Combining simple patient-oriented tests with state-of-the-art molecular diagnostics for early diagnosis of cancer

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Abstract

Early diagnosis is an important strategy to improve outcomes from cancer. Oesophageal adenocarcinoma is an example of a cancer that presents late, with very poor outcomes, and for which the presence of the precursor lesion Barrett's oesophagus provides the opportunity to intervene at an early stage. In this review, I describe the challenges in the field and the work that we have done to devise a conceptually novel approach to early diagnosis, using a cell collection device (Cytosponge), coupled with molecular assays. This is a personal perspective in which I also describe the career pathway that led me into academic gastroenterology, and the rewards and challenges of translational research in molecular diagnostics. There are fantastic opportunities for clinicians wishing to pursue academic medicine, because it is a time when massive strides are being made in a whole number of areas; for example: imaging, sequencing technology and targeted therapies. Clinicians who can straddle the laboratory and the clinic are essential, to maximise the progress that can be made for the benefit of patients.

Keywords

Adenocarcinoma, Barrett's oesophagus, biomarker, cancer, cell collection, Cytosponge, early diagnosis, medical device, oesophagus, outcome, Trefoil factor

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Screening tests for cancer have had bad press in recent years, due to concerns about over-diagnosis on the one hand and false reassurance on the other, coupled with the costs entailed for an over-stretched health service.¹ This debate has been most vociferous around the pros and cons of the established breast cancer screening service's use of mammography.^{2,3} On the other hand, clinicians are frequently faced with managing a patient whom has an advanced cancer diagnosis, which requires toxic systemic and surgical treatment that, depending on the cancer type, all too often results in poor outcomes. There is, therefore, a need to determine accurate and cost-effective strategies for early diagnosis of cancer, which can be applied to the relevant higher-risk groups.

My interest in the early detection of gastrointestinal cancer was fuelled by my early exposure to jobs in the areas of gastroenterology and oncology, as a junior doctor. The enthusiasm of the gastroenterologists for whom I worked, coupled with the challenge of

achieving an accurate diagnosis in a timely fashion, led me to pursue gastroenterology as a career, rather than oncology. The requisite skills of careful observation, coupled with a precise understanding of the pathogenesis of a disease process in order to reach a diagnosis, were for me a fascinating challenge in detective medicine. Oncologists seemed to me to be prescribing drugs after the difficult diagnostic work had been done for them, but perhaps I should not broadcast this sentiment too widely!

Through serendipity, my husband's job took us to Stanford, CA, USA and I found a research project in Barrett's oesophagus, under the charismatic supervision of professors George Triadafilopoulos and Bishr

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Omary, which was just the opportunity that I sought. We researched how acid and bile reflux altered the physiological processes of oesophageal cells; and thus, how these might cause a malignant phenotype. Imagine my horror when exposing tissue explants to low pH (the supposed mutagen) led to cells proliferating more slowly, and assuming a more differentiated state. My compulsory Monday morning supervision was nerve-racking, as I explained my apparently contradictory findings. An early lesson as a scientist is to believe in the data and to repeat the experiment several times over. The results held true.

I also came to realise that a more physiological experiment might be pulsatile acid exposure, more akin to reflux episodes. Then came the result that we had been looking for, an unstable phenotype with more rapidly proliferating cells, which we later demonstrated occurred secondary to effects on membranous proton pumps and thence, to changes in the cell cycle.^{4,5} These results led to the acceptance of my first research paper for publication, with virtually no corrections! I was left with the impression that scientific research was easy, not a feeling that lingered for long, but the buzz of discovering something new, followed by publication, was addictive.

When I returned to the UK, I continued with specialist training in gastroenterology, along with post-doctoral laboratory research. I was impressed time and again through my clinical work for the need for better strategies for early diagnosis. I was confronted too often with breaking bad news to young patients diagnosed with oesophageal cancer too late, whom died in my care despite our best efforts. There was also the challenge of identifying patients with dysplasia who were confined to oesophagectomy, as a result of a lack of endoscopic therapy at that time. I recall patients with high-grade dysplasia whom had an oesophagectomy that revealed no invasive cancer; yet they were left with life-changing morbidity. Therefore, the questions that concerned me were: How could we identify patients with early cancer, without endoscopy for a large proportion of the population? How could we achieve a more objective cancer risk profile than dysplasia? What treatments could be offered to patients who had an early cancer, but were asymptomatic?

Endoscopic treatments evolved quickly with the advent of endoscopic resection and ablation techniques; however, an accurate and timely diagnosis was still a clinical concern. With my research team in Cambridge, UK, I set about devising a molecular diagnostic strategy that could be implemented on a population-wide scale. The result was an easy-to-swallow device coupled with an assay specific to Barrett's oesophagus.

The Cytosponge technique

The device, which we called the Cytosponge, was modelled on previous devices used in high-risk areas for squamous cell cancer. It comprises a small, spherical sponge tethered to a string and compressed into a capsule that dissolves quickly on contact with the moist, warm environment of the stomach. The patient swallows the capsule along with the string. It enters the stomach, where the capsule dissolves and 5 minutes later, the string is withdrawn pulling the sponge with it to retrieve one-half million superficial cells. The specimen is placed into a universal container containing preservative. The whole test takes 10 minutes, can be done in the primary care setting and has the potential to be very inexpensive. Developing the device taught me all sorts of things about materials, knots and device regulations! It brought me into contact with manufacturers, engineering workshops and management consultants, in a way that I would never have envisioned, which led to a huge amount of fun, as well as some stressful and hard-learned lessons along the way.

The diagnostic assay

The molecular assay was in a way straightforward, because I had been trained in biomarker research. A gene expression profiling experiment enabled us to distinguish between genes that were highly expressed in Barrett's oesophagus, compared with the gastric cardia and squamous oesophagus. After whittling down the possible candidates based on the antibody's availability and specificity, we chose Trefoil Factor 3 (TFF3), a clinically applicable assay with a binary read-out. It was clear from the outset that diagnosing Barrett's oesophagus would not be sufficient, in order to avoid increasing the burden on controversial Barrett's surveillance programmes even further. Hence, we sought to determine markers of malignant risk that could also be applied to the Cytosponge. We had characterised the mutational landscape of oesophageal adenocarcinoma and high-grade dysplasia; and a pilot study showed that testing for the most recurrently mutated gene, Tumour protein 53 (TP53), was promising for identifying dysplasia.⁶

BEST trials

One of the most exciting and nerve-racking experiences for any team developing a diagnostic test is to see how effective the strategy is in a clinical trial setting. An initial primary care study of 500 individuals (BEST) was extremely encouraging, and demonstrated that the device was safe and acceptable to patients.⁷

Subsequently, a trial of over 1000 individuals (BEST2) demonstrated the accuracy of the TFF3

assay, with an overall sensitivity of approximately 80% that increased to 87% for Barrett's segments within ≥ 3 cm circumferential to Barrett's oesophagus, which is known to confer a higher cancer risk. The specificity in this trial was 92.4%. Again, our device was also extremely safe and well tolerated, compared with endoscopy.⁸

Health economics model

In order for such a strategy as this to be effective, it must be coupled with sound health economics. It has been a pleasure to collaborate with experts in public health and statistics, to create a micro-simulation model to examine the economic implications of a Cytosponge-based versus endoscopic strategy for the diagnosis, coupled with either surgical or endoscopic management.⁹ This analysis suggested that the Cytosponge could be a cost-effective way forward.

There is still work to be done, and anyone working in diagnostics appreciates that one needs to be in it for the long haul! It is a good thing that devices are now being subjected to rigorous evaluation prior to implementation, in a similar way to that required for drugs. In order to achieve clinical implementation, commercialisation is essential: We have been very fortunate to find a commercial partner with a long track record and expertise in devices, and an established network of clinicians managing Barrett's oesophagus. On the other hand, it has been a new experience for me, to be viewed as a clinician scientist with a potential conflict of interest. It is also interesting to observe that my clinical colleagues often need a lot of persuading to adopt a change in clinical practice.

The UEG prize was a huge boost. This recognition of our efforts so far on this, and other Barrett's oesophagus and oesophageal adenocarcinoma projects, means that one is more determined than ever to strive to make more progress; and their generous funding enables one to pursue research strategies that could lead to a breakthrough, but are on the other hand higher risk than some funding agencies are willing to take. Our next goal is to develop an innovative DNA-based strategy for diagnosis and risk stratification of Barrett's oesophagus that doesn't rely on a histo-cytopathologist's manual review of slides.

Our ambitious long-term vision is to increase the proportion of cases treated at the early, treatable stage; and thus, reduce the deaths from oesophageal adenocarcinoma. On a more general note, I hope that this approach can be extended to other tissue types. We live in a fantastic era of molecular diagnostics, made possible through advances in the chemistry required to rapidly assess nucleic acid states. To date, screening has relied on relatively blunt imaging tools or biomarkers that lack specificity. Much work is going on

to risk stratify patients much more accurately, from less invasive sampling. These should not necessarily be applied to the population *en masse*, but be implemented in high-risk groups and those with symptoms, in whom investigations may currently be delayed, due to the burden on our healthcare system.¹⁰ Earlier diagnosis is what we should strive for, to achieve the improvements in cancer survival that we crave.

So do I have any tips to those starting out on their research career, from what I have learned thus far? I would say that a career in academic gastroenterology is a most exciting, varied and rewarding one. I have made great friends and work colleagues. I have had chance to travel and to meet people in a way I would never have imagined. My sincere hope that all this work might lead to a small improvement in the management of Barrett's oesophagus and oesophageal cancer sustains me, when it gets tough. I would recommend this path to others. The sky is the limit for making progress in research with the tools that we have available today and organisations like UEG are a fantastic source of support and inspiration.

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Conflict of interest

The Cytosponge™ technology has been licensed by the medical research council to Covidien GI Solutions. Rebecca Fitzgerald is named on relevant patents for the Cytosponge™.

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