

Oesophageal disease

S, M, L, XL . . .

T Wang, G Triadafilopoulos

Methods of surveillance for Barrett’s oesophagus

Although the early detection of high grade dysplasia, the precursor of oesophageal adenocarcinoma, remains a primary task in the management of patients with Barrett’s oesophagus, several other key end points of screening and surveillance need to be considered (table 1). As dysplasia is rarely visually recognised during routine fiberoptic or video endoscopy, extensive four quadrant biopsy sampling every 1–2 cm of the entire mucosal surface using jumbo biopsy forceps (Seattle protocol) has been extensively practised, validated, and is currently recommended.<sup>1</sup> In a recent report by the pioneers of this approach, the use of this systematic jumbo biopsy protocol every 1 cm in patients with high grade dysplasia who eventually developed cancer, 100% of cancers were detected.<sup>2</sup> However, because the technique is labour intensive

and requires a therapeutic endoscope, it is used by less than 20% of US gastroenterologists.<sup>3</sup> Recently, many strategies and innovative techniques have been developed to improve the sensitivity of dysplasia detection and to overcome the problems of sampling error. Such approaches aim at enhancing the image contrast of the mucosa, detecting biochemical changes associated with dysplasia, and increasing the image resolution. The purpose of all of these approaches has been to improve from the small degree (S) of resolution and tissue penetration to medium (M), large (L), or even extra large (XL) levels of refinement, sensitivity, and specificity not only for dysplasia but also for a wider range of end points of screening and surveillance (table 1).

For the uninitiated endoscopist, these novel techniques are briefly described

here and summarised in table 2. Chromoendoscopy uses a standard video endoscope to visualise the mucosal surface after application of a dye, such as methylene blue or indigo carmine.<sup>4</sup> High magnification endoscopy employs a special endoscope that has additional lenses in the distal tip for enlarging the image, using a special control knob to allow conversion from a standard (S) to high level (L-XL) magnification.<sup>5-7</sup> Light induced fluorescence (autofluorescence) is generated by exciting endogenous biomolecules such as collagen, NADH, FAD, and porphyrins, which have greater accumulation in dysplasia than in normal mucosa.<sup>8</sup> Photodynamic diagnosis involves collection of fluorescence images after administration of an exogenous agent, such as 5-aminolevulinic acid (5-ALA).<sup>9</sup> 5-ALA is a prodrug that concentrates in dysplasia and is converted to the highly fluorescent protoporphyrin IX.<sup>10</sup> Optical coherence tomography (OCT) is a method of detecting backscattered infrared light from microstructures within the tissue layers of the oesophagus.<sup>11</sup> High resolution ultrasound uses a 20 MHz transducer to evaluate mucosal changes by detecting the backscattering of acoustic waves.<sup>12</sup> Confocal microscopy with a miniature probe can visualise subcellular structures below the surface of the mucosa by optical sectioning to reduce the effects of light scattering.<sup>13</sup> Table 2 lists the relative strengths of different imaging methods, including sensitivity for detection of high grade dysplasia and Barrett’s metaplasia, resolution, field of view, light penetration depth, cost, and time.

Despite the wide range and potential of all of these approaches, an adequate method of surveillance of Barrett’s oesophagus has remained elusive to date because of the complexity and variable evolution of this epithelium. Firstly, the architecture of mucosa containing dysplasia in the oesophagus is flat, and its

**Table 1** End points of screening and surveillance in Barrett’s oesophagus

- (1) Distinguish Barrett’s oesophagus from specialised intestinal metaplasia of the oesophagogastric junction (SIM-OGJ).
- (2) Recognise Barrett’s oesophagus within areas of erosive or ulcerative oesophagitis or stricture.
- (3) Identify high grade dysplasia or carcinoma within the Barrett’s oesophagus surface.
- (4) Detect intestinal metaplasia, dysplasia, or carcinoma underlying neosquamous islands or extensive re-epithelialisation after ablation or long term proton pump inhibitor use.
- (5) Minimise the number of biopsies to target areas only.
- (6) Sample greater surface areas of Barrett’s mucosa.
- (7) Avoid biopsies in patients with bleeding diathesis (anticoagulant use, underlying oesophageal varices).
- (8) Safely and reliably increase the surveillance interval.

**Table 2** Comparison of methods for Barrett’s oesophagus surveillance

Method	Description	S for SIM	S for HGD	Res	FOV	Depth (µm)	Cost	Time
Four quadrant biopsy	Random biopsy at 4 sites every 1–2 cm with jumbo forceps	++++	++	+	++++	~1000	+++	+++
Chromoendoscopy	Standard surface view of oesophageal mucosa enhanced with stain or dye	++++	+/-	++	++++	None	+	++
High magnification endoscopy	Magnified surface view of oesophageal mucosa, may be enhanced by acetic acid	+++	+/-	+++	+	None	++	+++
Light induced fluorescence	Fluorescence from endogenous molecules	++	+/-	+	++++	~200	+++	+++
Photodynamic diagnosis	Fluorescence from exogenous molecules	++	+/-	+	++++	~200	+++	+++
Optical coherence tomography	Backscattered infrared light from cellular microstructures	++	+/-	+++	++	~500	++++	++++
High frequency ultrasound	Backscattered acoustic waves from cellular microstructures	+	+/-	++	++	~1000	+++	++++
Confocal microscopy	Miniature microscope that images with subcellular resolution in thick tissue	?	?	++++	+	~500	++	++

S, Sensitivity; SIM, specialised intestinal metaplasia; HGD, high grade dysplasia; Res, image resolution; FOV, field of view. ?, unknown; +/-, inconclusive; +, low; ++, moderate; +++, high; +++++, very high.

appearance on conventional endoscopy is indistinct from that of specialised intestinal metaplasia. Secondly, the distribution of dysplasia over the mucosal surface can be quite variable—that is, focal, patchy, or diffuse. Thus the method of surveillance must be sensitive over a wide area. Thirdly, dysplasia is a histopathological diagnosis that requires subcellular image resolution to visualise nuclear and perinuclear morphology. This level of detail cannot be achieved in practice by conventional imaging methods, such as ultrasound, computed tomography, or magnetic resonance imaging. The best resolution so far has been obtained by optical methods, but these techniques have yet to achieve subcellular resolution in vivo. Fourthly, detection of dysplasia is frequently needed in the setting of an oesophagus that may contain erosions, strictures, and inflammation. Inflammatory changes in the mucosa may obscure methods sensitive to tissue biochemistry such as fluorescence. Fifthly, neosquamous epithelialisation of the oesophagus can occur after prolonged acid suppression or ablative injury and thus methods of surveillance must be able to identify Barrett's metaplasia present below the new (squamous) mucosal surface. Finally, for practical purposes, screening must be performed in a time and cost efficient manner. This set of diagnostic requirements is quite rigorous and is unlikely to be satisfied by any single technique of surveillance. Moreover, because the identification of high grade dysplasia may result in an oesophagectomy for the patient, a conventional biopsy is desired for confirmation. Thus a new technique that serves as a guide for biopsy as an adjunct to conventional endoscopy is greatly needed.

Since the early days of fiberoptic endoscopy, many techniques have been used to identify specific epithelia or to enhance mucosal surface characteristics. Magnification chromoendoscopy alone or combined with methylene blue or indigo carmine has been used to detect intestinal metaplasia in Barrett's oesophagus since 1994 and characteristic patterns (villous, ridged) have since been described. Using an adjustable image magnification in a continuous range up to 35× (M), combined with 1.5% acetic acid instillation, Guelrud *et al* described four different mucosal surface patterns and a sensitivity for specialised intestinal metaplasia of up to 100% when the ridged pattern was noted.<sup>5</sup> Highly magnified images (80× at maximum, L) with or without methylene blue using a magnifying endoscope fitted with a transparent cap allowed Endo *et al* to classify the superficial mucosal appearance of Barrett's epithelium by histological (gastric or intestinal) phenotypes.<sup>6</sup> In this issue of *Gut*,<sup>7</sup> magnification chromoendoscopy (115×, XL) is

proposed as a useful tool not only for the diagnosis of intestinal metaplasia but also for detection of high grade dysplasia [see page 24]. Also in this issue of *Gut*,<sup>8</sup> high resolution standard video endoscopy combined with methylene blue staining and tissue autofluorescence imaging proved inferior to stepwise four quadrant biopsies for surveillance in Barrett's oesophagus [see page 18]. How does the clinician reconcile these observations and incorporate the findings in their everyday practice? What does the future have to offer? Should endoscopists implement the newly proposed techniques? If yes, which one?

Chromoendoscopy has the advantages of simplicity, low cost, and safety but adds to the procedure time, requires reagents and supplies, and the volume, concentration, and dwell time for reagent use have not yet been standardised. Also, this method looks only at the mucosal surface and misses important subepithelial pathology. Furthermore, the interpretation of staining is still subjective due to differing definitions and staining criteria.<sup>14</sup> Methylene blue selectively stains intestinal metaplasia with up to 90% accuracy.<sup>15</sup> However, results of methylene blue directed biopsy were similar to conventional biopsy in detecting specialised intestinal metaplasia and low grade dysplasia.<sup>16</sup> Light or absent methylene blue staining with heterogeneity of uptake is associated with high grade dysplasia or cancer.<sup>17</sup> High magnification endoscopy also looks only at surface features and magnifies the image at the expense of reducing the field of view. A significant amount of additional time may be needed to adjust the image into focus, and a special endoscope adds cost to the procedure.

Light induced fluorescence is a promising method for guiding biopsy because it provides wide area surveillance and can visualise below the mucosal surface.<sup>18</sup> Fluorescence detects the presence of biomolecules associated with dysplasia rather than subcellular morphology, and thus extra high (XL) resolution is not required. In a promising case series, autofluorescence endoscopy using the LIFE-II imaging system in which blue light excites tissue autofluorescence, identified focal high grade dysplasia in Barrett's mucosa.<sup>19</sup> However, the image contrast with endogenous fluorescence alone may not be sufficient to obtain high sensitivity for detecting high grade dysplasia, as found by Egger and colleagues<sup>8</sup> in this issue of *Gut*, and further refinements may be needed. For example, addition of agents that label dysplasia such as 5-ALA<sup>15</sup> or optical reporter peptides<sup>20</sup> have the potential to significantly enhance high grade dysplasia detection. OCT has a sensitivity of 97% and a specificity of 92% for detection of intestinal metaplasia using specific

architectural criteria.<sup>21</sup> However, the resolution of this instrument is not sufficient to characterise subcellular structures, such as nuclei, that are important for histopathological evaluation. Higher resolution OCT systems are being developed but at a cost that is not practical for clinical use. High frequency ultrasound is good for detecting submucosal invasion and lymph node involvement, but also does not have sufficient resolution to detect dysplastic cells at an early stage. Confocal microscopy is an intriguing option that can image with subcellular resolution but this instrument is still in an early stage of development.

At this time, endoscopists and clinicians should resist the temptation to use these very promising technologies in making management decisions on their Barrett's oesophagus patients. The available data, although highly encouraging, are insufficient to allow us to draw conclusions about the best way to screen and survey these patients. As these technologies develop, the sensitivity for detection of intestinal metaplasia and high grade dysplasia will improve. The optimum method for surveillance of Barrett's oesophagus and dysplasia will likely evolve in the form of an imaging instrument that has wide area surveillance and penetration below the mucosal surface. A combination of approaches (autofluorescence enhanced by 5-ALA, magnification chromoendoscopy, etc) may be applied in the interim. It will take years before validation, comparison, and standardisation of all of these technologies brings the level of confidence that biopsy currently provides for all the end points listed in table 1. For the time being, in the deadly game of Barrett's oesophagus surveillance, the American actor and comedian Mae West (1892–1980) reminds with wit and wisdom that: “It is better to be looked over than overlooked . . .”

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Small intestine

## HLA-DQ8 as an Ir gene in coeliac disease

K E A Lundin

DQ8 restricted gliadin peptide is immunogenic in the intestinal mucosa of HLA-DQ8 positive patients, representing the first demonstration that a given peptide may be of pathogenic significance only for a subset of coeliacs, and strongly suggests that DQ2 and DQ8 act as immune response (Ir) genes in this disease

Coeliac disease (CD) is an excellent model for dissecting human immunological disease. It is one of the few human non-infectious diseases where the triggering agent can be removed and thereby a causal treatment exists—the gluten free diet. Removal of the harmful agent is followed by complete normalisation of the small intestinal mucosa. The subsequent introduction *in vivo* and *in vitro* of the disease process offers a unique possibility to dissect this process. Clearly, the *in vivo* short or long term challenge most directly addresses the range of possible pathological processes that eventually lead to the overt enteropathy. However, the *in vitro* challenge of the coeliac mucosa stands as an excellent surrogate system.

In this issue of *Gut*, Mazzarella and colleagues,<sup>1</sup> representing some of the leading groups in coeliac disease, present work on the coeliac mucosa in an *in vitro* challenge system [see page 57]. Such a challenge can be done on the damaged

mucosa from untreated patients (readout: lack of normalisation on withdrawal of gluten) or on a normalised mucosa from treated patients (readout: induction of pathology on introduction of gluten). The group chose the latter and investigated several aspects of immunological activation in biopsies following a 24 hour challenge. One of the few critical comments of this excellent study is that a bucket full of synthetic peptide was used for the challenge. As a putative immunodominant epitope, a concentration of 1 mg/ml is certainly very high and determination of a dose-response could clearly have been included.

The first important observation provided in this paper is that a peptide identified as an epitope for small intestinal T cells can also induce a mucosal response, strongly suggesting that the epitope is of immunobiological and immunopathological importance. The outcome of the challenge is identical regardless of the peptide being used in its native form or

in its deamidated form. The process of deamidation results in conversion of glutamines in the primary gliadin sequence to glutamic acid, leading to better binding of the peptides to HLA and more efficient recognition by gliadin specific T cells.<sup>2</sup> The present findings corroborate other data suggesting that the enzyme tissue transglutaminase deamidates gliadin *in situ*.<sup>3</sup> The study of Mazzarella *et al* is an important step forward, although regrettably it did not address in a dose-response assay if the already deamidated peptide was more potent than the native peptide. Similar experiments involving any of the other gliadin T cell epitopes have not been reported. As a natural extension of these studies, one could envisage that all newly described T cell epitopes should be studied in similar experiments. This would be a daunting task but certainly much more amendable than complete *in vivo* toxicity testing.<sup>4</sup> Such a combination of epitope identification using T cell clones and epitope confirmation using *in vitro* biopsy challenge will generate a valuable map of the gliadin fragments involved in disease pathogenesis.

The second important observation the authors make sheds light on a long-standing battle in progress within the inner circle of scientists and clinicians working with CD. Two mutually exclusive pathways have been suggested to contribute to the disease, as is clearly discussed in the paper. One is thought to be a rapid effect on the epithelium and intraepithelial T cells that involves several aspects of the innate immune system. The other is a delayed-type hypersensitivity-like reaction involving CD4<sup>+</sup> T cells that recognise processed gliadin peptides presented by HLA-DQ2 and HLA-DQ8. By and large, this pathway represents the adaptive immune

system. At the recent 10th International Symposium on Coeliac Disease in Paris, the present status of this battle was summarised by one speaker as: "It is the epithelium, after all!" However, good evidence that these two pathways are in reality distinct from one another is lacking. The present paper suggests that even an innate-type immune response such as induction of Fas on enterocytes is controlled by a distinct interaction between gliadin peptides, lamina propria, HLA-DQ8, and HLA-DQ8 restricted T cells. In fact, no signs of innate immune system activation were observed in DQ8 negative patients. Maybe "it is the adaptive immune system, after all?"

The third important aspect to this manuscript should arouse the immunological society. We know that whereas the vast majority of coeliacs carry HLA-DQ2, the rest carry DQ8. Individuals without DQ2 or DQ8 are extremely unlikely to develop CD. One important aspect that must be remembered is that most individuals with DQ2 and DQ8 will not go on to develop CD. However, the HLA association per se strongly suggests that DQ2 and DQ8 act as immune response (Ir) genes for the mucosal immune response. The term Ir genes was defined when

immunology as a science was in its infancy.<sup>3</sup> Later it was established that the Ir gene phenomenon was due to specific binding and presentation by antigenic peptides by the major histocompatibility complex encoded molecules.<sup>6</sup> Moreover, demonstration that the Ir gene phenomenon is operable in humans has been very difficult. Mazzarella *et al* demonstrated that a DQ8 restricted peptide acts pathogenically only in DQ8 positive and not in DQ8 negative coeliacs. This represents the first demonstration that a given peptide may be of pathogenic significance only for a subset of coeliacs, and strongly suggests that DQ2 and DQ8 act as Ir genes in the disease.

At the end of the day, our efforts will not be measured by how much we understand of CD but to what extent we can translate our knowledge for the benefit of patients. Through basic medical science we have learned a lot about the disease over the past 10 years. The present paper represents both good news and not so good news. On the positive side, our understanding of the disease has made a significant step forward. On the negative side, this paper clearly illustrates that any attempt to design methods of specifically intervening with the

disease process must take the HLA type of the patient into account. This confirms the fears that this difficult task has just become more complex.

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#### Cancer

## A rat virus visits the clinic: translating basic discoveries into clinical medicine in the 21st century

**C R Boland, A Goel**

Mutant forms of the KRAS2 gene are present in the serum of patients who have undergone putatively curative surgery for colorectal cancer and may be used to guide novel therapies in the future by identifying those individuals at greatest risk of recurrence

One of the holy grails of biomedical research is to identify markers of occult disease that might lead to early treatment of that disease before the manifestations are overt—and ipso facto incurable. In this issue of *Gut*, Ryan and colleagues<sup>1</sup> from Dublin report that one more application of basic science discovery might be ready for use in the management of patients with colorectal cancer [see page 101]. These investigators have found that mutant forms of the KRAS2 gene are present in the serum of patients who have undergone putatively curative surgery

for colorectal cancer. This information can predict tumour recurrences and, by inference, might be used to guide novel therapies in the future by identifying those individuals at greatest risk of recurrence. We have all waited patiently for the fruits of the molecular biology revolution that began nearly two decades ago, and it may be worthwhile to assess the tempo by which these discoveries make their way from the bench to the clinic.

The RAS gene family encodes for a series of at least 50 guanosine triphosphatases (GTPases) which are small proteins involved in the regulation of

growth and other biological activities.<sup>2</sup> Their link to cancer required a circuitous route through what might have seemed at the time to be obscure basic research. In the 1960s and 1970s, several viruses had been identified that cause sarcomas in rodents and birds. By the late 1970s, it was found that some of these viruses carried single copies of altered genes—viral oncogenes—that were responsible for the transformation process.<sup>3,4</sup> By 1980, two members of the RAS family were identified as oncogenes in rats; in fact, the family name for these genes was derived from *rat sarcoma*.<sup>5</sup> The genes had been hijacked from the mammalian genome by the Harvey (HRAS) and Kirsten (KRAS) transforming viruses but in mutant forms that abrogated the GTPase activity of the protein, and converted them from regulators of signal transduction to oncogenic proteins. Finding mutant RAS genes in oncogenic viruses led to a search for mutant forms in the cellular DNA from human tumours. By 1987, two laboratories reported that approximately half of all colon cancers had mutant copies of KRAS.<sup>6,7</sup> The technical challenges involved were not trivial, and in one of these papers was a very early application of the polymerase chain reaction (PCR) to solve a problem that was limited by the modest amount of DNA that could be retrieved from a surgically excised tumour sample.<sup>6</sup>

By 1992, KRAS mutations had been found in the stools of patients with colorectal neoplasia,<sup>8</sup> and by 1996, mutant forms of KRAS were reported from the plasma or serum of some cancer patients.<sup>9</sup> It had been known for several decades that circulating tumour cells could be seen in the blood of patients undergoing colorectal cancer surgery; interestingly, this did not predict tumour recurrence.<sup>10</sup> Before long, groups reported that mutated forms of the tumour suppressor genes APC and p53 were also found in the blood of colorectal cancer patients.<sup>11</sup> The attractiveness of KRAS mutations is that the hunt for mutations can be limited, and is clinically more feasible.

What have Ryan *et al* added to this several decade long saga that stretches from mouse sarcomas to human colon cancers? There is a prospective study in which KRAS mutations were used to predict tumour recurrence. Firstly, even control subjects have wild-type (that is, normal) copies of KRAS in serum. The investigators have focused upon mutant forms of the gene in cancer patients, which is detected by strategic designing of the PCR primers. They demonstrated that 53% of the group of 78 patients who could be studied preoperatively had KRAS mutations in their tumour sample, and that in 76% of these patients a matching mutation was found in serum (which was 41% of all preoperative sera). In the larger group of 94 patients in which there was a putatively curative resection, 64% had KRAS mutations in the tumour. The serum became persistently KRAS mutation positive in the postoperative period in 27% of these 60 patients. The novel finding was that 63% of the mutation positive group developed recurrent cancer whereas only 2% of those whose blood remained negative did so. There was no predictive value

for those tumours that did not have a KRAS mutation. One can only speculate on what is occurring in patients with these mutations in the postoperative blood, but do not develop tumour recurrences.

What is the clinical significance of this study? This is a suitably large study, with a prospective design. Persistently positive mutant KRAS in the blood was valuable in predicting tumour recurrence although, as the authors note, not substantially better than using carcinoembryonic antigen. However, a gene based approach has the potential to be made more sensitive. Time will tell. KRAS mutations were also found in the blood of patients with large or villous adenomas but the clinical utility of a blood test for benign lesions is not likely to be high. Moreover, there is no avoiding the issue that about half of colorectal cancers do not harbour KRAS mutations, and the blood test is of no value in these cases. The principal value implied here is that one may be able to detect recurrences earlier, and more sensitively than with other diagnostic modalities, but that remains to be proved.

Perhaps the most illuminating issue is how long it takes for basic science discoveries to be translated to clinical medicine. The use of KRAS for colorectal cancer diagnostics was limited perhaps by those who were discouraged by the fact that only half of colon cancers carry these mutations. As we understand more about the complexity of tumour development, we may discover that a battery of markers will be required to manage our patients, and we may eventually not only accept this limitation but embrace the fact that the mutant KRAS approach may be useful fully for half of our patients at risk of tumour recurrence. These approaches will be more valuable

when we finally develop more effective means for treating early signs of recurrence. Optimists will gamely await progress on both fronts.

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