intestinal development was studied only in mice and there is a different pattern along the horizontal intestinal axis. CDX2 expression arises in the proximal intestine and declines distally, whereas CDX1 expression arises in the distal intestine with overlap of both in the midgut.¹⁴ It is possible that in humans, injurious agents present in GORD activate ectopic expression of CDX-1 through NF- κ signalling which in turn initiates the development of the intestinal phenotype. Wong *et al.*¹⁵ have found CDX1 mRNA and protein expression in all samples of Barrett's metaplasia tested but not in normal oesophageal squamous or gastric body epithelia. Conjugated bile salts and inflammatory cytokines tumour necrosis factor α (TNF α) and interleukin 1b (IL-1b) were found to increase CDX2 mRNA expression in vitro through NF- κ B signalling.¹⁶ CDX2 may cooperate with CDX-1 in inducing and maintaining a complete intestinal phenotype. The presence of CDX2 protein and mRNA has been shown in oesophageal adenocarcinoma, in Barrett's metaplasia cells of the intestinal type with goblet cell specific Muc2 mRNA, and also in squamous epithelium of a proportion of patients with Barrett's metaplasia, and may precede the morphological changes seen in Barrett's oesophagus.^{17–19} This was not the case in patients with gastric type metaplastic epithelium or patients with GORD without Barrett's metaplasia.¹⁹

Molecular changes in the metaplasia-dysplasia-adenocarcinoma sequence in biomarker studies

Further progression leading to dysplasia and adenocarcinoma development in Barrett's metaplasia is a multistep process that probably takes many years.^{20–21} It is driven by genomic instability and evolution of clones of cells with accumulated genetic errors that carry selection advantage and allow successive clonal expansion.²² Some chromosomal aberrations,

including aneuploidy and loss of heterozygosity (LOH), genetic mutations and epigenetic abnormalities of tumour suppressor genes that are the hallmarks in cancer development have been already identified. They may be categorised according to Hanahan and Weinberg's²³ hypothesis of essential changes in carcinogenesis. Cancer cells need to provide growth signals, ignore growth inhibitory signals, avoid apoptosis, replicate without limit and invade and proliferate.

Cell cycle and proliferation

Cell cycle regulatory genes known to be implicated in oesophageal adenocarcinoma development include p16 and cyclin D1. Inactivation of p16 located on chromosome 9p or overexpression of cyclin D1 promote hyperphosphorylation of the retinoblastoma protein Rb which controls the transition between phase G1 of the cell cycle and phase G0 or S (synthesis). Hypophosphorylated (active) Rb normally blocks progression of the cycle whereas phosphorylation inactivates Rb and stimulates proliferation.

Loss of this control mechanism including p16 lesions allows affected cells to be selected, grow and spread within a Barrett's segment, a process called clonal expansion. It is believed that subsequently such clones accumulate further genetic abnormalities that confer proliferative and survival advantage over normal cells and progress to oesophageal adenocarcinoma.^{24–25} p16 lesions are very common in Barrett's metaplasia and include loss of heterozygosity, mutation and methylation of the promoter.^{26–28} Inactivation of one of both alleles occur in 85% to 90% of patients and progression from monoallelic to biallelic damage in the course of the disease in individual patients occurs. Prospective evaluation of predictive significance of p16 status is underway but the early and widespread prevalence of its abnormalities means that it will probably not be useful for selection of patients at increased risk and for outcome prognosis. p16 as a biomarker in Barrett's oesophagus as well as other cell cycle abnormalities have been broadly described in the review by Reid *et al.*²¹

Histochemically assessed cyclin D1 overexpression has been documented in Barrett's oesophagus and oesophageal adenocarcinoma.²⁹ One prospective study has shown that Barrett's metaplasia patients with cyclin D1 overexpression were at increased risk of cancer development compared to patients in whom this its expression was normal.³⁰

Hyperproliferation has been consistently observed in Barrett's metaplasia by many assays including immunohistochemistry staining for division markers such as proliferating cell nuclear antigen (PCNA) and Ki67, and flow cytometry for DNA content,^{31–34} but there were no advanced phase studies showing that proliferation indices have any predictive value in progression to cancer.

Telomerase

Telomeres are long fragments of non-coding DNA repeats that protect chromosome ends from degradation and aberrant fusion. Due to incomplete replication at the 3' ends of chromosomes some fragments of telomeric DNA are lost with each cell division. After a certain number of divisions telomeres become too short to protect chromosomes and that mechanism causes growth arrest preventing the cells from further mitoses. In the majority of human cancers reactivation of telomerase enzyme is observed, which is a ribonucleoprotein reverse transcriptase that stabilises telomeres and maintains the proliferative capacity of cancer cells.³⁵ Telomerase RNA (hTR) was found in 100% of oesophageal adenocarcinomas and a pronounced increase in its level from low grade to HGD was observed in patients with Barrett's oesophagus.³⁶ Also the level of telomerase reverse transcriptase catalytic sub-unit (hTERT) mRNA in histologically normal squamous oesophageal tissues

from cancer patients was significantly higher than in normal oesophageal tissues from patients with no cancer.³⁷ More recently a molecular functional assay evaluating actual telomerase activity (telomerase repeat assay protocol, TRAP)³⁸ was used to discover zonal distribution of telomerase activity gradient in biopsies from different anatomical levels of Barrett's oesophagus.³⁹

Aneuploidy

Increased tetraploid DNA and aneuploid DNA content detected by flow cytometry reflect abnormal proliferative capacities of a studied population of cells. In Barrett's epithelium ploidy abnormalities are associated with progression to dysplasia and rarely occur in normal tissue.⁴⁰ Its predictive value has been extensively studied by the Seattle group who have shown in prospective studies that tetraploidy is a strong and significant predictor of progression to aneuploidy, dysplasia and cancer.^{41–43} The authors found that when histology and flow cytometry results were considered together, flow cytometry was most predictive in patients negative for dysplasia and with indefinite or low grade dysplasia. The 5 year cumulative incidence of cancer was 28% in patients with either aneuploidy or tetraploidy compared to 0% in those with normal cytometric results. Based on these results, the Seattle group has included flow cytometry analysis into their assessment protocol of Barrett's oesophagus patients and offer annual endoscopic surveillance to those with cytometry abnormalities detected, even if no HGD is present. However, there have been no studies published yet from other centres reproducing these results.

Apoptosis

The p53 protein gene localised on chromosome 17p prevents cells with DNA damage, including karyotypically abnormal tetraploid and aneuploid cells, from dividing and activates apoptosis pathways. Damage of p53 disrupts this apoptosis process and therefore allows expansion of abnormal cells. Genomic abnormalities of this oncogene usually result in the accumulation of the inactivated protein, which can be visualised as an overexpression in immunohistochemistry. Certain types of mutations produce a truncated protein that fails to stain immunochemically that may produce false negative results. Therefore genetic studies detecting LOH and mutations are more reliable to confirm p53 damage. p53 lesions occur frequently in oesophageal adenocarcinomas (85–95%) and almost never in normal tissue from the same patients; their prevalence increases with advancing histologic grade of dysplasia which makes them appropriate candidates for further studies.^{44–48} So far only Reid *et al* have evaluated 17p (p53) LOH in a large phase 4 study⁴⁸ with prospective observation of 256 patients and oesophageal adenocarcinoma as the primary end point. In this study 17p (p53) LOH was a strong and significant predictor of progression to oesophageal adenocarcinoma with the relative risk of 16 in patients with this lesion compared to the patients without.

Invasion

E-cadherin

Cadherins are a family of calcium dependent cell-cell adhesion molecules essential to the maintenance of intercellular connections, cell polarity and cellular differentiation. Adhesion occurs via "cadherin" domains in the extracellular portion, and an internal conserved catenin-binding domain which binds to the cytoskeleton. Germline mutation of the E-cadherin gene (CDH1) causes familial gastric cancer.^{49–50} Loss of E-cadherin expression is associated with many non-familial human cancers, including oesophageal adenocarcinoma.⁵¹ E-cadherin is further implicated in development of sporadic colorectal cancer through its interaction with the APC gene (adenomatous

polyposis coli) and β -catenin through Wnt signalling. The expression of E-cadherin is significantly lower in patients with Barrett's oesophagus compared with normal oesophageal epithelium and the reduction of its expression is observed when the sequence metaplasia-dysplasia-adenocarcinoma progresses.⁵² These findings suggest that E-cadherin may serve as a tumour suppressor early in the process of carcinogenesis.

Growth signals

TNF α

Along with decreasing E-cadherin expression in metaplastic tissue, loss of membranous β -catenin expression and an increase in cytoplasmic and nuclear β -catenin localisation in oesophageal cancer⁵² is observed. Levels of free cytosolic β -catenin, which is not bound to E-cadherin at the membrane, are regulated by a complex of APC, Axin and GSK-2 β that drives degradation of β -catenin. Free cytoplasm β -catenin binds to nuclear transcription factors and promotes transcription of many target genes, including several oncogenes such as c-myc and cyclin D1. TNF α , an inflammatory cytokine that can be detected in cells in ovarian, breast, prostate bladder and colorectal cancers, can down-regulate the expression of E-cadherin at a transcription level.⁵³ In Barrett's metaplasia the expression of epithelial TNF α , most pronounced in regions with intense lymphocytic infiltration, increases during the progression from metaplasia to dysplasia and carcinoma.⁵⁴ β -catenin mediated transcription following TNF α stimulation of cell lines was also shown, including c-myc gene. Increased level of this oncogene had directly stimulated proliferation of these cells.

COX-2

Cyclooxygenase-2 (COX-2) is normally found in the kidney and brain but in other tissues its expression is inducible and rises during inflammation, wound healing and neoplastic growth in response to interleukins, cytokines, hormones, growth factors and tumour promoters. COX-2 and derived prostaglandin E2 (PGE2) appear to be implicated in carcinogenesis because they prolong the survival of abnormal cells that favours accumulation of genetic changes. They reduce apoptosis and cell adhesion, increase cell proliferation, promote angiogenesis and invasion and make cancer cells resistant to the host immune response.⁵⁵

COX-2 is expressed in the normal oesophagus but its expression was found to be significantly increased in Barrett's oesophagus and even more in HGD and oesophageal adenocarcinoma.^{56–58} Some authors suggested that COX-2 expression might be of prognostic value in oesophageal adenocarcinoma as the COX-2 immunoreactivity study in cancer tissues showed that patients with high COX-2 expression were more likely to develop distant metastases and local recurrence and had significantly reduced survival rates when compared to those with low expression.⁵⁸

These data illustrate how chronic inflammation can contribute to the carcinogenesis process in the gastrointestinal tract, but the prognostic value of overexpression of TNF α and COX-2 in Barrett's metaplasia have not been documented in prospective studies.

Epigenetic abnormalities

Genetic alterations do not always fully account for the inactivation of both alleles of tumour suppressor genes. For example, although reduced levels of E-cadherin expression are frequent in Barrett's oesophagus and oesophageal adenocarcinoma, and LOH of CDH1 locus was found in 65% cases of oesophageal adenocarcinoma, mutations are rare.⁵⁹ This suggests that other, non-genetic events could contribute to gene inactivation in carcinoma development. One of such epigenetic mechanisms that can lead to transcription silencing is

abnormal hypermethylation of CpG rich regions, called CpG islands, found in promoter regions of many genes. Hypermethylation of promoters of tumour suppressor genes has been observed in nearly every type of human tumour, with unique patterns of individual gene methylation exhibited by each tumour type. In oesophageal adenocarcinoma Wong *et al*²⁶ have shown that hypermethylation of the CDKN2A promoter of p16 was present in tumours with CDKN2A LOH but lacking mutations in the remaining allele. Hypermethylated APC DNA in plasma of patients with oesophageal adenocarcinoma was found to be associated with reduced survival.⁶⁰ Another study by Eads *et al*⁶¹ confirmed that promoters of these two genes and ESRI (oestrogen receptor α) were hypermethylated in oesophageal adenocarcinoma and Barrett's dysplastic and non-dysplastic tissue which may indicate that these changes can occur in early stage tissues associated with dysplasia and malignancy. Within a particular profile of genes susceptible to promoter hypermethylation, individual tumours would exhibit different patterns of hypermethylation and its characteristic may be potentially predictive of a patient's clinical outcome. A retrospective analysis of aberrant methylation patterns of an even broader panel of seven genes in oesophageal adenocarcinoma samples and Barrett's oesophagus from patients who underwent oesophagectomy compared with clinical outcomes suggest that positive methylation status for multiple genes in oesophageal adenocarcinoma is a predictor of poor prognosis.⁶² Hypermethylation of secreted frizzled-related proteins (SFRP) genes, which normally counteract persistent or excessive Wnt signalling,⁶³ has been also confirmed in oesophageal carcinoma and Barrett's metaplasia⁶⁴ after it was found in several other cancers. A prospective longitudinal study is needed to establish whether any of these molecular alterations could be used as early predictors of neoplastic disease.

Dysplasia

Definition and criteria

Dysplasia is the only biomarker that entered routine clinical practice in Barrett's oesophagus management. According to current guidelines the diagnosis of Barrett's metaplasia warrants regular endoscopic surveillance for dysplasia with four-quadrant biopsy at 2 cm intervals in the affected oesophageal portion. Gastrointestinal dysplasia is defined microscopically as replacement of the native intestinal epithelium by an unequivocally neoplastic but as yet non-invasive epithelium.⁶⁵ The classification and criteria of dysplasia in Barrett's mucosa are the same as those used in inflammatory bowel disease. The mucosa can be negative for dysplasia, indefinite, or positive, when it is further graded into low grade dysplasia (LGD) or HGD. The last, fifth category is cancer. HGD in Barrett's oesophagus is an indication for oesophagectomy or endoscopic therapies.

Diagnostic difficulties

The limitations of this method of surveillance are sampling problems and histopathologic interpretation problems. Routine endoscopy cannot reliably identify macroscopically pre-malignant lesions and biopsies are usually taken randomly. Even rigorous biopsy protocols can miss HGD and cancer.⁶⁶⁻⁶⁷ The inter-observer variation in dysplasia diagnosis is high due to subjectivity of evaluation and difficulties to distinguish dysplastic from inflammatory and regenerative changes.⁶⁸

Dysplasia as a predictive marker

The prognostic significance of dysplasia in Barrett's oesophagus has been extensively studied for several decades and results are inconsistent. In four prospective phase 4 studies in patients with HGD followed-up for a mean of 3–7.3 years,⁴³⁻⁶⁹⁻⁷¹ cumulative cancer incidence ranged from 14–56% with the

relatively benign course of HGD observed in the longest and the most powerful study from the Hines VA (79 patients).⁷¹ HGD regression, especially in short (<3 cm) Barrett's oesophagus, is not infrequent.⁶⁹ The absence of HGD on two subsequent biopsies does not exclude progression to cancer in a relatively short time.⁷² While in clinical guidelines LGD detection is an indication to increased frequency of surveillance endoscopy with repeated pathologic assessment, long term observations have shown that cancer in patients with LGD develops with a frequency similar to non-dysplastic Barrett's metaplasia.⁴³⁻⁷³⁻⁷⁴ Clearly dysplasia alone is not an entirely reliable biomarker and more specific progression predictors need to be included in surveillance recommendations to increase their efficacy and cost effectiveness.

ASPECT STUDY

The largest prospective intervention study in patients with Barrett's metaplasia was started in April 2004 in the UK. It is organised by the National Institute for Cancer Research and sponsored by Cancer Research UK. ASPECT, a phase IIIB randomised study of aspirin and esomeprazole chemoprevention in Barrett's metaplasia, is a national, multicentre, randomised controlled 2×2 factorial trial of low or high dose esomeprazole with or without low dose aspirin for 8 years.⁷⁵

Its primary aim is to study if intervention with aspirin can result in a decreased mortality or conversion rate from Barrett's metaplasia to adenocarcinoma or HGD and if high dose proton pump inhibitor (PPI) treatment can decrease the cancer risk further. The background for chemoprevention use in Barrett's oesophagus has been described in detail elsewhere.⁷⁶ Secondary objectives are identification of clinical and molecular risk factors for the development of Barrett's adenocarcinoma and the evaluation of cost effectiveness of aspirin and/or PPI treatment in the prevention of Barrett's adenocarcinoma.

A molecular substudy will use ASPECT to investigate early mechanisms of the progression from Barrett's oesophagus to oesophageal adenocarcinoma and the role of chemoprevention in modifying this sequence. Biomarker studies in this project include characterisation and validation of p16, TP53 (gene expression and LOH and mutation analysis) and also aneuploidy/ploidy changes (by flow cytometry), currently the best characterised biomarkers for prediction of progression. Other

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Main points

- Barrett's metaplasia, a mosaic of metaplastic epithelium of intestinal, gastric, colonic and pancreatic types that replaces oesophageal squamous epithelium, is the most common premalignant lesion in the UK.
- Biomarkers that indicate neoplastic process in its early phases may be used as surrogate end points in intervention trials.
- The process of biomarker development is analogous to the process used in drug evaluation.
- Accumulations of genetic errors that carry selection advantage in clones of metaplastic cells allow successive clonal expansion.
- Numerous abnormalities in cell cycle and apoptosis of Barrett's metaplastic cells, their adhesive properties and response to growth signals have been observed, but only a few of them were assessed in more advanced clinical trials.
- Prospective studies showed that dysplasia presence alone is not an entirely reliable biomarker and its absence does not exclude progression to cancer.
- The ASPECT study in the UK is a large long-term intervention study aimed to establish whether chemoprevention with aspirin and proton pump inhibitor will reduce adenocarcinoma development and mortality in patient with Barrett's oesophagus. This trial will be able to validate biomarkers for progression, clinical response, disease pathogenesis, genetic predisposition, environmental mutagenesis and histological sampling.
- Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK, REporting of tumour MARKer studies) have been developed for researchers studying biomarkers, and are available at <http://www.cancerdiagnosis.nci.nih.gov/assessment/progress/clinical.html>

changes in protein expression will also be studied including CDX2,¹⁷ COX-2,⁷⁷ protein kinase C- ϵ (PKC ϵ)⁷⁸ and minichromosome maintenance protein 2 (Mcm2)⁷⁹ expression in 2000 cases. The aim is to address whether these markers are reproducible and if they are clinically informative (sensitivity and specificity) at 2 and 4 years. In addition microarray analysis is underway as well as identification of novel DNA SNP (single nucleotide polymorphism) signatures and target regions for expression analysis and investigation of clonality.

The expected number of subjects is 5000. The population studied are males and females >18 years old with circumferential Barrett's metaplasia at least 2 cm from the gastro-oesophageal junction (histologically proven by intestinal metaplasia in at least one sample). Patients receive either continuous PPI at a dose of 20 mg/day or 80 mg/day with or without aspirin 300 mg/day. Subjects undergo endoscopy at baseline and thereafter at 2 yearly intervals. Each time any macroscopic abnormality of the Barrett's metaplasia is biopsied with systematic quadrant biopsies taken using from the lower, middle and upper levels of the Barrett's zone.

The study protocol and details are available on the Digestive Diseases Centre website (<http://www.digestivediseases.org>) and the American National Cancer Institute registered trials website (<http://www.cancer.gov/clinicaltrials>).

CONCLUSION

The dramatic increase in oesophageal adenocarcinoma incidence over the last few decades has stimulated research interest in the earliest molecular phases of its development. The precancerous oesophageal lesion, Barrett's metaplasia, is accessible through endoscopic biopsy, and the long progression time from metaplasia through dysplasia to adenocarcinoma provides an opportunity to identify biomarkers of neoplastic transformation and to validate their predictive value in prospective studies.

Although dysplasia has been a traditionally accepted risk factor and its presence is an indication for more vigilant surveillance or ablative therapies, advanced phase 4 studies on HGD predictive significance have brought conflicting results. Ongoing prospective studies validate the predictive value of mutations of tumour suppression genes, including p16 and p53, as well as aneuploidy of Barrett's metaplastic tissue. Many other biomarkers involved in various aspects of oesophageal carcinogenesis undergo earlier phases of development. The ASPECT study in UK is the largest prospective study on the Barrett's metaplasia population and aims to evaluate whether aspirin and PPIs may prevent Barrett's oesophagus from progression to oesophageal adenocarcinoma as well as to study further the molecular biology of both conditions.

Emerging methods of global gene and protein profiling using bioinformatics are increasingly employed for molecular evaluation of Barrett's metaplasia but their results are preliminary and do not have clinical relevance yet. Mitas *et al*⁸⁰ developed a quantitative mathematical algorithm based on the expression levels of a panel of three genes including TSPAN (tetraspan1), ECGF1 (endothelial growth factor 1) and SPARC (secreted protein, acidic, cysteine-rich) to discriminate between Barrett's oesophagus and oesophageal adenocarcinoma. cDNA microarray used for gene expression profiling provides abundant information on differences between normal and metaplastic tissue regarding hundreds of genes,^{81 82} which possible role in neoplastic progression needs further studies, and methods of analysis of these data which still need to be developed. Proteomic studies using mass spectrometry enable direct analysis of epithelial protein expression patterns, as used in combination with microdissection by Streitz.⁸³

Identification of specific biomarkers is necessary to select patients who need intensified surveillance, to better characterise populations for intervention studies including chemoprevention and to improve outcomes and reduce care costs. In order to facilitate evaluation of the appropriateness and quality of further studies' design, methods, analyses and to improve the ability to compare results across studies, NCI-EORTC developed and published Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK, REporting of tumour MARKer studies),⁸⁴ available at <http://www.cancerdiagnosis.nci.nih.gov/assessment/progress/clinical.html>

MULTIPLE CHOICE QUESTIONS (TRUE (T)/FALSE (F); ANSWERS AFTER THE REFERENCES)

1. Regarding the epidemiology of Barrett's oesophagus and oesophageal adenocarcinoma:

- Barrett's metaplasia occurs in about 1.5% of the population of UK
- Gastro-oesophageal reflux disease severity, white race, male sex, obesity and smoking strongly correlate with oesophageal adenocarcinoma incidence
- Decreasing prevalence of *Helicobacter pylori* infection may result in lower incidence of oesophageal adenocarcinoma
- Aspirin and NSAID use correlates with lower incidence of oesophageal adenocarcinoma

2. Regarding the molecular features of Barrett's tissue:

- (A) Expression of cyclooxygenase-2 enzyme is usually lowered in Barrett's metaplastic tissue
- (B) Lesions of p16 that functions as a tumour suppressor gene are among the most frequent abnormalities in Barrett's tissue
- (C) Abnormalities in apoptosis controlled by p53 gene correlate with severity of dysplasia
- (D) The E-cadherin gene that is mutated in familial gastric cancer and is involved in development of sporadic colorectal cancer may also play a role in the development of Barrett's adenocarcinoma

3. Which of the following biomarkers are currently used as predictive factors in clinical practice in patients with Barrett's oesophagus:

- (A) Hyperproliferation and dysplasia
- (B) p16 gene lesions and dysplasia
- (C) Aneuploidy and dysplasia
- (D) Cyclooxygenase-2 expression and dysplasia

4. Regarding dysplasia in Barrett's oesophagus:

- (A) Current surveillance guidelines for patients with Barrett's metaplasia include regular endoscopic biopsying of any suspicious areas within the metaplastic tissue
- (B) Due to histopathologic interpretation problems the reproducibility of dysplasia diagnosis is unsatisfactory
- (C) Most patients with dysplasia in Barrett's oesophagus will develop cancer
- (D) Dysplasia of any grade is currently considered as an indication for oesophagectomy or endoscopic therapies

5. Regarding the ASPECT study currently being conducted in UK:

- (A) This is a chemoprevention trial that will also characterise and validate a panel of biomarkers
- (B) Patients receive continuously proton pump inhibitor in standard or high dose with or without aspirin
- (C) The population studied are men between 40 and 75 years old with circumferential Barrett's oesophagus at least 2 cm long
- (D) An inclusion criterion is dysplasia in Barrett's oesophagus

Authors' affiliations

Edyta Zagorowicz, Department of Gastroenterology, Institute of Oncology, Warsaw, Poland

Janusz Jankowski*, Department of Clinical Pharmacology, University of Oxford, Oxford, UK

*Also Department of Gastroenterology, Institute of Oncology, Warsaw, Poland, and Digestive Diseases Centre, University Hospitals of Leicester, Leicester, UK

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ANSWERS

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