Biomarkers in Barrett’s oesophagus

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Abstract

Biomarkers are needed to screen multiple stages in the clinical pathway of Barrett’s oesophagus patients; from disease diagnosis to risk stratification and predicting response to therapy. Routes to the identification of biomarkers have been recognized by known molecular features of the disease and more recently through transcriptomic, methylation and proteomic screening approaches. The majority of Barrett’s oesophagus patients remain undiagnosed in the general population. In order to develop a tool to screen Barrett’s oesophagus in the primary care setting, minimally invasive sampling methods coupled with immunocytoLOGY-based biomarkers are currently being assessed. Biomarkers may also have utility in surveillance programmes by allowing endoscopic interval to be adjusted according to individual neoplastic risk. Many individual biomarkers have been proposed in this regard, but have frequently been assessed in studies of limited power, or have lacked sufficient sensitivity or specificity when assessed in wider population-based studies. Biomarker panels may provide a route forward. In this regard, a panel of methylation markers has shown promise in a multicentre, double-blind, validation study. Biomarkers are also being developed to improve detection of high-grade dysplasia and oesophageal adenocarcinoma, utilizing brush cytology combined with FISH (fluorescence in situ hybridization), and to assess therapeutic success and risk of complication during photodynamic therapy. Finally, we outline progress in identifying alternative sources of biomarkers for this condition.

Introduction

Barrett’s oesophagus is characterized by the replacement of the normal squamous lining of the oesophagus with a columnar intestinal epithelium, usually in response to longstanding GORD (gastro-oesophageal reflux disease). The clinical significance of Barrett’s oesophagus lies in its potential to progress to OAC (oesophageal adenocarcinoma). Barrett’s oesophagus patients are estimated to be at 30–125-fold increased risk of OAC compared with the general population, and, in recent decades, OAC has dramatically increased in incidence in the West [1]. Biomarkers are required to inform clinical practice at multiple points in this disease pathway, ranging from detection of Barrett’s oesophagus in the general population to identifying those at greatest risk of disease progression and optimizing their therapy.

Biomarkers can be defined as biological variables that correlate with biological outcome [2]. Consistent with other cancer-related biomarkers, those applied in Barrett’s oesophagus (and OAC) should have high sensitivity and specificity [3,4], be stable over time, reproducible, cost-effective [3], minimally invasive and readily applied in the clinical or primary care setting [4–6]. A five-phase structure to guide the process of cancer biomarker development and validation has been proposed by the EDRN (Early Detection Research Network) of the NCI (National Cancer Institute), involving cross-sectional, retrospective and prospective studies, and large population-based clinical trials [6] (Table 1). Guidelines for the reporting of tumour marker studies (REMARK) have also been published [7].

In the present review, we outline the current state of biomarker development in Barrett’s oesophagus and highlight those with greatest promise.

Identifying Barrett’s oesophagus in the general population

It is generally accepted that the majority of Barrett’s oesophagus cases in the general population remain undiagnosed [8]; a Swedish population-based study suggests that as many as 1.6 % of the general population may have Barrett’s oesophagus [9]. Identification of these undiagnosed individuals would provide an opportunity for surveillance and possibly chemoprevention strategies to limit disease progression. Recently, Lao-Sirieix et al. [4] demonstrated that non-invasive capsule sponge sampling of the oesophagus combined with TFF3 (trefoil factor 3) immunostaining of recovered cells, resulted in a high sensitivity (78 %) and specificity (94 %) to detect confirmed Barrett’s oesophagus cases compared with GORD controls [4]. Such promising results require independent validation and assessment in the context of a prospective study in the primary care setting.

Key words: adenocarcinoma, Barrett’s oesophagus, biomarker, screening, surveillance, therapy.

Abbreviations used: AKR, aldo–keto reductase; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; GORD, gastro-oesophageal reflux disease; HGD, high-grade dysplasia; IND, indefinite dysplasia; LGD, low-grade dysplasia; LOH, loss of heterozygosity; ND, no dysplasia; OAC, oesophageal adenocarcinoma; PDT, photodynamic therapy; RR, risk ratio; TFF3, trefoil factor 3.

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Biomarkers of disease progression

Barrett’s oesophagus diagnosis is usually followed by endoscopic surveillance in order to detect disease progression at an early stage. However, the value of such surveillance programmes, including their cost-effectiveness, is questioned because of a range of issues, including sampling error, inter- and intra-observer variation in histology grading, lead time bias and low yield of cancer cases [2,3]. Informative biomarkers applied in this setting have the potential to address some of these issues. One method which is being considered in relation to reducing endoscopic sampling error for dysplasia and OAC is the collection of brush cytology specimens, to improve sampling area, coupled with probes directed against chromosomal abnormalities. Using this FISH (fluorescence in situ hybridization)-based cytology approach coupled with probes directed against chromosomes 7 and 17, Rygiel et al. [10] reported high sensitivity (85%) and specificity (84%) to detect and distinguish HGD (high-grade dysplasia)/OAC from LGD (low-grade dysplasia)/IND (indefinite dysplasia) and ND (no dysplasia) [10].

It is estimated that OAC develops in Barrett’s oesophagus patients at a rate of approx. 0.5% per year [11]. Therefore, for most Barrett’s oesophagus cases identified, the vast majority will not progress to OAC and further endoscopic assessment is unnecessary. Biomarkers are therefore required to stratify according to risk, resulting in surveillance which is targeted and cost-effective.

The pathogenesis of OAC is a co-ordinated accumulation of genetic and epigenetic abnormalities. Pivotal molecular gatekeeper events driving Barrett’s oesophagus progression have the potential to serve as biomarkers to predict disease progression in advance of histological change. Our current understanding of this process is limited, but inactivation of p53 through LOH (loss of heterozygosity) and mutation is frequent in Barrett’s oesophagus and usually occurs on a background of p16 loss [12]. In a cohort of Barrett’s oesophagus patients, Reid et al. [13] reported the prevalence of 17p (p53) LOH was 6% in patients with ND and 57% in HGD. 17p (p53) LOH in Barrett’s oesophagus patients with ND/IND/LGD was associated with increased risk of progression to HGD [RR (risk ratio) = 3.6] and OAC (RR = 16). Murray et al. [14] investigated the utility of p53 protein overexpression as a marker of progression in an Irish population-based nested case-control study of Barrett’s oesophagus patients [14]. As a single marker, it was not sufficiently sensitive to predict OAC in Barrett’s patients without dysplasia (sensitivity 32% and specificity 88.3%).

Development of tetraploid and aneuploid cell populations are thought to occur later in Barrett’s oesophagus progression, typically appearing after p53 inactivation [15]. Biallelic inactivation of p53 is associated with elevated 4N (tetraploid) fraction [16], and, in one study, Barrett’s oesophagus patients with tetraploid fractions exceeding 6% were 11.7-fold more likely to progress to OAC than patients below this cut-off [15]. Patients with aneuploid DNA contents exceeding 2.7N were also at elevated risk of progression (9.5-fold) [15]. These results are informative, but given the technical challenges associated with such precise assessment of tetraploid and aneuploid fractions, the introduction of these markers into routine practice will be difficult to achieve outside specialized centres. Positive cyclin A and Mcm2 (minichromosome maintenance 2) immunostaining have also been highlighted as potential biomarkers of progression in Barrett’s oesophagus. These markers could be translated into clinical practice more...
readily, but lack sufficient sensitivity and specificity to be applied as stand-alone markers [17,18].

Given the aforementioned limited understanding of events involved in progression, an array of discovery technologies has subsequently been applied in the search for informative biomarkers. Dahlgren et al. [19] analysed expression of 12000 genes in OAC and found that 64 genes were up-regulated (≥4-fold) and 110 genes were down-regulated (≥4-fold) compared with normal oesophageal samples. Further results from transcriptional profiling have shown that the gene-expression pattern in Barrett’s oesophagus tissue bears greater similarity to OAC than does normal squamous oesophagus, supporting the notion of Barrett’s oesophagus as an intermediate stage in OAC genesis [20]. Breton et al. [21] used a proteomic approach to screen cell lines at different stages in OAC development and identified dysregulation of cathepsin D, AKR1B10 (where AKR is aldo–keto reductase) and AKR1C2 proteins in Barrett’s oesophagus and OAC [21]. Utilizing methylation arrays, Schulmann et al. [22] demonstrated that hypermethylation of p16, RUNX3 (runt-related transcription factor 3), HPP1 (hyperplastic polyposis coli 1), NELL1 (NEL-like protein 1), TACI (tachykinin 1), SST (somatostatin), AKAP12 (A-kinase-anchoring protein 12) and CDH13 (cadherin 13) genes occurs early in Barrett’s oesophagus and predicts progression [22]. These results have recently been taken forward in a retrospective, multicentre, double-blind validation study. Applying this panel of eight methylation biomarkers, 60.7% of individuals who progressed within 2 years to HGD/OAC were detected with 90% specificity [23].

In addition to acquired molecular changes, numerous studies have sought to identify genetic markers of disease development and progression, with both protective and predisposing associations reported in pathways associated with immune function, detoxification, DNA repair and cell-cycle control [24–27]. Genome-wide association studies are currently underway in relation to Barrett’s oesophagus and OAC development and will provide an opportunity to validate these reports and, importantly, will highlight novel genetic markers which may contribute to assessing patient risk.

Biomarkers related to therapy

In addition to Barrett’s oesophagus diagnosis and predicting OAC development, there is also a role for biomarkers to predict response and to serve as targets for therapy. Endoscopic therapy has emerged over the last few years as an alternative treatment for HGD and OAC because of the high mortality and morbidity associated with oesophagectomy. The use of PDT (photodynamic therapy) has recently been approved by the FDA (U.S. Food and Drug Administration) for the treatment of HGD in Barrett’s oesophagus. Evidence from clinical trials has indicated that a significant proportion of patients either do not respond (23%), or progress to OAC following PDT (13%) [28]. In addition, oesophageal strictures have been reported in up to 36% of patients following PDT [29]. Identification of biomarkers which predict poor response to PDT or elevated risk of complication could allow its use to be targeted to those patients who would benefit most. In a prospective study reported by Prasad et al. [30], FISH cytology was used to assess response to PDT [30]. The results showed that p16 allelic loss predicted decreased response to PDT with an odds ratio of 0.32 (0.10–0.96). In another study by the same group, approx. 20% patients remained positive for FISH-based chromosomal markers (including loss of 9p21 and 17p13.1 loci, gains of 8q24, 17q and 20q13 loci, and multiple gains) despite the elimination of HGD after PDT. Among them, two patients [one with amplification at the 8q24 (c-myc) locus and the other one with multiple gains] developed recurrent HGD [31].

In individuals who progress to OAC, neoadjuvant multimodality treatment is frequently applied to improve survival of patients with locally advanced disease. However, only patients with a major histopathological response will benefit from this therapy. Predictive markers to allow individualization of the treatment could be very helpful. Expression of the DNA-repair gene, ERCC1 (excision repair cross-complementing 1), or the gene for the DNA-synthesis enzyme TS (thymidylate synthase), have been shown to be inversely associated with major response to treatment and improved survival [32]. Other potentially informative biomarkers include VEGF (vascular endothelial growth factor), proliferative activity, survivin, p53, p21, COX-2 (cyclo-oxygenase 2) and NF-κB (nuclear factor κB) [33–36]. In peripheral blood, increased expression of ERCC1 [37] and survivin [38] have been associated with minor response to chemoradiotherapy and could represent non-invasive predictive markers. Genetic polymorphisms in drug pathways have also been investigated [39]. Alakus et al. [40] reported that 80% of patients homozygous for the C allele at GNAS1 (guanine-nucleotide-binding protein, α-stimulating 1 complex locus) T393C had a major response to therapy, contrasting with individuals with a T-allele (TT or CT) where the majority of individuals were minor responders (63%).

Molecular features of the disease may also have the potential to be used as biomarkers to target therapy. EGFR (epithelial growth factor receptor) is overexpressed in a high proportion of OAC, and results from limited trials of EGFR tyrosine kinase inhibitors, such as Gefitinib and Cetuximab, suggest that a proportion of patients may derive benefit from EGFR-targeting therapies (reviewed by Dragovich and Campen [41]). Villanacci et al. [42] recently reported that treatment with anti-HER2 monoclonal antibody (Trastuzumab) in HGD and OAC for 6 months resulted in down-regulation of HER2 and EGF expression and stimulated tumour apoptosis.

Alternative sources of biomarkers

Whereas endoscopic biopsies are utilized primarily as the tissue of choice for biomarker studies in Barrett’s oesophagus (and OAC), there are increasing attempts to identify informative biomarkers in alternative biological samples. The
advantages of testing biomarkers utilizing a less invasive method are low cost, ease of performance and greater acceptability. This would be particularly beneficial in the context of screening in the primary care setting where endoscopic examination is not practicable.

As mentioned above, Lao-Sirieix et al. [4] have recently applied a capsule sponge device to sample the oesophagus for Barrett’s oesophagus, with TFF3 showing promise as a biomarker. Chao et al. [43] reported that bleomycin sensitivity in peripheral blood lymphocytes was associated with increased risk of cancer development in Barrett’s oesophagus, particularly in those with 17p LOH. Recently, Xing et al. [44] demonstrated that short telomere lengths on chromosome 17p and 12q in peripheral blood lymphocytes was associated with a dose-dependent increase in OAC risk [44]. Tumour DNA/RNA has been reported in the circulation of cancer patients from a number of primary tumours, including lung and colorectal sites [45,46]. In a study by Eisenberger et al. [47], matched normal, tumour and serum samples were subject to microsatellite analysis using 12 markers. Microsatellite abnormalities in serum were found in 81 % of OAC patients (including patients without lymph node involvement), and all serum samples from normal controls were negative. Hypermethylation of DAPK (death-associated protein kinase) and APC (adenomatous polyposis coli) genes has been detected in peripheral blood in OAC cases pre-operatively and shown to be significantly associated with unfavourable prognosis [48]. The combination of both markers allowed survival beyond 2.5 years to be predicted with 99.9 % sensitivity and 57.1 % specificity [48]. Using proteomic tools, Hammond et al. [49] sought to identify a serum protein pattern which could serve as a reliable blood test for the diagnosis of OAC. Using a hydrophotib chip surface, three peaks were identified that correctly diagnosed all OAC patients (including patients without lymph node involvement), and ten of 11 normal controls; using an immunocytology as a risk stratification tool for Barrett’s esophagus [50].

Concluding remarks
There is great potential to incorporate biomarkers at multiple points in the clinical pathway of Barrett’s oesophagus patients; from early disease diagnosis, to assessing risk of progression and personalizing therapy. Current studies suggest that panels of biomarkers are likely to be required to achieve the sensitivities and specificities required in clinical practice. Although many candidate biomarkers have been proposed, as yet few biomarkers have progressed to being assessed in multicentre or large population-based studies. Translation of promising biomarkers into clinical practice could be greatly facilitated by co-ordinated consortium approaches and by developing shared biobank resources for validation studies.

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References


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