Studies of the Pathophysiology of Barrett's Oesophagus

Lisa Helen Moyes

MBChB, BSc (Hons)

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1) **Moyes LH**, McEwan H, Radulescu S, Pawlikowski J, Lamm CG, Nixon C, Sansom OJ, Going JJ, Fullarton GM, Adams PD. Activation of Wnt signalling promotes development of dysplasia in Barrett's oesophagus. *Journal of Pathology 2012;* 228(1):99-112.

2) **Moyes LH**, Going JJ. Goblet cells in Barrett's oesophagus: cancer precursor, risk marker or irrelevance? *Diagnostic Histopathology 2012*; 18(12); 503-512.

3) **Moyes LH**, Going JJ. Still waiting for predictive biomarkers in Barrett's oesophagus. *Journal of Clinical Pathology 2011*; 64:742-750.

4) **Moyes LH**, Going JJ, Stuart RC, Fullarton GM. Deprivation and dysplasia are associated with disease progression in Barrett's oesophagus. *In submission*

List of Presentations

1) The role of Wnt signalling and p16INK4a in Barrett's oesophagus and oesophageal cancer. LH Moyes, C Nixon, S Radelescu, OJ Sansom, GM Fullarton, PD Adams. Presented at Mouse Model Symposium, Beatson Institute for Cancer, September 2010

2) The role of Wnt signalling in the development and progression of Barrett's oesophagus. LH Moyes, C Nixon, JJ Going, GM Fullarton, PD Adams
Presented at International Cancer Meeting, University of Glasgow, July 2011

List of Abbreviations

AC	Adenocarcinoma
ACG	American College of Gastroenterology
AF	Autofluorescence
AFI	Autofluorescence imaging
AGA	American Gastroenterological Association
APC	Adenomatous polyposis coli
ASGE	American Society of Gastroenterological Endoscopy
BMI	Body mass index
BSG	British Society of Gastroenterologists
Cdk	Cyclin dependent kinase
CLO	Columnar lined oesophagus
CM	Columnar mucosa
CT	Computed tomography
EMR	Endoscopic mucosal resection
EUS	Endoscopic ultrasonography
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridisation
FSDE	French Society of Digestive Endoscopy
GI	Gastrointestinal
GO	Gastroesophageal
GSK3	Glycogen synthase kinase 3
HET	Heterozygous
HGD	High grade dysplasia
HOM	Homozygous
HRE	High resolution endoscopy
IM	Intestinal metaplasia
IQR	Interquartile range
LGD	Low grade dysplasia
LOH	Loss of heterozygosity
NBI	Narrow band imaging
NS	Not significant
OA	Oesophageal adenocarcinoma
OGD	Oesophagogastroduodenoscopy
P	p value
PET	Positron emission tomography
PPI	Proton pump inhibitor
RFA	Radiofrequency ablation
SC	Squamocolumnar
TCF	T cell factor
WT	Wild type

Summary

Barrett's oesophagus is a common condition in which the normal stratified squamous oesophageal epithelium is replaced by metaplastic reflux-induced glandular ("columnar") mucosa (Jankowski, Barr, Wang et al. 2010;Playford 2005). Over the last three decades, the incidences of oesophageal adenocarcinoma (OA) and Barrett's oesophagus have risen to the point that OA is now common in the United Kingdom, with Scotland having one of the highest rates in the world (Jankowski, Provenzale, & Moayyedi 2002). Unfortunately most cancers present at an advanced stage with five year survival less than 30% (Holmes and Vaughan 2007). Barrett's oesophagus is associated with malignant progression via a recognised metaplasia-dysplasia-carcinoma sequence (Jankowski, Wright, Meltzer et al. 1999). The premalignant nature of Barrett's oesophagus has powered intense clinical interest in the hope of eventually having an impact on the earlier diagnosis and treatment of dysplasia, and ultimately the prognosis of oesophageal adenocarcinoma.

Despite years of research interest, Barrett's oesophagus remains an enigmatic condition. The exact incidence is unknown, and it is recognised that not all patients with Barrett's oesophagus will progress to adenocarcinoma. Current strategies aim to ascertain the presence of dysplasia, the current gold standard marker of malignant progression. However although Barrett's mucosa is visible at endoscopy, the presence of dysplasia is difficult to diagnose as these areas tend to be focal and inconspicuous to the naked eye. Current systematic biopsy regimes are recommended, but can be fraught with sampling errors. Furthermore, the molecular mechanisms underlying Barrett's metaplasia and progression to dysplasia remain unclear. Molecular risk biomarkers have been sought with modest success, and at present dysplasia remains the most reliable clinical marker. However dysplasia itself is not without limitations: focal dysplasia can be difficult to ascertain, with many biopsies sometimes necessary to detect it reliably (Abela, Going, Mackenzie et al. 2008). Inter-observer variability may cause over or under diagnosis, especially regarding LGD (Flejou 2005). Moreover, although patients with HGD are at elevated risk of progression to OA, few studies provide reliable data on rates of progression

from HGD to OA, with estimates varying between 16-59% at five years (Reid, Blount, Feng et al. 2000;Schnell, Sontag, Chejfec et al. 2001;Shaheen and Richter 2009;Spechler SJ 2011). There is a real need, therefore, to be able to identify and treat those patients at greatest risk of malignant transformation, and reassure those at low risk. Without an improved molecular understanding of Barrett's metaplasia and progression to neoplasia, clinically useful prognostic biomarkers (allowing appropriate targeting of surveillance and therapy) will be delayed.

The current challenges associated with Barrett's oesophagus are 1) to accurately determine the rate of malignant progression of Barrett's oesophagus and identify clinical risk factors, 2) to improve the endoscopic detection of dysplasia and early neoplasia allowing earlier diagnosis and treatment and, 3) to understand the molecular mechanisms involved in the initiation of Barrett's metaplasia, and the pathways involved in disease progression.

In an attempt to improve the care of patients with Barrett's oesophagus within the West of Scotland, my thesis will address each of the main challenges associated with this puzzling condition at clinical, endoscopic and molecular levels. The hypotheses of my thesis are threefold -

a) Patients with Barrett's oesophagus in the West of Scotland have high rates of progression to high grade dysplasia and oesophageal adenocarcinoma.
b) The WavSTAT optical biopsy system will be able to correctly identify non-dysplastic and dysplastic Barrett's oesophagus.
c) The Wnt signalling pathway is upregulated in Barrett's oesophagus and dysplasia.

The aims of my thesis are as follows:

1) To present a general overview of the Barrett's literature highlighting current clinical challenges
2) To examine the incidence of dysplasia and oesophageal adenocarcinoma in the West of Scotland by analysing a cohort of patients undergoing surveillance endoscopy
3) To review the current endoscopic imaging adjuncts for the diagnosis of Barrett's oesophagus and dysplasia, and assess the role of optical biopsy forceps in determining the presence of dysplasia

4) To evaluate the role of Wnt signalling in Barrett's oesophagus, from metaplasia to carcinoma in a mouse model, with complementary human studies

Chapter 1 introduces the reader to Barrett's oesophagus and highlights current areas of clinical challenge and debate. A universal definition of Barrett's oesophagus does not exist and Chapter 2 explores the need for the presence of intestinal metaplasia in the diagnosis of Barrett's oesophagus. Chapters 3 and 4 present original data from a West of Scotland Barrett's oesophagus database, specifically analysing rates of dysplasia and adenocarcinoma and cause of death. This study suggests patients with Barrett's oesophagus in the West of Scotland are at high risk of disease progression with almost 10% of patients dying from oesophageal adenocarcinoma. The results highlight the importance of a comprehensive surveillance in our "high risk" population - an ideal niche for future chemopreventative and molecular studies. In an attempt to improve the diagnosis of dysplasia in our West of Scotland population, Chapter 5 reviews current endoscopic imaging adjuncts used in research and clinical practice while Chapter 6 presents original data from a pilot study assessing the use of innovative optical biopsy forceps in the endoscopic diagnosis of dysplasia. While this technology is in its infancy and further changes in the algorithm are required, the optical forceps could be a promising tool for ongoing surveillance in high risk Barrett's patients. Chapter 7 summarises the role of biomarkers in Barrett's oesophagus, reviewing the literature and highlighting the lack of clinically useful markers of disease progression to date. The Wnt signalling pathway plays an important role in normal oesophageal (and intestinal) development, yet when aberrantly activated leads to carcinogenesis. To date, very little is known about the role of Wnt signalling in Barrett's oesophagus. Chapter 8 presents the results of a mouse model of upregulated Wnt signalling and the interesting finding of dysplasia within the oesophageal mucosa. Chapter 9 therefore translates these results to the human population by assessing the role of Wnt signalling in Barrett's metaplasia and dysplasia by immunohistochemical analysis of a panel of markers. The results suggest Wnt signalling is upregulated in Barrett's dysplasia, particularly in high grade, and this may have a future role as a biomarker. Chapter 10 summarises the main findings of the thesis, and presents future directions.

Chapter 1

Barrett's Oesophagus : A review

Barrett's oesophagus is an acquired condition in which the normal stratified squamous lining of the oesophagus is replaced by a glandular ("columnar") epithelium (Jankowski, Barr, Wang, & Delaney 2010). Barrett's "columnar lined" oesophagus is a common condition and is of clinical significance due to its predisposition to oesophageal adenocarcinoma. Over the last three decades the incidences of adenocarcinoma and Barrett's oesophagus have risen to the point that oesophageal adenocarcinoma is now one of the commonest cancers in the United Kingdom, with Scotland having one of the highest rates in the world (Jankowski, Provenzale, & Moayyedi 2002;www.cancerresearchuk.org 2006). Unfortunately most cases of adenocarcinoma present at an advanced stage and the prognosis is poor with five year survival rates of less than 30% (AUGIS, BSG, & NCASP 2010;Holmes & Vaughan 2007). The premalignant nature of Barrett's oesophagus has powered an intense clinical interest within this field, in the hope of eventually having an impact on the earlier diagnosis and treatment of dysplasia and ultimately the prognosis of oesophageal adenocarcinoma. This first chapter provides a review of the literature, highlighting current clinical challenges and areas requiring further study.

1.1 The History of Barrett's Oesophagus

In 1906, Wilder Tileston a Boston pathologist described three cases of peptic ulceration of the oesophagus, noting "the close resemblance of the mucous membrane about the ulcer to that normally found in the stomach" (Tileston 1906). In 1950, Norman Barrett proposed that clinicians should define the oesophagus as "that part of the foregut, distal to the cricopharyngeal sphincter, which is lined by squamous epithelium" (Barrett 1950). He went on to describe a case of peptic ulceration within a tubular organ arising from a gastric type epithelium associated with an oesophageal stricture. From his previous observations that the oesophagus was lined with squamous epithelium, he concluded that the tubular viscus was a segment of stomach that had been tethered within the chest, presumably a congenitally short oesophagus. This paper was one of the first to describe the association between oesophagitis, reflux disease and hiatus hernia.

In 1953 Allison and Johnstone argued that the tubular, columnar lined structure described by Barrett was actually "an oesophagus lined with gastric mucous membrane" (Allison and Johnstone 1953). They showed that the tubular structure was not covered by peritoneum, and contained submucosal glands as would be found in the oesophagus. Perhaps to placate Barrett, the editor of Thorax, they suggested that ulceration in the columnar lined oesophagus be termed "Barrett's ulcers". By 1957, Barrett accepted Allison and Johnstone's theory and suggested the disease be called "lower oesophagus lined by columnar epithelium". However, the name "Barrett's oesophagus" has stuck even though the present concept is not as Barrett initially described (Barrett 1957).

Over the following decade, further studies supported the view that "columnar lined oesophagus" (CLO) was an acquired sequel of reflux. Moersch et al reviewed 36 specimens from patients who had undergone oesophageal resection for oesophagitis, concluding that the columnar mucosa of Barrett's oesophagus was acquired following repeated exposure of the distal oesophagus to gastric refluxate (Moersch, Ellis, & McDonald 1959). Bremner and colleagues confirmed the theory in an animal model in 1970 and the congenital theory was globally discarded (Bremner, Lynch, & Ellis 1970).

1.2 The Diagnosis of Barrett's Oesophagus

1.2.1 The definition of Barrett's oesophagus

Norman Barrett commenced his landmark paper of 1957 with the following words: "*This paper concerns a condition whose existence is denied by some, misunderstood by others, and ignored by the majority of surgeons*" (Barrett 1957). These words still apply today as no universally accepted definition of Barrett's oesophagus currently exists.

The American College of Gastroenterology (ACG) suggest "*Barrett's oesophagus is a change in the distal oesophageal epithelium of any length that can be recognized as columnar type mucosa at endoscopy and is confirmed to have intestinal metaplasia by biopsy of the tubular oesophagus*" (Wang and Sampliner 2008). The American Gastroenterological Association (AGA), the American Society of Gastrointestinal Endoscopy (ASGE) and the French Society of Digestive Endoscopy (FSDE) have similar definitions requiring the presence of intestinal metaplasia on biopsy (Boyer, Laugier, Chemali et al. 2007;The AGA Institute Medical Position Panel 2011;The Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy 2006). The requirement for identification of intestinal metaplasia on biopsy is not demanded by the British Society of Gastroenterology (BSG) who define Barrett's oesophagus as "*an endoscopically apparent area above the oesophagogastric junction that is suggestive of Barrett's which is supported by the finding of columnar lined oesophagus on histology"* (Playford 2005). The Montreal workshop (the Global Evidence Based Consensus Workshop on the Definition and Classification of Reflux Disease) recently proposed that the term "Barrett's oesophagus" should be applied if any type of oesophageal columnar metaplasia is confirmed histologically, with qualification if an intestinal-type metaplasia is present (Vakil, van Zanten, Kahrilas et al. 2006).

1.2.2 The endoscopic assessment of Barrett's oesophagus

The diagnosis of Barrett's oesophagus requires two components

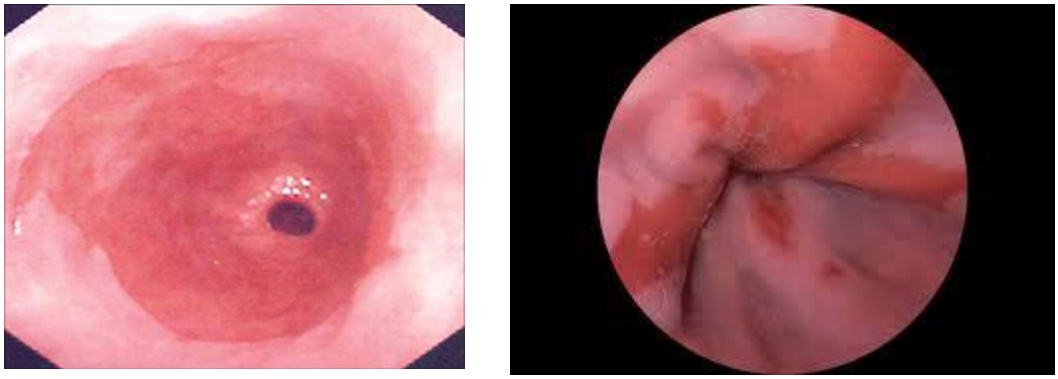
a) the direct visualisation of the oesophagus by endoscopy and

b) the histological assessment of biopsy samples.

Under normal conditions the squamocolumnar (SC) junction (the intersection between the squamous lining of the oesophagus and the columnar lining of the stomach) and gastroesophageal (GO) junction are located at the same level, whereas in Barrett's oesophagus the SC junction migrates proximally. Endoscopic assessment of the normal oesophagus reveals a white or light pink colouring of the squamous mucosa, while in Barrett's oesophagus the salmon pink columnar epithelium extends proximally into the oesophagus (Figure 1.1). Using high resolution white light endoscopy the oesophagus should be carefully examined paying particular attention to appropriate landmarks, namely the SC junction and GO junction. The length of the Barrett's segment is noted from the level of the most proximal gastric folds on minimal insufflation to the level of the proximally placed SC junction, and whether the columnar mucosa is circumferential or projecting as tongues of Barrett's mucosa. In order to standardise the endoscopic reporting of Barrett's oesophagus, the Prague classification should be used to assess the circumferential (C) and maximum (M) extent of the endoscopically visualised segment (Figure 1.2) (Sharma, Dent, & Armstrong 2006).

Figure 1.1 Barrett's oesophagus at endoscopy

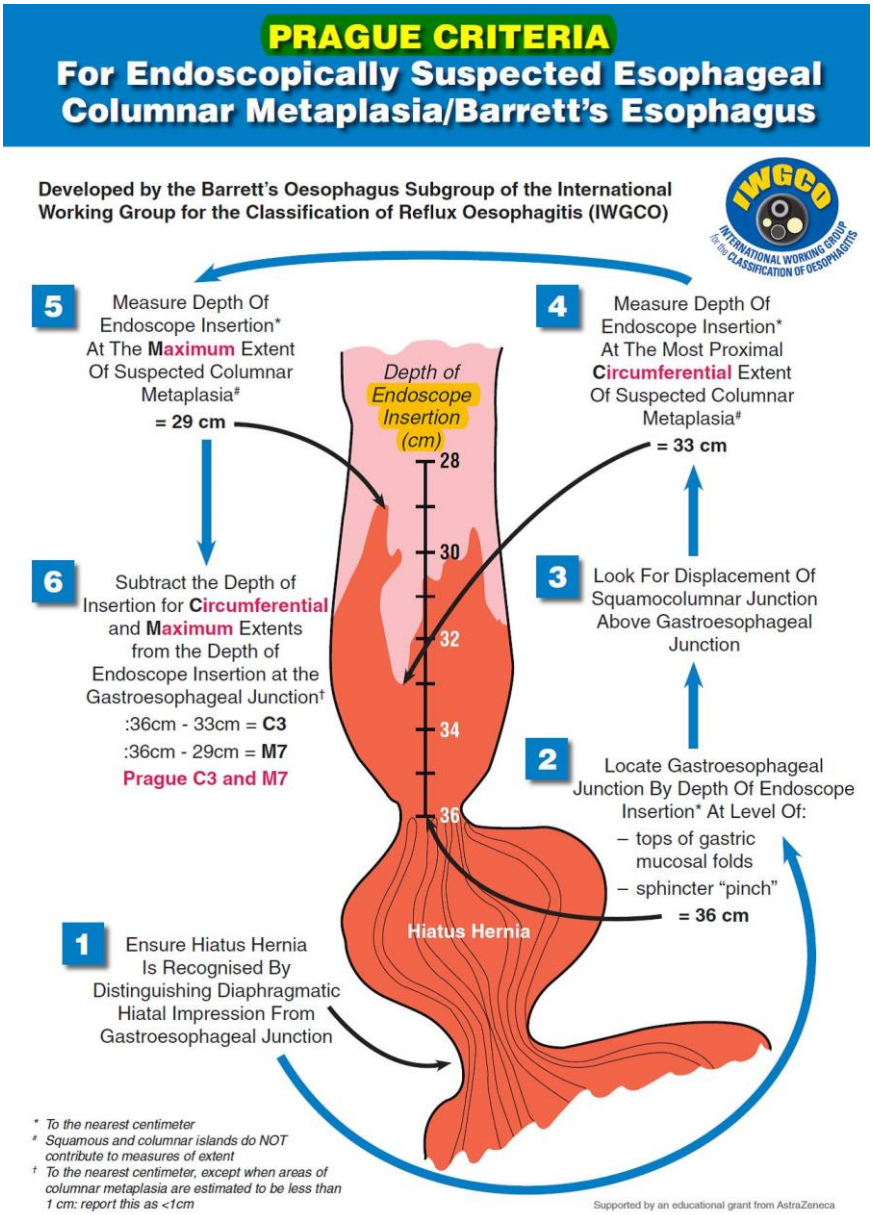
A B



(A) Endoscopic view of oesophagus noting salmon pink colour of Barrett's segment.

(B) Endoscopic view of Barrett's tongues at oesophagogastric junction.

Figure 1.2 The endoscopic assessment of Barrett's oesophagus



Endoscopists should start at Box 1 working anticlockwise finishing at Box 6. This allows standardised assessment and reporting of Barrett's oesophagus. Taken with permission from International Working Group for the Classification of Oesophagitis (www.iwgco.com).

The Barrett's segment should be carefully assessed with image adjunct tools such as narrow band imaging for any mucosal nodularity, ulceration or discrete lesions with extensive biopsies of these areas as there is evidence these lesions are associated with dysplasia and intramucosal cancer (Reid, Blount, Feng, & Levine 2000). Following careful endoscopic inspection, most centres recommend a systematic biopsy approach, with four quadrant biopsies taken every two centimetres within the Barrett's segment (Playford 2005;Wang & Sampliner 2008). Barrett's oesophagus, and in particular dysplasia can be focal and patchy, and a systematic approach has been shown to detect more dysplasia and early cancer than a random biopsy approach (Abela, Going, Mackenzie, McKernan, O'Mahoney, & Stuart 2008;Fitzgerald, Saeed, Khoo et al. 2001).

1.2.3 The histological assessment of Barrett's oesophagus

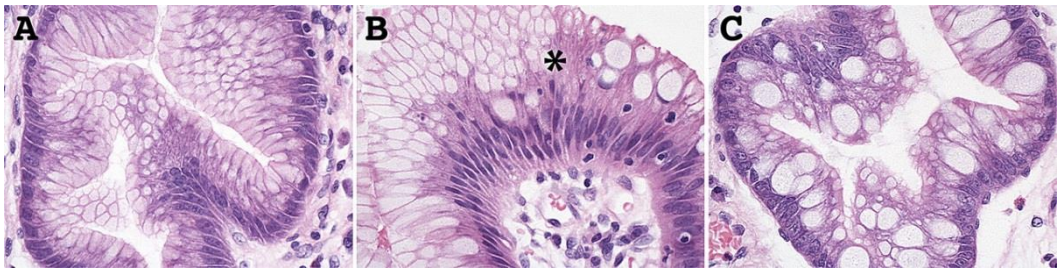
The second component in establishing the diagnosis of Barrett's oesophagus is the accurate histological assessment of biopsy material. Oesophageal biopsies from patients with Barrett's oesophagus are assessed by a specialist upper gastrointestinal pathologist ideally with an interest in the condition. The histological diagnosis of Barrett's oesophagus is made by the identification of an oesophageal columnar epithelium on sections stained with haematoxylin and eosin. It is vital to ensure that biopsy specimens are labelled appropriately, defining the exact location of the biopsy, as intestinal metaplasia of the cardia is a different entity from intestinal metaplasia of the oesophagus.

The histology of Barrett's mucosa is complex and heterogenous. Following Allison and Johnstone's initial description of an oesophagus lined with a columnar epithelium, other clinicians have discovered a mosaic of histological types. In 1976 Paull and colleagues provided a clear histological description of columnar lined oesophagus from a case series of 11 patients with Barrett's epithelium, concluding there were three types of columnar epithelia above the lower oesophageal sphincter: 1) gastric fundic type with parietal and chief cells, 2) junctional type with cardiac mucous glands and 3) a distinctive so called "specialised" type with mucous glands and intestinal-like goblet cells (Paull, Trier, Dalton et al. 1976) (Figure 1.3). Goblet cells can be identified as barrel-shaped cells containing an acidic mucin which stains with Alcian blue pH2.5. The staining largely depends on the type of

mucopolysaccharide within the crypt: sialylated mucins stain blue and sulphated mucopolysaccharides a brown-black colour (Figure 1.4). This special stain can help pathologists clearly identify the presence of goblet cells.

At the time of Barrett's original description and other early studies, Barrett's oesophagus was an endoscopic diagnosis and its histology of purely academic interest. However events changed in the 1970s when it became apparent an association between Barrett's oesophagus and oesophageal adenocarcinoma existed, and the histological findings, particularly of intestinal metaplasia with its neoplastic potential, became paramount (Haggitt, Tryselaar, Ellis et al. 1978).

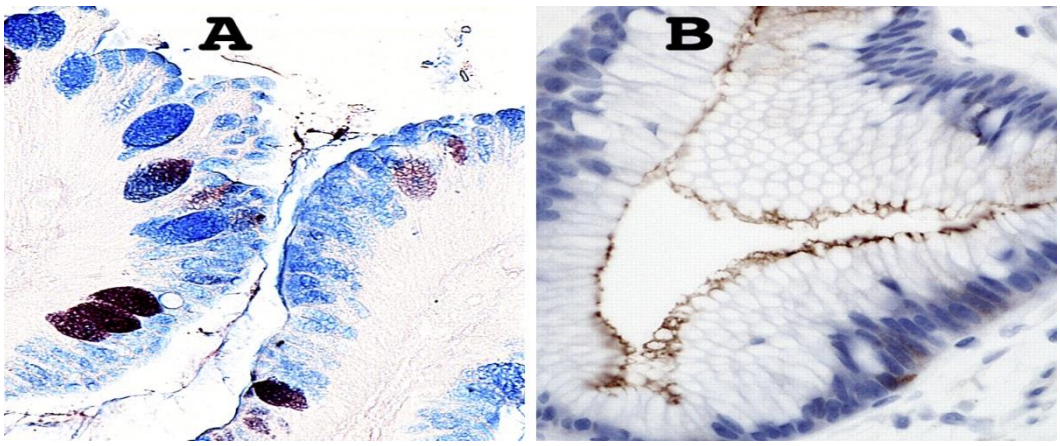
Figure 1.3 The histological subtypes of Barrett's metaplasia



Display of the characteristic histological diversity found within Barrett's oesophagus.

A. Cardiac like crypt without goblet cells. B. Point (*) marks area on mucosal surface where cells from crypt in A meet cells from crypt in C. C Intestinal crypt with numerous goblet cells, in keeping with intestinal metaplasia. Figure taken from (Moyes and Going 2011)

Figure 1.4 Mucosal phenotypic diversity in Barrett's oesophagus using Alcian blue stain



A. Alcian blue (pH2.5) staining of goblet cells. The sialylated mucopolysaccharides stain blue, and sulphated stain brownish-black. This section clearly shows the presence of goblet cells, while specimen B demonstrates a cardiac-like phenotype with an absence of immunostaining. Figure taken from (Moyes & Going 2011)

1.3 Dysplasia

Barrett's oesophagus appears to progress in a stepwise manner with cellular changes ranging from metaplasia, to low grade dysplasia, to high grade dysplasia and ultimately invasive cancer – known as the metaplasia-dysplasia-carcinoma sequence (Jankowski, Wright, Meltzer, Triadafilopoulos, Geboes, Casson, Kerr, & Young 1999). Dysplasia is one of the most important features of Barrett's oesophagus as it currently remains the gold standard marker of risk for disease progression. The presence of dysplasia also determines the frequency of surveillance endoscopy and guides management options.

Dysplasia may be viewed as "the histological expression of genetic changes which favour cell growth and neoplasia" (Noffsinger 2008;Spechler 2001). Dysplasia is diagnosed in the presence of cytological and architectural changes such as nuclear enlargement, hyperchromatism, surface maturation, atypical mitosis and loss of cytoplasmic maturation (Goldblum 2003). These changes suggest that the epithelium is damaged, after undergoing a series of genetic and epigenetic changes resulting in the clonal proliferation of cells with a predisposition to malignancy. Dysplasia is generally categorized as low or high grade depending on the degree of genetic damage, the potential for carcinogenesis and is diagnosed according to histological changes. Most pathologists with an interest in Barrett's oesophagus would classify dysplasia according to the Vienna classification system (Table 1.1) (Schlemper, Riddell, & Kato 2000).

Studies have shown that the extent of dysplasia is an important risk factor for disease progression. A recent large scale study from Seattle showed that the extent of low grade dysplasia was a significant risk factor for the development of oesophageal adenocarcinoma (Srivastava, Hornick, Li et al. 2007). They also found that although high grade dysplasia is associated with a greater risk of neoplasia, the actual extent of high grade dysplasia was not an independent risk factor for progression.

Table 1.1 Vienna classification of gastrointestinal epithelial neoplasia

Category		Subclassification
1	Negative for neoplasia/dysplasia	
2	Indefinite for neoplasia/dysplasia	
3	Non-invasive low grade neoplasia	
4	Non-invasive high grade neoplasia	4.1 High grade dysplasia
		4.2 Non-invasive carcinoma (carcinoma in situ)*
		4.3 Suspicion of invasive carcinoma
5	Invasive neoplasia	5.1 Intramucosal carcinoma $
		5.2 Submucosal carcinoma or beyond

* Non-invasive indicates absence of evident invasion and $ intramucosal indicates invasion into the lamina propria or muscularis mucosae. (Adapted from original manuscript by RJ Schlemper, Gut 2000 (Schlemper, Riddell, & Kato 2000))

1.3.1 The challenges of dysplasia in Barrett's oesophagus

The diagnosis of dysplasia itself however can be challenging as it may be difficult for pathologists to distinguish between reactive changes associated with reflux oesophagitis and low grade dysplasia. Interobserver agreement between pathologists regarding low grade dysplasia can be less than 50%, although the rates of concordance are higher with high grade dysplasia (Kerkhof, Kusters, van Dekken et al. 2007;Spechler 2001). The diagnosis of low grade dysplasia is often inaccurate when made by pathologists without expertise in Barrett's oesophagus. Studies from the Netherlands have shown that 85% of low grade dysplasia cases diagnosed by general pathologists were downgraded to "no dysplasia" on review by expert pathologists (Curvers, ten Kate, Krishnadath et al. 2010). These findings are in keeping with results from Germany and the United States (Alikhan, Rex, Khan et al. 1999;Vieth 2007).

The second difficulty with diagnosing dysplasia is due to sampling error. Unlike the colon where early cancers often take the form of polyps, early dysplasia and carcinoma in Barrett's oesophagus may be difficult to identify as it is flat, patchy and relatively inconspicuous (Montgomery, Goldblum, & Greenson 2001). Endoscopists are encouraged to perform extensive four quadrant biopsy to maximize the chance of identifying any inconspicuous lesion. However this technique is not without fault and areas of dysplasia or indeed a focus of invasive cancer can be missed. In a series of patients undergoing oesophagectomy for high grade dysplasia, invasive cancer was found in 30-40% of cases after pathological examination of the resected specimen (Spechler SJ 2011).

The third challenge which dysplasia presents is the lack of correlation between the presence of dysplasia and the clinical outcome. Even if the diagnosis of dysplasia is accurately made, not all patients will progress through the metaplasia-dysplasia-carcinoma sequence (Coppola, Nasir, & Turner 2010). Indeed most patients with Barrett's oesophagus will never develop oesophageal cancer. Furthermore, some patients with previously documented evidence of dysplasia, often reveal no dysplasia on subsequent endoscopies. It is unclear if this phenomenon is purely due to sampling error, or whether the dysplasia has actually regressed. Adenocarcinomas have been found in patients whose previous endoscopies have never shown any dysplasia (Reid, Li, Galipeau et al. 2010). In this case it is not known if this too is due to sampling error, or a result of rapid progression between surveillance

endoscopies, or if the cancer has completely bypassed the dysplastic stage (Montgomery, Goldblum, & Greenson 2001). Additional techniques to improve the diagnosis of dysplasia are clearly required.

1.4 The Epidemiology of Barrett's Oesophagus

1.4.1 The incidence and prevalence of oesophageal adenocarcinoma and Barrett's oesophagus

The incidence of oesophageal adenocarcinoma has risen over the last three decades in Western Europe and the United States, with the United Kingdom now having the highest incidence of cancer in the world (12-16/100 000 cases) (Jankowski, Provenzale, & Moayyedi 2002). Oesophageal adenocarcinoma is more common in males although the reasons for this remain unclear (Bollschweiler, Wolfgarten, Gutschow et al. 2001;Derakshan, Liptrot, Paul et al. 2009). The survival from oesophageal adenocarcinoma remains poor as patients often present at an advanced stage with overall survival rates of less than 30% at five years (Jamieson, Mathew, Ludemann et al. 2004). Studies have shown that survival is inversely related to tumour stage and the presence of lymph node metastases, with T1 stage disease carrying five year survival rates of more than 90% (Farrow and Vaughan 1996;Liu, Hofstetter, Rashid et al. 2005). Therefore detection of oesophageal adenocarcinoma at an earlier stage may improve the survival among patients.

Barrett's oesophagus is a common condition with 10-20% of patients undergoing endoscopic examination for reflux symptoms found to have the disease, but the true incidence of Barrett's oesophagus in the general population remains unknown (Spechler 2002). One of the most accurate population studies from Sweden suggests that the incidence of Barrett's oesophagus in 3000 adults sampled was 1.6% (Ronkainen J, Aro, Storskrubb et al. 2005). However these results may be a little low as the study group was highly selective, with patients included if they had only intestinal metaplasia and the number of biopsies taken was limited. Nevertheless, similar results have been found in Italy and China (Zagari, Fuccio, Wallander et al. 2008;Zou, He, & Ma 2011). An American study invited 961 patients undergoing colonoscopy to undergo elective gastroscopy to detect Barrett's oesophagus. The overall prevalence of Barrett's oesophagus was 6.8% with 5.2% of the asymptomatic population having short segment disease, and only 5.7% of those complaining of heartburn with short segment disease (Rex, Cummings, & Shaw 2003). One of the challenges associated with unravelling the true incidence of Barrett's oesophagus within the general population is that the condition is often asymptomatic with 46.2% of patients reporting no reflux symptoms (Reid, Li, Galipeau, & Vaughan 2010).

1.4.2 Risk factors associated with Barrett's oesophagus

The development of oesophageal adenocarcinoma is associated with four main risk factors – gastroesophageal reflux disease, obesity, smoking and a diet low in fruit and vegetables (Reid, Li, Galipeau, & Vaughan 2010). The conventional risk factors for the development of Barrett's oesophagus include male sex, advancing age, history of reflux disease, ethnicity (white) and tobacco use (Shaheen & Richter 2009). The symptom severity of gastroesophageal reflux disease does not seem to relate to the presence of Barrett's oesophagus, although symptom frequency and chronicity (total years with reflux symptoms) are better predictors of the presence of the disease (Conio, Filiberti, & Blanchi 2002). The use of non-steroidal anti-inflammatory drugs (NSAIDS) may be associated with a decreased risk of Barrett's oesophagus and disease progression, and the awaited results of the AspECT trial will help to evaluate the role of low dose aspirin and proton pump inhibitors as chemopreventative agents (Jankowski and Barr 2006;Nguyen, Richardson, & El-Serag 2010).

Some reports suggest that a higher proportion of first degree relatives of patients with Barrett's oesophagus have the condition and studies suggest a genetic component to oesophageal adenocarcinoma (Reid, Li, Galipeau, & Vaughan 2010). However further research is required to fully understand and identify the "at risk" genes. An increasing body mass index (BMI) is associated with reflux disease, Barrett's oesophagus and oesophageal adenocarcinoma. A population study reported an association between Barrett's oesophagus and increasing waist-to-hip ratio, suggesting that the high risk of Barrett's was due to central adiposity (Edelstein, Farrow, Bronner et al. 2007).

Unfortunately these risk factors only point to the presence of Barrett's oesophagus and are neither sensitive nor specific enough for identifying individuals at high risk of progression to cancer. Therefore at present, all patients with Barrett's oesophagus are enrolled in an endoscopic surveillance programme.

1.4.3 Risk Factors Associated with Disease Progression

The vast majority of patients with Barrett's oesophagus will not develop oesophageal adenocarcinoma. Over the last decade many groups have investigated factors which may predict progression to dysplasia and adenocarcinoma, with variable success. Predictors of progression in Barrett's oesophagus can be classified into patient factors, endoscopic predictors, pathological predictors and biomarkers.

Patient Factors

Although several groups suggest increasing age is a risk factor for dysplasia and adenocarcinoma, there is insufficient data from well conducted studies to support this. It is recognised that a male predominance is associated with adenocarcinoma, and this also seems to be a risk factor for progression to dysplasia (Prasad, Bansal, Sharma et al. 2010). The association between smoking and Barrett's associated adenocarcinoma is not as strong as the link between smoking and squamous oesophageal cancer, nor is the role of dietary factors.

Endoscopic Factors

There is evidence that increasing segment length may be associated with an increased risk of progression to dysplasia and cancer (Anandasabapathy, Jhamb, & Davila 2007). The presence of nodules or visible abnormalities during endoscopy is associated with disease progression (Montgomery, Bronner, & Greenson 2002). Patients with Barrett's oesophagus often have a hiatus hernia, and there is some evidence that the length of the hiatus hernia (more than six centimetres) is predictive of progression to high grade dysplasia and adenocarcinoma (Weston, Sharma, Mathur et al. 2004).

Pathological Predictors

The grade of dysplasia remains the current gold standard for risk stratification in patients with Barrett's oesophagus. The presence of low grade dysplasia, particularly when confirmed by an expert pathologist is now a recognised risk factor for progression (Prasad, Bansal, Sharma, & Wang 2010). Studies have suggested the extent of dysplasia, whether focal or diffuse, may predict disease progression although assessing the extent of dysplasia is a very labour intensive technique preventing its widespread use in clinical practice (Buttar, Wang, & Sebo 2012;Srivastava, Hornick, Li, Blount, Sanchez, Cowan, Ayub, Maley, Reid, & Odze 2007).

1.5 The Pathogenesis of Barrett's Oesophagus

The induction of Barrett's metaplasia is really rather speculative as there are no reliable, physiological animal models. Allison and Johnstone believed that Barrett's oesophagus developed from gastric columnar cells migrating from the stomach into the oesophagus in response to the reflux damaged squamous epithelium (Allison & Johnstone 1953). However more recent work suggests that chronic exposure to gastric and duodenal juices results in luminal damage to the squamous lining, exposing oesophageal stem cells lying in the basal layers of the epithelium (Dvorak, Payne, & Chavarria 2007;Pera and Pera 2002). The refluxate stimulates abnormal differentiation in a genetically susceptible host leading to formation of a more "robust" columnar lined oesophagus. Although the progenitor cell in humans remains unknown, metaplastic transformation arises as a consequence of changes in cellular gene expression and these changes are induced by gastroesophageal reflux, a key pathogenic factor.

The events resulting in the metaplastic transformation of squamous to columnar epithelium are not clear, nor is the source of the columnar cells (Spechler, Fitzgerald, Prasad et al. 2010). It has been proposed that the cardiac mucosa itself is an acquired metaplastic mucosa resulting from reflux damage to the squamous epithelium (Chandrasoma, Der, & Ma 2000). pH and manometry studies suggest that the extent of cardiac mucosa is increased in those with increased levels of acid reflux and is absent in children with no reflux. Chandrasoma has proposed that the entire oesophagus is normally lined by squamous epithelium with an abrupt transition to gastric oxyntic mucosa at the gastro-oesophageal junction (Chandrasoma 1997). Reflux of acid and bile damages the squamous lining and transforms it to the glandular columnar epithelium. Cardiac mucosa can develop above the anastomosis of a gastric pull-up in patients who have undergone oesophagectomy, supporting the view this may be an acquired condition (Lord, Wickramasinghe, & Johansson 2004).

Reflux of acid, pepsin and bile has little or no effect on gastric fundic mucosa. However it has two effects on squamous epithelium – oesophagitis and metaplastic transformation from squamous into cardiac type glandular mucosa (Chandrasoma 1997). Glandular metaplasia of the squamous epithelium first produces the simplest type of columnar mucosa which contains only mucous cells (ie cardiac mucosa) (Chandrasoma 1997). Other types of glandular mucosa evolve from this mucosa by developing specialised cells such as parietal cells and goblet cells. Studies have

shown severity of reflux is associated with length of the columnar lined segment (Clark, Ireland, & Chandrasoma 1994). Incomplete intestinal metaplasia is associated with gastric adenocarcinoma (Correa, Piazuelo, & Wilson 2010). It may be that the intestinal metaplasia of the oesophagus is premalignant, but there are few original studies showing that this is indeed the case (Haggitt, Tryselaar, Ellis, & Colcher 1978).

1.5.1 Stem cells

Jankowski and many other experts believe Barrett's metaplasia arises from stem cells within the native oesophagus or adjacent oesophageal glandular tissue (Jankowski, Harrison, Perry et al. 2000). However the origins of the stem cells are unclear although three theories have been put forward. The *de novo metaplasia* theory suggests that stem cells in the inflamed oesophageal squamous mucosa are exposed and damaged by reflux, producing metaplastic changes within the stem cells. These cells undergo phenotypic changes resulting in Barrett's stem cells capable of surviving in a hostile reflux environment. This is a similar process to metaplasia of the vagina (Sodhani, Gupta, Prakash et al. 1999). The second theory is the *transitional zone* theory. Cells from the gastro-oesophageal junction, the transitional zone, or the gastric cardia colonise the distal oesophagus in response to noxious luminal agents in an attempt to re-epithelialize the damaged mucosa. These cells express a columnar phenotype and may be the progenitors for the Barrett's cell population. This theory is supported by metaplasia seen in other transitional zones in the body such as the cervix and the prostate gland (Jankowski, Harrison, Perry, Balkwill, & Tselepsis 2000;Wallner, Syvan, Stenling et al. 2000). An interesting paper studying p63 null mice embryos suggested, upon programmed damage to the squamous epithelium, mice developed an intestinal metaplasia with gene expression profiles similar to Barrett's oesophagus (Wang, Ouyang, Yamamoto et al. 2011). The last theory, *the duct-cell metaplasia* theory, is that stem cells in the oesophageal ducts selectively colonise the oesophagus in response to reflux induced damage. Other studies suggest the progenitor cells may be circulating stem cells from bone marrow (Spechler, Fitzgerald, Prasad, & Wang 2010).

1.5.2 Local environment

Stem cells are essential in the metaplastic transformation of the native squamous oesophageal mucosa, but the extrinsic forces resulting in the changes in stem cells remain unknown. It is generally accepted that a clear association exists between host susceptibility (genetic and epigenetic abnormalities) and the intraluminal environment (mucosal inflammation and acid/bile acid reflux).

Recent studies have shown that molecular events within the squamous epithelium, triggered by reflux disease, may cause Barrett's oesophagus. In cell culture, acid and bile induce the expression of caudal homeobox genes, CDX1 and CDX2 which are responsible for intestinal development during embryogenesis (Souza, Krishnan, & Spechler 2008). Gastroesophageal reflux may therefore induce aberrant expression of Cdx genes as a first stage in the metaplastic process. It is well recognised that acid and bile reflux leads to oxidative stress within oesophageal cells causing DNA damage and free radical formation which can initiate the process of apoptosis, cellular senescence or carcinogenesis (Dvorak, Payne, & Chavarria 2007).

Inflammation in the oesophagus in response to gastroesophageal reflux disease, was first linked to cancer in 1863 and is now considered to be one of the hallmarks of cancer (Colotta, Allavena, Sica et al. 2009;Lao-Sirieix and Fitzgerald 2010). An inflammatory infiltrate is often seen in the oesophageal mucosa in response to reflux, and even when gastroduodenal reflux disease is corrected by acid suppressing medication, a mild chronic inflammatory infiltrate persists. Inflammation can generate free radicals and this may lead to increased expression of various cytokines and upregulation of genes, propagating the metaplastic transformation (Reid, Li, Galipeau, & Vaughan 2010).

1.5.3 Molecular abnormalities in Barrett's oesophagus

Unlike colon cancer, Barrett's oesophagus and oesophageal adenocarcinoma are genetically heterogenous conditions, in which there are many abnormalities involving tumour suppressor genes and key pathways. Chapter 7 reviews the literature concerning biomarkers and Barrett's oesophagus.

1.6 Endoscopic Surveillance Programmes

Barrett's oesophagus is a complex condition in which the pathophysiology of the disease remains enigmatic. The malignant potential of Barrett's epithelium is important and the future management of patients with Barrett's oesophagus should concentrate on improving risk stratification of patients and initiating early appropriate treatments. This will allow targeted surveillance and treatment of those at higher risk of progression to dysplasia and cancer, and reassurance and avoidance of regular unnecessary endoscopies for those at no/low risk of progression.

Most centres advocate endoscopic surveillance of Barrett's oesophagus, aiming to identify cancer at an early stage when it is curable, and detect dysplasia in order that endoscopic treatments may be offered. However there continues to be debate surrounding the usefulness and cost effectiveness of endoscopic surveillance programmes as the yield is often low. Barrett's mucosa is often heterogenous containing various epithelia, and areas of dysplasia found alongside areas of metaplasia. This leads to one of the main problems in carrying out endoscopic surveillance as small neoplastic lesions may be missed as they are often not visible. Despite these concerns, Barrett's oesophagus is a disease which is amenable to surveillance as it is a common condition, is associated with the development of oesophageal adenocarcinoma and tends to have a stepwise progression from metaplasia to dysplasia and carcinoma (Spechler S.J 2011). Survival from oesophageal adenocarcinoma is dependent on the depth of invasion and the presence of lymph node metastases. Patients with T1 stage have survival rates of more than 90% at five years, whilst patients with T4 disease or lymph node metastases have rates of less than 25% (Kim, Grobmyer, Smith et al. 2011). Observational studies suggest that patients with surveillance detected cancers have a better prognosis as they are diagnosed at an early stage compared with those who present with symptoms (Corley, Levin, & Habel 2002;Spechler S.J 2011). Endoscopic surveillance is therefore recommended to detect dysplasia and early cancer.

1.6.1 Entry Criteria To Surveillance Programme

Patients with a clear diagnosis of Barrett's oesophagus should be invited to participate in endoscopic surveillance programmes. As discussed the general consensus definition of Barrett's oesophagus is a condition in which the normal squamous lining of the oesophagus is replaced by a columnar metaplasia at risk of malignant progression. However the diagnostic criteria required to make such a diagnosis vary among gastroenterological and surgical societies.

Patients should be counselled regarding the risks and benefits of endoscopic surveillance, and the limitations associated with such a programme. Other important criteria may depend upon patient age (perhaps surveillance in those over 80 is not appropriate), other comorbidities and life expectancy (likelihood of survival over the next few years) and the ability to undergo repeated endoscopic procedures or other treatment options. Barrett's oesophagus is a common condition with population estimates suggesting a disease prevalence of 2-7%. Endoscopic surveillance therefore is a time and resource consuming process with significant cost implications.

1.6.2 Surveillance Protocols

The aim of endoscopic surveillance in patients with Barrett's oesophagus is to detect and treat dysplasia and cancer at an early stage. Most centres advocate the use of high resolution white light endoscopy with the use of image adjuncts such as narrow band imaging if available.

Endoscopic assessment

At endoscopy, the oesophagus is inspected and the presence of important landmarks noted, namely the oesophagogastric junction (the level of the proximal gastric folds on minimal insufflation) and the squamocolumnar junction. The presence of a columnar lining of the oesophagus is noted (salmon pink colour compared to normal white/pink of squamous epithelium) and any subtle mucosal abnormalities. The Barrett's segment should be described using the Prague C&M classification system, where C describes the circumferential extent of the columnar lining, and M applies to the whole segment of metaplasia, including any tongues or islands of Barrett's mucosa (Sharma, Dent, & Armstrong 2006). Most guidelines suggest obtaining four quadrant biopsies at two centimetre intervals along the Barrett's segment (Playford 2005;Wang & Sampliner 2008). Any mucosal lesion such as nodules, ulcers,

erosions or mucosal irregularity should be separately biopsied as there is evidence that these lesions are linked to the presence of dysplasia or cancer (Reid, Blount, Feng, & Levine 2000). The rationale for a comprehensive biopsy protocol arises from the focal nature of dysplasia and often the lack of associated mucosal abnormalities (Abela, Going, Mackenzie, McKernan, O'Mahoney, & Stuart 2008). There has been debate whether the use of jumbo forceps improves endoscopic sampling by providing the pathologist with larger pieces of tissue (Gonzalez, Yu, Smith et al. 2010). However there is no persuading evidence to suggest these are necessary for routine endoscopic surveillance.

There is some evidence that the Seattle protocol, using a smaller interval of one centimetre in addition to biopsy of visible lesions, was more effective at detecting adenocarcinoma in patients with high grade dysplasia (Reid, Blount, Feng, & Levine 2000). However the increasing use of endomucosal resection for patients with dysplasia or visible lesions has resulted in very few centres now adhering to the Seattle protocol. Indeed a study of patients undergoing oesophagectomy for high grade dysplasia showed no difference in undiagnosed cancer incidence between those undergoing traditional quadrantic biopsies every two centimetres compared with those using the Seattle protocol (Kariv, Plesec, & Goldblum 2009).

Histological Assessment

Biopsies taken from each two centimetre level within the Barrett's segment, and any focal abnormality should be placed in individual specimen pots and clearly labelled. It is important that the pathologist is aware of the location of the biopsy as metaplasia of the distal oesophagus can mimic changes seen in the cardia of the stomach. Biopsy specimens should ideally be assessed by a gastrointestinal pathologist with expertise in Barrett's oesophagus. Each biopsy is cut and stained with haematoxylin and eosin to allow initial assessment of the tissue and classification according to the Vienna classification system, see Table 1.1 (Schlemper, Riddell, & Kato 2000). The columnar lining is assessed for the presence of intestinal metaplasia, junctional type mucosa or fundic type mucosa. The pathological assessment of Barrett's biopsy samples is vitally important, as it is the presence (or absence) of dysplasia which determines further surveillance intervals and/or the need for treatment.

1.6.3 Surveillance Intervals

The time interval between surveillance procedures is based on the presence or absence of dysplasia, and our limited understanding of the biology of Barrett's oesophagus. The recommendations from each gastrointestinal society are not based on results from randomised clinical trials and it seems that the societies do not agree on surveillance intervals (Boyer, Laugier, Chemali, Arpurt, Boustiere, Canard, Dalbies, Gay, Escourrou, Napoleon, Palazzo, Ponchon, Richard-Mollard, Sautereau, Tucat, & Vedrenne 2007;Hirota W, Zuckerman, Adler et al. 2006;Playford 2005;Wang & Sampliner 2008;Wang, Wongkeesong, & Buttar 2005). Table 1.2 summarises the important points within each guideline.

The general principles of the surveillance programme advise patients without metaplasia to undergo regular endoscopic assessment every 2-3 years. The French Society of Digestive Endoscopy is the only body to suggest surveillance intervals should depend on the length of Barrett's segment as shown in Table 1.2 (Boyer, Laugier, Chemali, Arpurt, Boustiere, Canard, Dalbies, Gay, Escourrou, Napoleon, Palazzo, Ponchon, Richard-Mollard, Sautereau, Tucat, & Vedrenne 2007). In the presence of dysplasia the surveillance interval is shortened. It is recommended that patients with low grade dysplasia are treated with eight weeks of high dose PPI therapy to reduce inflammation which is often associated with dysplasia. If the repeat biopsy continues to suggest the presence of low grade dysplasia after review by an expert pathologist, the surveillance interval is reduced to six months. In the absence of low grade changes on two successive endoscopies, the surveillance period is lengthened to an annual endoscopy initially then every two years.

In the presence of high grade dysplasia, most institutions would recommend treatment with high dose PPI therapy and repeat endoscopy with further biopsy or endomucosal resection of any abnormal area. Should high grade dysplasia be confirmed on repeat biopsy, the patient should be referred to a clinician with expertise in Barrett's oesophagus and treatment offered. Other imaging modalities may be required (EUS, CT, PET) to stage the disease as high grade dysplasia is often associated with the presence of intramucosal cancer.

Table 1.2 Summary of Surveillance Guidelines for Barrett's Oesophagus

Organisation	Diagnostic Criteria	Metaplasia	Low Grade Dysplasia	High Grade Dysplasia
BSG (2005)	Columnar metaplasia	Every 2 years	Acid suppression, repeat OGD If LGD persists, 6 monthly If LGD absent, 2 negative OGDs and repeat 2 years	OGD every 6 months or refer for treatment
ACG (2008)	Intestinal metaplasia	2 OGDs in first year If negative for dysplasia, every 3 years	Repeat OGD 6 months If LGD absent, annual until no dysplasia for 2 years	Repeat OGD 3 months If HGD persists, refer for intervention
AGA (2005)	Intestinal metaplasia	2 OGDs in first year If negative for dysplasia, every 5 years	Repeat OGD 1 year If LGD present, annual OGD	Repeat OGD 3 monthly
ASGE (2006)	Intestinal metaplasia	2 OGDs in first year If negative for dysplasia, every 3 years	Repeat OGD 6 months If LGD persists, repeat 6 months then annual endoscopy	Repeat OGD 3 monthly If HGD absent after 2 OGD, lengthen interval to 1 year
FSDE (2007)	Intestinal metaplasia	Short segment (<3cm) 5 years Long segment (3-6cm), 3 years Long segment (>6cm), 2 years	Repeat OGD 2 months If LGD persists, repeat 6 months then annual endoscopy	Repeat OGD If HGD persists, refer for intervention

1.6.4 Limitations of Barrett's Oesophagus Surveillance

The current endoscopic surveillance programme carries limitations. The endoscopic procedure can be uncomfortable for patients, and time consuming especially in those with long segment disease in whom many biopsies should be performed. Many dysplastic or early neoplastic lesions are not visible to the naked eye, resulting in missed lesions on biopsy. The vast majority of patients with Barrett's oesophagus will not develop cancer (0.5% annual cancer risk), but continue to undergo the anxiety often associated with repeated endoscopies.

The interpretation of dysplasia, particularly low grade dysplasia is often difficult, and many specimens when reviewed by expert pathologists are often downgraded. Patients and clinicians are also fairly poor at rigid adherence to the surveillance protocols and this may have an impact on the diagnosis of dysplasia (Waxman 2011). Lastly, some oesophageal adenocarcinomas may arise as *de novo* cancers without any evidence of previous dysplasia, or present as interval cancers.

1.6.5 Improving the Surveillance Programme

The endoscopic surveillance programme could be improved. Firstly, a larger area could be sampled by using more aggressive biopsy protocols, or other techniques such as brush or sponge cytology. Targeting areas of dysplasia may allow more rigorous sampling within areas of interest. In order to perform this, new technologies need to be applied, such as confocal microscopy or autofluorescence. Perhaps the most promising improvement would be risk stratification of patients using a variety of markers, such as clinical risk factors and biomarkers. In the meantime we await with interest the results of a randomised clinical trial assessing the role of endoscopic surveillance (versus no surveillance) for the prevention of early mortality in patients with Barrett's oesophagus (Jankowski & Barr 2006).

1.7 Aims of thesis

In an attempt to improve the care of patients with Barrett's oesophagus within the West of Scotland and understand the molecular mechanisms underlying the disease, my thesis will address the main challenges associated with this enigmatic condition at clinical, endoscopic and molecular levels. The aims of my thesis are as follows:

1) To present a general overview of the Barrett's literature highlighting current clinical challenges and discuss the role of intestinal metaplasia in the diagnosis of Barrett's oesophagus
2) To examine the incidence of dysplasia and oesophageal adenocarcinoma in the West of Scotland by analysing a cohort of patients undergoing surveillance endoscopy and study the effects of deprivation on disease progression
3) To review the current endoscopic imaging adjuncts for the diagnosis of Barrett's oesophagus and dysplasia, and assess the role of optical biopsy forceps in determining the presence of dysplasia
4) To evaluate the role of Wnt signalling in Barrett's oesophagus, from metaplasia to carcinoma in a mouse model, with complementary human studies

Chapter 2

Intestinal metaplasia – the only cancer precursor?

It is generally accepted that the cancer risk in Barrett's oesophagus is conferred by intestinal metaplasia, characterised by the presence of goblet cells. This belief is difficult to test, as it is often impossible to prove an absence of goblet cells in a particular oesophagus. Furthermore, little is known about the distribution and temporal drift of the intestinal phenotype. However studies suggest genetic and epigenetic abnormalities are present within the non-intestinal mucosa making it difficult to believe the intestinal phenotype alone confers malignant risk. In order to determine patient-specific cancer risk, a consensus definition is required and an understanding of the metaplastic nature of the columnar mucosa and the evolution of dysplasia is needed. It is unlikely malignant risk can be limited to whether intestinal metaplasia is present or absent.

Publication

Moyes LH, Going JJ. Goblet cells in Barrett's oesophagus: cancer precursor, risk marker or irrelevance. Diagnostic Histopathology 2012; 18 (12): 503-510.

2.1 Background

It is widely considered that metaplasia to a mucosa with goblet cells is associated with premalignant dysplasia and risk of progression to oesophageal adenocarcinoma. Intestinal metaplasia (IM) is regarded as a prerequisite for the diagnosis of Barrett's oesophagus and enrolment into endoscopic surveillance programmes in the USA and Germany. On the other hand, the British Society of Gastroenterologists and the Japan Esophageal Society do not require IM for the diagnosis which can be made when columnar mucosa of cardiac, oxyntic or intestinal types are found in a mucosal biopsy of oesophageal origin.

Studies over the recent decades have shown that Barrett's dysplasia and adenocarcinoma develop in the presence of, and perhaps even from IM. However, there is molecular evidence to suggest Barrett's "columnar lined" oesophagus, even without IM may have malignant potential (Riddell and Odze 2009).

The original evidence for a specific role of IM as the usual precursor of oesophageal adenocarcinoma was reviewed, along with whether its presence or absence is really an appropriate precondition for a diagnosis of Barrett's oesophagus.

2.2 Literature search

A literature search was performed using PubMed, Embase and Ovid databases searching for English literature available since 1900. The search was performed with MESH terms "Barrett", "Barrett's esophagus/oesophagus", "columnar lined esophagus/oesophagus", "oesophageal adenocarcinoma", "intestinal metaplasia", "metaplasia" and "goblet cells". All related reference articles were examined and included in the analysis if relevant. In this chapter, the terms "Barrett's oesophagus" and "columnar lined oesophagus" are synonymous.

2.3 Barrett's associated adenocarcinoma – the original studies

By the 1970s when the association between gastro-oesophageal reflux, hiatus hernia and columnar lined oesophagus (CLO) had been accepted (DeMeester and DeMeester 2000), surgeons and pathologists recognised the link between CLO and oesophageal adenocarcinoma motivating research and justifying endoscopic surveillance programmes (Reid and Weinstein 1987).

The first recorded case of oesophageal adenocarcinoma in an aberrant gastric type mucosa was described by Morson and Belcher in 1952 (Morson and Belcher 1952). Since then numerous studies of patients with resected adenocarcinomas arising in Barrett's oesophagus have been published, although most are limited by small patient numbers with few detailed descriptions of the pathology and histology (Adler 1963;Armstrong, Blalock, & Carrera 1959;Berenson, Riddell, & Skinner 1978;Haggitt, Tryselaar, Ellis, & Colcher 1978;Hamilton and Smith 1987;Hawe, Payne, Weiland et al. 1973;Lortat-Jacob, Maillard, Richard et al. 1968;Maas, Katz, & Pascale 1974;McCorkle and Blades 1955;Murray, Watson, Johnston et al. 2003;Naef, Savary, & Ozzello 1975;Nilsson, Skobe, Johansson et al. 2000;Paraf, Flejou, Pignon et al. 1995;Rosenberg, Budev, Edwards et al. 1985;Ruol, Parenti, Zaninotto et al. 2000;Skinner, Walther, Riddell et al. 1983;Smith, Hamilton, Boitnott et al. 1984;Spechler SJ, Robbins, Rubins et al. 1984).

Haggitt originally suggested that if atrophic gastritis with intestinal metaplasia predisposed to gastric carcinoma, the columnar epithelium of the oesophagus with its intestinal metaplasia may signify a similar predisposition in the oesophagus (Haggitt, Tryselaar, Ellis, & Colcher 1978). Dr Haggitt's study of 14 patients with oesophageal adenocarcinoma showed that 12 cancers arose within an epithelium resembling gastric cardiac mucosa with mucous glands but no parietal cells. Earlier studies identified adenocarcinoma in the presence of only a glandular mucosa and it was not until Paull's histological description of the columnar lined oesophagus that most studies identified intestinal metaplasia as the predominant mucosa associated with dysplasia and cancer.

The original reports and studies which suggest a link between Barrett's oesophagus and adenocarcinoma are summarised in Table 2.1. Many of these describe few patients and varied inclusion/exclusion criteria make interpretation difficult. Early reports often exclude lesions near the cardia and short segments of columnar lined

oesophagus, allowing a diagnosis of Barrett's oesophagus only if the columnar lining is more than 3cm above the gastroesophageal junction. Another difficulty with earlier studies was determining the exact cellular origin of the tumour, most presenting at an advanced stage. However despite these diagnostic difficulties, most studies concluded that adenocarcinomas developed within an area of columnar lined oesophagus, often in association with intestinal metaplasia.

Sjogren's review of the literature (121 cases of adenocarcinoma associated with Barrett's oesophagus) found many reports did not describe the adjacent epithelial type (Sjogren and Johnson 1983). However, by the 1990s "specialised" intestinal metaplasia was widely recognised as the most common epithelium associated with Barrett's oesophagus and by inference, conferred its malignant potential (Haggitt 1994). The annual cancer risk was quoted around 0.8% in patients with endoscopically obvious disease. Later a metaplasia-dysplasia-adenocarcinoma sequence was proposed and endoscopic surveillance programmes for patients with Barrett's oesophagus commenced to identify dysplasia and allow earlier recognition and treatment of cancers (Reid, Blount, Rubin et al. 1992).

Table 2.1 Summary of original studies describing carcinoma in the presence of columnar lined oesophagus

Reference	*Patients*	*Sex*	*Location*	*Histology*	*Intestinal metaplasia*	*Comments*
Morson BC (1952)	1	M	Mid oesophagus	Glandular Distal limit – cardiac type Intestinal type including goblet cells	Yes	
McCorkle RC (1955)	1	M	Mid	Glandular	No comment	
Armstrong RA (1959)	1	M	Mid	Glandular	No comment	
Adler RH (1963)	1	M	Cardia	Glandular	No comment	
Lortat-Jacob JL (1968)	16					3 cases – arising in oesophageal/cardiac glands 2 cases – derive from glandular components 6 cases – in association with peptic ulceration
Hawe A (1973)	5	4M 1F	Mid/lower	Glandular	No comment	
Maas LC (1974)	1	M	Mid	Columnar epithelium without parietal cells		
Naef AP (1975)	12	Unknown	Mid/lower	Columnar epithelium	No comment	
Berenson MM (1978)	2	2M	N/A	Gastric and intestinal type	Yes	
Haggitt (1978)	12	8M 4F	Mid/lower	Cardiac type (12/12) 100% Intestinal type (9/12) 75% Fundic type (2/12) 17% Dysplasia (10/12) 80%	Present Proximal location	"Specialised intestinal type similar to atrophic gastritis which is associated with gastric cancer, so perhaps intestinal type may signify a similar predisposition". In this cohort, most common type was cardiac mucosa.
Smith RR (1984)	21	23M 3F	Mid/lower	Specialised type (21/21) 100% Dysplasia (26/26) 100%	Present	
Rosenberg JC (1985)	9	8M 1F	N/A	Specialised type (9/9) 100% Dysplasia (9/9) 100%	Present	
Hamilton SR (1987)	14	N/A	Mid/lower	Dysplasia occurred in	Present	Intestinal metaplasia most commonly

				• Specialised type (93%) • Cardiac type (21%)		associated with dysplasia and cancer
Skinner DB (1983)	20	18M 2F	Mid/lower	Intestinal type alone (60%) Cardiac type alone (40%) Fundic type alone (10%) Intestinal and cardiac (30%) Intestinal all cases (100%)	Present	Intestinal metaplasia present in all cancer cases (100%) whereas only present in 74% benign cases. IM most common type to undergo malignant change, although dysplasia seen in all epithelial types.
Spechler SJ (1984)	8	N/A	N/A	Intestinal type (8/8) 100%	Present	67% patients displayed IM and all 8 cancers in this cohort. 33% did not have IM with no cancers in this cohort.
Paraf F (1995)	67	61M 6F		Intestinal type (32/67) 48% Cardiac type (25/67) 38% Fundic type (2/67) 3% All types (7/67) 11% Dysplasia (50/67) 76% Dysplasia and intestinal 98% Dysplasia and cardiac 2%	Present	Intestinal metaplasia most commonly associated with dysplasia.
Nilsson J (2000)	5	4M 1F	Mid/lower	Specialised type (5/5) 100%	Present	All cancers arose on a background of long segment IM. "The risk of missing small areas of SCM within columnar mucosa when obtaining specimens is far from negligible".
Ruol A (2000)	26	25M 1F	Mid/lower	Intestinal type 25/26 (96%) Dysplasia present 22/26 (85%)	Present	
Murray L (2003)	29	22M 7F	N/A	Intestinal type 26/29 (90%) Cardiac type 1/29 (3%) Unknown 3/29 (6%)	Present	"Risk of cancer almost exclusively in patients with SIM"

2.4 Are goblet cells really necessary for progression to cancer?

The association between CLO and adenocarcinoma has been recognised for many years, with an acceptance that the mucosal type surrounding the malignancy tends to be intestinal, and often dysplastic. However, patients without intestinal metaplasia on index endoscopy, may progress to intestinal metaplasia and subsequent adenocarcinoma. The first paper to suggest that goblet cells, a histological marker for intestinal metaplasia, were not always detected in patients with CLO suggested that 20% of patients did not exhibit intestinal metaplasia on two endoscopic examinations (Kim, Waring, Spechler et al. 1994). These results have been corroborated by Harrison who found that even if 15 or 16 biopsies were performed, there was still no guaranteed detection of goblet cells within a columnar segment (Harrison, Perry, Haddadin et al. 2007).

Several factors influence the identified prevalence of intestinal metaplasia - age, number of endoscopies, number of biopsies, length of reflux symptoms and length of the columnar lined segment (Csendes, Smok, & Burdiles 2003;Harrison, Perry, Haddadin, McDonald, Bryan, Abrams, Sampliner, Talley, Moayyedi, & Jankowski 2007;Oberg, Johannson, & Wenner 2001;Oberg, Peters, & DeMeester 2000;Qualman, Murray, McClung et al. 1990). A large prospective study analysed 3568 biopsies from 1751 patients with non-dysplastic CLO, demonstrating intestinal metaplasia in 65.8% of cases (Gatenby, Ramus, Caygill et al. 2008). Increased prevalence of intestinal metaplasia detection was correlated with male sex, increasing age and number of biopsies and longer segment lengths, in keeping with previous studies. At the start of the study 12.7% of patients displayed intestinal metaplasia on initial biopsies, with 72% at 5 years, and 90.8% at 10 years. This study had 322 patients with CLO (negative for goblet cells) and 612 patients with CLO (positive for goblet cells). Whilst the results were not statistically significant, there was a trend towards a higher risk of progression to dysplasia and adenocarcinoma in the group with intestinal metaplasia. Of the 322 patients without intestinal metaplasia, ten patients developed adenocarcinoma and two patients, high grade dysplasia. These patients would have been excluded from surveillance programmes according to the American College Guidelines. The authors do not deny that detection of intestinal metaplasia is associated with increased malignancy, but point out that failure to

detect intestinal metaplasia does not mean it is not present or is associated with malignant potential.

If malignant risk is specifically conferred by intestinal metaplasia, patients with a columnar "glandular" mucosa alone (i.e. no intestinal metaplasia) on biopsy should be less likely to develop adenocarcinoma. Kelty and colleagues classified 688 patients into three groups – no glandular metaplasia, glandular metaplasia without intestinal metaplasia and glandular metaplasia with intestinal metaplasia (Kelty, Gough, van Wyk et al. 2007). On index endoscopy, 379 patients (55.1%) had intestinal metaplasia and 309 patients (44.9%) only glandular with each group having equal numbers of biopsies (average 4, range 2-15). 28 patients developed cancer in the follow up period, 17 in the intestinal metaplasia group and 11 in the glandular only group (p=NS). The overall cancer risk was 0.34% per year with no significant difference between groups with intestinal metaplasia and those with glandular mucosa alone. This study adds weight to the argument that patients with glandular mucosa, without identifiable goblet cells, also carry a malignant risk, should be diagnosed as Barrett's oesophagus and invited to participate in endoscopic surveillance programmes.

Takubo reviewed 141 endomucosal resection specimens (German patients) specifying the background mucosa as squamous, cardiac, fundic and intestinal with goblet cells (Takubo, Aida, Naomoto et al. 2009). Cardiac mucosa was seen in the presence of adenocarcinoma: it was claimed 75% of cases had a mucosal background without goblet cells. Approximately one third of these small tumours were completely surrounded by CLO; one third had non-neoplastic CLO on one aspect with dysplastic/neoplastic CLO on the other; and one third non-neoplastic CLO on one side and squamous on the other. Cardio-oxyntic mucosa was more common than intestinal type than oxyntic alone (70.9% vs 22% vs 3.5%). It is unclear the extent to which this lack of peritumoral goblet cells could be a consequence of tumour overgrowth, due to sampling errors or whether the disease in Japan is a different entity to CLO of the western world.

However, a recent study by Chandrasoma and colleagues assessing the role of a systematic biopsy protocol in patients with Barrett's oesophagus and the prevalence of intestinal metaplasia disagrees with the above studies (Chandrasoma, Wijetunge, DeMeester et al. 2012). At index endoscopy, 187 of 214 patients presented with

intestinal metaplasia with 55 patients progressing to dysplasia or cancer. In the group with no intestinal metaplasia on systemic biopsy, no patient progressed. They conclude that systematic biopsy is necessary to adequately assess patients with Barrett's oesophagus and those patients without intestinal metaplasia carry an insignificant cancer risk and should be discharged from further endoscopic follow up. However, the total number of patients in this biopsy study was moderated with only 27 non-IM cases.

Most pathologists and clinicians accept the view that IM is associated with the development of oesophageal adenocarcinoma. Dysplasia invariably arises within areas of IM, and Barrett's adenocarcinoma is nearly always preceded and accompanied by dysplasia. However, there are some cases in which a "non-goblet" epithelium carries risk of malignant progression. Neoplasia is unlikely to arise directly from the goblet cells as these are terminally differentiated cells. Goblet cell density varies. Is risk proportional to density? It appears even areas of low density carry some malignant risk, and therefore it is unlikely a non-goblet mucosa would be risk-free.

2.5 Non-goblet columnar epithelium – at risk of progression?

Several research groups have looked for evidence that a non-goblet epithelium may also carry malignant potential. Metaplastic oesophageal columnar epithelium without goblet cells shows similar chromosomal and DNA content abnormalities to those with goblet cells (Kelty, Gough, van Wyk, Stephenson, & Ackroyd 2007;Takubo, Aida, Naomoto, Sawabe, Arai, Shiraishi, Matsuura, Ell, May, Pech, Stolte, & Vieth 2009). Patients with CLO without goblet cells have a similar risk of neoplastic progression compared with those patients in whom goblet cells are present. However there are clearly other factors besides genetics involved in the development of Barrett's oesophagus and the local milieu plays an important role.

Hahn assessed the expression of CDX2 and other markers of intestinal differentiation in non-goblet epithelium (Hahn, Blount, Ayub et al. 2009). Patients without goblet cells had a lower percentage of CDX2 staining (43%) compared with those expressing goblet cells (98%) but did show phenotypic evidence of intestinal differentiation (immunohistochemical positivity of markers such as Das-1, villin and MUC-2). It has been suggested that squamous epithelium converts initially to a non-goblet columnar epithelium before goblet cell metaplasia. It may appear the unstable metaplastic epithelium is associated with malignant transformation.

There is a proportion of patients with columnar lined oesophagus who do not show goblet cells on biopsies regardless of the site and number of biopsies performed (Riddell & Odze 2009). This could be due to sampling errors but there is now evidence to suggest a non-goblet cell mucosa, such as cardiac type, also carries DNA and chromosomal abnormalities and is associated with malignant transformation and neoplastic progression (Liu, Hahn, Odze et al. 2009). However the actual risk of this "non-goblet" epithelium is currently unknown. There are two retrospective follow up studies suggesting both types of epithelium carry a risk of malignant transformation but due to their retrospective nature it is difficult to quantify such risk (Gatenby, Ramus, Caygill, Shepherd, & Watson 2008;Kelty, Gough, van Wyk, Stephenson, & Ackroyd 2007).

2.6 Conclusion

In conclusion, the phrase "no goblet cells implies no cancer risk" needs to be challenged. Studies suggest that patients without goblet cells are at risk of cancer and should not be excluded from surveillance programmes. This is in line with current British guidelines and clinical practice within the UK. On a more practical note, it seems that the issue of whether goblet cells are present or not may become less important if the non-goblet epithelium is also at risk of malignant transformation (Riddell & Odze 2009). It is still unclear how the squamous epithelium undergoes its initial metaplastic transformation to a columnar mucosa, and whether cardiac type mucosa is indeed a precursor to intestinal metaplasia. Metaplastic transformation appears to be the key event leading to an unstable epithelium which is capable of neoplastic progression. In order to improve patient care, the key focus should be risk stratification based on clinical (duration, length of symptoms), phenotypic composition and molecular markers. It is unlikely the presence (or absence) of goblet cells will be sufficient for effective risk management.

Chapter 3

High Risk of Dysplasia and Adenocarcinoma: The Glasgow Experience

Recent meta-analyses suggest the rates of malignant progression in patients with Barrett's oesophagus are lower than originally reported. However Scotland has one of the highest rates of oesophageal adenocarcinoma in the world, and the incidence of Barrett's oesophagus has risen in parallel. The West of Scotland population appears to be a high risk population and there is a concern that the low rates of malignant progression in lower risk populations may lull physicians, health economists and politicians into complacency concerning this potentially lethal condition. Barrett's oesophagus, and particularly dysplasia can be treated with endoscopic intervention (endoscopic resection and radiofrequency ablation) and specialist centres now have access to excellent endoscopic facilities allowing appropriate and timely intervention. A clear understanding of the natural history of Barrett's oesophagus is required to allow surveillance of "at risk" patients, and improve resource management. The aim of this study was to assess the incidence of progression to dysplasia and/or adenocarcinoma in a cohort of patients with Barrett's oesophagus undergoing surveillance endoscopy in a Glasgow hospital.

3.1 Introduction

Oesophageal adenocarcinoma is the fifth most common cause of cancer death in Scotland. The incidence of oesophageal adenocarcinoma (OA) has continued to rise over the last two decades, currently 16.9/100 000 person years follow up (Jankowski, Provenzale, & Moayyedi 2002;NHS National Services Scotland 2012). Although survival rates have improved with more accurate staging and better perioperative support, survival remains low with only one in four patients surviving five years (Clinical Resource and Audit Group (CRAG) 2005).

Barrett's oesophagus is a recognised precursor of oesophageal adenocarcinoma. About 12% of people undergoing endoscopy for reflux symptoms have Barrett's oesophagus although the true incidence of Barrett's oesophagus in the general population remains unknown (Spechler 2002). One of the most accurate population studies from Sweden suggests the incidence of Barrett's oesophagus in 3000 adults sampled was 1.6% (Ronkainen J, Aro, Storskrubb, Johansson, Lind, Bolling-Sternevald, Vieth, Stolte, Talley, & Agreus 2005). Similar results were found in an endoscopic study from Italy (Zagari, Fuccio, Wallander, Johansson, Fiocca, Casanova, Farahmand, Winchester, Roda, & Bazzoli 2008). One of the challenges associated with unravelling the true incidence of Barrett's oesophagus is that the condition is often asymptomatic with 46.2% of patients reporting no reflux symptoms (Reid, Li, Galipeau, & Vaughan 2010). Furthermore the vast majority of oesophageal adenocarcinomas arise in patients with no prior diagnosis of Barrett's oesophagus (Dulai, Guha, Khan et al. 2002).

Dysplasia is one of the most important features of Barrett's oesophagus and it currently remains the clinical gold standard marker of disease progression. The presence of high grade dysplasia is associated with the highest risk of malignant progression with cancer rates of 16-59%, although it is well recognised that not all patients progress to dysplasia (Reid, Blount, Feng, & Levine 2000;Schnell, Sontag, Chejfec, Aranha, Metz, O'Connell, Seidel, & Sonnerberg 2001;Spechler SJ 2011). With an improvement in the endoscopic therapies available for patients with dysplasia, it is necessary to know the incidence rates of dysplasia and adenocarcinoma to allow earlier and appropriate treatment for patients. Ultimately the hope is this would have an impact on the incidence and outcome of oesophageal adenocarcinoma.

Recent studies from Europe and the US have suggested that malignant progression in Barrett's oesophagus is sufficiently uncommon to call into question cost-effectiveness of endoscopic surveillance and even dysplasia treatment (de Jonge, van Blankenstein, Looman et al. 2010;Hur, Choi, Rubenstein et al. 2012;Sikkema, de Jonge, & Steyerberg 2010). However there are questions about ascertainment of true Barrett's oesophagus and segment length in these studies. There are still not enough good studies of rates of incidence and progression to dysplasia and adenocarcinoma in well characterised Barrett's oesophagus.

3.2 Study Aims

The West of Scotland has one of the highest incidences of Barrett's oesophagus in the United Kingdom but little is known about the rates of progression to dysplasia and adenocarcinoma within this population (Caygill, Watson, Reed et al. 2003). Therefore, the aims of this cohort study were:

1) to determine the incidence and progression of dysplasia in a well-defined cohort of patients undergoing surveillance of Barrett's oesophagus in the West of Scotland.

2) to assess the effect of segment length on malignant progression in Barrett's oesophagus.

3.3 Methods

Database

Patients with endoscopically visible and histologically proven Barrett's oesophagus identified between January 1994 and December 2009, and undergoing endoscopic and biopsy surveillance at Glasgow Royal Infirmary (GRI) or Stobhill Hospital were reviewed and added to a computer database (Microsoft Access). All patients were resident within the catchment area of GRI, an inner city teaching hospital covered a deprived population of 560 000 in NE Glasgow. Any tertiary referrals of patients non-resident within the catchment area of GRI were excluded from the database.

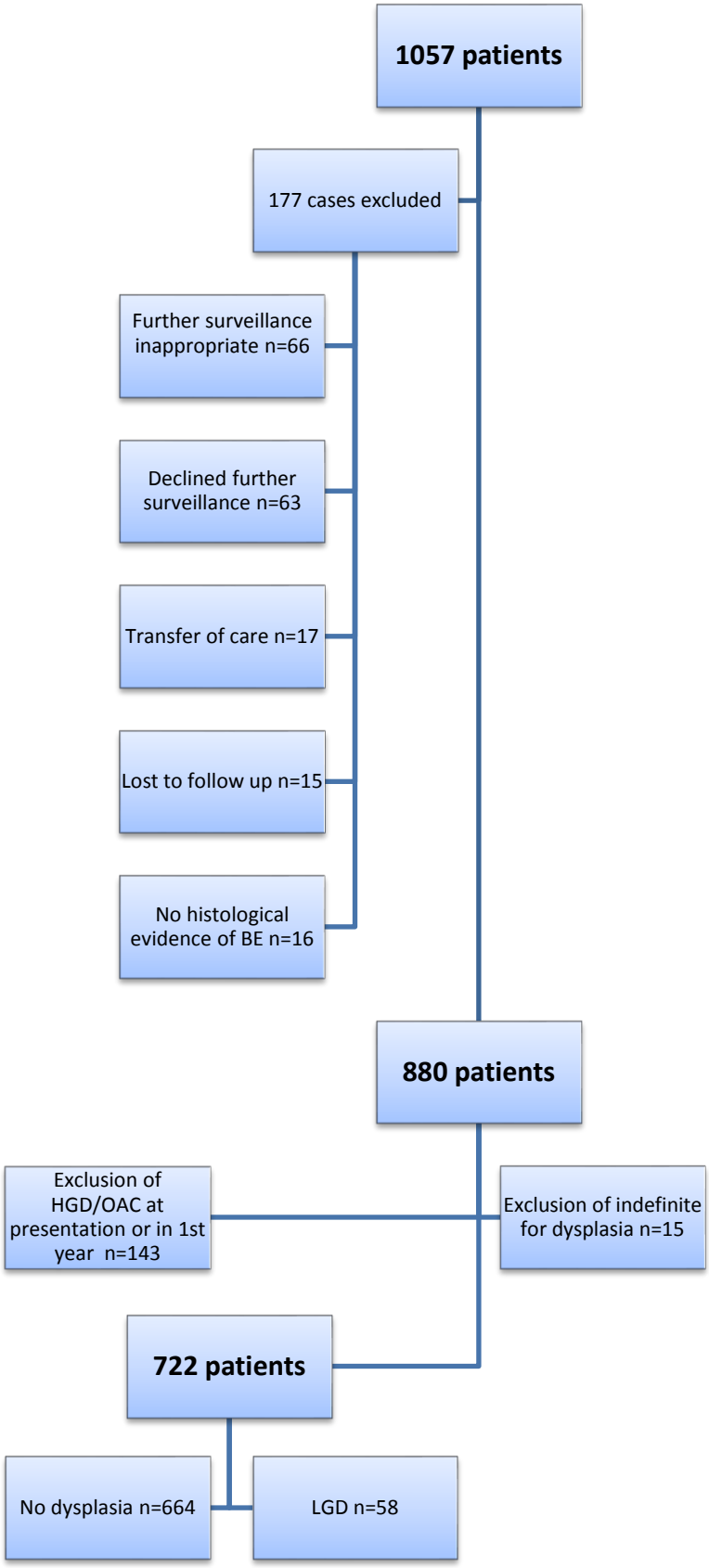
Definition of Barrett's oesophagus

Patients were diagnosed with Barrett's oesophagus in the presence of an endoscopically visible columnar lined oesophagus above the gastroesophageal junction, located by the most proximal margin of the gastric folds AND oesophageal columnar (glandular) epithelium on histology. In keeping with British Society of Gastroenterology guidelines, the presence of goblet cells (intestinal metaplasia) was not a prerequisite, although goblet cells were present in the majority of patients diagnosed with Barrett's oesophagus.

Patients

All patients with Barrett's oesophagus were added to the database (n=1057). Patients in whom further endoscopy was considered inappropriate due to age and comorbidities were excluded as were those who decline further surveillance or were lost to follow up. Following the initial exclusions, 880 patients were left, of whom a further 143 with oesophageal adenocarcinoma or high grade dysplasia at presentation or within one year of their index endoscopy were excluded. Fifteen patients initially classified "indefinite for dysplasia" were also excluded as not assignable to either group (low grade dysplasia or no dysplasia). Seven hundred and twenty two patients were included in the final analysis (Figure 3.1).

Figure 3.1 Flow diagram of patient selection



Patients with short (≤ 3cm) and long (> 3cm) Barrett's segment were included. Patients underwent surveillance endoscopy with or without intended implementation of the Seattle protocol (quadrantic biopsies every 2cm throughout the length of the Barrett's segment) (Reid, Blount, Feng, & Levine 2000). In the absence of dysplasia, patients were offered surveillance endoscopy every two years, and the surveillance interval decreased to 3-6 months in those with low grade dysplasia. All patients with high grade dysplasia or invasive adenocarcinoma were discussed at a multidisciplinary oesophagogastric oncology meeting. Patients with high grade dysplasia were offered surgery (if medically fit) or endoscopic treatment, including argon coagulation or photodynamic therapy, moving towards endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA) of the residual segment from 2008. All patients continued acid suppression with prescribed proton pump inhibitors throughout surveillance.

Histology

Oesophageal biopsy specimens were interpreted and reported by staff at the pathology laboratory (GRI) with supervision by three GI specialist histopathologists, one with a research interest in Barrett's oesophagus (Dr James J Going). Two of the pathologists were in post over the entire recruitment period. Dysplasia was classified according to the Vienna classification and any difficult cases discussed among consultant pathologists for consensus opinion (Odze 2006). Persistent dysplasia was defined as dysplasia present at two or more endoscopies at least one year apart while non-persistent dysplasia was identified at one surveillance endoscopy and not subsequently after a minimum follow up of one year. In this series, Barrett's high grade dysplasia and oesophageal adenocarcinoma were only diagnosed in patients with a previous endoscopic and histological diagnosis of Barrett's oesophagus.

Histology

Deaths were identified between 1994 and 2010 inclusive, and the cause of death obtained from the regional Registrar's Office using patient name and date of birth. Only the primary cause of death was considered. Person years follow up were calculated for each patient from the date of entry into the database (1994-2009) until the date of death or 31st December 2012, whichever was earlier.

Statistical analysis

Data are presented as median (range). Variables were partitioned using standard thresholds: age <65, 65-74, >75; male, female; segment length ≤3cm, >3cm. Groups were compared using Chi square or Fisher's exact test, with a p value of <0.05 deemed significant. Student t-test analysed continuous variables. Kaplan-Meier survival analysis was used to assess the effect of risk factors such as segment length on the development of dysplasia and cancer. Multivariate regression analysis was used to examine the effects of variables on progression to HGD or adenocarcinoma with adjustment for confounders such as age and sex. The analysis was performed using SPSS software (SPSS for Windows v18.0, SPSS Inc, Chicago, IL, USA).

3.4 Results

Patient demographics

A total of 880 patients with Barrett's oesophagus were identified by endoscopy with biopsy confirmation between January 1994 and December 2009 (summarised in Table 3.1). There were 562 males (median age 62 years ± 13, range 17-96 years) and 318 females (median age 69 years ± 12, range 31-97 years). The median length of Barrett's segment was 6.6 ± 3.7 (range 1-22cm).

Patients with oesophageal adenocarcinoma (n=95) or high grade dysplasia (n=48) either at index endoscopy or within one year of the initial diagnosis of Barrett's oesophagus were excluded from further analysis (Figure 3.1). Fifteen patients labelled as "indefinite for dysplasia" were also excluded. The remaining cohort of 722 patients, with no dysplasia or low grade dysplasia at presentation, was analysed. This group underwent a total of 6249 years follow up (median 8.6 years).

Biopsy protocol

Of the 664 patients with no dysplasia on index endoscopy, adherence to the Seattle biopsy protocol (352 patients, 53%) yielded substantially greater individual biopsy numbers (median number of sites biopsied 54 v 17, $p<0.001$). Implementation of the biopsy protocol was higher among the four surgeons than the eight physicians (78% vs 22%, $p<0.05$) and was associated with higher numbers of biopsies with dysplasia (surgeons 103/141 patients with dysplasia (73%) vs physicians 38/141 patients with dysplasia (27%), $p<0.01$) as previously described (Abela, Going, Mackenzie, McKernan, O'Mahoney, & Stuart 2008). Six hundred and four patients (91%) had histological evidence of goblet cells (intestinal metaplasia).

Table 3.1 Summary of patient characteristics (n=880)

Characteristic	Category	n	
Sex	Male	562	64%
	Female	318	36%
Age (years)	Male	62	Range 17-96
	Female	69	Range 31-97
Length of segment (cm)	Metaplasia	6.5	Range 1-17
	LGD	6.8	Range 1-22
	HGD	7.9	Range 3-22
	Cancer	8.3	Range 1-22
Carstairs deprivation index	Deprived (5-7)	576	66%
	Moderate (3&4)	172	19%
	Affluent (1&2)	132	15%
Pathology at index endoscopy	Metaplasia	664	75.4%
	Indefinite for dysplasia	15	1.7%
	Low grade dysplasia	58	6.6%
	High grade dysplasia	48	5.5%
	Adenocarcinoma	95	10.8%

Carstairs deprivation index is an area-based measure (derived from 2001 census data) and uses postcode of residence at diagnosis to assign patients to one of seven categories which are amalgamated into affluent (categories 1&2), intermediate (categories 3&4) and deprived (categories 5-7). The role of deprivation and Barrett's oesophagus will be discussed in Chapter 4.

Patient mortality

During the study period 223 (n=223/722, 31%) patients died: 126 (57%) men and 97 (43%) women. The mean age at death was 74 years with men dying younger than women (70 years vs 77 years, t=3.9 95% CI 3.5-9.2, p<0.001 Student t-test). Twenty four deaths (11%) were directly related to Barrett's associated oesophageal adenocarcinoma and 93 deaths (42%) from myocardial infarction (MI) or other cardiovascular event (Table 3.2). Deaths from oesophageal adenocarcinoma were more common in those with LGD rather than glandular metaplasia at presentation (16 patients with LGD (67%) vs 8 patients with metaplasia alone (33%), p=0.01).

Table 3.2 Cause of death in patients with Barrett's oesophagus (n=223)

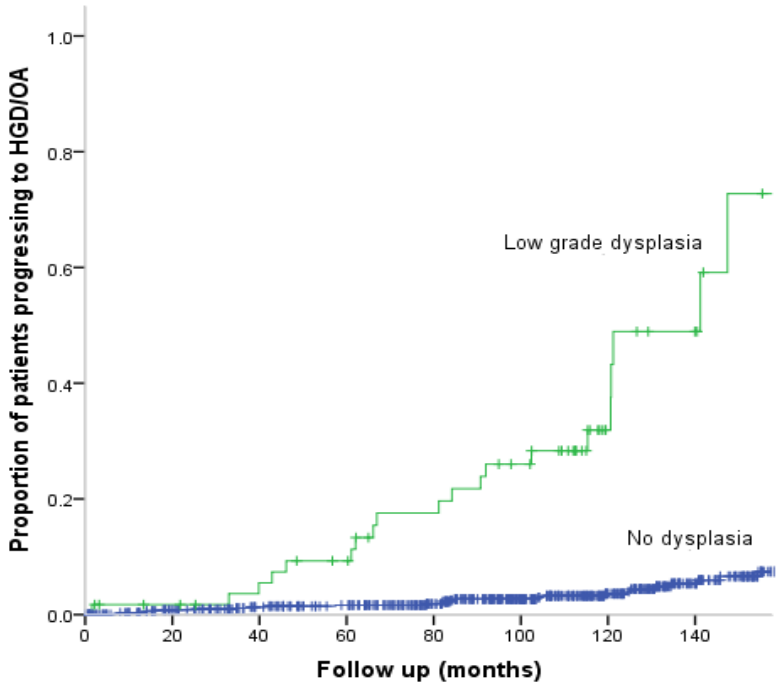
Death		**Number**	**%**
Cancer related	Oesophageal cancer	24	11
	Urological (bladder, kidney, prostate)	5	2
	GI (stomach, colon, HCC, pancreas)	19	9
	Gynaecological (uterus)	3	1
	Lung	11	5
	ENT (including lymphoma)	5	2
Non cancer	Cardiac event (inc MI)	93	42
	Respiratory (pneumonia, PE)	41	18
	Sepsis	12	5
	Alcohol related	10	5

Risk of progression to high grade dysplasia and cancer

Within the 722-patient cohort, the median age of patients developing HGD or OA was 69 years compared to non-progressors (64 years, $p<0.05$). At diagnosis of HGD/OA, male patients were younger than females (65 years vs 75 years, $p<0.01$).

The annual risks of HGD, OA and combined HGD/OA were 0.17%, 0.36% and 0.53% respectively (Table 3.3). These risks were substantially higher in patients with LGD at baseline endoscopy (2.0%, 2.7% and 4.7%, $p<0.001$). Figure 3.2 illustrates the proportion of patients developing either HGD or OA according to the presence or absence of LGD at baseline endoscopy. Within 2, 5 and 10 years from initial diagnosis of Barrett's oesophagus, either HGD or OA was diagnosed in 1%, 2% and 6% patients without baseline LGD dysplasia, compared with 5%, 10% and 40% patients with LGD at presentation ($p<0.001$). On regression analysis, male sex ($p<0.05$), the presence of LGD at baseline endoscopy ($p<0.001$) and being older ($p<0.01$) were associated with an increased risk of malignant progression.

Figure 3.2 The progression of patients with Barrett's oesophagus to HGD/OA according to the presence or absence of baseline LGD



Survival plot illustrating the proportion of patients progressing to HGD or adenocarcinoma over time. Patients with low grade dysplasia (LGD) at baseline endoscopy carry higher rates of disease progression compared with patients with columnar metaplasia alone at baseline ($p<0.001$)

Table 3.3 Annual incidence rates of HGD, OA, and combined HGD/OA in patients with Barrett's oesophagus (n=722)

	Follow up (years)	HGD cases (n)	OA cases (n)	Combined HGD/OA (n)	Annual risk HGD (%)	Annual risk OA (%)	Combined HGD/OA (%)
All cases	**6249**	**19**	**33**	**52**	**0.30 (0.18-0.46)**	**0.53 (0.42-0.61)**	**0.83 (0.73-0.93)**
Male	3813	15	20	35	0.39 (0.22-0.58)	0.52 (0.48-0.56)	0.91 (0.79-1.02)
Female	2436	4	13	17	0.16 (0.09-0.24)	0.82 (0.77-0.90)	0.98 (0.82-1.22)
No dysplasia at baseline	**5802**	**10**	**21**	**31**	**0.17 (0.08-0.28)**	**0.36 (0.22-0.44)**	**0.53 (0.41-0.66)**
Male	3495	7	13	20	0.20 (0.13-0.30)	0.37 (0.23-0.46)	0.57 (0.50-0.62)
Female	2307	3	8	11	0.13 (0.07-0.20)	0.35 (0.25-0.45)	0.48 (0.41-0.55)
LGD at baseline	**447**	**9**	**12**	**21**	**2.01 (1.08-3.15)**	**2.68 (2.00-3.10)**	**4.69 (3.55-5.55)**
Male	318	8	7	15	2.52 (1.80-3.20)	2.20 (1.72-2.72)	4.72 (3.70-5.70)
Female	129	1	5	6	0.78 (0.14-1.20)	3.88 (2.10-4.86)	4.66 (2.80-6.68)

HGD (high grade dysplasia), OA (oesophageal adenocarcinoma), n (number of patients)

Outcomes for patients with columnar metaplasia but no baseline dysplasia

Of the 664 patients with columnar metaplasia only on baseline endoscopy, 85 (13%) subsequently progressed to LGD, HGD or invasive adenocarcinoma with a male preponderance (52 men, 33 women).

Of these, 54 of 664 patients (8%; 32 men, 22 women) developed LGD during follow up, with an incidence rate of 0.93% per year. Median time to development of LGD was 36 months (range 12-96). Ten patients (1.5%, 7 men, 3 women) developed HGD during the study period, with an incidence rate of 0.17% per year. Twenty one patients (3.2%, 13 men, 8 women) developed oesophageal adenocarcinoma, an overall incidence rate of 0.36% per year.

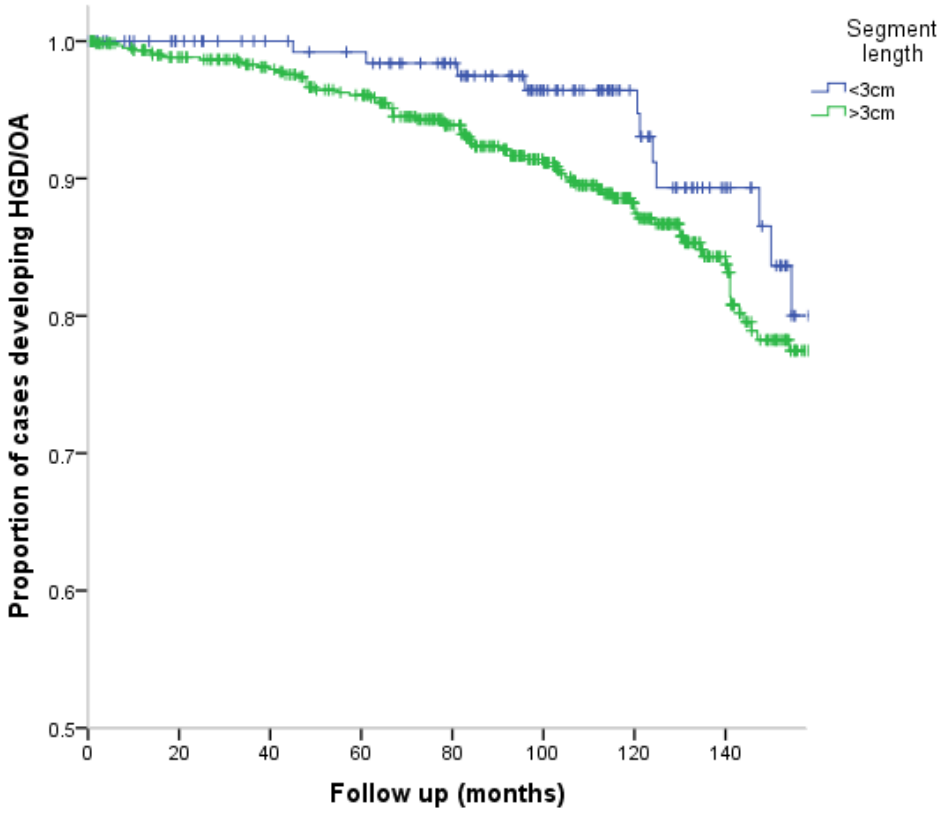
Outcomes for patients with prevalent low grade dysplasia

Fifty eight patients presented with LGD at baseline endoscopy (40 men, 18 women; median age 67 years, range 49-96). Of these, 21 (36%) progressed to HGD or OA (HGD n=9, OA n=12) with annual incidence rates of HGD and OA of 2.0% and 2.7% respectively. Patients with low grade dysplasia on index endoscopy were older than those without (67 years vs 64 years, $p<0.05$) and more likely to be male ($p<0.02$).

Segment length and progression to high grade dysplasia or adenocarcinoma

The length of the Barrett's segment was collected for all 85 patients who progressed to dysplasia and adenocarcinoma. Patients with long segment Barrett's oesophagus (>3cm) had higher rates of progression to dysplasia and adenocarcinoma than patients with short segments (long segment, n=73 (87%); short segment, n=12 (13%), 95% CI short 174-190 vs long 180-193, p=0.04, Figure 3.3).

Figure 3.3 Time to high grade dysplasia and/or adenocarcinoma according to segment length



Survival plot displaying the progression to dysplasia and/or adenocarcinoma according to segment length (short vs long). Patients with long segment disease tended to progress to dysplasia or adenocarcinoma more quickly than those with short segment disease (p=0.04).

3.5 Discussion

The cohort study of patients with Barrett's oesophagus in the West of Scotland has shown patients with LGD at baseline endoscopy are at increased risk of progression to dysplasia and oesophageal adenocarcinoma. Older age (>75 years) and male sex were independent risk factors for disease progression.

The risk of adenocarcinoma in Barrett's oesophagus has been long debated. Early studies suggested high incidence rates with annual rates of malignant progression of 1.9% per year in the 1980s (Hammeeteman, Tytgat, & Houthoff 1989). Over time, larger studies suggested lower risks with systematic reviews estimating annual risk of progression to adenocarcinoma to vary between 0.5% and 0.63% per year (Hammeeteman, Tytgat, & Houthoff 1989;Shaheen, Crosby, & Bozymski 2000;Sikkema, de Jonge, & Steyerberg 2010). Several recent studies have suggested that Barrett's oesophagus is still less hazardous with annual risk of progression around 0.15-0.2% (de Jonge, van Blankenstein, Looman, Casparie, Meijer, & Kuipers 2010). One concern when interpreting studies is the potential lack of a clear definition of Barrett's oesophagus which may lead to overdiagnosis and subsequent dilution of the dysplasia/ adenocarcinoma progression rate. Therefore for inclusion in our study, all patients required a prospective endoscopic and histological diagnosis of Barrett's oesophagus rather than relying solely on retrospective endoscopic or pathological records, ensuring a well-defined Barrett's cohort.

There is significant variation in the reported rates of dysplasia and adenocarcinoma in patients with Barrett's oesophagus but the UK appears to have a higher burden of oesophageal adenocarcinoma compared with the US (12-16 per 100 000 in UK compared with 3-5 per 100 000 in US) (www.cancerresearchuk.org 2006). Furthermore, there has been a rise in the incidence of Barrett's associated adenocarcinoma in the UK over the last three decades, increasing by more than 5% per year, with Scotland now having the highest rates (Jankowski, Provenzale, & Moayyedi 2002;McKinney, Sharp, Macfarlane et al. 1995). Presumably this trend is related to the increased prevalence of Barrett's oesophagus within the UK (Prach, MacDonald, Hopwood et al. 1997).

The rates of progression to HGD and adenocarcinoma in our study are comparable with recent systematic reviews. A recent US multicentre cohort of patients with Barrett's oesophagus reported similar results to our study although the rate of

progression to adenocarcinoma was lower (LGD 3.6% per year; HGD 0.48% per year and 0.27% per year adenocarcinoma) (Wani, Falk, Hall et al. 2011). A population study (the Danish Pathology Registry) from Denmark reported lower annual incidence rates of high grade dysplasia and adenocarcinoma than previously described (HGD 0.19% and adenocarcinoma 0.12%) (Hvid-Jensen, Pedersen, Drewes et al. 2011). A large-scale Dutch population study of pathology records from 16325 patients reported an annual cancer risk of 0.4%, and incidence rates of 4.3 per 1000 person years for OA and 5.8 per 1000 person years for combined HGD and adenocarcinoma (de Jonge, van Blankenstein, Looman, Casparie, Meijer, & Kuipers 2010).

Our study describes the presence of LGD at baseline endoscopy as an independent risk factor for disease progression, with 40% of patients developing HGD or adenocarcinoma within 10 years. This has been confirmed by a recent risk assessment study from a Northern Ireland population where LGD was the strongest predictor of progression (Bird-Leiberman, Dunn, Coleman et al. 2012).

At 22 per 100,000 per annum Scotland has one of the highest rates of oesophageal adenocarcinoma in the world, compared with Denmark and The Netherlands (17 per 100,000 and 12 per 100,000) (Anon 2008). The reasons for our higher mortality rates are difficult to define at present. Although deprivation and obesity may play a part, other environmental factors (such as diet, vitamin D and nitrate levels) and epi/genetic factors may be involved. Several genes and biomarkers have been studied in the development and progression of Barrett's oesophagus but further research using gene sequencing is required (Moyes & Going 2011). Of course, our higher rates could be linked to "the Glasgow effect". There has been considerable amount of attention recently in relation to this epidemiological phenomenon – the high levels of mortality in Scotland (and Glasgow) compared to other UK cites which cannot be solely explained by differences in socioeconomic status (Hanlon, Lawder, Buchanan et al. 2005).

Study limitations

There are limitations within this study. The analysis was retrospective within a local cohort. However our unit is currently establishing a prospective database of all patients with Barrett's oesophagus throughout the 1.2 million population within the West of Scotland to determine which risk factors (clinical and molecular) may play a role in malignant progression. This will provide an excellent opportunity to assess the effects of deprivation and Barrett's epithelium and to understand the molecular and pathophysiological abnormalities associated with this unstable oesophageal mucosa. In the future combined staging of Barrett's oesophagus with clinical and pathological markers may aid in appropriate risk stratification of patients, targeting surveillance endoscopy and therapeutic interventions in those at high risk.

3.6 Conclusion

Patients with Barrett's population in the West of Scotland have high rates of malignant progression confirming the need for careful endoscopic surveillance. Low grade dysplasia is a significant risk factor for progression to HGD or adenocarcinoma, with 40% of patients with LGD at presentation progressing within 10 years. This is a high risk group of patients and an interesting cohort on which to focus further research. Additional work is required to assess the reasons why patients have a tendency towards dysplasia and understand the molecular and physiological abnormalities associated with this unstable oesophageal mucosa. In the future, we would anticipate other factors (clinical and molecular) will aid in appropriate risk stratification of patients, targeting surveillance endoscopy and therapeutic interventions in those at high risk. For the present time, we have a "high risk" Barrett's population in the West of Scotland providing an ideal niche for molecular and chemopreventative studies.

Chapter 4

Deprivation and Barrett's oesophagus: an observational study

The West of Scotland, and in particular the city of Glasgow, is renowned for its high mortality rates from ischaemic heart disease and cancer. The incidence of oesophageal adenocarcinoma has risen in Scotland over the last thirty years, increases not wholly explained by changes in diagnostic or reporting practices (Brewster, Fraser, McKinney et al. 2000). An observational study of patients with Barrett's oesophagus within the catchment area of Glasgow Royal Infirmary was performed to assess any relationship between Barrett's oesophagus, dysplasia and adenocarcinoma and deprivation. The results show a clear association between deprivation and higher rates of disease progression and cancer related death, with patients in deprived areas at greater risk than those in affluent areas. Although the reasons for this remains unclear, further research assessing environmental (smoking, alcohol, diet) and genetic factors within this "at risk" population is required.

4.1 Introduction

For many years, Glasgow has been renowned for its high mortality rates, specifically from ischaemic heart disease and cancer, compared with the rest of the world. In 2010, the media described this as the "Glasgow Effect". A recent study by Professor Walsh and his colleagues assessing mortality in three UK cities (Glasgow, Liverpool and Manchester) found that although the deprivation profiles were almost identical, premature deaths in Glasgow were more than 30% higher and all deaths approximately 15% higher (Walsh, Bendel, Jones et al. 2010). The relationship between deprivation and poor outcomes has been recognised for many years. In 1985, Hume acknowledged that Greater Glasgow Health Board mortality rates varied between different communities, with greater mortality in areas of socioeconomic deprivation (Hume and Womersley 1985).

The association between poor health, cancer outcomes and deprivation in the West of Scotland has been previously described. Coleman and colleagues reported a 5% difference in survival between patients from the most deprived areas compared with the most affluent areas within the UK (Coleman, Babb, Damiecki et al. 1999). More recently, Hole and McArdle confirmed that cancer specific survival rates following colorectal cancer were lower in deprived patients residing in the West of Scotland (Hole and McArdle 2002). As the West of Scotland has a spectrum of socioeconomic groups, residing in close proximity to one another, and a high incidence of Barrett's oesophagus and adenocarcinoma, it appears to be an ideal setting to examine the effects of deprivation on these conditions.

There is no single universally agreed definition of deprivation. Material deprivation reflects the accessibility of material goods and resources to people and is a useful marker of deprivation in clinical studies. Material deprivation is measured by the Carstairs and Morris index, originally developed in the 1980s using the 1981 census data (Carstairs and Morris 1991). It is a scale composed of four variables which when combined creates a composite score.

The variables are:

1) Overcrowding – persons in private households living at a density of more than one person per room as a proportion of all persons in private households
2) Male unemployment – proportion of economically active males who are seeking work
3) Social class 4 or 5 – proportion of all persons in private households with head of household in social class 4 or 5
4) No car – proportion of all persons in private households with no car

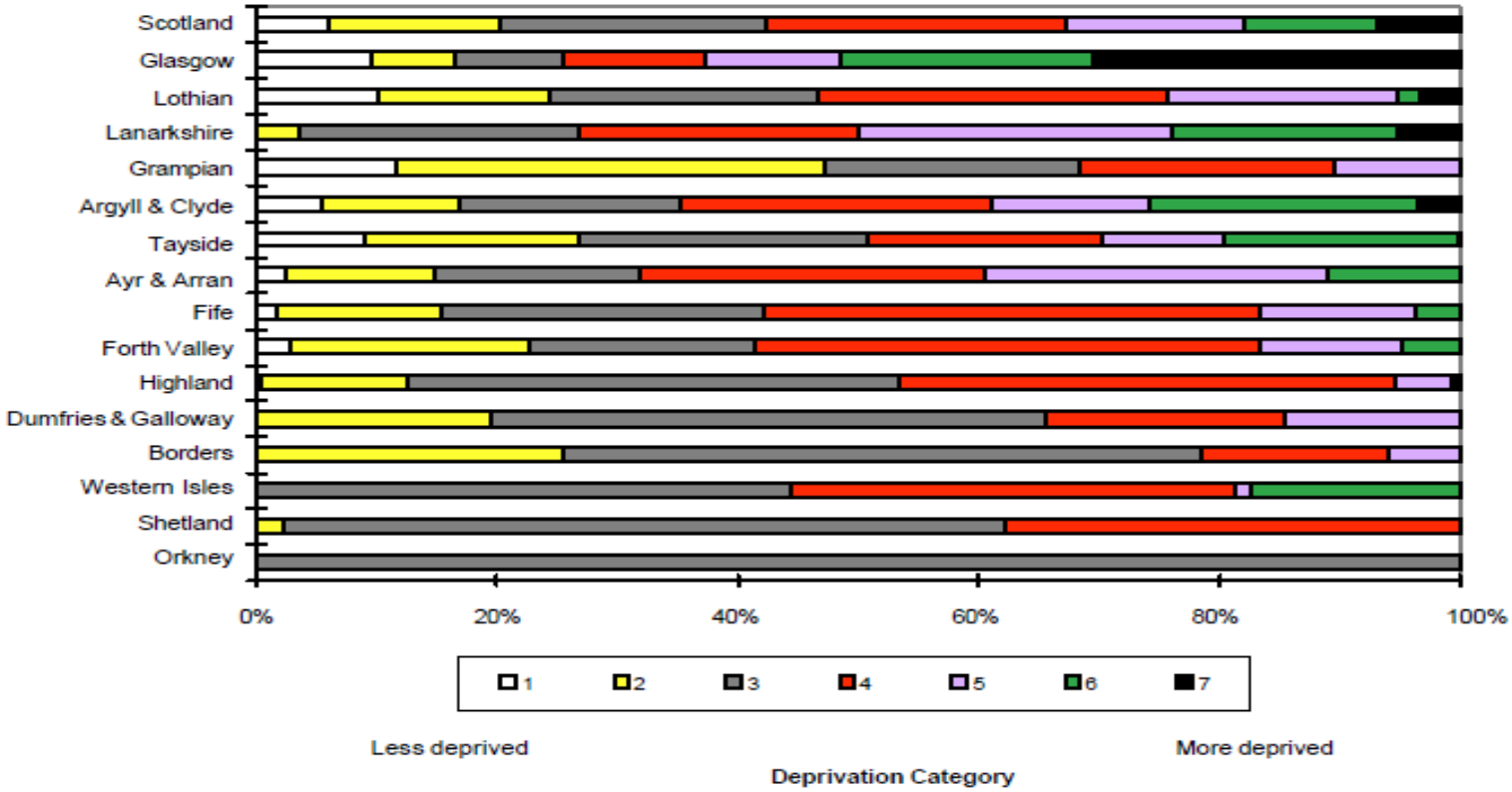
Deprivation is often associated with smoking, increased alcohol consumption, poor nutrition with diets lacking in fruit and vegetables and perhaps family history.

The Carstairs deprivation score is divided into seven categories ranging from very high deprivation (category 7) to very low (category 1) deprivation. Figure 4.1 summarises the proportions of people in Scotland living in postcode sectors assigned to deprivation categories according to health board. It clearly shows Greater Glasgow has the largest number of people living in deprivation categories 6 and 7. Furthermore of all those living in deprivation category 7 areas, 80% reside in Glasgow. The Carstairs deprivation index has been utilised in cancer patients and is particularly appropriate for use in our population (Hole & McArdle 2002).

4.2 Study Aims

The aim of this study was to assess any relationship between the presence of Barrett's oesophagus, in particular dysplasia and adenocarcinoma, and deprivation using the postcode sector of residence. A secondary aim was to examine the number of deaths in our cohort according to postcode sector.

Figure 4.1 Deprivation at health board level



Distribution of health board populations by Carstairs deprivation category (1991 census). Reproduced from *www.show.scot.nhs.uk/publications/isd/deprivation_and_health/images*

4.3 Methods

Database

The previously described database of all patients diagnosed with Barrett's oesophagus from January 1994 to December 2009 (Chapter 3) was used for this study. All patients with histologically proven Barrett's oesophagus resided within the catchment area of Glasgow Royal Infirmary, an inner-city teaching hospital in the north east of the city, covering a population of 560,000. Tertiary referrals of patients non-resident within the catchment area were excluded.

Postcode

The postcode of residence at the time of diagnosis was obtained and the Carstairs deprivation index used to categorise each postcode into one of seven groups. For illustrative purposes the seven categories were amalgamated into three groups: affluent (categories 1 and 2), intermediate (categories 3 and 4) and deprived (categories 5-7). The postal town associated with each postcode was also noted. Table 4.1 lists each postal town and postcode within the Glasgow Royal Infirmary catchment area.

Outcome measures

The two main outcome measures were 1) cause of death and 2) number of cases of high grade dysplasia and/or oesophageal adenocarcinoma.

1) Cause of death

Deaths were identified between 1994 and 2011 inclusive, and the cause of death obtained from the regional Registrar's Office for Deaths and Births using patient name and date of birth. For the purposes of the study, only the primary cause of death was considered. Deaths were categorised into four groups:

(1) Barrett's related oesophageal adenocarcinoma,

(2) myocardial infarction (or ischaemic related event),

(3) other cancer death (colon, lung, pancreatic, prostate)

(4) other non-cancer related death (pneumonia, alcohol related death, renal failure, sepsis or pulmonary embolism)

2) Diagnosis of dysplasia/adenocarcinoma

All cases diagnosed with high grade dysplasia and Barrett's related oesophageal adenocarcinoma were included (as previously described in Chapter 3).

Statistical analysis

Data are presented as mean values ± standard deviation. Comparison between groups was performed using Chi square or Fisher's exact test, with a p value of <0.05 deemed significant. The analysis was performed using SPSS for Windows v18.0, SPSS Inc, Chicago, IL, USA.

Table 4.1 List of postcodes and postal towns of Barrett's patients

Postcode	Postal town	Postcode	Postal town
G1	City centre	PA1	Paisley (central)
G12	Dowanhill	PA13	Kilmalcolm
G15	Drumchapel	PA14	Port Glasgow
G2	City centre (south east)	PA15	Greenock
G20	Maryhill	PA16	Greenock
G21	Springburn	PA19	Gourock
G3	Finnieston	PA2	Paisley (south)
G31	Dennistoun	PA20	Isle of Bute
G32	Shettleston	PA23	Dunoon
G33	Stepps	PA28	Campbeltown
G34	Garthamlock	PA29	Tarbert
G4	City centre (north)	PA3	Paisley (north)
G40	Rutherglen	PA34	Oban
G41	Bellahouston	PA38	Appin
G42	Queen's Park	PA4	Renfrew
G43	Thornliebank	PA42	Isle of Islay
G44	Cathcart	PA5	Johnstone
G45	Carmunnock	PA78	Isle of Coll
G46	Giffnock	PA8	Erskine
G5	City centre (south)	ML1	Newarthil
G51	Shieldhall	ML10	Strathaven
G52	Renfrew	ML12	Douglas
G53	Nitshill	ML4	Bellshill
G60	Old Kilpatrick	ML5	Coatbridge
G61	Bearsden	ML6	Caldercruix
G62	Milngavie	ML7	Shotts
G63	Balfron	ML8	Carluke
G64	Torrance	ML9	Stonehouse
G65	Kilsyth	KA1	Hurlford
G66	Lennoxtown	KA10	Troon
G67	Cumbernauld	KA12	Dreghorn
G68	Croy	KA13	Kilwinning
G69	Muirhead	KA15	Beith
G71	Uddingston	KA18	Cumnock
G72	Cambuslang	KA2	Dundonald
G73	Rutherglen (south)	KA22	Ardrossan
G74	Stewartfield	KA25	Kilbirnie
G76	Eaglesham	KA26	Girvan
G77	Newton Mearns	KA30	Largs
G78	Neilston	KA4	Galston
G81	Clydebank	KA5	Mauchline
G83	Inverbeg	KA6	Dalmellington

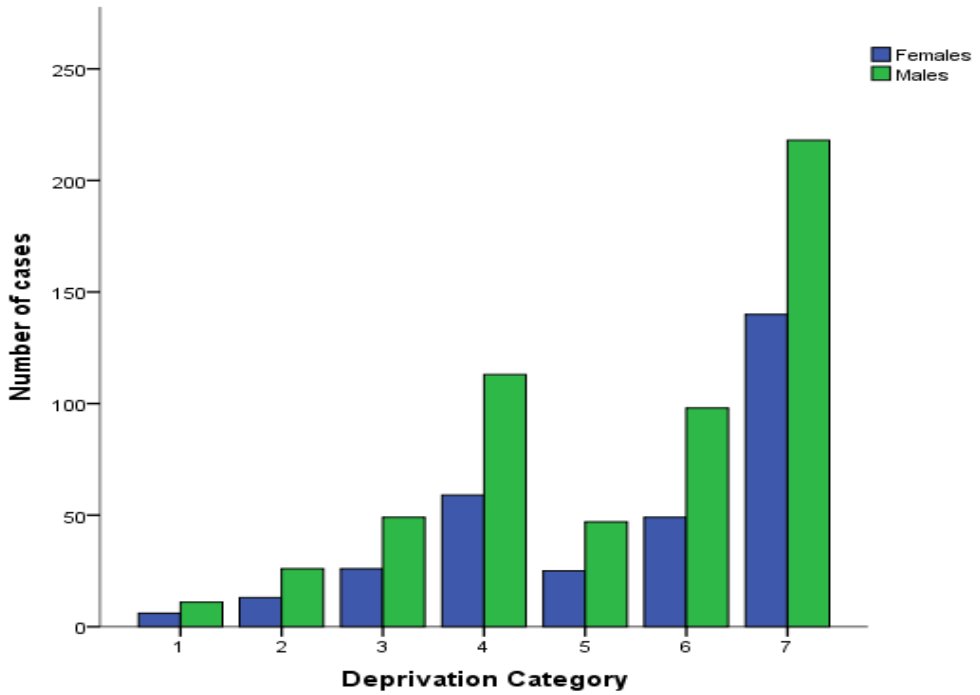
4.4 Results

Patient demographics

A total of 880 patients underwent endoscopic surveillance for Barrett's oesophagus between January 1994 and December 2009 (summarised in Table 3.1). Of these, 91 patients (10%) resided out with the local catchment area of Glasgow Royal Infirmary being referred from the Isles, Lanarkshire, Stirling or Dumfries for further endoscopic assessment and/or treatment. 50 postcode sectors constitute the "G" (Glasgow) postcode area, and 39 (78%) of these sectors were represented in this cohort of Barrett's patients.

In the study population, the proportion of Barrett's oesophagus appeared to be higher in deprived areas compared to affluent areas with 65% of patients living in deprived areas (Figure 4.2). The majority of patients with Barrett's oesophagus lived in the postal towns of Springburn, Shettleston, Stepps, Dennistoun and the Gallowgate. Although there was a male preponderance across all categories, the majority of patients within the cohort were deprived males.

Figure 4.2 Cases of Barrett's oesophagus by deprivation category and sex

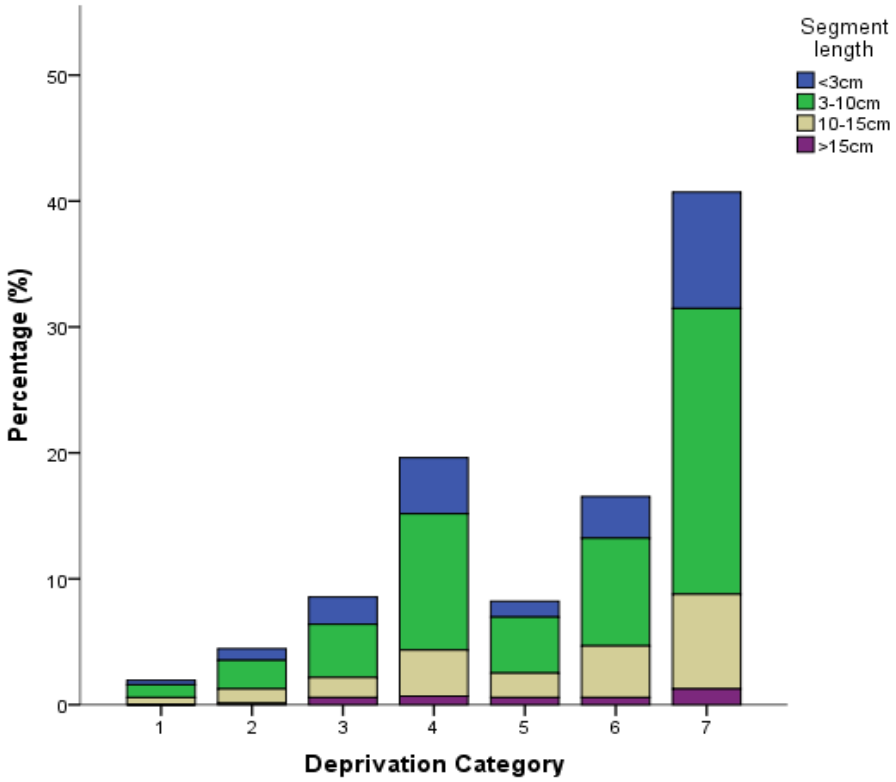


Bar chart displaying number of cases of Barrett's oesophagus (n=880) according to sex and Carstairs deprivation category codes. There was a preponderance of male patients and a higher number of patients with Barrett's oesophagus residing in deprived areas (categories 5-7).

Deprivation and segment length

The mean segment length in the Barrett's cohort was 6.6cm (range 2-22cm). Although the ratio of short: long segment disease was comparable across all deprivation categories, there was a greater proportion of patients with long segment disease living in deprived areas, than those in affluent areas (Figure 4.3). Patients with Barrett's segments >15cm tended to live in deprived areas (categories 5-7) whereas there were no patients with >15cm segment residing in an affluent area (categories 1&2, p=0.07).

Figure 4.3 Barrett's oesophagus and deprivation and segment length



Bar chart displaying percentage of all patients with Barrett's oesophagus (n=880) according to Carstairs deprivation category and length of Barrett's segment.

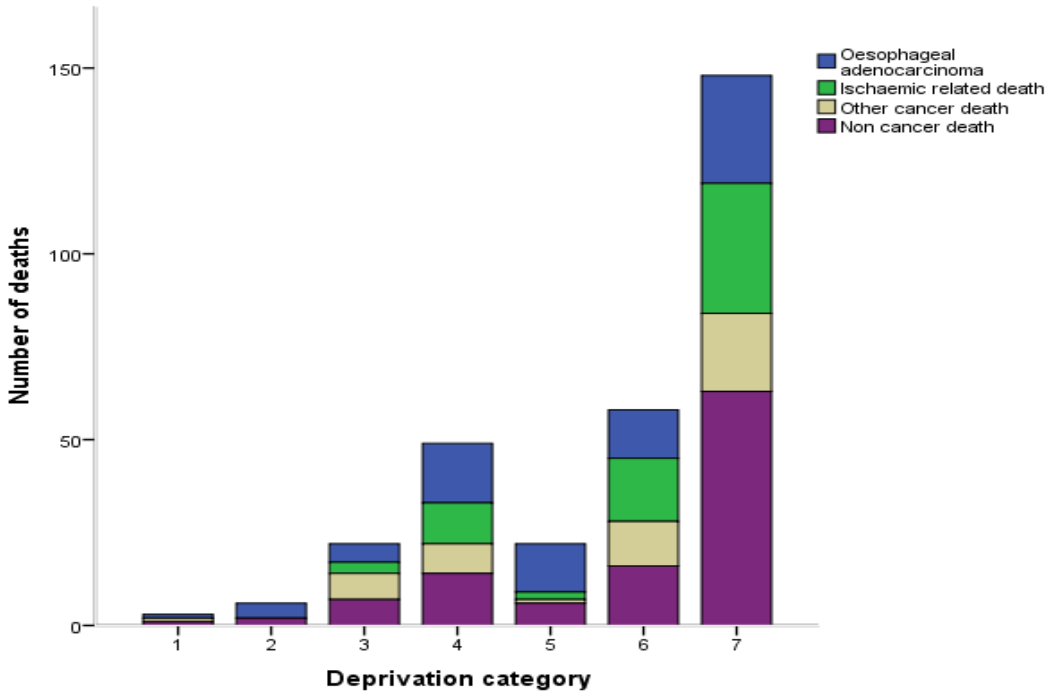
Deprivation and cause of death

There were 308 deaths with 74% patients living in deprived inner city areas, with the highest number of deaths occurring in Stepps, Shettleston and Dennistoun ($p<0.001$). Figure 4.4 illustrates the spectrum of postcode sectors found within the Barrett's cohort, and highlights the areas with highest number of deaths (purple and black stars).

Of the 308 deaths, there were 81 deaths from oesophageal adenocarcinoma (27%), 61 deaths from ischaemic heart disease (22%), 50 deaths from other cancers (16%) and 109 deaths from other causes (35%). Figure 4.5 is a histogram displaying the cause of death grouped by deprivation. The majority of deaths (all cause) were found in patients who resided in deprived areas. Deprivation category 7 had twice the number of deaths compared with all other areas: and the greatest proportion of OA related deaths. Moreover 20% of the deaths within this area were from other non-cancer causes such as alcohol (liver disease), suicide or pneumonia and 15% from ischaemic heart disease. Deaths from oesophageal adenocarcinoma occurred in 32% of those living within category 7 compared with only 7% in those in affluent categories 1 and 2 ($p<0.001$).

The distribution of deaths from oesophageal adenocarcinoma and ischaemic heart disease was similar to the distribution of all-cause deaths (Figure 4.6 and 4.7). There was a clear association between deprivation and higher oesophageal cancer-specific and ischaemic-related deaths (OA $p<0.001$ and IHD $p<0.05$) (Table 4.2).

Figure 4.5 Cause of death in Barrett's patients by deprivation category



Bar chart displaying cause of death in patients with Barrett's oesophagus (n=308) according to Carstairs deprivation category. All cause and oesophageal adenocarcinoma mortality rates were higher in those patients residing in deprived areas (deprivation category 5-7, $p<0.001$).

Legends to Figures overleaf

Figure 4.4 Illustrative map of Greater Glasgow showing distribution of deaths (all-cause) within the Barrett's cohort (n=308). Black and purple stars related to highest number of deaths.

Figure 4.6 Illustrative map of Greater Glasgow displaying number of oesophageal adenocarcinoma related deaths according to postal towns (n=81)

Figure 4.7 Illustrative map of Greater Glasgow displaying number of deaths from ischaemic heart disease according to postal town of residence (n=61).

Figure 4.4 Number of all-cause deaths within population with Barrett's oesophagus according to postal town

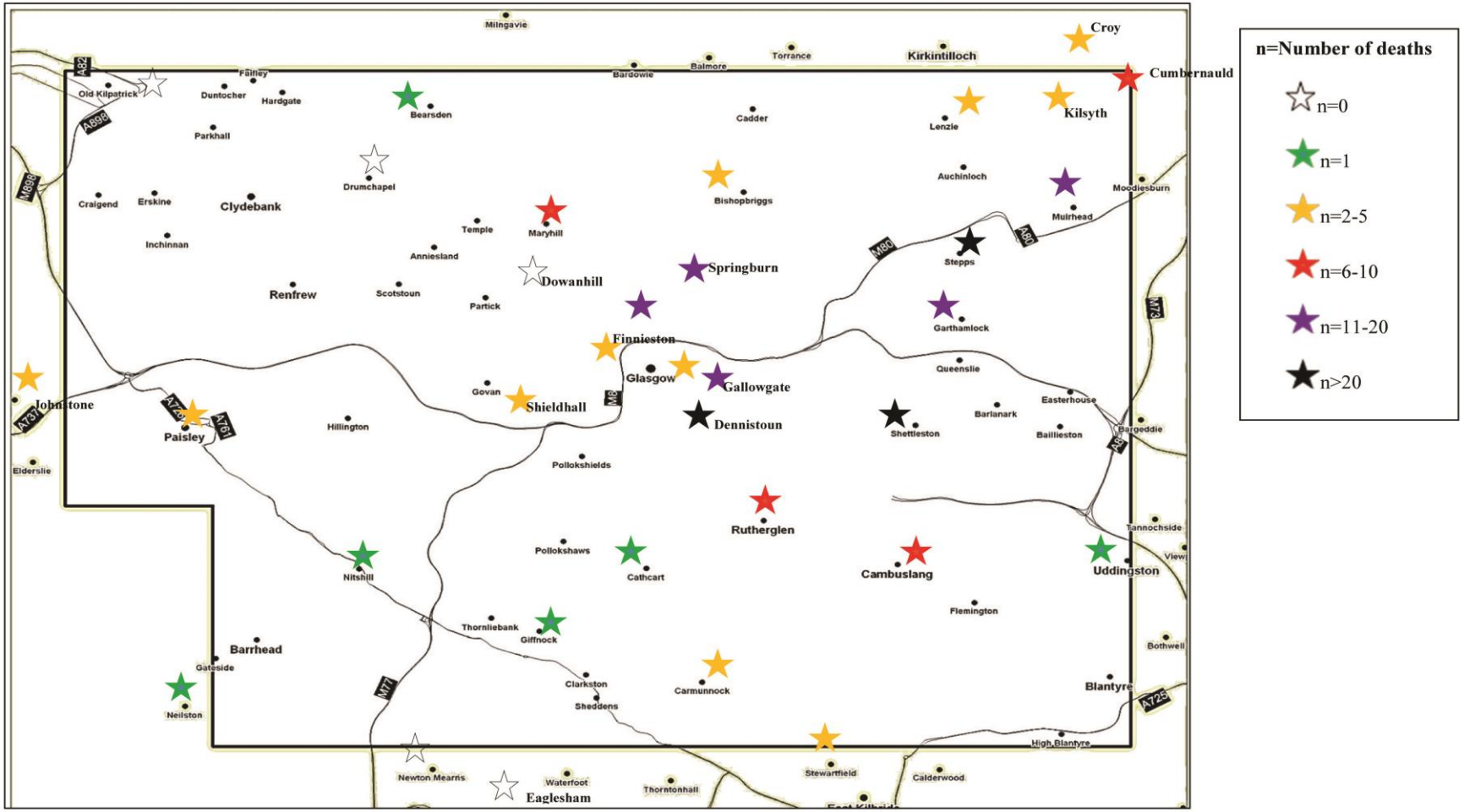


Figure 4.6 Number of deaths from oesophageal adenocarcinoma within Barrett's cohort according to postal town

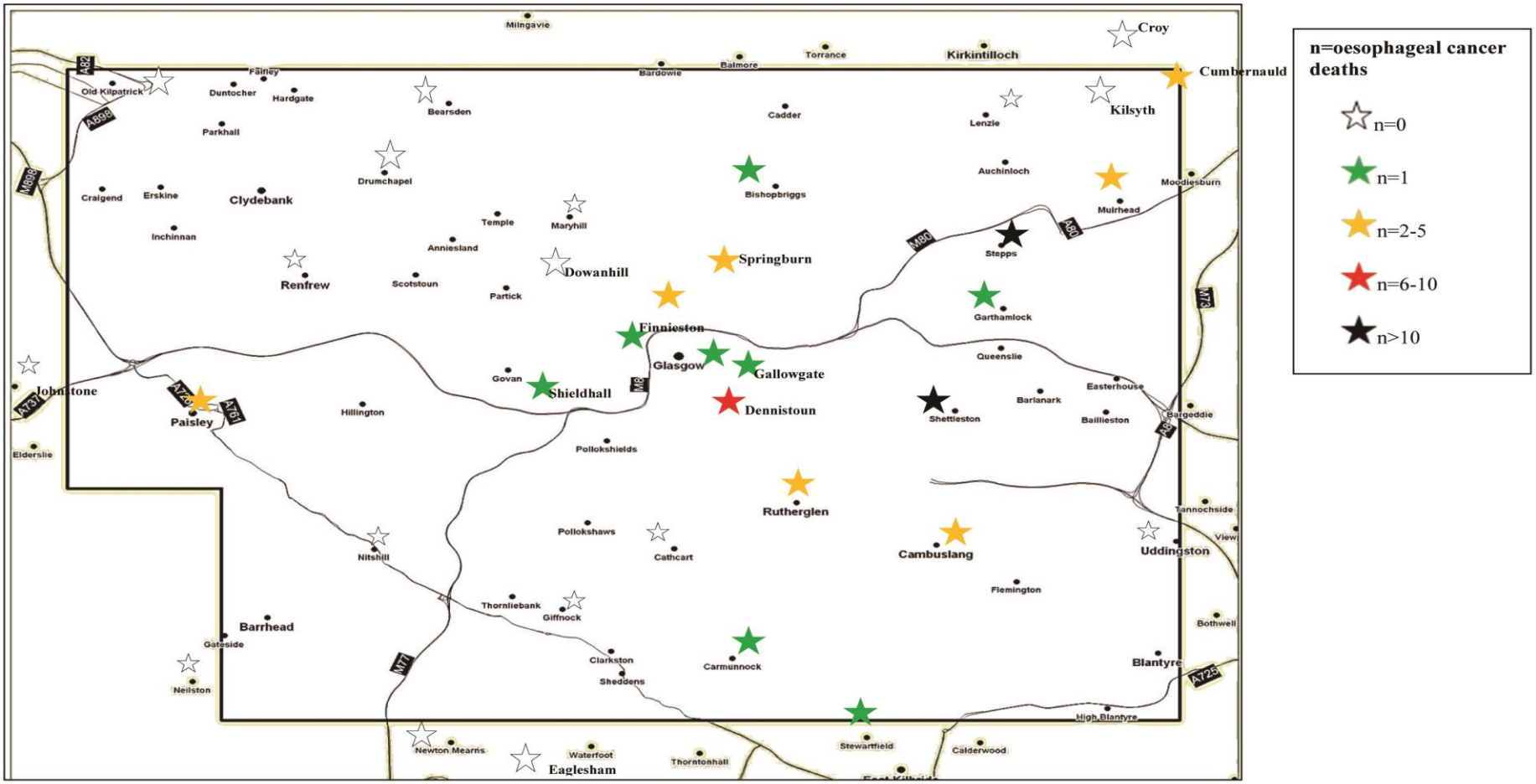
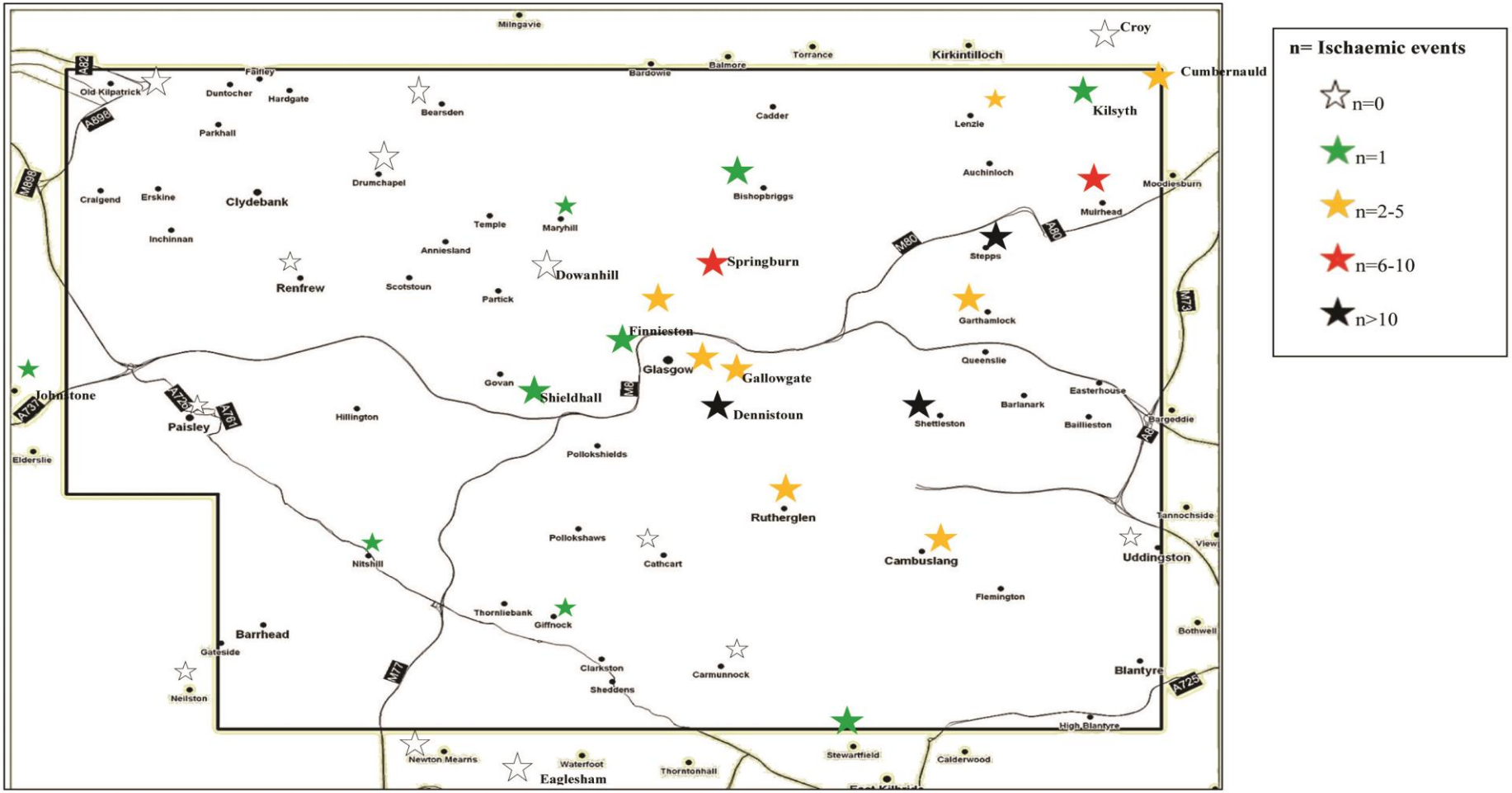


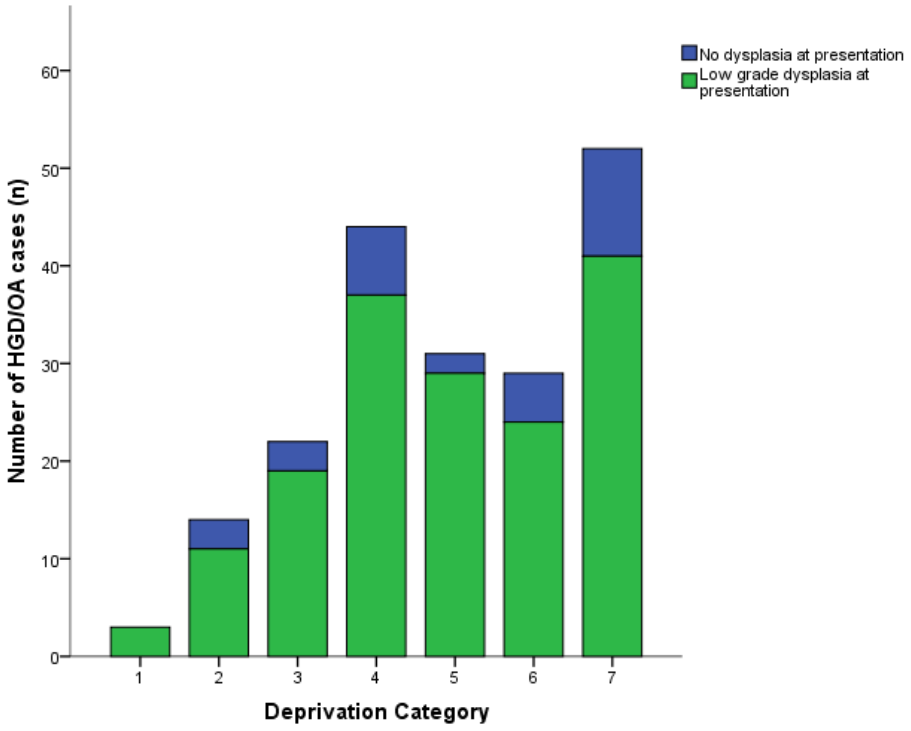
Figure 4.7 Number of deaths from ischaemic heart disease within population with Barrett's oesophagus according to postal town



High grade dysplasia and adenocarcinoma by postal sector

Malignant change, defined as the presence or development of HGD, OA or combined HGD/OA, was present in 197 cases (22%). Deprivation was associated with higher rates of progression to HGD and OA ($p<0.001$). Patients living in deprived areas were more likely to present with initial dysplasia (48% deprived vs 7% affluent areas, $p<0.05$) and develop dysplasia during follow up (57% deprived vs 9% affluent areas, $p<0.01$).

Figure 4.8 Progression to HGD/OA by deprivation category and presence of low grade dysplasia at initial presentation (n=197)



Bar chart displaying the number of cases of HGD and oesophageal adenocarcinoma (n=197) according to Carstairs deprivation category and the presence (green) or absence (blue) of low grade dysplasia at presentation.

Of 197 patients progressing to HGD and OA, the majority of patients lived in Shettleston, Dennistoun, Muirheard and Stepps – the most "at risk" and deprived areas within this Glasgow cohort.

4.5 Discussion

This observational study has shown a clear relationship between socioeconomic deprivation and an increase in the number of cases of Barrett's oesophagus, with higher numbers of deprived patients progressing to HGD and oesophageal cancer and cancer related death.

The National Records of Scotland reported higher death rates in Glasgow, compared with the rest of Scotland with the main cause of death due to circulatory disease, followed by cancer (ISD Scotland 2012). Higher mortality rates are associated with lower socioeconomic status, poor diet and deprivation. This study has shown that the "at risk" areas within the catchment of the Royal Infirmary are those with the highest deprivation index, namely Shettleston, Dennistoun and Stepps. This is true for all-cause mortality, oesophageal cancer death and ischaemic heart mortality. These areas also carried the highest rates of malignant progression. This is one of the first studies to show a clear association between deprivation and Barrett's adenocarcinoma.

It is generally believed that Barrett's oesophagus and its associated adenocarcinoma are more common in white Caucasian males of higher socioeconomic status although there are limited studies to support this. In 2005, Ford and colleagues studied a large population (20,412 patients) with Barrett's oesophagus in England. They suggested white race and higher socioeconomic status were risk factors for the development of oesophagitis and Barrett's oesophagus (Ford, Forman, Reynolds et al. 2005). Other studies have assessed the relationship between deprivation and oesophageal adenocarcinoma, but the results are variable. A study in Scotland, using national data suggested that in contrast to the association between squamous oesophageal cancer and deprivation, there was no clear association between deprivation and oesophageal adenocarcinoma (Brewster, Fraser, McKinney, & Black 2000). However, a case control study from the US, with level of education as a marker for socioeconomic status, showed those with higher education were at lower risk of developing Barrett's oesophagus (Kubo, Levin, Block et al. 2009). This was corroborated by a large population study in Sweden (HUNT study) which suggested the risk of reflux increased with decreasing socioeconomic status, based on occupation, education and material deprivation (Jansson, Nordenstedt, Johansson et al. 2007).

The dramatic increase in incidence of oesophageal adenocarcinoma, particularly in Caucasian males appears to be in response to environmental and lifestyle factors. Factors such as alcohol intake (volume and type of alcohol), smoking, diet and exercise may play a role (Kubo, Levin, Block, Rumore, Quesenberry, Buffler, & Corley 2009). Our high risk areas of Shettleston, Stepps and Dennistoun have patients with alcohol and smoking addictions. Although the relationship between smoking and squamous carcinoma of the oesophagus is clearly demonstrated, and has recently been linked with Barrett's oesophagus, an association with oesophageal adenocarcinoma has not been well-defined (Cook, Shaheen, Anderson et al. 2012). Deprivation is associated with smoking, higher alcohol intake and a diet poor in fruit and vegetables (Hanlon, Lawder, Buchanan, Redpath, Walsh, Wood, Bain, Brewster, & Chalmers 2005). These effects of deprivation may play a part on the development of Barrett's oesophagus and progression to adenocarcinoma although further work is required.

Although studies assessing relationships between deprivation and oesophageal adenocarcinoma are limited, there are numerous studies reporting a link between socioeconomic status and cancers such as breast, colon and lung cancer. A review studying the effect of deprivation and cancer outcome leaves little doubt that socioeconomic status is an important factor, although the underlying explanations are not so well documented (Woods, Rachet, & Coleman 2006). One reason may be the stage of presentation of disease with patients from higher social classes being more aware of symptoms and presenting earlier. However a systematic review did not find convincing evidence for patient delay among deprived women with breast cancer (Ramirez, Westcombe, & Burgess 1999). Tumour biology may play a role with lifestyle factors, such as alcohol and diet influencing the biology of the tumour. However this needs further research. Patient factors such as comorbidity and psychosocial factors (marital status and level of social support) are known to influence survival from cancer (Macleod, Ross, Fallowfield et al. 2004). In a study of patients with bowel cancer, comorbidity increased the risk of cancer death, although it did not vary with socioeconomic status (Munro and Bentley 2004). The role of nutrition and obesity is an interesting factor and although little work has been performed in this area, obesity and poor nutritional state is more common among deprived women (Woods, Rachet, & Coleman 2006).

Study limitations

The study does carry some limitations. It is an observational study looking at patients within the catchment area of Glasgow Royal Infirmary, a tertiary referral centre for cases of Barrett's oesophagus, particularly for specialist investigation and endoscopic treatments, and is the regional oesophagogastric unit. However in an attempt to remove potential bias, only patients with Barrett's oesophagus attending the Royal Infirmary as the "local" hospital, were included in the analysis, removing the bias associated with an increased number of malignant cases from other centres. The number of persons residing within catchment areas of the different deprivation categories would be key to understanding the true prevalence of Barrett's oesophagus and cancer death within the West of Scotland population. Furthermore, there are three other hospitals covering the southern and western areas of Glasgow and these patients have not been included in the analysis and these should be included in future work to provide "true" population data. Additional work is currently underway to develop a prospective database which will include all patients with Barrett's oesophagus within Greater Glasgow, allowing further studies to accurately identify the prevalence of the disease, and incidence of malignant progression. Evaluation of other factors such as BMI, smoking status, alcohol intake and detailed nutritional assessment, besides socioeconomic status and deprivation, will be performed.

In conclusion, this observational study of patients with Barrett's oesophagus in Glasgow has shown a clear association between deprivation and Barrett's oesophagus and adenocarcinoma. The underlying factors are unclear but our "at risk" population is an ideal niche for further studies.

Chapter 5

Image adjuncts for the assessment of Barrett's oesophagus

There has been a vast improvement in the quality of endoscopic systems over the last decade. The current standard high resolution white light endoscope provides the endoscopist with high definition and a clear picture of the oesophageal epithelium, improving visualisation of the Barrett's segment. As a result, small cancers and areas of abnormality are more readily visualised at endoscopy, yet the diagnosis of dysplasia and early malignant transformation still remains difficult. The role of the "expert" endoscopist in improving diagnostic yield of dysplasia and early neoplasia appears well defined (Curvers, Singh, Song et al. 2008). To maximise the endoscopic diagnosis of dysplasia and early cancer several image adjuncts have been developed. This chapter summarises the literature regarding current endoscopic imaging techniques used in the diagnosis of Barrett's oesophagus and highlights areas for improvement and further research.

5.1 Introduction

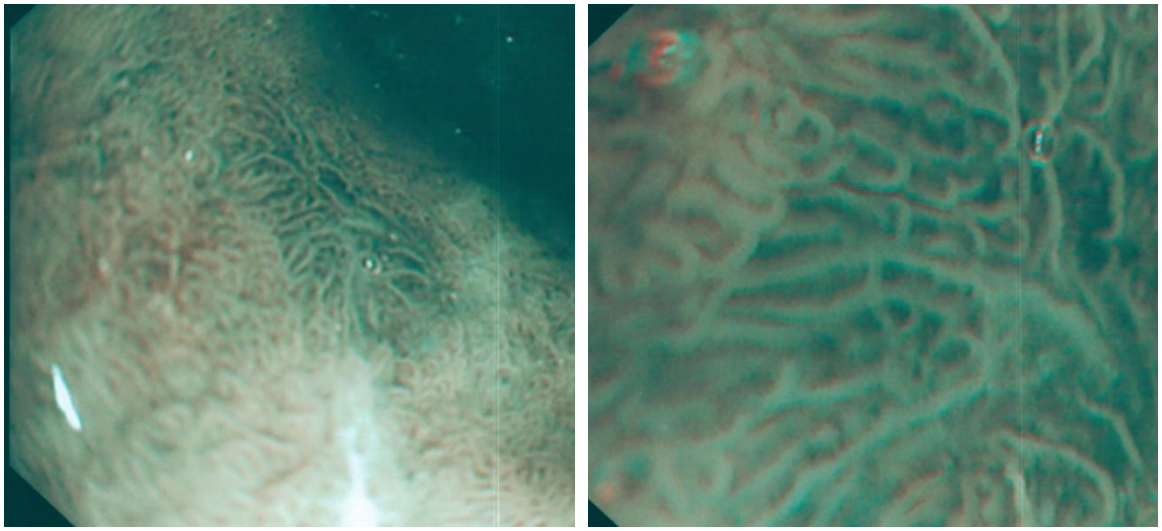
In the United Kingdom, Barrett's oesophagus is diagnosed at endoscopy by the presence of columnar epithelium lining the oesophagus above the proximal gastric folds and by histological confirmation of a metaplastic columnar epithelium (Playford 2005). Barrett's mucosa is often heterogenous with areas of fundic, gastric and intestinal epithelia found alongside areas of dysplasia. Dysplasia is often difficult to identify at endoscopy as lesions are often flat and inconspicuous, leading to one of the main problems in carrying out endoscopic surveillance of patients with Barrett's oesophagus, as small neoplastic lesions may be missed (Montgomery, Goldblum, & Greenson 2001).

Current surveillance programmes advocate rigorous biopsy sampling of areas where there are no visible lesions, and targeted biopsy of any abnormal mucosa using white light endoscopy (Playford 2005). Most clinicians dealing with patients with Barrett's oesophagus take four quadrant biopsies for every two cm along the length of the Barrett's segment (Fitzgerald, Saeed, Khoo, Farthing, & Burnham 2001;Wang & Sampliner 2008). This procedure can be labour intensive, tedious and uncomfortable for patients particularly in those with long segment disease, and may be associated with sampling error. Studies have suggested poorer compliance with increasing segment lengths, and indeed even in the best circumstances only 4-6% of the Barrett's epithelium is actually sampled (Sharma, Hawes, Bansal et al. 2013).

5.2 High resolution magnification endoscopy

Standard endoscopes were designed to view the mucosa from a focus distance of 1-2cm and the combination of low pixel density and low resolution monitors often compromised the quality of the image when the tip of the scope was advanced to an area of interest. More recently, new endoscopic systems have been developed to improve the focus and image quality of the visualised mucosa. High resolution endoscopes (HRE) are equipped with high quality charge-coupled device chips with moveable lenses that allow the endoscopist to focus in on an area of interest without blurring the image compared with standard video endoscopes (Wolfsen 2009). Magnification endoscopy (HRME) provides the endoscopist with a zoom function which is excellent in detecting minute abnormalities in mucosal and vascular structures within the area of interest. When combined with narrow band imaging, HRME is a very useful tool for assessing specific lesions (Figure 5.1).

Figure 5.1 High resolution magnification image of Barrett's nodule



(A) Low magnification NBI HRE view of BE with focal HGD. Note raised area with irregular pattern

(B) High magnification NBI HRE view of focal HGD. Note abnormal vascular and mucosal pattern

5.3 Chromoendoscopy

In order to overcome some of the constraints associated with standard endoscopy, high resolution endoscopy was combined with chromoendoscopy using various dyes in an attempt to improve detection of mucosal abnormalities. Chromoendoscopy agents can be classified into three broad groups based on their mechanism of action: absorptive (Lugol's iodine, methylene blue), reactive (congo red) and contrast (acetic acid, indigo carmine). The two essential steps of chromoendoscopy are firstly to remove any surface mucus (N-acetylcysteine or water are successful mucolytics) followed by the application of the dye either directly flushing it through the working channel or using a spray catheter (Singh, Mei, & Sethi 2011). Table 5.1 summarises the mode of action, uses and limitations of the most common agents used in clinical practice.

The first chromoendoscopy agent within the oesophagus was Lugol's iodine. Lugol's iodine binds to glycogen within the normal squamous epithelium of the oesophagus and stains it brown. Absence of staining from glycogen depletion is indicative of inflammation, squamous high grade dysplasia and carcinoma. A study from the Linxian region of China, known for its high rates of squamous cell carcinoma, showed Lugol's iodine was sensitive and specific for high grade squamous dysplasia and carcinoma (96% and 63% respectively) (Wei, Abnet, Lu et al. 2005). Barrett's epithelium does not stain with Lugol's iodine, and currently its most useful clinical role is the assessment of residual squamous tissue after mucosal ablation therapy.

Studies using methylene blue, acetic acid or indigo carmine dyes with magnification endoscopy have identified mucosal glandular patterns aiding the diagnosis of intestinal metaplasia, rather than simple colour changes within the mucosa (Canto 2005;Sharma, Weston, Topalovski et al. 2003). Methylene blue is the most studied, and perhaps controversial, adjunct in detecting Barrett's epithelium. It is selectively taken up by goblet cells within the intestinalised mucosa, and not absorbed by gastric or squamous epithelia. Initial studies suggested methylene blue targeted biopsies resulted in a higher yield of Barrett's epithelium and dysplasia compared with random standard biopsies (Canto, Setrakian, Willis et al. 2000). However these results have not been reproducible and follow up studies have shown mixed results. The main issues seem to be absence of staining in dysplastic tissue and different staining patterns between long and short segment disease. These discrepancies may

be due to differences in application technique, operator skill and interpretation of results (Horwhat, Maydonovitch, & Ramos 2008). There are also concerns that the combination of methylene blue and white light may lead to DNA damage (Olliver, Wild, Sahay et al. 2003). A recent meta-analysis of 450 patients showed no benefit of methylene blue chromoendoscopy compared with random biopsy in detecting intestinal metaplasia, dysplasia or oesophageal carcinoma (Ngamruengphong, Sharma, & Das 2009). In the light of these results and limitations, methylene blue may only be of historical interest.

Acetic acid is not a chromoendoscopy agent per se as it has no specific colour, yet it has been used in the diagnosis of squamous dysplasia of the cervix for some time (Sauvaget, Fayette, Muwonge et al. 2011). Acetic acid whitens the squamous epithelium of the oesophagus and gives Barrett's epithelium a reddish hue. Acetic acid acts by breaking the bonds of surface glycoproteins resulting in a transient disruption of the cell barrier leading to swelling and reddening of the mucosa, enhancing pit patterns (Lambert, Rey, & Sankaranarayanan 2003). The mucosal pit patterns can be assessed using magnification endoscopy – round patterns typifying gastric epithelia, whereas villous and ridged patterns represent Barrett's epithelium. A large UK series of 190 patients with Barrett's oesophagus undergoing acetic acid chromoendoscopy (2.5% dye spray) show the acid assisted evaluation detected higher rates of dysplasia compared with white light endoscopy alone and excellent correlation with histology (Longcroft-Wheaton, Duku, Mead et al. 2010). The use of acetic acid is inexpensive, easy to perform/interpret and can be useful in the assessment of Barrett's epithelium.

Table 5.1 Common chromoendoscopy agents

Agent	Mode of action	Tissue stain	Limitations
Lugol's iodine	Absorptive agent – absorbed into cellular glycogen	Lugol's iodine stains to glycogen of normal squamous epithelium (black/green) Lack of staining with glycogen found in inflammation/dysplasia/neoplasia	Not specific to Barrett's oesophagus Unable to differentiate metaplasia from dysplasia
Methylene blue	Absorptive agent – absorbed by goblet cells within the intestinal epithelium	Intestinal type epithelium blue Normal squamous absent stain	Mucolytic before application Vigorous washing of dye No universal application method No universal interpretation of results
Acetic acid	Contrast agent - cellular barrier breakdown to enhance borders and surface architecture of mucosa	Normal squamous stains white Barrett's epithelium has reddish hue	Short stain life and needs repeated applications

5.4 Narrow band imaging

Narrow band imaging (NBI) is a method of optical chromoendoscopy developed in Japan in 1999, allowing enhanced visualisation of mucosal abnormalities without the messy and time consuming problems associated with dye chromoendoscopy (Gono, Obi, Yamaguchi et al. 2004). NBI is based on the principle that the depth of light penetration depends on its wavelength – the longer the wavelength, the deeper the penetration.

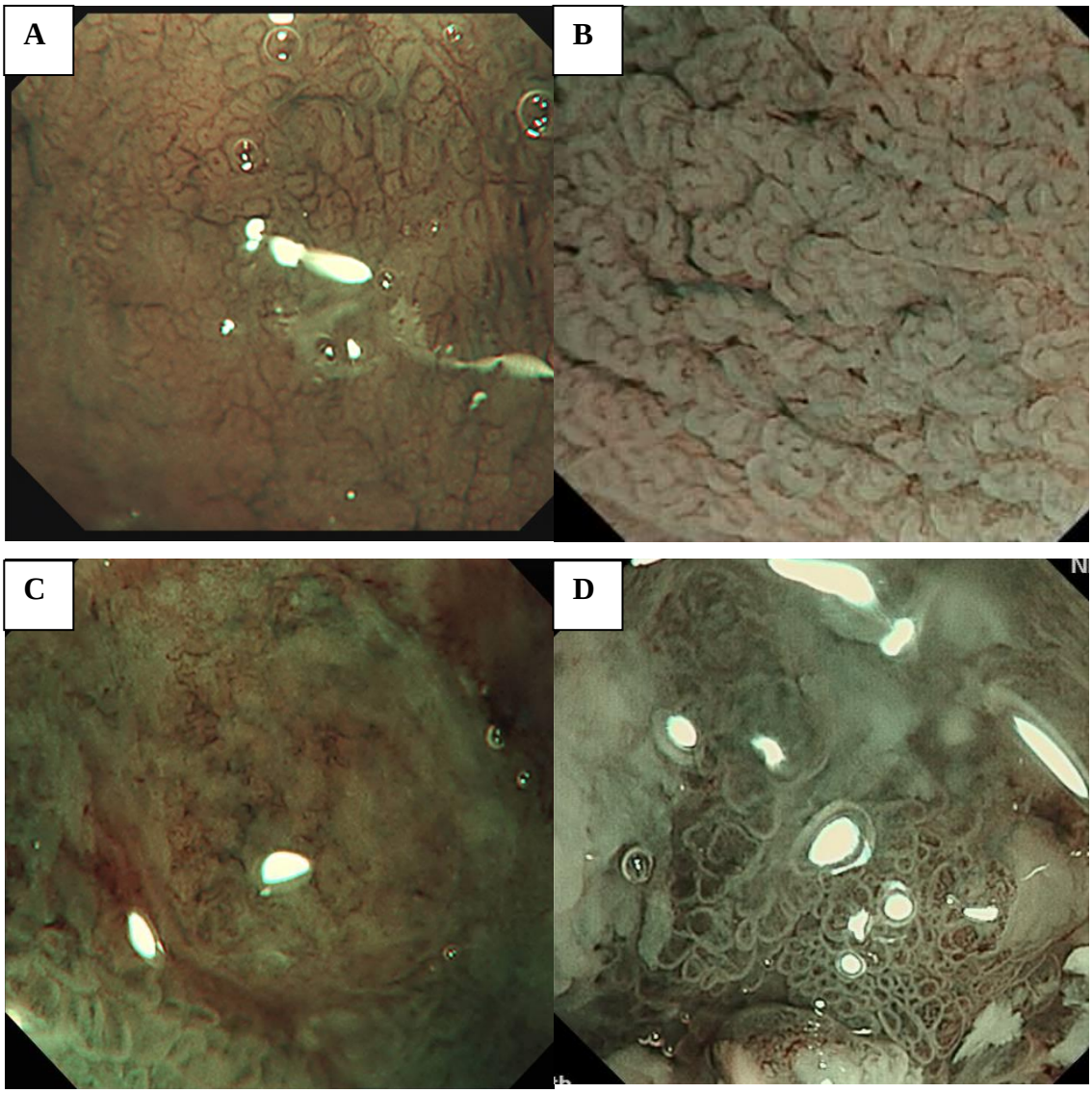
The first prototype NBI system (Olympus, Tokyo, Japan) used a light source with sequential red-green-blue (RGB) illumination. This allowed standard white light images to be passed through red, green and blue band pass filters, illuminating the mucosa. In NBI mode, all bandwidths are narrowed resulting in a relative increase in the intensity of blue light which improves visualisation of the superficial mucosa. Mucosal blood vessels in particular are enhanced because blue light excitation is highly absorbed by haemoglobin. A metaplastic epithelium is associated with regular mucosa and vascular patterns, while dysplasia can be identified by irregular patterns and the presence of abnormal blood vessels (Kara, Ennahachi, Fockens et al. 2006).

NBI has several advantages compared with chromoendoscopy. No staining agents are required, it is less messy and more user friendly, and it allows inspection of the whole endoscopic field. It also allows the endoscopist to easily switch between standard and NBI modes as required during a procedure.

Various groups have proposed classification systems for the use of NBI in the assessment of Barrett's mucosa (Herrero, Weusten, & Bergmann 2010). Raganuth and colleagues recently described a grading system which was simple to use, accurate at predicting the presence of metaplasia and high grade dysplasia, and was associated with good interobserver agreement (Singh, Anagnostopoulos, Yao et al. 2008). The four categories of pit/microvasculature patterns were recognised as illustrated in Figure 5.2. A recent meta-analysis of eight studies (446 patients with 2194 lesions) suggested that NBI was useful for the detection of Barrett's mucosa (sensitivity 95%, specificity 65%) and high grade dysplasia (sensitivity 96%, specificity 94%) (Mannath, Subramanian, Hawkey et al. 2010). However the majority of these studies were conducted in high volume units by expert endoscopists

and further work may be required to standardise the classification of mucosal/vascular patterns to allow its incorporation in routine clinical practice.

Figure 5.2 Mucosal patterns within Barrett's oesophagus as identified on NBI-zoom



(A) Round/oval pits with regular microvasculature. (B) Villous/ridge pits with regular microvasculature as found in metaplasia while (C) and (D) demonstrate irregular vasculature and pits associated with high grade dysplasia. *Images reproduced with permission from Professor Raganuth (personal communication).*

Several studies have compared high resolution endoscopy (HRE) alone, or combined with dye chromoendoscopy, versus HRE-NBI in identifying areas of dysplasia or early cancer. Early studies concluded that NBI is an excellent tool for detailed inspection of suspicious areas within a Barrett's segment (Canto 2005). Kara and colleagues compared NBI and chromoendoscopy for the detection of HGD and early adenocarcinoma. The results showed no difference in the detection achieved by both techniques, although only 28 patients were included in the study (Kara, Peters, Rosmolen et al. 2005). More recently, an international randomised controlled trial compared the use of HRE and NBI in relation to the diagnosis of IM, the number of biopsies required, and the detection of dysplasia (Sharma, Hawes, Bansal, Gupta, Curvers, Rastogi, Singh, Hall, Mathur, Wani, Hoffman, Gaddam, Fockens, & Bergmann 2013). The results confirmed no difference in the detection of IM using either NBI or HRE, although NBI required significantly fewer biopsies (mean 3.6 vs 7.6, $p<0.0001$). All cases of HGD or adenocarcinoma were correctly identified by NBI having irregular mucosal/vascular patterns. Furthermore, no area with a normal pattern by NBI had dysplasia. However the accuracy of low grade dysplasia was limited.

These results are very encouraging and NBI clearly has a role in the assessment of Barrett's oesophagus and the diagnosis of HGD and early neoplasia. However these results are from tertiary referral centres and may not be reproduced in general hospitals. There remains a lack of consensus regarding the interpretation of mucosal/vascular patterns and intense training would be required in an attempt to reduce interobserver variability.

Despite these challenges, there are now several commercially available electronic chromoendoscopy systems – NBI (Olympus, Southend-on-Sea, UK), Fujinon Intelligent ChromoEndoscopy (Fujinon Inc, Saitama, Japan) and Pentax I-Scan (Pentax Inc, Tokyo, Japan). NBI (Olympus endoscopic system) is now more widely available throughout endoscopy units in the UK, and should be used as an adjunct for the routine assessment of patients with Barrett's oesophagus.

5.5 Autofluorescence

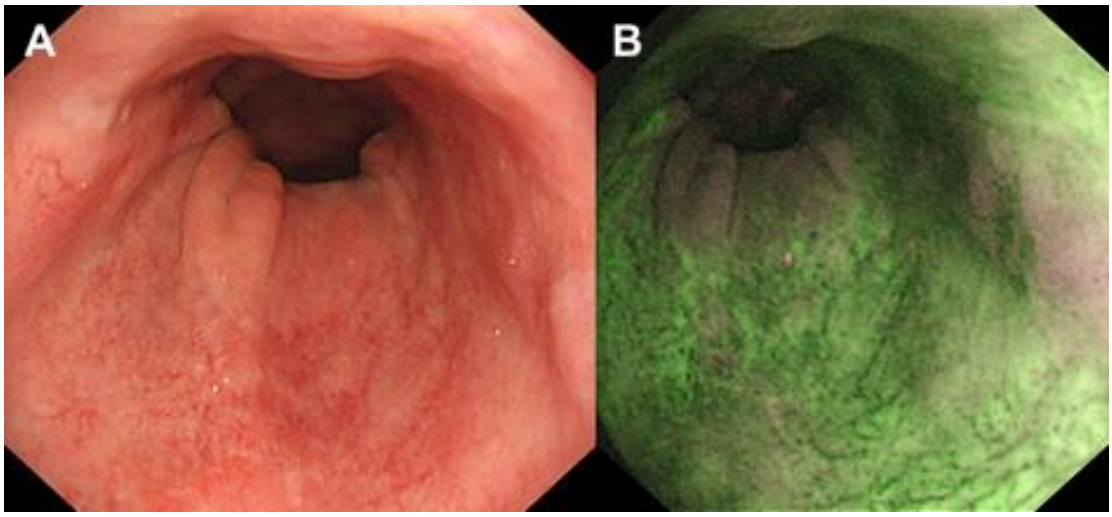
Fluorescence endoscopy may become a useful adjunct in the diagnosis of Barrett's dysplasia although the literature is still in its infancy. Fluorescence imaging differentiates tissue types based on differences in their fluorescence emissions. Tissues are exposed to short wavelength light, either ultraviolet or blue light, which leads to excitation of endogenous biological molecules (fluorophores) that emit fluorescent light of longer wavelengths. As these signals are generated within the tissue itself, this phenomenon is known as autofluorescence (AF) (Haringsma and Tytgat 1999).

The fluorophores responsible for tissue autofluorescence include connective tissue matrix (collagen, elastin), cellular metabolic coenzymes (NADH, FAD), aromatic amino acids (tryptophan, tyrosine, phenylalanine), byproducts of the heme pathway (porphyrins) and lipopigments (lipofuscin) (DaCosta, Wilson, & Marcon 2007). Each group of fluorophores is characterised by individual excitation and emission wavelengths. Normal and neoplastic tissues have different autofluorescent characteristics because of differences in the concentration and distributions of fluorophores and chromophores. The incident and fluorescent light can also be absorbed by other tissues, haemoglobin being the main chromophore in gastrointestinal tissues.

Although each fluorophore has its individual fluorescence spectrum, most tissues contain a mixture of different fluorophores which are found in various concentrations and depths. Therefore identifying individual fluorophores is problematic. Furthermore, the use of autofluorescence in the detection of dysplasia or early neoplasia is dependent on changes within the normal oesophageal mucosa (DaCosta, Wilson, & Marcon 2007).

Autofluorescence imaging (AFI) is an endoscopic system which detects differences in the natural endogenous fluorescence of normal, dysplastic and neoplastic tissues using blue light illumination, which is detected through a charge coupled device (CCD). The image processor incorporates the CCD signals into a real time image of normal mucosa (green) and dysplasia or neoplasia (varying tones of blue/purple) (Kara, Peters, ten Kate et al. 2005) (Figure 5.3).

Figure 5.3 Autofluorescence in Barrett's oesophagus

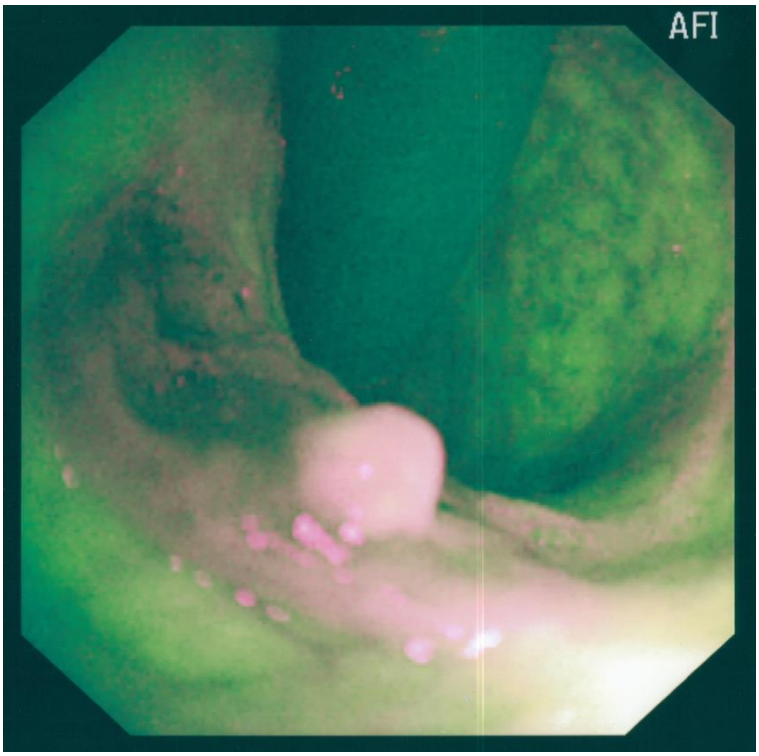


Autofluorescence image of Barrett's oesophagus (B) with corresponding white light image (A). Dysplasia at 3'o'clock position appears purple. Reproduced from (Herrero, Weusten, & Bergmann 2010)

Initial studies of AFI added little to the diagnosis of dysplasia as first generation AF systems did not have clear contrast between dysplastic and non-dysplastic tissue as the fibreoptic endoscopes provided relatively poor white light. However the development of newer prototypes and white light HRE has produced excellent images which can guide the clinician. In a study from Amsterdam the addition of AFI increased the detection of high grade dysplasia by 10% in addition to detecting a substantial number of other lesions not identified by white light (Kara, Peters, ten Kate, van Deventer, Fockens, & Bergmanm 2005). However there were high false positive rates with AFI (PPV 49%) as some of the suspicious areas did not have dysplasia on histological assessment.

AFI does increase the targeted detection of HGD/early neoplasia and may be of use in tertiary referral centres to aid location of lesions for further therapy (Figure 5.4). However compared with standard Seattle protocol, it does not result in higher dysplasia detection rates. AFI alone has not clinically been as useful as NBI due to the poorer detection of dysplasia/early neoplasia and the high false positive rates.

Figure 5.4 Autofluorescence image of nodule in Barrett's oesophagus



High magnification autofluorescence view of high grade nodule with early neoplasia. Note surrounding normal tissue (green) with dysplastic nodule and surrounding raised edges in keeping with HGD/early cancer.

5.6 Trimodal imaging endoscopy

A further improvement in endoscopic imaging began with the introduction of endoscopic trimodal imaging (ETMI), combining the use of white light HRE, AFI and NBI. This system enhances and improves the combined accuracy of the three modalities allowing better detection of mucosal abnormalities. The results from a large multicentre study in Europe and the United States assessing the trimodal imaging system were encouraging (Curvers, Singh, Song, Wolfsen, Ragunath, Wang, Wallace, Fockens, & Bergmanm 2008). At endoscopy, HRE was used to examine and describe the Barrett's segment, AFI was used to identify areas suspicious of dysplasia and NBI was then used to examine the mucosal and vascular pattern of suspicious lesions before obtaining random and image-targeted biopsies. The addition of AFI increased the number of patients with high grade dysplasia from 53% to 90%, and the use of NBI reduced the false positive rate of AFI from 81% to 26%. These results are indeed promising but the majority of new endoscopic imaging systems are prototypes and not widely available outwith study centres. The systems are used in specialised centres which tend to have a high risk patient population and experienced endoscopists with an interest in Barrett's oesophagus.

More recently, a similar study was performed in the community, comparing endoscopic trimodal imaging (ETMI) versus standard video endoscopy (Curvers, van Vilsteren, Baak et al. 2011). There was no significant difference in the overall histological yield between ETMI and standard endoscopy. They concluded that ETMI performed in the community setting did not improve detection of dysplasia.

5.7 Optical coherence tomography

Optical coherence tomography (OCT) is a novel non-invasive technique that uses infrared light to excite oesophageal tissue, with reflected light being subsequently analysed according to the delay and intensity of reflection, producing black/white cross-sectional images of the microanatomy (Li, Boppart, van Dam et al. 2000). Real time images are obtained by a miniprobe which can be placed through the working channel of the endoscope. The images strongly resemble those obtained by histological microscopy with an image depth of 1-3mm and high resolution (1-10μm). The potential advantage of OCT is an "optical biopsy" – an in situ image which may obviate the need for tissue sampling and improve the detection of malignancy. Recent studies have assessed the efficacy of OCT in the diagnosis of

dysplasia in Barrett's oesophagus. While OCT is able to detect dysplasia (78% diagnostic yield), there were limitations with sensitivity rates of only 68% and high interobserver variability (Isenberg, Sivak, Chak et al. 2005). With the emergence of ablative therapies to treat high grade dysplasia and intramucosal cancer, there is a concern that current endoscopy is unable to identify subsquamous Barrett's epithelium. An elegant ex vivo study by Cobb and colleagues has shown OCT is a promising tool for the recognition and surveillance of intestinal metaplasia (subsquamous and surface) in patients who have undergone ablative therapies (Cobb, Hwang, Upton et al. 2010).

One of the main concerns is that OCT remains a bench technique and cannot be applied to clinical practice at the present time. Despite technological advances, image resolution is still insufficient to compete with standard histological images. Technical challenges include movement artefacts due to breathing and peristalsis, and the compressibility of the tissues can distort optical images. There are also concerns that OCT cannot adequately differentiate metaplasia and dysplasia (Bergmann and Tytgat 2005).

While OCT is an exciting concept, further work will be required before it has a role in the clinical assessment of patients with Barrett's oesophagus. The ability to survey a wider area of mucosa would be beneficial for endoscopic surveillance and improvements in resolution may aid diagnosis of occult malignancy.

5.8 Conclusion

There have been significant advances in the endoscopic assessment of Barrett's oesophagus with improved video systems and particularly the development of NBI. NBI is an exciting new development and an excellent adjunct in the diagnosis of HGD/early neoplasia but further research is required to translate this adjunct into daily clinical practice. Trimodal imaging carries the greatest potential for use in clinics, as the combined advantages of the three modalities outweigh the use of each modality on its own.

Chapter 6

Endoscopic detection of dysplasia in Barrett's oesophagus: a novel technique

Barrett's oesophagus can be visualised at endoscopy, but the diagnosis must be confirmed by the histological findings of a metaplastic columnar epithelium and/or dysplasia. Dysplasia can be difficult to identify at endoscopy as lesions are often flat, inconspicuous and easily missed during surveillance endoscopy. Laser induced tissue fluorescence has been used to differentiate normal and malignant tissue with varying degrees of success. However the recent improvement in high definition endoscopy systems and other image adjuncts has led to a renewed interest in the use of laser autofluorescence in Barrett's oesophagus. The aim of this study was to assess the use of "optical" biopsy forceps to identify the presence of metaplasia and dysplasia in patients with Barrett's oesophagus.

6.1 Introduction

The West of Scotland has the highest rates of oesophageal cancer and the incidence has risen over the last thirty years (NHS National Services Scotland 2012). Early accurate detection and treatment of precancerous Barrett's epithelium may reduce the incidence of oesophageal cancer over time. As the diagnosis of dysplasia has such a crucial impact on disease management and subsequent patient outcomes, a system that could aid identification of dysplasia by the endoscopist in real time would have great clinical utility. Due to the heterogeneity of Barrett's mucosa and the small endoscopic biopsy size only a small area of the oesophageal mucosa is generally sampled. Dysplastic areas therefore may be missed with current biopsy techniques.

White light endoscopy (WLE) and narrow band imaging (NBI) are currently used for imaging Barrett's mucosa. Recent advances in light sources, detectors and fibre optics have encouraged development of optical systems to visualise and evaluate epithelia in vivo (Wang and van Dam 2004). An "optical biopsy" uses point measurements from an optical probe allowing in vivo assessment of mucosa without the need for tissue removal, or targeted biopsies in areas likely to contain dysplasia. This could reduce the number of "normal/non-dysplastic" biopsies taken during surveillance endoscopy and focus on areas of interest. All methods of optical imaging collect backscattered photons from the mucosa after initial stimulation with light. Optical techniques which have been used to date in gastrointestinal epithelia include light induced fluorescence, Raman spectroscopy and elastic scattering spectroscopy (Lovat, Johnson, Mackenzie et al. 2006;Panjehpour, Overholt, Vo-Dinh et al. 1996). Each technique has its own features and specific applications, but all depend on architectural and biochemical changes within the mucosa.

All tissues display endogenous fluorescence when exposed to light of certain wavelength. Different excitation wavelengths activate different fluorophores within tissues. Examples of fluorophores within oesophageal tissue are collagen, nicotinamide adenine dinucleotide (NAD), flavins and porphyrins. When these biomolecules absorb light, electrons elevate to higher energy states. Subsequent relaxation leads to emission of fluorescence (DaCosta, Wilson, & Marcon 2007). The wavelength of the emitted signal is longer than the wavelength of the excitation light, allowing the difference in excitation/emission wavelengths to be measured. Pattern recognition analysis is used to develop algorithms aided by computer

software which can classify tissue as non-dysplastic, dysplastic or neoplastic (Panjehpour, Overholt, Vo-Dinh, Haggitt, Edwards, & Buckley 1996).

The first Laser-Induced Fluorescence Spectroscopy (LIFS) study in gastrointestinal tissue was performed by Kapadia and colleagues (Kapadia, Cutruzzola, O'Brien et al. 1990). In an *ex vivo* study, sixteen colonic adenomas were differentiated from hyperplastic polyps with a sensitivity and specificity of 100% and 94% respectively. Cothren performed the first *in vivo* study during colonoscopy, showing adenomas could be distinguished from normal colonic tissue in 97% cases (Cothren, Richards-Kortum, Sivak et al. 1990). Panjehpour and colleagues performed the first LIFS study in 32 patients with oesophageal adenocarcinoma (Panjehpour, Overholt, Schmidhammer et al. 1995). They classified tissue into normal or malignant with sensitivity and specificity of 100% and 98% respectively. A follow up study of 36 patients with Barrett's oesophagus accurately identified high grade dysplasia, but not low grade dysplasia (Panjehpour, Overholt, Vo-Dinh, Haggitt, Edwards, & Buckley 1996).

The WavSTAT® Optical Biopsy System is based on the principles of laser induced fluorescence and was approved by the FDA in November 2000 as safe and effective for adjunctive use during endoscopy of the colon to improve the endoscopist's clinical sensitivity to identify adenomatous polyps.

6.2 Study Aim

The aim of this pilot study was to assess the feasibility of the WavSTAT® Optical Biopsy System to identify dysplasia or neoplasia within Barrett's oesophagus.

6.3 Methods

6.3.1 Patients

Patients with Barrett's oesophagus were invited to participate in the study if they were scheduled for endoscopy for the following reasons: (1) upper gastrointestinal symptoms suggestive of reflux disease, (2) regular surveillance for Barrett's metaplasia or low grade dysplasia, and (3) follow up after endoscopic therapy for high grade dysplasia or early cancer. The patients were enrolled on a first-come first-enrolled system. The study protocol was approved by the South East Scotland Research Ethics Committee. Information packs were delivered to all patients and a telephone discussion available on request. Written informed consent was obtained before the procedure.

6.3.2 Endoscopic system

The standard endoscopy system used during the study consisted of a high resolution white light endoscope with optical zoom (GIF-H180, Olympus Inc, Tokyo, Japan), a light source with two filters, one for white light imaging and one for NBI (CLV-180, Olympus Inc, Tokyo, Japan) and a processor (CV-180, Olympus Inc, Tokyo, Japan).

6.3.3 Optical biopsy system

The WavSTAT® Optical Biopsy System (SpectraScience Inc, San Diego, USA) was gifted to the endoscopy unit of Glasgow Royal Infirmary, to allow a pilot study to be performed among patients with Barrett's oesophagus, residing in the West of Scotland. The WavSTAT® Optical Biopsy System consists of a laser light source console with optical fibre embedded biopsy forceps, statistical tissue recognition algorithm software, activation foot pedal and a touch screen user-interface console (Figure 6.1). The device contains an internal flash memory to store data. The WavSTAT® system uses modified standard biopsy forceps containing an optical fibre to send light (337 nanometre wavelength pulsed nitrogen laser) to the tissue, scanning the area in question. The tissue reacts to the light, emitting a fluorescence signal (autofluorescence) which on returning via the optical fibre is analysed by the computer's statistical software algorithm. Non-dysplastic tissue generates a different fluorescence signal to dysplastic or cancerous tissue. The algorithm determines in

real time whether the tissue in question is non-dysplastic, dysplastic or cancerous. The user-interface console then displays the classification in green "non-dysplastic" or red "dysplastic".

The optical biopsy is performed by first touching the oesophageal tissue with the tip of the WavSTAT® optical biopsy forceps. The foot pedal is pressed, triggering firing of the laser down the optical fibre. The proper placement of the probe against the tissue is verified on the WavSTAT® monitor, and a warning issued if the probe placement is not suitable. The fluorescent signal was returned by the same probe and analysed by the software programme. The process takes 1-2 seconds for each optical biopsy.

Figure 6.1 The WavSTAT system

The WavSTAT System provides physicians two powerful tools by combining an optical fiber within customized forceps.

Image of new generation WavSTAT monitor and optical biopsy forceps. The forceps are single use and able to take an optical and physical biopsy sample.

6.3.4 Methodology

Participants were prepared for the endoscopic examination using routine outpatient protocols, namely fasting from midnight and cessation of any oral anticoagulants. Patients underwent upper endoscopy either under conscious sedation with intravenous midazolam, or application of topical anaesthetic (lidocaine spray).

The oesophagus was initially examined using the standard endoscopy system as previously described. The presence and length of the Barrett's segment was noted and recorded according to the Prague C&M classification (Sharma, Dent, &

Armstrong 2006). The presence of oesophagitis, hiatus hernia and any visible abnormalities suspicious of dysplasia or early neoplasia were noted. The location of any suspicious lesions (distance from the incisors and endoscopic quadrant) was recorded.

After initial assessment of the Barrett's segment, an optical biopsy was performed, followed by a physical biopsy (taken from the same piece of tissue as the optical biopsy). The whole Barrett's segment was sampled in this manner according to the Seattle protocol – four quadrant biopsies (optical followed by physical) taken every two centimetres. Any suspicious areas were also individually targeted. Biopsies of normal stomach and normal squamous oesophagus were taken as control tissue. The results from the optical biopsy (red-dysplastic, green-non dysplastic) were noted on pre-printed endoscopy proformas.

6.3.5 Tissue Handling and Analysis

Each physical biopsy was placed alone in standard labelled specimen pots containing 10% buffered formalin and sent to histology. All pots were numbered, corresponding to the optical biopsy identification number on the endoscopic proforma, thus ensuring a direct correlation would be made between the physical and optical biopsy (1:1 correlation).

The physical biopsies were processed, stained with haematoxylin and eosin, and assessed by an expert GI histopathologist with an interest in Barrett's oesophagus (Dr James Going, Royal Infirmary, Glasgow). All slides were read by the site pathologist and the presence of dysplasia or carcinoma recorded on standardised pathology forms, based on the Vienna classification of GI neoplasia – specifically, non-dysplastic Barrett's oesophagus, indefinite for dysplasia, low grade dysplasia, high grade dysplasia or invasive cancer (Schlemper, Riddell, & Kato 2000). All pathology reports were reviewed independently by a second pathologist (Dr ten Kate, Amsterdam Medical Centre, Amsterdam), blinded to the first pathologist's findings. On rare occasions, any discrepancies were sent to a third pathologist for another independent review. There was good agreement between the pathologists from two sites (Glasgow and Amsterdam) in the interpretation of all biopsy samples (kappa value=0.95). The results of the physical biopsies were then married to the optical fluorescence results.

6.3.6 Follow up

Each patient was informed of their physical biopsy results and any further surveillance or treatment carried out in their local hospital. Patient involvement in the study ended at this point. All clinicians involved in the care of the patients were alerted to the results and outcomes.

6.4 Results

Patient demographics

One hundred patients consented to participate in the study over a twelve month period. Between March 2010 and May 2010, 31 patients underwent surveillance endoscopy with WavSTAT® optical and standard biopsy of the Barrett's oesophagus segment. There were 20 males and 11 females with a mean age of 67 years (range 46-80 years). All patients had a previous diagnosis of Barrett's oesophagus, were participants of the endoscopic surveillance programme and were prescribed regular proton pump inhibitor therapy. Although there was no past history of oesophageal adenocarcinoma, 11 patients (35%) had a previous diagnosis of low grade dysplasia.

The mean segment length was 5.4cm (range 4-15cm) and mean number of biopsies/patient was 15 (range 6-33 biopsies). A visible nodule was identified in one case but all other oesophageal sites biopsied appeared endoscopically to be normal columnar mucosa.

Histology

A total of 384 matched optical and histological biopsies were taken. Eighty seven biopsies were excluded due to histology; squamous epithelium (n=51) or gastric mucosa (n=36); data was incomplete for 15 biopsies and 20 biopsies were excluded due to acquisition errors during optical biopsy. Two hundred and sixty two biopsy sites were included in the final analysis. Table 6.1 summarises the histology of all biopsy samples.

Table 6.1 Histology of oesophageal biopsy sites

	Number of biopsies
Vienna 5 (Cancer)	4
Vienna 4 (HGD)	0
Vienna 3 (LGD)	20
Vienna 2 (Indefinite for dysplasia)	5
Vienna 1 (No dysplasia)	233
Total	262

Vienna classification of dysplasia was used to classify oesophageal biopsy specimens (Schlemper, Riddell, & Kato 2000). Total number analysed (n=262) by two experienced pathologists.

LGD=low grade dysplasia, HGD=high grade dysplasia

Correlation between optical and physical biopsies

The number of biopsies taken from each patient, and the results from the optical and physical biopsies are presented in Table 6.2. Results from patients 23, 27, 29 and 30 were excluded due to problems with acquisition of the optical biopsy results. The preliminary results demonstrate no clear correlation between the optical biopsy result and the histological assessment of the physical biopsy (r=0.103, Pearson correlation coefficient, p=0.95).

Table 6.2 Results of optical and physical biopsy (n=262)

		OPTICAL BIOPSY RESULTS		PHYSICAL BIOPSY RESULTS	
Patient	**Number of biopsies (n=)**	**Green (not suspect)**	**Red (suspect)**	**No dysplasia**	**Dysplasia**
1	2	2	0	2	0
2	10	3	7	10	0
3	3	1	2	3	0
4	9	4	5	9	0
5	14	0	14	9	5
6	8	0	8	8	0
7	8	4	4	8	0
8	15	10	5	15	0
9	5	2	3	3	2
10	13	3	10	7	6
11	2	2	0	1	1
12	17	11	6	16	1
13	12	0	12	12	0
14	22	5	17	21	1
15	7	2	5	7	0
16	17	5	12	15	2
17	12	9	3	12	0
18	7	5	2	7	0
19	12	2	10	12	0
20	2	0	2	1	1
21	12	5	7	8	4
22	12	9	3	10	2
24	20	4	16	20	0
25	3	3	0	0	3
26	12	1	11	12	0
28	2	2	0	2	0
31	6	4	2	3	3

6.5 Discussion

Current endoscopic programmes often yield large numbers of normal "non-dysplastic" biopsies in low risk patients (Lovat, Johnson, Mackenzie, Clark, Novelli, Davies, O'Donovan, Selvasekar, Thorpe, Pickard, Fitzgerald, Fearn, Bigio, & Bown 2006). The presence of dysplasia remains the current gold standard marker of disease progression, yet some patients may develop adenocarcinoma without a previous diagnosis of dysplasia (Montgomery, Goldblum, & Greenson 2001). In these cases, it is thought dysplasia was present at previous endoscopy but not adequately detected. Furthermore, there is often interobserver variability among pathologists particularly in the diagnosis of low grade dysplasia and in the presence of reactive atypia and inflammation (Goldblum 2010). Standard endoscopy and biopsy is time consuming, uncomfortable for the patient, and places significant time and financial burdens on endoscopy and histopathology services. These challenges have led to development of new optical technologies.

The WavSTAT® Optical Biopsy system is a laser induced fluorescence system based on the principles of light induced fluorescence, which is simple to use and allows real time histological assessment of Barrett's mucosa. Disappointingly, this pilot study failed to show any correlation between the WavSTAT® optical biopsy and the physical biopsy in Barrett's oesophagus. The system failed to differentiate non dysplastic Barrett's epithelium from dysplastic or early neoplastic tissue.

The WavSTAT® Optical Biopsy system has several potential advantages. It is relatively cheap, easy to use and provides the endoscopist with instant results, rather than a delay of days for histology results from physical biopsy. The optical biopsy forceps do not need to be removed from the endoscope unless a physical biopsy is required, allowing the endoscopist to survey long segments of Barrett's mucosa in a shorter time compared with the standard Settle protocol (Lovat, Johnson, Mackenzie, Clark, Novelli, Davies, O'Donovan, Selvasekar, Thorpe, Pickard, Fitzgerald, Fearn, Bigio, & Bown 2006). Typically 6 or 7 optical biopsies can be performed in the time taken to perform one physical biopsy (Panjehpour, Overholt, Vo-Dinh, Haggitt, Edwards, & Buckley 1996). The non-invasive nature of the optical biopsy reduces bleeding which often interferes with visual assessment of the mucosa during surveillance endoscopy. In time the WavSTAT® Optical Biopsy system could reduce the number of physical biopsies taken, reducing the number of low risk biopsy specimens sent for pathology review (Panjehpour, Overholt, Vo-Dinh et al.

2012). Finally, the technique has the potential to reduce variability in histological interpretation of standard biopsy samples.

LIFS has been used for detection of premalignant lesions within the gastrointestinal tract (colonic polyps and oesophageal adenocarcinoma) for two decades with promising results. However despite encouraging preliminary results, optical techniques remain at an investigational stage and further work is required before they can be used in clinical practice. These are summarised in Table 6.3.

The presence of reactive atypia and inflammation within Barrett's biopsies produces diagnostic challenges for the pathologist, particularly in differentiating non-dysplastic Barrett's mucosa from low grade dysplasia (Odze 2006). This is also a concern for optical technology – would the presence of atypia and inflammation produce false positive results during optical biopsy. A recent study from Panjehpour and colleagues showed no difference in sensitivity between oesophageal biopsies with and without inflammation (162/175 biopsies with inflammation, sensitivity 92.6% vs 118/128 biopsies without inflammation, sensitivity 92.2%) (Panjehpour, Overholt, Vo-Dinh, & Coppola 2012). These results are very encouraging but from a high volume, experienced centre and clinical studies in other units are necessary to corroborate these results.

Table 6.3 Summary of Light-induced Fluorescence Studies

Study	Excitation wavelength	Tissue analysed	Results
(Kapadia et al 1990)	325nm	Colon Normal/hyperplastic polyps vs adenomas	Correct diagnosis of 34 normal mucosa/hyperplastic polyp specimens and 16 adenomatous polyps.
(Cothren et al 1990)	370nm	Colon Normal vs adenomas	Adenomas distinguished from nonadenomatous tissue 97% cases
(Panjehpour et al 1995)	410nm	Oesophagus Normal vs adenocarcinoma	Sensitivity 100% and specificity 98%
(Panjehpour et al 1996)	410nm	Oesophagus Normal vs LGD vs HGD	96% no dysplasia classified as benign All LGD samples classified as benign 90% HGD samples classified as premalignant 28% LGD with focal HGD classified premalignant
(Vo-Dinh, Panjepour, Overholt et al. 1995)	410nm	Oesophagus Normal vs adenocarcinoma	DNF (differential normalised fluorescence) signal Sensitivity 96% no dysplasia, 90% HGD Low specificity for focal HGD and unable to detect LGD
(Georgakoudi, Jacobson, van Dam et al. 2001)	Several excitation 337nm-620nm	Oesophagus Non dysplastic vs LGD/HGD	HGD vs LGD and no dysplasia: sensitivity 100% and specificity 97% LGD and HGD vs no dysplasia: sensitivity 79% and specificity 88%

Study limitations

The main limitation with this study was the lack of correlation between the optical and physical biopsies. The small number of biopsies with dysplasia and cancer reduced the amount of data suitable for analysis. Within this cohort of patients, there were no cases of high grade dysplasia. The volume of tissue sampled just beneath the optical probe is small (1-3mm^3). Although multiple readings can be taken very quickly, the technique is still prone to sampling error as it does depend on the endoscopist identifying visible lesions. This inherently reduces the sensitivity and clinical utility of the technique.

The current wavelength spectrum using 337nm excitation failed to discriminate between dysplastic and non-dysplastic oesophageal tissue. It is unlikely the failure resulted from issues with histological interpretation of the physical biopsies as there was strong interobserver agreement among the pathologists (Glasgow and expert pathologists in Amsterdam). It would appear the excitation wavelength of 337nm is not capable of assessing the qualities of dysplastic and non-dysplastic tissue in the oesophagus and alternative excitation wavelengths, such as 405nm could be studied in future studies.

Future improvements

Optical biopsy sampling has the potential to provide real-time diagnosis of premalignant and early neoplastic tissue in Barrett's oesophagus. Unfortunately the WavSTAT® Optical Biopsy system in its current state does not meet the necessary criteria. However further work is in progress and once the optimum excitation wavelength has been identified, the results may be more favourable. The principle of targeted oesophageal biopsies is good. Techniques to enhance the oesophageal mucosa may aid detection of dysplasia and neoplasia, such as mucosal enhancement using chromoendoscopy dyes or tumour markers. A study by von Holstein used low dose Photofrin (fluorescent tumour marker composed of porphyrins) to enhance laser induced fluorescence in detection of adenocarcinoma in Barrett's oesophagus. They found Photofrin related signals in malignant tissue did not significantly differ from normal tissue, and the difference between malignant and normal tissue was due to the presence of endogenous fluorophores (von Holstein, Nilsson, Andersson-Engels et al. 1996).

More recently, visually tagged probe molecules which selectively bind neoplastic cells have been used with success. Lu and colleagues developed a peptide that binds specifically to high grade dysplasia and adenocarcinoma of the oesophagus (Lu and Wang 2008). The peptide was labelled using a fluorescin-tagged antibody and delivered topically. The oesophagus was then washed to remove any unbound antibody and imaging techniques used to visual the mucosa. Confocal endomicroscopy found a 3.8-fold greater fluorescence signal in neoplastic tissue compared with non-dysplastic Barrett's oesophagus with sensitivity and specificity rates of 75% and 97% respectively (Strum, Joshi, Lu et al. 2013). Bird-Leiberman and colleagues have shown cell-surface glycan alteration in progression of Barrett's oesophagus to adenocarcinoma leading to changes in lectin binding. Selective binding of lectin (wheat germ agglutin) improved visualisation of high grade dysplasia in ex vivo oesophageal tissue (Bird-Leiberman, Neves, Lao-Sirieix et al. 2012). Targeted imaging agents have potential to aid optical biopsy imaging techniques, but further work to identify novel targets and improving sensitivity and specificity is required before implementation in clinical practice.

6.5 Conclusion

The role of future endoscopic biopsy technology may lie in the use of the "optical biopsy", offering real time in situ histological diagnosis allowing targeted tissue removal. Although laser induced spectroscopy (and point spectroscopy) is still in its infancy, it has the potential to be an excellent imaging adjunct. Further studies developing fluorescence endoscopy may render the optical biopsy system an excellent method for the detection of dysplasia and early neoplasia.

Chapter 7

Predictive biomarkers in Barrett's oesophagus

Barrett's oesophagus is important as a precursor of oesophageal adenocarcinoma via a metaplasia-dysplasia-carcinoma sequence. In the absence of glandular dysplasia the risk of progression to cancer is low but ascertainment of dysplasia is not always straightforward. Sparse mucosal sampling may miss dysplasia, or reactive changes may be over-interpreted due to inter and intraobserver variation. Low and even high grade dysplasia do not necessarily progress, provided prevalent cancer has been rigorously excluded. This indeterminacy motivates an ongoing search for clinically useful predictive biomarkers. Although many genetic and epigenetic abnormalities have been associated with neoplastic progression in Barrett's mucosa no molecular tests have as yet been accepted into routine pathology practice. Challenges of assay definition remain and many marker studies lack statistical power or have other methodological flaws. Even where strong evidence of clinically relevant predictive value does exist (in the case of ploidy analysis by flow or image cytometry) adoption into clinical practice has been minimal, likely reflecting technological and possible reimbursement obstacles. Well-designed multi-centre studies are likely to be required to translate improved knowledge of Barrett's carcinogenesis into clinically significant progress on predictive testing, and will require a degree of cooperation not so far widely seen in the field.

Publication

Moyes LH, Going JJ. Still waiting for predictive biomarkers in Barrett's oesophagus. *Journal of Clinical Pathology 2011*; 64:742-750.

7.1 The Potential for Biomarkers

It has already been established that Barrett's oesophagus is an acquired precursor of oesophageal adenocarcinoma. Characteristically, the squamous epithelium of the lower oesophagus is replaced by a metaplastic glandular mucosa. It appears that reflux of gastric and/or duodenal contents leads to mucosal injury, cellular proliferation and healing resulting in columnar/glandular metaplasia of the normal squamous mucosal lining, although the details are not fully elucidated (Manjunath and Jankowski 2000). A metaplasia-dysplasia-carcinoma sequence is characteristic of progression to Barrett's adenocarcinoma. Current guidelines on both sides of the Atlantic suggest that patients with Barrett's oesophagus should undergo periodic surveillance endoscopy and biopsy in the hope of detecting dysplasia and cancer at an early stage. However as most patients with Barrett's oesophagus never develop adenocarcinoma, most patients undergoing surveillance derive no benefit and the added psychological concern regarding cancer development may have a significant effect on quality of life (Kyrgidis, Kountouras, Zavos et al. 2005;Wright, Gray, & Morris 1996). In addition the massive health economic issues related to surveillance programmes make the identification and targeting of selected Barrett's cases a health care priority.

In this context, a clinical or laboratory marker which did actually predict progression to dysplasia (itself a marker of cancer risk), or to cancer in the case of patients with dysplasia already, would be extremely valuable, allowing targeting of screening to those most at risk. Currently, high grade dysplasia is the most reliable indicator of increased risk of progression to malignancy, and indeed it is often already associated with invasive cancer when detected (Collard 2002;Spechler SJ 2005).

However, estimates of the incidence of progression from dysplasia to carcinoma are variable, and the diagnosis of dysplasia, particularly low grade dysplasia, can be difficult owing to sampling errors, disagreement between observers, and the difficulty of discriminating inflammatory and reactive changes from true dysplasia (Spechler SJ 2005). Despite a substantial literature on genetic and molecular changes in Barrett's oesophagus and Barrett's adenocarcinoma, practically nothing has translated so far into clinical practice. This chapter shall discuss the current roles of candidate molecular and other biomarkers in Barrett's oesophagus, and prospects for progress.

7.2 Methods

A search of the English language literature since 1975 was performed using PubMed, Embase and Cochrane databases with MESH terms "Barrett's oesophagus/ epithelium/ metaplasia", "oesophageal cancer/ adenocarcinoma", "biomarkers", "disease progression" and "dysplasia". Abstracts were examined and relevant articles from reference lists of other papers retrieved.

7.3 Biomarkers

A biomarker is defined as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention*".

Biomarkers should detect a state already established, predict a future state, or both. Few achieve either. Good biomarkers require high sensitivity and specificity for the state or event they purport to detect: cardiac troponins, for example, are excellent markers of myocardial injury but are not highly specific for myocardial infarction. In Barrett's oesophagus we might wish for markers of the diagnosis *per se*; but especially useful would be predictors of premalignant or malignant progression. *A priori* such markers are likely to be concerned with key pathways in the development of oesophageal adenocarcinoma, and would distinguish clearly between people with low and high cancer risk. Ideally a test would be minimally invasive, cost effective, and could be used on its own or in conjunction with other techniques (Preston and Jankowski 2006).

A formal model of phased biomarker development has been proposed, analogous to the process in therapeutic drug studies (Pepe, Etzioni, & Feng 2001). There are five phases of development, summarised as follows -

I. Preclinical exploratory studies to identify potential markers.
II. Clinical assay development to determine sensitivity and specificity of markers in subjects with the disease, compared to normal control subjects.
III. Retrospective studies on specimens from subjects prior to their diagnosis, to test capacity of the marker to detect preclinical disease.
IV. Prospective screening studies.
V. Cancer control studies to address whether screening with biomarkers reduces the population burden of cancer.

Biomarkers never studied beyond phases one or two vastly outnumber those taken to phases three and four, and there are few established, clinically useful predictive biomarkers in Barrett's oesophagus, other than histomorphology. Even so, there are markers for which evidence of predictive power does exist, but which are used only in a few centres, in particular ploidy, assessed by flow or image cytometry. In ploidy studies relatively complex technology and reimbursement issues may have impeded wider adoption.

Some believe panels of biomarkers may eventually provide more useful clinical information than any single marker, but the validation challenges for marker combinations will be at least as great as for single markers. To evaluate the prospects for new biomarkers, we need first to understand and define morphological molecular and genetic abnormalities associated with Barrett's oesophagus.

7.4 Morphological Features of Barrett's Oesophagus

7.4.1 Intestinal metaplasia

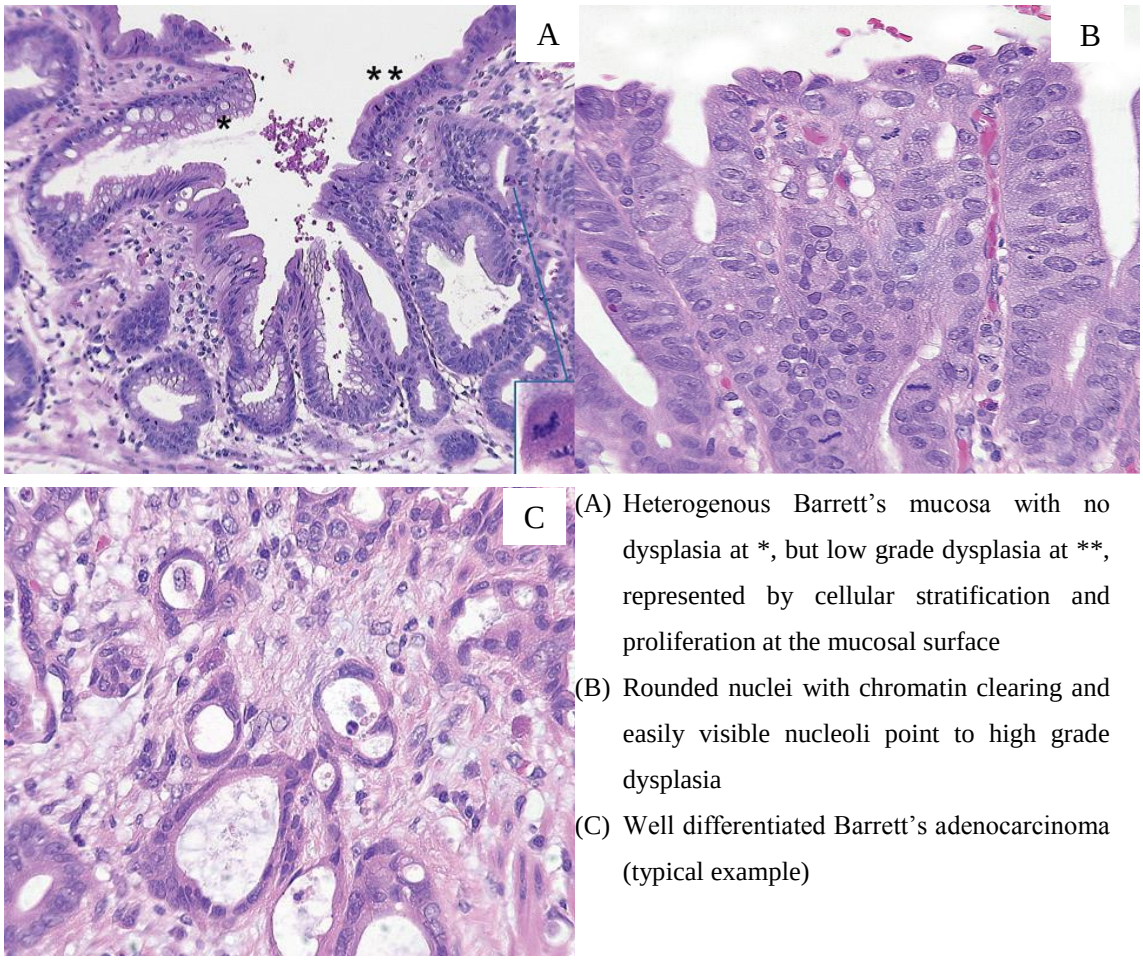
Intestinal metaplasia *per se* is not a useful marker of cancer risk, being present in most cases if not every case of Barrett's oesophagus, but the belief that oesophageal glandular dysplasia and adenocarcinoma usually develop on a background of intestinal metaplasia (IM) has led to a perception that without IM cancer risk may be low. Recent data suggests this is an over-simplification (Takubo, Aida, Naomoto, Sawabe, Arai, Shiraishi, Matsuura, Ell, May, Pech, Stolte, & Vieth 2009). Studies suggest that a columnar metaplasia of the oesophagus carries the same malignant risk whether an intestinal phenotype is present or not, and therefore the original association of intestinal metaplasia carrying malignant risk has been brought under scrutiny (Dent 2011;Kelty, Gough, van Wyk, Stephenson, & Ackroyd 2007). Also, even in the absence of detectable goblet cells, Barrett's mucosa still expresses markers and has ultrastructural features of intestinal differentiation. As previously discussed in Chapter 2, the perception that an absence of goblet cells negates a diagnosis of Barrett's oesophagus may therefore not survive and will not be considered further here (Riddell & Odze 2009).

7.4.2 Dysplasia

As in other situations dysplasia is a marker of cancer risk in Barrett's oesophagus. In the gastrointestinal tract it is synonymous with intraepithelial neoplasia, and implies architectural and cytological changes commonly associated with carcinomas, and from which the latter are presumed, at least sometimes, to have evolved (Figure 7.1). As a marker of risk, however, dysplasia is far from perfect. There is significant intra and interobserver variation in assessing Barrett's oesophagus (Spechler SJ 2005). Pathologists are not good at agreeing on the presence of mild and moderate (low grade) dysplasia, although agreement on severe (high grade) dysplasia is better (Goldblum 2003;Goldblum 2010). Dysplasia may be patchy, and many biopsies may be necessary to detect it reliably, creating a burden for patient, endoscopist, and pathologist (Abela, Going, Mackenzie, McKernan, O'Mahoney, & Stuart 2008). Not all dysplasia will progress to a higher grade or invasive adenocarcinoma; in some cases it may regress.

Even high grade dysplasia, provided invasive adenocarcinoma is not already present, may persist for years before progression to invasion. All of these considerations emphasize the limitations of dysplasia as a risk biomarker. That no better biomarker has yet emerged is only to restate the challenge. It is likely that dysplasia will remain a mainstay of risk assessment in Barrett's oesophagus for some time, with newer technologies complementing it initially, not least because morphology may usefully allow targeting of marker studies such as ploidy assessment by image cytometry, immunohistochemistry, or FISH (fluorescent in situ hybridisation). Different patterns of dysplasia are also coming to be recognised and in time may prove to have different behaviours (Brown, Whiteman, & Lauwers 2010;Rucker-Schmidt, Sanchez, Blount et al. 2009). Until then, dysplasia agreed on by more than one experienced gastrointestinal pathologist may be more robust than an uncorroborated diagnosis. Where three pathologists agree on a diagnosis of low grade dysplasia, an elevated risk of progression exists perhaps because dysplasia on which any three GI pathologists can agree is close to being high grade (Skacel, Petras, Gramlich et al. 2000).

Figure 7.1 Examples of dysplasia associated with Barrett's epithelium



(A) Heterogenous Barrett's mucosa with no dysplasia at *, but low grade dysplasia at **, represented by cellular stratification and proliferation at the mucosal surface

(B) Rounded nuclei with chromatin clearing and easily visible nucleoli point to high grade dysplasia

(C) Well differentiated Barrett's adenocarcinoma (typical example)

7.5 Molecular Abnormalities of Barrett's Oesophagus

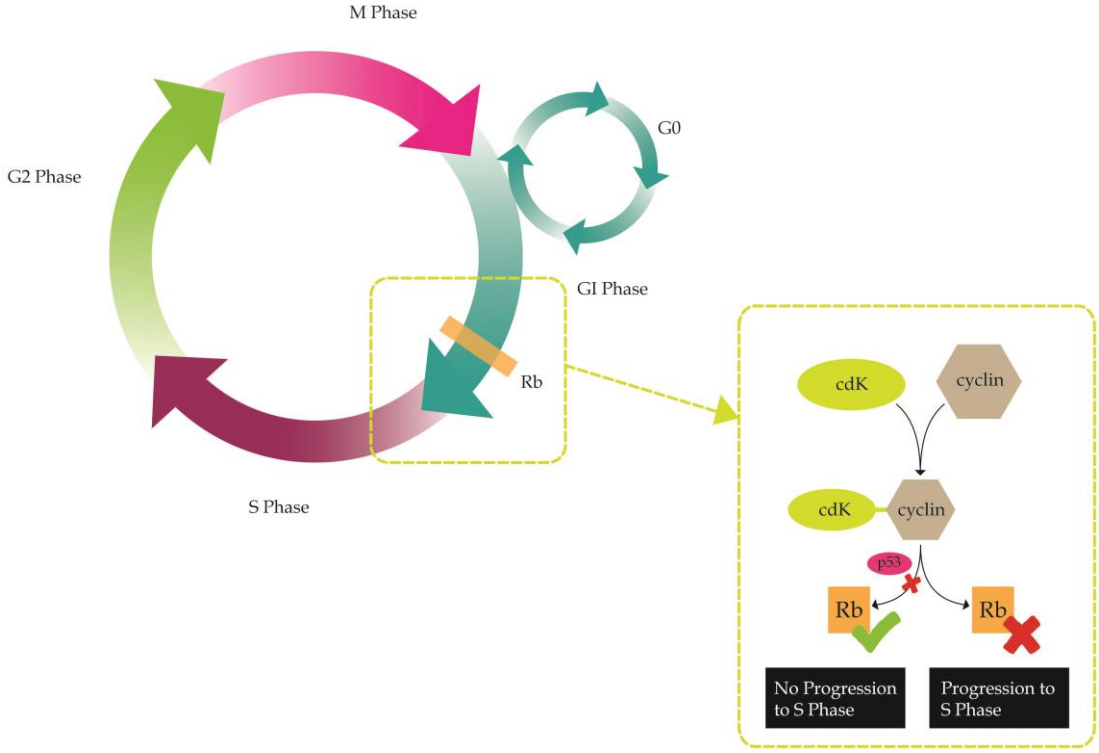
Mutations accumulating in premalignant tissue lead to evolution of cellular clones with increasing genomic instability and abnormal cell behaviour until clones of cells emerge with invasive and metastatic potential (Barrett, Sanchez, Prevo et al. 1999;Nowell 1976). Epigenetic events and aspects of the host environment such as inflammation are also important, and cancer can be promoted by factors not known to be genotoxic.

Genomic instability is a fundamental property of neoplastic progression, developing before the onset of cancer and characterised by chromosomal instability (aneuploidy), epigenetic instability, loss of heterozygosity (LOH) affecting tumour suppressor genes and microsatellite instability. The targets of genomic instability are usually seen to include proto-oncogenes, tumour suppressor genes, DNA mismatch repair genes and mitotic checkpoint genes (Koppert, Wijnhoven, van Dekken et al. 2005). Hanahan and Weinberg's popular taxonomy of properties required by cancer cells - namely, growth and self-sufficiency, insensitivity to growth inhibitory signals, avoidance of apoptosis, replication without limit, sustained angiogenesis, invasion and metastasis and more recently inflammation, provides a convenient framework for an examination of potential biomarkers (Hanahan and Weinberg 2011;Morales, Souza, & Spechler 2002). However over the last decade the cancer stem cell hypothesis has caused a shift in thinking about the key events of carcinogenesis. Stem cells and cancer cells share several important properties and there is now evidence to suggest dysregulation of the self renewal process of stem cells may be the key event in early carcinogenesis rather than random mutation. This hypothesis, if adopted, carries significant implications for diagnosis and therapeutic options in cancer (Wicha, Liu, & Dontu 2006).

7.5.1 Growth Self-Sufficiency

The cell cycle is an ordered series of events, resulting in cell growth and division into two daughter cells. Normal cells require exogenous growth signals to move from G_0 (quiescence) into the cell cycle (Figure 7.2). A key mechanism controlled by the retinoblastoma protein p185 Rb, late in G_1, restricts progression into S phase and DNA synthesis to cells without DNA damage, which may trigger cell cycle arrest and DNA repair, or if the damage cannot be repaired, apoptosis and cell death (Souza, Morales, & Spechler 2001).

Figure 7.2 The key regulators of the cell cycle



Key to figure: (M) mitosis, (G_1) growth phase 1, (S) DNA synthesis, ($G_{2)}$ growth phase 2. Retinoblastoma (Rb) is the molecular switch controlling the G_1 R point. The cdk-cyclin complex phosphorylates Rb, inactivating it and allowing the cell to progress into S phase. However in the presence of p53 (a tumour suppressor gene), the cyclin-cdk complex is inhibited and Rb remains unphosphorylated preventing onward progression. This holds the cell in G_1 preventing replication of cells containing damaged DNA.

Progression through the cell cycle is controlled by cyclins and cyclin dependent kinases (cdks). Different cdks and cyclins are required at various stages of the cell cycle. There are two main structurally related groups of cdk inhibitors. The Ink4 family (inhibitors of cdk4) consists of proteins (p15, p16, p18, p19) that inhibit cyclin D-cdk 4/6 complexes. Mutations, deletions or silencing through DNA methylation of p15 and p16 have been reported in various human malignancies. The other group is the Cip/Kip family including p21, p27 and p57 which preferentially target Cdk2. p21 (also known as Cip1 or Waf1) is regulated by p53, and although there are many mutations in p53, no molecular alterations of p21 have yet been reported.

Cyclin D

Cyclin D_1 is a proto-oncogene that controls the G_1-S transition by activating Cdks 4 and 6, which phosphorylate p185 Rb (thereby inactivating it) and stimulate progression through the cell cycle. A single base polymorphism in a variant known as cyclin D_1b has been implicated in overexpression and neoplastic transformation, and immunohistochemistry shows cyclin D_1 overexpression in Barrett's oesophagus and oesophageal adenocarcinoma (Casson, Zheng, Evans et al. 2005). It has been claimed that patients with Barrett's metaplasia and cyclin D_1 overexpression are 6-7 times more likely to develop adenocarcinoma although other studies do not support this (Bani-Hani, Martin, Hardie et al. 2000;Murray, Sedo, Scott et al. 2006). Increased expression of cyclin D_1 is an early event in carcinogenesis and may of itself predispose to malignant transformation (Zagorowicz and Jankowski 2007). At present abnormalities of cyclin expression cannot be confirmed as markers of progression risk.

Cyclin A

Cyclin A is also a proto-oncogene expressed in the proliferative compartment in normal gastrointestinal mucosae. Cyclin A immunohistochemistry from oesophageal brushings in Barrett's patients showed similar localization in the proliferative compartment in 76% of samples. However with increasing grades of dysplasia, the expression of cyclin A shifted toward the upper crypts and surface epithelium. In non-dysplastic tissue, only 24% of patients express cyclin A at the mucosal surface compared with 59% of low grade dysplasia patients, 87% of high grade dysplasia patients and 100% of adenocarcinoma patients (Lao-Sirieix, Lovat, & Fitzgerald 2007).

Cyclin/CDK Inhibitors

The tumour suppressor p27 inhibits cyclin E/cdk2 complexes, blocking cell cycle progression into S phase. p27 knockout mice have increased risk of oesophageal cancer compared to wild type mice and low levels or absence of p27 are associated with a worse prognosis in human colon, stomach, lung and prostate cancers (Ellis and Loda 2004;Ellis, Xu, & Kulke 2001;Singerland and Pagano 2000). In Barrett's oesophagus and oesophageal adenocarcinoma lack of p27 expression is associated with malignant transformation and a poorer prognosis (Ellis & Loda 2004). In non-dysplastic Barrett's mucosa p27 expression is nuclear but in dysplastic mucosa, staining is often cytoplasmic. Low levels of p27 expression correlate with higher

histological grade, depth of invasion and lymph node metastasis in patients with oesophageal adenocarcinoma (Singh, Lipman, Goldman et al. 1998). Nuclear localization of p27 is essential for its growth-inhibiting function, and loss of expression or altered localization in adenocarcinoma are associated with tumour progression and adverse prognosis, suggesting that p27 has a role in preventing progression of Barrett's epithelium to adenocarcinoma.

7.5.2 Insensitivity to Anti-growth Signals

Normal cell growth is restrained by inhibitory signals which block proliferation by inducing quiescence or permanent growth arrest (cellular senescence). Most anti-growth signals are controlled by the retinoblastoma gene protein (p185 Rb) at the G_1 checkpoint. However, tumour cells can overcome this inhibition by inactivating tumour suppressor genes via mutation, allelic deletion (loss of heterozygosity) or promoter hypermethylation. Loss of the retinoblastoma gene itself seems to be rare in Barrett's metaplasia, but abnormalities in genes such as *CDKN2A* (encoding p16) and *TP53*, which normally block Rb phosphorylation and its activation, are relatively frequent.

p16, tumour suppressor gene

p16 (*INK4* or *CDKN2A*) is a tumour suppressor gene on chromosome 9p21. p16 protein binds to and inhibits cdk4/6, resulting in reduced phosphorylation of the retinoblastoma protein and inhibition of cell cycle progression through G_1. Many studies have analysed p16 in cancers but fewer have examined premalignant lesions. Paulson and colleagues suggest that inflammation caused by exposure of oesophageal mucosa to acid and bile is a potential source of oxidative damage (Paulson, Galipeau, Xu et al. 2008). Reactive oxygen and nitric oxide species may mediate mutations, including inactivation of p16, with subsequent uncontrolled cellular proliferation and disease progression (Kerkhof, Kusters, van Dekken, Kuipers, & Siersema 2007). Early loss of heterozygosity appears to be a common mechanism of p16 inactivation associated with subsequent clonal expansion along the Barrett segment, favouring further mutations and facilitating disease progression (Kerkhof, Kusters, van Dekken, Kuipers, & Siersema 2007;Maley, Galipeau, & Li 2004). Other genetic and epigenetic events leading to loss of p16 include hypermethylation of CpG islands or allelic deletions. Immunohistochemistry has shown abnormalities of p16 expression in all grades of dysplasia. In Barrett's mucosa without dysplasia, p16 staining is nuclear. As dysplasia progresses, nuclear staining

wanes while cytoplasmic positivity increases - an early signal and a potential mechanism of further genetic changes (Shi, Bhagwandeen, & Leong 2008). A prospective study has shown that 9pLOH, 17pLOH and aneuploidy together predict progression to adenocarcinoma, but further studies are needed (Galipeau, Li, Blount et al. 2007).

7.5.3 Avoidance of Apoptosis

TP53, tumour suppressor gene

Neoplastic cells must avoid apoptosis to expand their numbers. Loss of p53 allows cells to bypass apoptosis and proliferate. *TP53* is a tumour suppressor gene which encodes the protein (p53) involved in regulation of cell cycle progression, DNA repair, cellular senescence and apoptosis. It induces expression of p21 and mediates both G_1 and G_2/M arrest. Both p53 and p21 prevent cells with DNA breaks from entering DNA synthesis, holding them back until they are repaired, or if repair is not possible, directing them to undergo apoptosis (Vousden and Lane 2007). p53 has a central role in human malignancy, being mutated in at least 50% of all malignant tumours (Wijnhoven, Tilanus, & Dinjens 2000). Mutations in *TP53* have been reported in primary oesophageal adenocarcinomas and high grade Barrett's mucosa, in which both alleles are lost, one by point mutation (90%) and the second by LOH (Wijnhoven, Tilanus, & Dinjens 2000). LOH refers to the loss of normal function of the other allele of a gene when the first allele is already inactivated. Point mutations of TP53 in oesophageal adenocarcinomas are often G:C to A:T transitions resulting from endogenous mechanisms such as exposure to oxygen and nitric oxide radicals.

Mutations in *TP53* frequently increase the half life of p53, leading to increased levels of protein expression which can be detected by immunohistochemistry as unusually intense nuclear staining. In contrast normal (wild type) p53 has a short half life and is not readily detectable at all or is detected at low levels only (Keswani, Noffsinger, Waxman et al. 2006). Although p53 mutations are common in adenocarcinoma, they are relatively uncommon in non-dysplastic Barrett's oesophagus and low grade dysplasia. Patients with high grade dysplasia often overexpress p53, suggesting that TP53 mutation may play a role in the transition from low to high grade dysplasia (Ross, Kinney, Larghi et al. 2005;Younes, Lebovitz, Lechago et al. 1993). Younes showed that p53 accumulation increased along the metaplasia-dysplasia-carcinoma sequence (0%, 9% and 87% for no dysplasia, LGD and HGD). On follow up studies,

only one of 21 patients with p53-negative biopsies developed dysplasia (Younes, Ertan, Lechago et al. 1997).

Overexpression of p53 may therefore be a marker of progression in patients histologically indefinite for dysplasia or with LGD only. Sikkema showed that p53 overexpression and Ki67 were predictive of progression from metaplasia to cancer (Sikkema, Kerkhof, Steyerberg et al. 2009). However, some TP53 mutations produce a truncated p53 protein undetectable by immunohistochemistry (Keswani, Noffsinger, Waxman, & Bissonnette 2006). Therefore, protein expression is neither as sensitive nor as specific as gene analysis. Coggi et al showed that in patients with p53 mutations, there was no detectable accumulation by immunohistochemistry in 31% cases (Coggi, Bosari, Roncalli et al. 1997). In addition, inflammation, DNA damage and other cellular stresses can upregulate p53. So, not all p53 mutations result in p53 protein accumulation, and not all protein accumulation is due to mutations (Keswani, Noffsinger, Waxman, & Bissonnette 2006). In an attempt to overcome some of these difficulties, a study of 325 patients with Barrett's oesophagus investigated the prevalence of 17pLOH as a marker of dysplasia and risk of progression to cancer (Reid, Prevo, Galipeau et al. 2001). The prevalence of 17pLOH was 6% in non-dysplastic Barrett's mucosa, 57% in HGD, and it was an independent predictor of progression to adenocarcinoma. Of patients with baseline 17p LOH, 37% developed cancer whereas only 3% without 17p LOH progressed to cancer. These TP53 mutations seem to confer advantage to the mutant clone via three mechanisms: suppression of apoptosis, prevention of cell cycle arrest and senescence, permitting genetic instability (Maley 2007). Despite some of the limitations associated with the p53 protein expression, p53 is a well studied potential marker of neoplastic progression in Barrett's epithelium and newer genotyping technology may overcome some of the current limitations surrounding p53.

Alpha-methylacyl-CoA racemase

Alpha-methylacyl-CoA racemase (AMACR) is a protein expressed in peroxisomes and mitochondria of normal liver and kidney cells, and plays a role in the beta-oxidation of branched chain fatty acids (Maley, Galipeau, Finley et al. 2006). AMACR overexpression was initially reported in prostate cancer and high grade intraepithelial neoplasia but it is also expressed in dysplastic cases of Barrett’s oesophagus (Dorer and Odze 2006). Immunohistochemistry suggests AMACR is not expressed in non-dysplastic Barrett’s epithelium, but is present in low grade

dysplasia (38%), high grade (81%) and adenocarcinoma (72%) (Maley, Galipeau, Finley, Wongsurawat, Li, Sanchez, Paulson, Blount, Risques, Rabinovitch, & Reid 2006). The exact role of AMACR in the oesophageal epithelium is unclear but its overexpression may be a useful adjunct in diagnosing dysplasia in difficult cases. Future studies are required to fully explore the role of AMACR and its prognostic significance.

7.5.4 Invasion and Metastases

Wnt signalling is a key pathway in normal human organogenesis, but aberrant activation is implicated in carcinogenesis. Key genes and proteins in this pathway include the adenomatous polyposis coli (APC) gene, β-catenin and E cadherin and although much is known about these molecules, mechanisms by which they interact are still incompletely understood (Adams and Enders 2008). APC protein contains β-catenin degradation sites and E cadherin has β-catenin binding sites. Disturbance of normal interactions between these molecules can lead to loss of growth inhibition or increased tumour invasiveness.

Beta-catenin

β-catenin mediates cell-cell adhesion via the transmembrane glycoprotein E-cadherin. In carcinomas loss of the E-cadherin-catenin complex, which is involved in the maintenance of epithelial integrity, may confer increased invasiveness and metastatic ability on malignant cells (Washington, Chiappori, Hamilton et al. 1998). Beta-catenin is also an oncoprotein that can lead to carcinogenesis when APC-β-catenin-TCF/LEF (T-cell factor/lymphoid enhancer factor) signalling is disrupted. This is the so-called canonical Wnt signalling pathway. β-catenin and its integral role within the Wnt signalling pathway shall be discussed in greater detail in the following chapter.

E-cadherin

E-cadherin is a transmembrane protein essential for maintenance of cells during development. The extracellular domain of E-cadherin mediates adhesion with cadherins on neighbouring cells, while the intracellular domain interacts with cytoplasmic proteins linked to actin via catenins. It plays a crucial role in cell-cell adhesion and reduced expression is an important molecular event concerned with invasion and metastases (Bailey, Biddlestone, Shepherd et al. 1998). In conjunction with β-catenin several studies have shown decreased E-cadherin expression is

associated with progression from Barrett's metaplasia to adenocarcinoma (Clement, Jablons, & Benhattar 2007;Kerkhof, Kusters, van Dekken, Kuipers, & Siersema 2007). Reduced E-cadherin expression promotes epithelial cell invasiveness and metastasis in various human cancers (Nair, Naidoo, & Chetty 2005). Recent studies have shown that aberrant nuclear localisation of E-cadherin is present in some tumours (pancreatic endocrine tumours, oesophageal squamous and colorectal cancers) and associated with poor prognosis due to increased invasiveness of tumours (Chetty, Serra, & Asa 2008;Salahshor, Naidoo, Serra et al. 2008). Further research is required into the role of Wnt signalling in oesophageal adenocarcinoma.

7.5.5 Ploidy

Aneuploidy (abnormal cellular DNA content) is associated with increased risk of progression to dysplasia and adenocarcinoma. A large continuing phase 4 study by Reid et al has shown that patients with no dysplasia, indefinite or low grade dysplasia at baseline biopsy, and a diploid cell population (no aneuploidy) are at low risk of progression to adenocarcinoma (Reid, Levine, Longton et al. 2000). Patients in whom baseline biopsies demonstrated aneuploidy, tetraploidy (4N DNA content) or high grade dysplasia had five year cancer incidences of 43%, 56% and 59%, motivating more intense surveillance. In some centres, like Seattle, flow cytometry to assess aneuploidy is routinely undertaken in the assessment of Barrett's biopsy samples.

Despite good evidence in favour of ploidy as an early risk marker in Barrett's oesophagus, it is little used in clinical practice, probably because of its requirements in terms of costs and instrumentation and reimbursement issues. Flow cytometry has the disadvantage of divorcing DNA content measurements from morphology. Image cytometry of intact nuclei from thick sections partly addresses this issue by allowing histological control of the material submitted for analysis. Image cytometry on histological sections gives the best correlation of morphology and DNA content measurements but introduces its own problems with nuclear truncation and overlapping. Nevertheless, DNA content measurements are possible on sections around 7 microns thick, and further evaluation in this area would be desirable. Fleskens and colleagues showed that combining DNA content measurement with the cell cycle marker Ki67 facilitates detection of aneuploid cell populations in oral premalignant conditions, and demonstrated combined immunofluorescence and staining with a fluorescent DNA intercalating agent (DRAQ5) under stoichiometric

staining conditions (Fleskens, Takes, Otte-Holler et al. 2010). Aneuploidy is a biomarker which carries significant potential as a clinical biomarker, although cost and technical issues need reviewed before its routine use is implemented in hospital laboratories.

7.5.6 Inflammation

The association between inflammation and cancer was first described by Virchow in 1863 when he observed that leucocytes were present in cancerous tissues (Colleypriest, Palmer, Ward et al. 2009). Since then the role of systemic and local inflammation has been documented in many malignancies (Roxburgh and McMillan 2010). Reflux oesophagitis is often associated with Barrett's oesophagus and various cytokines (such as interleukins IL-1 and IL-8) are increased in Barrett's metaplasia. Two key factors which appear to link Barrett's oesophagus, inflammation and progression to dysplasia are nuclear factor κB (NFκB) and CDX genes (Colleypriest, Ward, & Tosh 2009). Inflammatory profiling as a biomarker for Barrett's oesophagus and risk of disease progression would be appealing, and an excellent target for therapeutic interventions. The role of anti-inflammatory agents and Barrett's oesophagus is currently being assessed by the AsPECT trial, the results of which are eagerly awaited (Jankowski & Barr 2006).

Nuclear Factor Kappa-B

Transcription factor NFκB regulates pro-inflammatory genes, differentiation and growth. It exists in the cytoplasm of most cells in an inactive form complexed to the inhibitory molecule IκB which prevents the migration of the heterodimer to the nucleus. Cytokines, oxygen free radicals and acid stimulate translocation of NFκB to the nucleus, where it binds specific DNA sites and upregulates transcription of genes involved in inflammatory processes and immune responses (Yamamoto and Gaynor 2001). NFκB has been linked to lung fibrosis, autoimmune arthritis and IBD (Rogler, Brand, Vogi et al. 1998).

Over the years there has been much interest in the role of inflammation, either local or systemic, in the development of cancer. The NFκB pathway is therefore of interest in Barrett's epithelium, where there is often an associated inflammation. O'Riordan et al showed a stepwise increase in expression of NFκB, IL-8 and IL- 1βin patients with Barrett's mucosa adjacent to adenocarcinoma (O'Riordan, Abdel-Latif, Ravi et al. 2005). They also showed that NFκB was upregulated in 60% of patients with

Barrett's oesophagus (all grades). In those with metaplasia but no dysplasia, 50% had NFκB overexpression; this rose to 63% in LGD and 100% in HGD. Adenocarcinoma patients with increased expression of NFκB had elevated levels of cytokines IL-8 and IL-1. Further studies are required to determine the role of these molecules in the metaplasia-carcinoma sequence.

Cyclooxygenase-2 (COX-2), an Oncogene

COX-1 and the oncogene COX-2 cyclooxygenases mediate synthesis of prostaglandins from arachidonic acid. COX-1 is generally expressed whereas COX-2 is undetectable in most tissues. It is induced by cytokines, gastric acid and bile acids. Overexpression of COX-2 in vitro has effects from increasing cell proliferation, reducing apoptosis, promoting angiogenesis, decreasing E cadherin expression and increasing the invasive and malignant potential of cells (Maley, Galipeau, & Li 2004;Shirvani, Ouatu-lascar, & Kaur 2000). COX-2 is detectable in metaplastic Barrett's mucosa and is overexpressed in high grade dysplasia and adenocarcinoma. Its expression in LGD is similar to that of metaplasia in the absence of dysplasia (Morris, Armstron, Bigley et al. 2001). Other studies have reported a progressive increase in COX-2 expression along the metaplasia-dysplasia sequence (Kuramochi, Vallbohmer, Uchida et al. 2004). Different techniques have been used to evaluate COX-2 expression such as immunohistochemistry, western blotting and polymerase chain reaction, with inconsistent results. While COX-2 overexpression may play a role in Barrett's oesophagus, at present there is not enough data to support a useful role as a biomarker.

7.6 Conclusions

Barrett's 'columnar lined' oesophagus is important as the precursor of oesophageal adenocarcinoma, which has the most rapidly rising incidence of any solid tumour in the western world, with Scotland having some of the highest rates in the world (Jankowski, Provenzale, & Moayyedi 2002). Advances in disease management over the last decade have seen improvements in endoscopic therapies to treat high grade dysplasia, better imaging and biopsy detection systems, and several candidate molecular biomarkers. At present, dysplasia develops in around 5% of patients with Barrett's oesophagus, with 10-50% progressing to high grade dysplasia and cancer over 2-10 years. The remainder remain static. Despite the risk of malignant progression, only 2-3% of the Barrett's oesophagus patients will die from oesophageal adenocarcinoma, and overall life expectancy is not very different from those without the disease.

The role of biomarkers in Barrett's oesophagus is potentially two fold. Firstly, to identify patients at risk of progression to high grade dysplasia and cancer, so they can be diagnosed and treated earlier with endoscopic therapies, minimising morbidity and avoiding the morbidity and mortality of oesophagectomy. Secondly, and almost equally useful, markers able to identify patients at little or no risk of progression would allow less frequent surveillance endoscopy and biopsy for low risk patients, minimising health care costs and patient anxiety. Being able to reassure a patient of a low progression risk is at least as important as to be able to assign a high risk, given limited evidence of effective risk management.

Barrett's oesophagus is a complex disease process with significant genetic heterogeneity, and greater heterogeneity identified within a Barrett's segment is itself a predictor of disease progression (Maley, Galipeau, Finley, Wongsurawat, Li, Sanchez, Paulson, Blount, Risques, Rabinovitch, & Reid 2006). Many individual mutations have been identified, but no one marker has yet been identified with ideal characteristics or the potential to fulfil clinical requirements on its own. It may be naïve to expect a single biomarker will fulfil all expectations in such a complex disease and many centres now think biomarker panels may be more likely to aid management.

Dysplasia, our 'gold' standard biomarker, and aneuploidy are at present the only markers routinely used in clinical practice. Many biomarkers have not passed through phase III or IV trials and much more work needs to be performed in this area before any of them is established on secure evidence as a basis for clinical practice. Multicentre trials will be required for assessment and integration of both clinical and molecular variables so comprehensive conclusions can be made. This will require a degree of cooperation rarely so far seen in the field, but without which greater understanding and appropriate management of Barrett's oesophagus will be further delayed.

Chapter 8

The Wnt Signalling Pathway –A mouse model

Cancer cells are characterised by their ability to proliferate outwith normal control mechanisms. Hanahan and Weinberg proposed seven hallmarks which are applicable to all cancer cells - (1) self-sufficiency in growth signals; (2) insensitivity to inhibitory growth signals; (3) evading apoptosis; (4) sustained angiogenesis; (5) limitless reproductive potential; (6) tissue invasion and metastasis and (7) inflammation (Hanahan & Weinberg 2011). A series of mutations and epigenetic changes are required for cancerous change within cells. Changes in the configuration, concentration or location of certain molecules involved in key signalling pathways can lead to malignant transformation (Doucas, Garcea, Neal et al. 2005). One example of such important signalling pathways involved in human cancer is the Wnt canonical pathway. Under normal conditions the Wnt pathway plays a key role in embryogenesis and organogenesis in humans, but when aberrantly activated is associated with carcinogenesis. More than 90% of colorectal cancers have a mutation which aberrantly activates the Wnt pathway but little is known about the role of Wnt signalling in oesophageal adenocarcinoma and Barrett's oesophagus (Giles, van Es, & Clevers 2003). The aim of the following two chapters is to further understand the role of Wnt signalling in Barrett's oesophagus and dysplasia in human tissue with a complementary mouse model.

8.1 Introduction

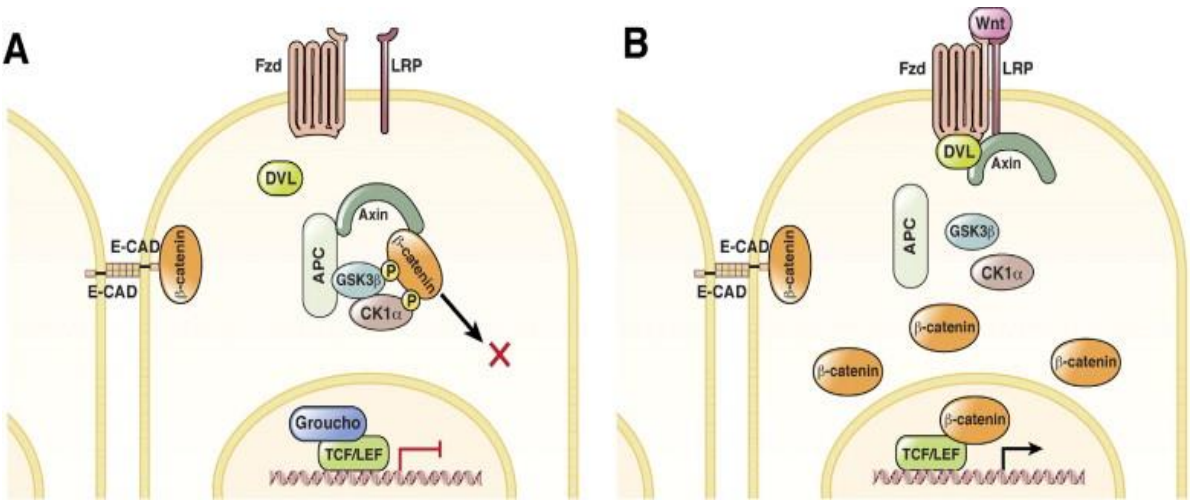
8.1.1 An Overview of Wnt Signalling Pathway

Wnt signalling is an ancient signalling pathway crucial to development and morphogenesis in embryos. In adults it is responsible for the maintenance of normal tissue and control of stem cell functions. The role of Wnt in cancer became clear more than 20 years ago in viral carcinogenesis experiments in mice, where the presence of Wnt1 was associated with the development of breast tumours (Shackleford, MacArthur, Kwan et al. 1993). In recent years, aberrant activation of the Wnt pathway is associated with many malignancies.

The Wnt family of genes code for a group of glycoproteins which are responsible for physiological responses within their target cells as a result of interactions with cell surface receptors (Reya and Clevers 2005). The canonical Wnt pathway describes the series of events that occur when extracellular Wnt ligands bind to their cell surface receptors (Frizzled proteins), causing activation of the intracellular Dishevelled proteins which ultimately results in changes in the amount of β-catenin which reaches the nucleus. β-catenin is a key component on the Wnt pathway as its presence in the nucleus allows transcription of various target genes involved in proliferation.

The canonical Wnt pathway shall be described in further detail as a clear understanding of this pathway is required to understand the role of β-catenin in carcinogenesis (Figure 8.1). In the absence of a Wnt signal, two scaffolding proteins axin and adenomatous polyposis coli (APC) form a destruction complex, which binds with newly synthesised β-catenin. Glycogen synthase kinase-3β (GSK3), a kinase also bound to the destruction complex then phosphorylates specific residues in the amino terminus of β-catenin. The phosphorylated β-catenin complex binds to the β-TrCP protein, and is tagged by a small protein called ubiquitin, ready for proteasomal degradation (Figure 8.1A). Wnt ligand receptor occupancy causes phosphorylation of the Dishevelled protein which leads to dissociation of the APC/axin/GSK complex. This releases β-catenin which accumulates in the cytoplasm and is transferred to the nucleus, where it binds to T cell factors (TCF) and activates a number of proliferative target genes such as cyclin D1, c-myc and c-jun (Figure 8.1B).

Figure 8.1 Overview of the canonical Wnt signalling pathway



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(A) In the absence of a Wnt signal, cytoplasmic β-catenin is phosphorylated by a destruction complex (Axin, APC and GSK3). It is targeted for ubiquitination and subsequent degradation. This maintains low levels of cytoplasmic and nuclear β-catenin.

(B) In the presence of a Wnt ligand, the Frizzled and Dishevelled proteins bind to axin, thus disrupting the destruction complex. β-catenin remains unphosphorylated and is translocated to the nucleus which it bind with TCF/LEF transcription factors, displaces Groucho and allows activation of nuclear transcription genes (eg cyclin D, c-myc).

8.1.2 Wnt and Cancer

The canonical Wnt signalling pathway is frequently activated in human cancers, either by activating mutations in β-catenin or inactivating mutations in the tumour suppressor genes (APC or axin) (Adams & Enders 2008). The most carefully studied model of Wnt signalling is found in colon cancer where mutations in APC and β-catenin are not uncommon (Pinto and Clevers 2005). Mutations of the APC gene occur early in colorectal cancer, leading to reduced β-catenin degradation and increased expression of transcription factors promoting carcinogenesis (Segditsas and Tomlinson 2006). β-catenin mutations, particularly common in epithelial tumours, suppress APC dependent binding and degradation of β-catenin. Tyrosine kinases such as epidermal growth factor, are commonly increased in cancer, resulting in hyperphosphorylation of tyrosine on β-catenin, making it unable to bind to cadherins. This reduces cell to cell adhesion and may account for invasion and metastases (Hoschuetzky, Aberle, & Kemler 1994).

Wnt ligands

Studies in human cell lines and animal models have shown that Wnt ligands play a role in carcinogenesis. A number of head and neck cancer cell lines have increased levels of Wnt1 mRNA, and blocking Wnt1 signalling leads to reduced cell proliferation and increased apoptosis (Rhee, Sen, & Lu 2002). The levels of β-catenin and cyclin D1 were also reduced. Wnt2 mRNA is detected in colon cancer but absent in normal colonic cells (Holcombe, Marsh, & Waterman 2002). In most human cancers, Wnt proteins themselves are not linked to carcinogenesis, but mutations mimicking Wnt stimulation leads to activation of the pathway and its downstream effects. The Wnt signalling pathway is now known to be involved in various tumours such as colon cancer, melanoma and leukaemia.

βeta-catenin

β-catenin is a protein involved in two independent processes - cell to cell adhesion and signal transduction. β-catenin is the key mediator of the canonical Wnt signalling pathway. In its unphosphorylated state, it migrates to the nucleus where it interacts with TCF and leads to the activation of tumour promoting genes such as c-myc, cyclin D1 and MMP-7.

β-catenin plays an important role in maintaining cell-cell adhesion by binding to the cytoplasmic domain of cadherins, particularly E-cadherin, and to α-catenin which links the cadherin-catenin complex to the actin cytoskeleton (Schuhmacher, Becker, & Oswald 1999). Disruption of β-catenin binding to cadherin results in loss of cell-cell adhesion and leads to loss of tissue architecture and invasion. Reduced expression of β-catenin in tumour cells may lead to impairment of the cadherin-catenin system and one study has shown a correlation between reduced expression of β-catenin and poor prognosis from oesophageal adenocarcinoma (Krishnadath, Tilanus, van Blankenstein et al. 1997). Unlike colon cancer, mutations in APC, axin and β-catenin are rare in oesophageal adenocarcinoma, and Wnt signalling appears to be activated upstream of β-catenin.

8.1.3 Wnt and Oesophageal Development

During embryonic development, the single endodermal tube is lined with a ciliated columnar epithelium before it differentiates into two separate tracts (the respiratory and upper gastrointestinal tracts) at 7 weeks gestation. The embryonic oesophagus remains covered with a columnar epithelium until 17 weeks when the oesophagus is progressively replaced by a squamous epithelium. The exact mechanisms underlying the mucosal transformation are unclear but it is thought that signalling pathways such as Hedgehog, Notch and Wnt may play a role. In the normal gut, activated Wnt signalling is required for the development and maintenance of the midgut and hindgut (small and large intestine), whereas formation of the foregut (oesophagus and stomach) is thought to result, at least in part, from a lower level of Wnt signalling (Gregorieff, Grosschedt, & Clevers 2004). Perhaps aberrant activation of Wnt signalling in adults may be involved in Barrett's metaplasia, or disease progression.

8.2 Study rationale and aims

Barrett's mucosa is predisposed to genetic and epigenetic changes, leading to low and high grade dysplasia, with high grade dysplasia carrying the greatest risk of progression to oesophageal adenocarcinoma (Shaheen & Richter 2009). Despite intensive research, the molecular mechanisms underlying Barrett's metaplasia and disease progression remain unclear. Previous chapters have shown molecular risk biomarkers have been sought with modest success and at present dysplasia remains the most reliable clinical marker of patients at risk of progression. There is therefore a real need to be able to identify those at greatest risk of malignant transformation. Without an improved molecular understanding of Barrett's metaplasia, clinically useful prognostic biomarkers will be delayed. With this in mind, the Wnt signalling pathway and its role in Barrett's oesophagus and dysplasia will be investigated in human tissue with a complementary mouse model.

The aim of this pilot study was to determine whether aberrant activation of Wnt signalling lead to phenotypic changes of metaplasia or dysplasia in a mouse model.

8.3 Methods

8.3.1 Mouse Model of Wnt Signalling

The Ah-Cre-ER^{+} β-catenin $^{\Delta ex3/\Delta ex3}$ mouse model with tamoxifen and β-napthoflavone-inducible gastrointestinal (GI) expression of activated β-catenin was used to upregulate Wnt signalling in the mouse oesophagus. This mouse model was chosen due to its availability in the Beatson Institute and its previous successful use in the upregulation of the Wnt signalling pathway in colorectal cancer. In this model, the Ah (aryl hydrocarbon) promoter directs expression of Cre-ER in the GI tract. Treatment with tamoxifen and β-napthoflavone activates Cre recombinase activity which generates the constitutively activated form of β-catenin lacking exon 3 and its associated inhibitory phosphorylation sites (Harada, Tamai, Ishikawa et al. 1999;Kemp, Ireland, Clayton et al. 2004).

Outbred mice segregating for the C57BL6J and S129 genomes were used from 6-10 weeks of age. The following alleles were used: Ah-Cre-ER and Catnb exon3fl. To induce recombination using the Ah-Cre-ER construct, mice were given four intraperitoneal injections of beta-napthoflavone (Sigma, Dorset, UK) and tamoxifen (Sigma, Dorset, UK) in corn oil at 80mg/kg over two days. At the appropriate time point, ie weight loss of 10%, or when showing signs of illness (hunched, pale feet), the mice were sacrificed and the oesophagus harvested, opened and fixed flat in 10% neutral buffered formaldehyde overnight. The oesophagus was embedded in paraffin and stained with H&E for initial pathological evaluation.

8.3.2 Tissue analysis

H&E Assessment

The H&E slides for each mouse were analysed with the assistance of Dr Catherine Lamm, a veterinary pathologist (University of Glasgow, Garscube Estate, Glasgow). Each slide was assessed for the type of mucosa present, any nuclear atypia, the presence of mitotic figures and any disorganisation within the tissue specimen. Each slide was assessed blindly, i.e the pathologist was unaware of whether the tissue came from a wild type mouse, or one containing the activated β-catenin$^{\Delta ex3}$ allele.

Immunohistochemistry

Serial sections (4μm thick) were cut from the paraffin embedded blocks of mouse oesophagus, and immunohistochemistry using the EnVision kit (Dako, Glostrup, Denmark) on a Dako autostainer was performed. The protocol used for immunohistochemistry was similar for most experiments, although the buffer and primary antibody varied.

Sections were initially deparaffinised in xylene and rehydrated in serial alcohols. Heat induced epitope retrieval was performed on a Thermo Pre-treatment module (25 minutes at 97^{o}C) with an appropriate buffer for each antibody (either 10MM sodium citrate (pH6), or 1 mM EDTA (pH8)). Endogenous peroxidase activity was blocked by incubation with hydrogen peroxidase (EnVision, Dako) for six minutes. All primary antibodies (see Table 8.1 for further details) - Ki67, β-catenin, cyclin D_1, c-myc, Sox9, villin, CK8, CLDN3 were incubated at room temperature for 45 minutes, followed by the appropriate secondary antibody. 3,3-diaminobenzidine tetrahydrochloride was used as chromagen. Sections were counterstained with haematoxylin, dehydrated in alcohol and mounted from xylene. The special stain, Alcian blue-periodic acid Schiff (PAS), a marker for the presence of goblet cells was used to stain the mouse oesophagus.

8.3.3 Ethical approval

The study was carried out at the Beaton Institute for Cancer Research under the supervision of Peter Adams and his lab staff. All mouse experiments were performed under the UK Home Office guidelines.

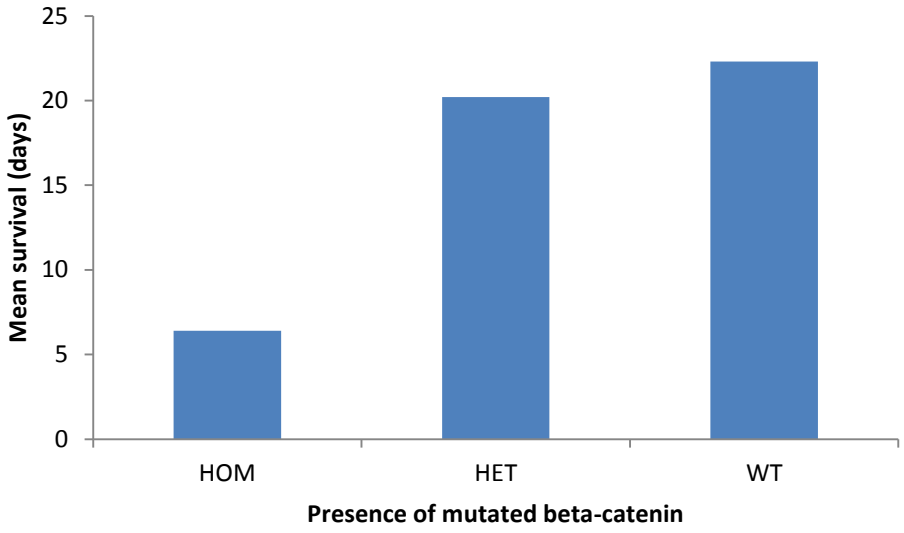
Table 8.1 Primary antibodies for immunohistochemistry on mouse oesophagus

Antibody	Clone	Dilution	Manufacturer	Catalogue Number
Anti-Sox9		1:700	Millipore, Livingstone, UK	AB5535
Cyclin D_1	SP4	1:50	Dako, Glostrup, Denmark	M3635
Ki67	SP6	1:200	Thermo Fisher Scientific, Cheshire, UK	RM-9106
β catenin	14	1:1200	BD Transduction Laboratories, San Jose, CA	610154
Villin			Abcam, Cambridge, UK	ab52102
MUC-5	CLH2		Leica, Milton Keynes, UK	NCL-HGM-45M1
CK8		1:250	University of Iowa, USA	Troma-1
CLDN-3		1:100	Thermo Fisher Scientific, Cheshire, UK	Rb9251

8.4 Results

Twelve Ah-Cre-ER β-catenin $^{\Delta ex3}$ mice were used in this initial study (wild type, n=3; heterozygous, n=4; homozygous, n=5). There were 6 males and 6 females with a mean life expectancy of 15 days (range 4-25 days). The life expectancy of the homozygous mice was significantly less than the heterozygous or wild type mice ($p<0.001$) (Figure 8.2). The wild type mice were culled if they were showing signs of illness, or at the end of the experiment.

Figure 8.2 Mean survival in Ah-Cre-ER β-catenin Δex3 mice



The mean survival in homozygous Ah-Cre-ER β-catenin $^{\Delta ex3}$ mice was 6.2 days (range 4-11), in heterozygous mice 20.2 days (range 18-21) and wild type mice 22.3 days (range 20-25). There was a significant reduction in survival in the homozygous Ah-Cre-ER β-catenin $^{\Delta ex3}$ mice ($p<0.001$).

Histological assessment of mouse oesophagus

The wild type mouse oesophagus (expressing Ah-Cre-ER only) showed a normal keratinised squamous epithelium with organised architecture and no nuclear atypia (Figure 8.3A). After induction with tamoxifen, the homozygous Ah-Cre-ER^{+} β-catenin $^{\Delta ex3}$ mice (harbouring two alleles of activated β-catenin $^{\Delta ex3}$) developed a very abnormal oesophageal mucosa (Figure 8.3C). The epithelium displayed nuclear atypia with increased numbers of suprabasal mitotic figures. The normal maturation pattern was disrupted with disorganisation of cells resulting in the formation of unusual intraepithelial clusters of cells. Many of these features, namely disruption of

maturation and increased proliferation, are classically associated with dysplasia but for the purposes of this study, these changes shall be labelled "quasi-dysplasia" as there is, as yet, little evidence to suggest these areas carry malignant potential. Little effect was observed on activation a single β-catenin $^{\Delta ex3}$ allele in the heterozygous mice, besides a slightly thickened basal layer (Figure 8.3B). Table 8.2 summarises the histological differences between the three groups of mice. The term "dysplasia counts" relates to the number of abnormal nuclei visualised within the section. There was no phenotypic change to a columnar type mucosa identified on H&E staining within this specific mouse model.

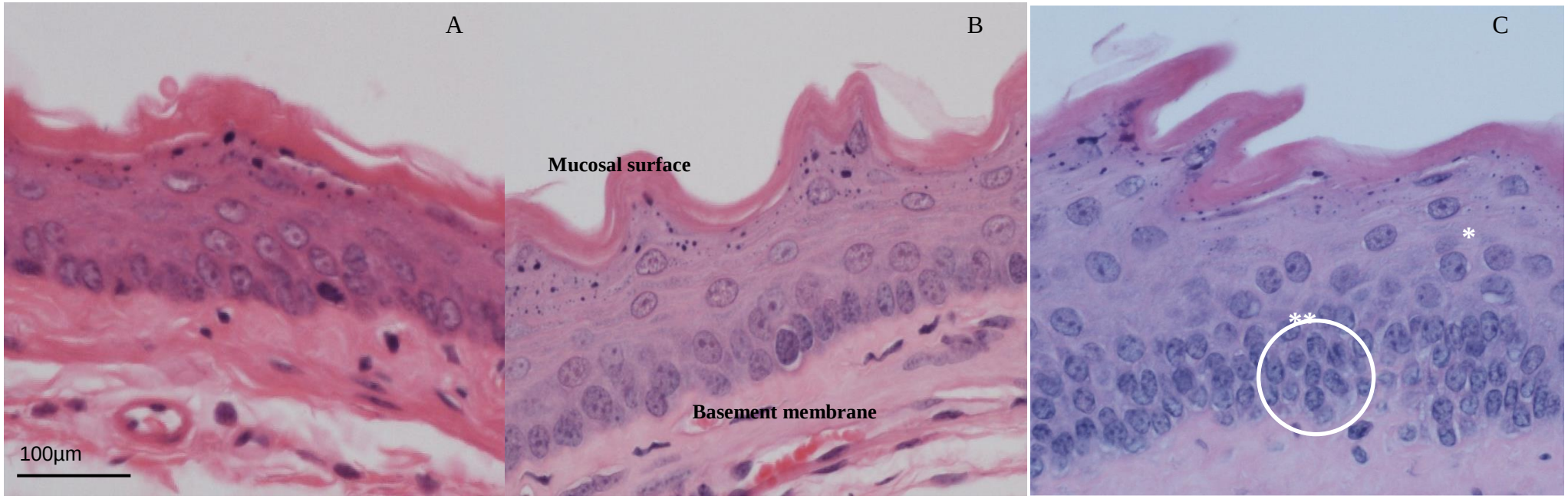
Table 8.2 Histological description of Ah-Cre-ER β-cateninΔex3 mice

	Wild type **n=3**	**Heterozygous** **n=4**	**Homozygous** **n=5**	**p value ***
Mitotic figures in basal layer	2 (1-3)	3.25 (2-6)	4 (3-8)	0.061
Nuclear disorganisation	Absent	Absent	Present	0.002
Nuclei (basal layer to surface)	3 (2-3)	4.5 (3-5)	11.5 (5-20)	0.023
Intraepithelial clusters	Absent	Absent	Present	0.002
Dysplasia counts	0	0	14 (2-50)	0.007

Table describes the histological features identified on H&E staining of mouse oesophagus (n=12).

* p value describes difference in histology between groups using Chi square test

Figure 8.3 Histological assessment of mouse oesophagus (H&E stain)



A - Ah-Cre-ER β-catenin$^{\Delta ex3}$ wild type demonstrating keratinised squamous mucosa of normal mouse oesophagus.

B - Ah-Cre-ER β-catenin$^{\Delta ex3}$ heterozygous mouse oesophagus with normal squamous mucosa similar to wild type. Note few more cells in basal layer.

C - Ah-Cre-ER β-catenin$^{\Delta ex3}$ homozygous mouse oesophagus demonstrating a thickened squamous mucosa with areas of nuclear atypia.

H&E staining of mouse oesophagus (magnification x20) * Cell with abnormal nucleus, ** Area of nuclear atypia forming cluster of disorganised cells (intraepithelial cluster) in keeping with abnormal maturation

β-catenin expression in Ah-Cre-ER$^{\Delta ex3}$ mouse oesophagus

β-catenin expression in the oesophageal mucosa of the wild type mouse was membranous with no evidence of nuclear staining (Figure 8.4 Panel A). After induction of activated β-catenin, the homozygous mouse mucosa showed a change in β-catenin expression with upregulation of nuclear β-catenin, particularly in the areas of "quasi-dysplasia". However, nuclear β-catenin was not distributed uniformly throughout the epithelium; instead, activation was more focal likely due to mosaic Cre-ER mediated activation of β-catenin$^{\Delta ex3}$ in cells throughout the epithelium (Figure 8.4 Panel B). Interestingly the focal activation of β-catenin coincided with areas of nuclear atypia with membranous staining preserved in "non-dysplastic" cells. There was no change in the expression of β-catenin in heterozygous mice.

Wnt target gene expression in Ah-Cre-ER$^{\Delta ex3}$ mouse oesophagus

The Wnt target genes cyclin D_1, and Sox9 were analysed by immunohistochemistry. In wild type mice (and heterozygous mice) cyclin D_1 was expressed in the nuclei of basal cells and some cells lining the basement membrane, while Sox9 was absent (Figure 8.4 Panel A). However in homozygous mice, upregulation of Wnt target genes, cyclin D1 and Sox9, in the oesophageal squamous mucosa coincided with the foci of nuclear β-catenin and apparent "quasi-dysplastic" areas (Figure 8.4 Panel B). Since Sox9 is a Wnt target gene that is normally expressed in the intestinal epithelium but not the oesophageal epithelium (Blache, van de Wetering, Duluc et al. 2004), its expression in the epithelium of mice expressing activated β-catenin, might be indicative of change from squamous oesophageal mucosa to an "intestinalised" metaplasia, at least at the molecular level.

Markers of intestinal phenotype in Ah-Cre-ER$^{\Delta ex3}$ mouse oesophagus

To test more completely for molecular changes of metaplasia in the mouse oesophagus, a panel of biomarkers and gene products normally associated with a columnar epithelium and/or Barrett's oesophagus was assessed. Alcian blue/PAS, a marker of goblet cells often associated with an intestinalised phenotype, did not reveal the presence of specific goblet cells within the mouse oesophagus. With the help of Mr Hamish McEwan, a fellow clinical research fellow, further immunohistochemistry was performed using villin, MUC-5, CDX2, cytokeratin 8 (CK8) and claudin-3 (CLDN-3). However, consistent with the histological analysis, there was no increased expression of villin, MUC-5 or CDX2 within this mouse

model. Interestingly there was increased expression of CK8 and CLDN-3 in the homozygous mouse, particularly in the areas of nuclear β-catenin expression. CK8 is often expressed in columnar mucosa and CLDN-3 is a marker of intestinal differentiation, associated with gastric cancer (Moll, Franke, & Schiller 1982;Satake, Semba, Matsuda et al. 2008).

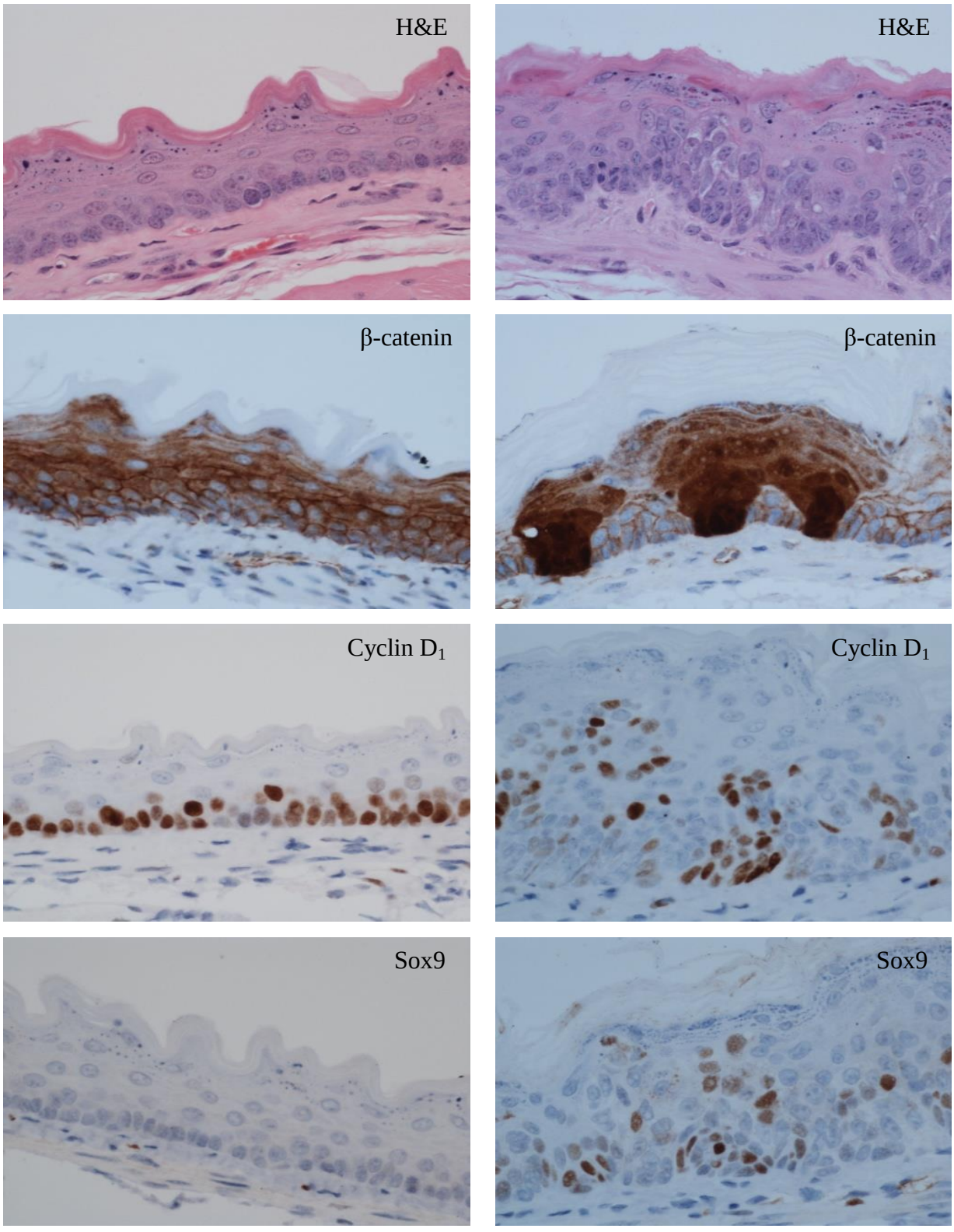
Figure 8.4 legend (Figure overleaf) Activation of Wnt signalling induces "dysplasia-like" features and intestinal gene expression (magnification x20)

Panel A Ah-Cre-ER$^{\Delta ex3}$ wild type mouse oesophagus. H&E stain demonstrating the keratinised squamous mucosa of normal oesophagus. β-catenin staining is membranous and cytoplasmic, with cyclin D_1 positive cells lining the basement membrane. There is no evidence of Sox9 expression in the wild type mouse.

Panel B Ah-Cre-ER$^{\Delta ex3}$ homozygous mouse oesophagus. H&E stain of homozygous mouse demonstrating a thickened squamous mucosa with areas of "dysplasia-like" features such as intraepithelial clusters nuclear atypia. Nuclear β-catenin expression is associated with areas of nuclear atypia with positive expression of the Wnt target genes, cyclin D_1 and Sox9.

Figure 8.4 Activation of Wnt signalling induces "quasi-dysplasia" features and intestinal gene expression

Panel A Wild type mouse **Panel B Homozygous Ah-Cre-ER β-catenin**



8.5 Discussion

This study has shown that upregulation of Wnt signalling by means of activated β-catenin expression, is associated with the development of quasi-dysplasia in the mouse oesophagus.

Wnt signalling is associated with the development of cancer, and increased expression levels of Wnt ligands and mutations within key genes are often found in colorectal cancer. However the underlying processes involved in Barrett's metaplasia and progression to dysplasia remain unclear. The Ah-Cre-ER β-catenin$^{\Delta ex3}$ mouse model has shown activation of Wnt signalling is sufficient to disrupt the normal architecture of the oesophageal mucosa, generating dysplasia-like features within the epithelium. At a molecular level, activation of Wnt signalling induced some features associated with an "intestinalised" mucosa, specifically by expression of Sox9, CK8 and CLDN3. Sox9 is not expressed in the normal mouse squamous oesophageal epithelium, but is expressed in the immature quasi-dysplasia cells harbouring nuclear β-catenin.

A recent paper by Kong and colleagues explored the role of Wnt signalling in human oesophageal keratinocytes, specifically focusing on the emergence of a Barrett's epithelium from squamous keratinocytes (Kong, Crissey, Stairs et al. 2011). They induced Wnt signalling by expression of CatCLef (a dominant Wnt effector) and showed that CatCLef-expressing cells were more proliferative, developed a thicker epithelium with cysts, filled with intestinal mucins (MUC5B and MUC17).

Unfortunately this mouse model failed to show any histological features in keeping with columnar metaplasia, or any intestinal mucin staining. One difficulty with the Ah-Cre-ER β-catenin$^{\Delta ex3}$ mouse model is the reduced life expectancy of the mice. The homozygous mice had a mean survival of only 6 days. It would appear the mice succumbed to other more serious diseases before the oesophageal mucosa had time to develop any further histological changes. A further limitation of the model may be a dosage phenomenon in the heterozygous mice, and further work would be required to explore this possibility. Nonetheless, it appears other factors besides Wnt signalling are required for the development of a columnar mucosa in the Ah-Cre-ER β-catenin$^{\Delta ex3}$ mouse model metaplasia within the oesophagus.

Taken together, these results indicate that activated Wnt signalling induced only selected molecular indicators of metaplasia and did not induce detectable metaplasia at the histological level. However, activation of canonical Wnt signalling did disrupt maturation of normal oesophageal squamous epithelium, creating an immature phenotype sharing quasi-dysplastic features. Further studies are required within this in vivo field to improve our understanding of the initial transformation from a squamous to a columnar epithelium, and while Wnt signalling is not the key driver, it may have a role in the disease progression associated with Barrett's oesophagus.

Chapter 9

The Wnt Signalling Pathway - A study in human oesophageal tissue

Barrett's oesophagus is a precursor of oesophageal adenocarcinoma with risk of cancer increasing substantially with dysplasia, particularly high grade. Thus there is a clinical need to identify and treat patients with early stage disease (metaplasia and low grade dysplasia) that are at risk of progression. Activated Wnt signalling is critical for normal intestinal development and homeostasis, but less so for oesophageal development. Therefore this study explored the interactions between increased Wnt signalling and the development of Barrett's oesophagus and/or dysplasia. Forty patients with Barrett's metaplasia, dysplasia or adenocarcinoma underwent endoscopy and biopsy. Immunohistochemistry of β-catenin, Ki67 and a panel of Wnt target genes and markers of intestinal metaplasia was performed. Expression of nuclear β-catenin was found in dysplasia, particularly high grade. Upregulation of Ki67 and Wnt target genes was also mostly associated with high grade dysplasia. Based on the results in human tissues and the mouse model, abnormal activation of Wnt signalling likely plays only a minor role in initiation of Barrett's metaplasia but a more critical role in progression to dysplasia.

Publication

Moyes LH, McEwan H, Radulescu S, Pawlikowski J, Lamm CG, Nixon C, Sansom OJ, Going JJ, Fullarton GM, Adams PD. Activation of Wnt signalling promotes development of dysplasia in Barrett's Oesophagus. *Journal of Pathology* 2012; 228 (11): 99-112.

9.1 Introduction

The role of Wnt signalling in the development and maintenance of normal tissue, and in carcinogenesis has been reviewed in previous chapters. In many clinical studies, β-catenin is the key molecule associated with Wnt signalling, with few studies considering other Wnt target genes. There are a handful of studies assessing the role of β-catenin in the development of oesophageal adenocarcinoma, with conflicting results. In some studies nuclear β-catenin was found in a limited subset of cancers, yet in other series there was positive staining in up to 80% of cases (Clement, Jablons, & Benhattar 2007). In one study of patients with adenocarcinoma, increased nuclear β-catenin expression was surprisingly associated with improved one year survival (Osterheld, Bian, Bosman et al. 2002). However there is paucity in the literature regarding the role of β-catenin in Barrett's metaplasia and dysplasia.

It is not known whether aberrant activation of Wnt signalling is causal in Barrett's metaplasia and the associated dysplastic or neoplastic transformation, or a passive bystander. Chapter 8 demonstrated aberrant activation of Wnt signalling in the Ah-Cre-ER β-catenin $^{\Delta ex3}$ mouse model resulted in areas of nuclear atypia and abnormal maturation, but failed to show a histological transformation from a squamous to a columnar epithelium in the oesophagus. It would appear that Wnt signalling is not a key player in the metaplastic transformation of the squamous oesophagus, but may be involved in the metaplasia-dysplasia-carcinoma sequence.

9.2 Study aims

On the background of the mouse model, the aim of this study was to assess whether increased Wnt signalling was associated with disease progression in patients with Barrett's oesophagus using immunohistochemical analysis.

9.3 Methods

9.3.1 Patients

Oesophageal mucosal biopsies from patients with endoscopically and histologically confirmed Barrett's oesophagus were collected at Glasgow Royal Infirmary from 2009 to 2010. For the purposes of this study, Barrett's oesophagus was defined endoscopically as a columnar lined oesophageal mucosa, and histologically by the presence of goblet cells. Patients were selected to obtain approximately twenty biopsies of each stage of the disease: 10 normal squamous mucosa, 26 Barrett's mucosa without dysplasia, 20 low grade dysplasia, 22 high grade dysplasia and 21 with Barrett's associated adenocarcinoma. Biopsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned (4µm) and stained with haematoxylin and eosin (H&E) for histological assessment by an experienced gastrointestinal pathologist (Dr JJ Going).

9.3.2 Immunohistochemistry

Serial sections (4µm thick) were cut from formalin fixed paraffin embedded (FFPE) blocks of human oesophagus, and immunohistochemistry using the EnVision kit (Dako, Glostrup, Denmark) was performed using a Dako autostainer. The protocol used for immunohistochemistry was similar for most experiments, although the buffer and primary antibody varied.

Sections were initially deparaffinised in xylene and rehydrated in serial alcohols. Heat induced epitope retrieval was performed on a Thermo Pre-treatment module for 25 minutes at 97^{o}C with an appropriate buffer for each antibody (either 10MM sodium citrate (pH6), or 1 mM EDTA (pH8)). Endogenous peroxidase activity was blocked by incubation with hydrogen peroxidase (EnVision, Dako) for six minutes. All primary antibodies (see Table 9.1 for further details), i.e. Ki67 (clone SP6, 1:200, Thermo Fisher Scientific, Cheshire, UK), p21 (clone M19, 1:800, Santa Cruz Biotechnology, Santa Cruz, CA), p53 (clone DO7, 1:1000, Dako, Glostrup, Denmark), p16 (clone 2D9A12, 1:2000, Abcam, Cambridge, UK), β-catenin (clone 14, 1:1200, BD Transduction Laboratories, San Jose, CA), cyclin D_1 (clone SP4, 1:50, Dako, Glostrup, Denmark), Sox9 (1:700, Millipore, Livingstone, UK) and c-myc (1:300, Abcam, Cambridge, UK) were incubated at room temperature for 45 minutes, followed by the appropriate secondary antibody. 3,3-diaminobenzidine tetrahydrochloride was used as chromagen. Sections were counterstained with

haematoxylin, dehydrated in alcohol and mounted from xylene. The special stain, Alcian blue-periodic acid Schiff (PAS), a marker for the presence of goblet cells was also used to stain the biopsy tissues.

9.3.3 Analysis of Immunohistochemistry

Immunohistochemistry of human tissues was assessed using light microscopy by two independent observers (LHM and Dr JJG). Weighted histoscores allowing semi-quantitative evaluation of epithelial protein expression were assigned at magnification x20 (Kirkegaard, Edwards, Tovey et al. 2006). Staining intensity was scored as negative (0), weak (1), moderate (2) or strong (3) before multiplication by the percentage of cells stained with that intensity. On each slide, each scorer scored 3 separate fields and the mean was calculated. For each slide, the mean of histoscores of LHM and JJG was calculated. The final histoscore ranges from 0 to 300. For some antibodies such as β-catenin, separate cellular locations (membranous, cytoplasmic and nuclear) were scored separately where appropriate. If there was any discrepancy between histoscores of >30, the slides were reviewed and a consensus reached. Figure 9.1 illustrates a general overview of the scores 0, 1, 2 and 3.

9.3.4 Statistical analysis

The mean histoscores obtained from combined LHM and JJG scoring were taken forward for analysis. Categorical data (ordering of ranks between histological grades) were compared by Kruskal-Wallis test and individual groups compared by Mann-Whitney test. A p-value of <0.05 was taken to be significant. The analysis was performed using SPSS for Windows v18.0, SPSS Inc, Chicago, IL, USA.

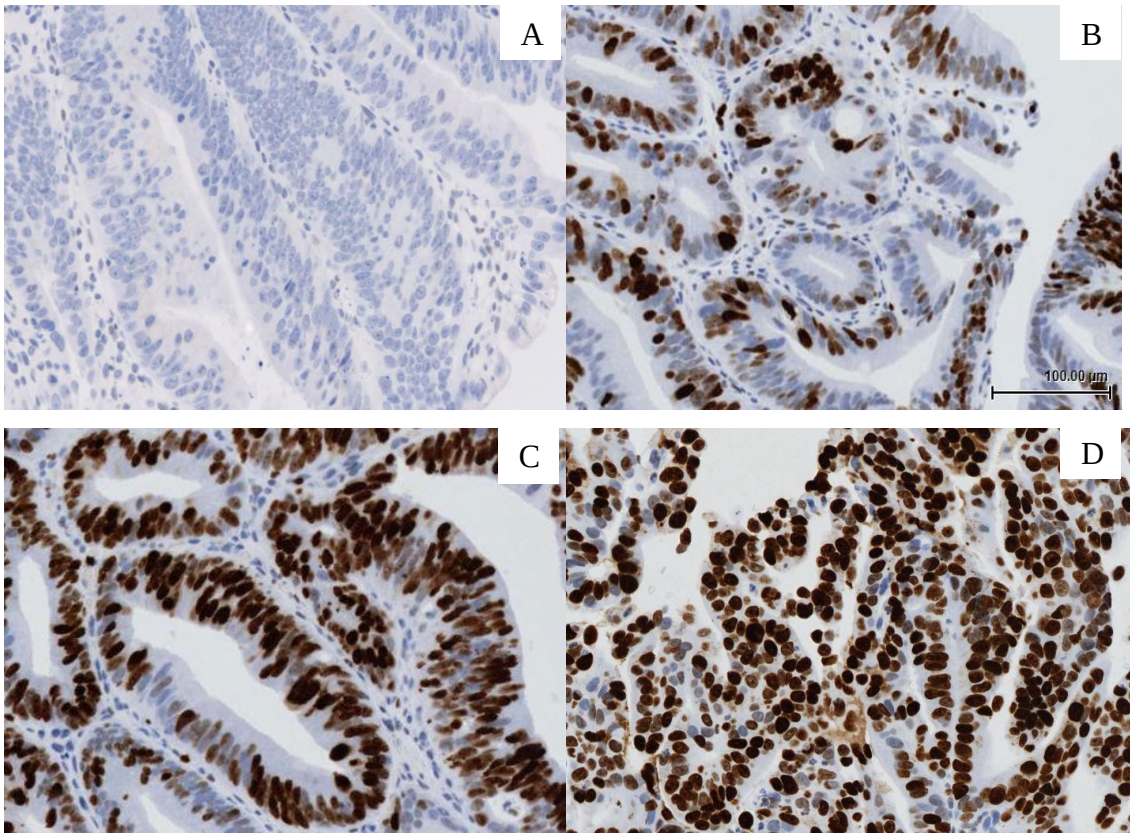
9.3.5 Ethical approval

Ethical approval for the study was given by West of Scotland Research Ethics Committee.

Table 9.1 Table of primary antibodies for immunohistochemistry studies on human oesophageal tissue

Antibody	Clone	Dilution	Buffer	Manufacturer	Catalogue Number
Anti-Sox9		1:700	Na Citrate pH6	Millipore, Dundee, UK	AB5809
CDX2		1:300	Na Citrate pH6	Abcam, Cambridge, UK	ab88129
c-myc	9E10	1:300	Na Citrate pH6	Abcam, Cambridge, UK	ab32
Cyclin D_1	SP4	1:50	Tris-EDTA pH9	Dako, Glostrup, Denmark	M3642
Ki67	SP6	1:200	Na Citrate pH6	Thermo Fisher Scientific, Cheshire, UK	RM-9106
p16	2D9A12	1:2000	Na Citrate pH6	Abcam, Cambridge, UK	ab54210
p16	CINtec®		Na Citrate pH6	mtm laboratories, Westborough, USA	9517
p21	M19	1:800	Na Citrate pH6	Santa Cruz Biotechnology, Santa Cruz, CA	SC-397
p53	CM5	1:200	EDTA pH8	Vector Laboratories, Peterborough, UK	VP-P596
p53	DO7	1:1000	EDTA pH8	Dako, Glostrup, Denmark	M7001
β catenin	14	1:1200	Na Citrate pH6	BD Transduction Laboratories, San Jose, CA	610154
E cadherin	36/E cad	1:300	Na Citrate pH6	BD Transduction Laboratories, San Jose, CA	610182
E cadherin	36B5	1:40	Na Citrate pH6	Vector Laboratories, Peterborough, UK	VP-E601

Figure 9.1 Histoscores – a general guide



Overview of slides for histoscores (magnification x10). (A) no staining, (B) weak staining, (C) moderate staining and (D) strong staining.

9.4 Results

Patient population

Table 9.2 summarises the basic demographics of the patients with Barrett's oesophagus in this study. The majority were males, with a mean age at presentation of 67 years (range 50-81 years). The length of Barrett's segment increased with the degree of dysplasia ($p<0.05$).

Table 9.2 Patient demographics according to histological grade

Histology	Sex (M:F)	Age (years)	Segment (cm)
Metaplasia (n=10)	9:1	64 (54-77)	6.0 (4-10)
LGD (n=10)	8:2	68 (50-79)	6.4 (4-10)
HGD (n=10)	8:2	70 (49-81)	7.1 (5-12)
Adenocarcinoma (n=10)	10:0	65 (54-78)	6.9 (3-10)

Wnt markers in Barrett's oesophagus

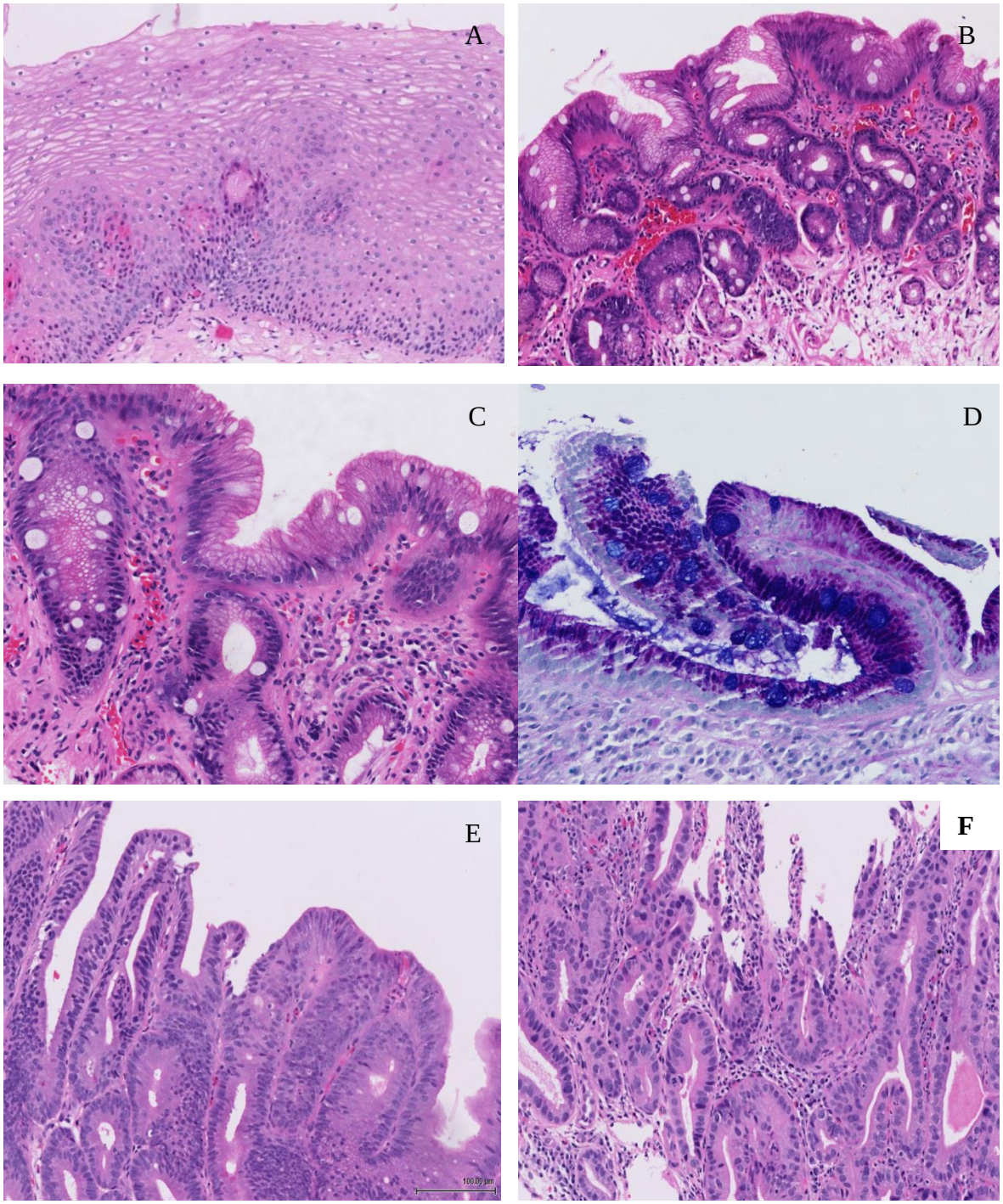
An initial immunohistochemical analysis of a panel of markers associated the p53 tumour suppressor pathway and Wnt signalling was used to initially assess the human oesophageal biopsy tissue. The p53 pathway plays a role in the progression of Barrett's oesophagus and therefore was a useful control pathway for future studies.

9.4.1 H&E and special stains

Ninety nine mucosal biopsies from 40 patients with Barrett's oesophagus or Barrett's associated adenocarcinoma were stained with H&E, complemented with Alcian blue-PAS. The normal oesophageal mucosa was composed of sheets of stratified squamous cells lying above a well-defined basement membrane (Figure 9.2A). Barrett's metaplasia was identified by the presence of a columnar epithelium with glandular crypts and the presence of goblet cells. The glands tended to be present in the middle zone of specimen, with columnar cells lined up regularly on the epithelial surface (Figure 9.2B&C). The Alcian blue stain helped to identify the acid mucin within the goblet cells, recognised by their purple/blue colour and characteristic shape (Figure 9.2D).

Low grade dysplasia can be recognised by cellular atypia. High grade dysplasia is associated with rounded nuclei with chromatin clearing and easily visible nucleoli. High grade dysplasia is associated with loss of normal tissue architecture and areas of necrosis. In some cases it can be difficult to differentiate between invasive adenocarcinoma and high grade dysplasia and expert pathologists are required in the accurate diagnosis in these challenging cases.

Figure 9.2 Histology of Barrett's oesophagus



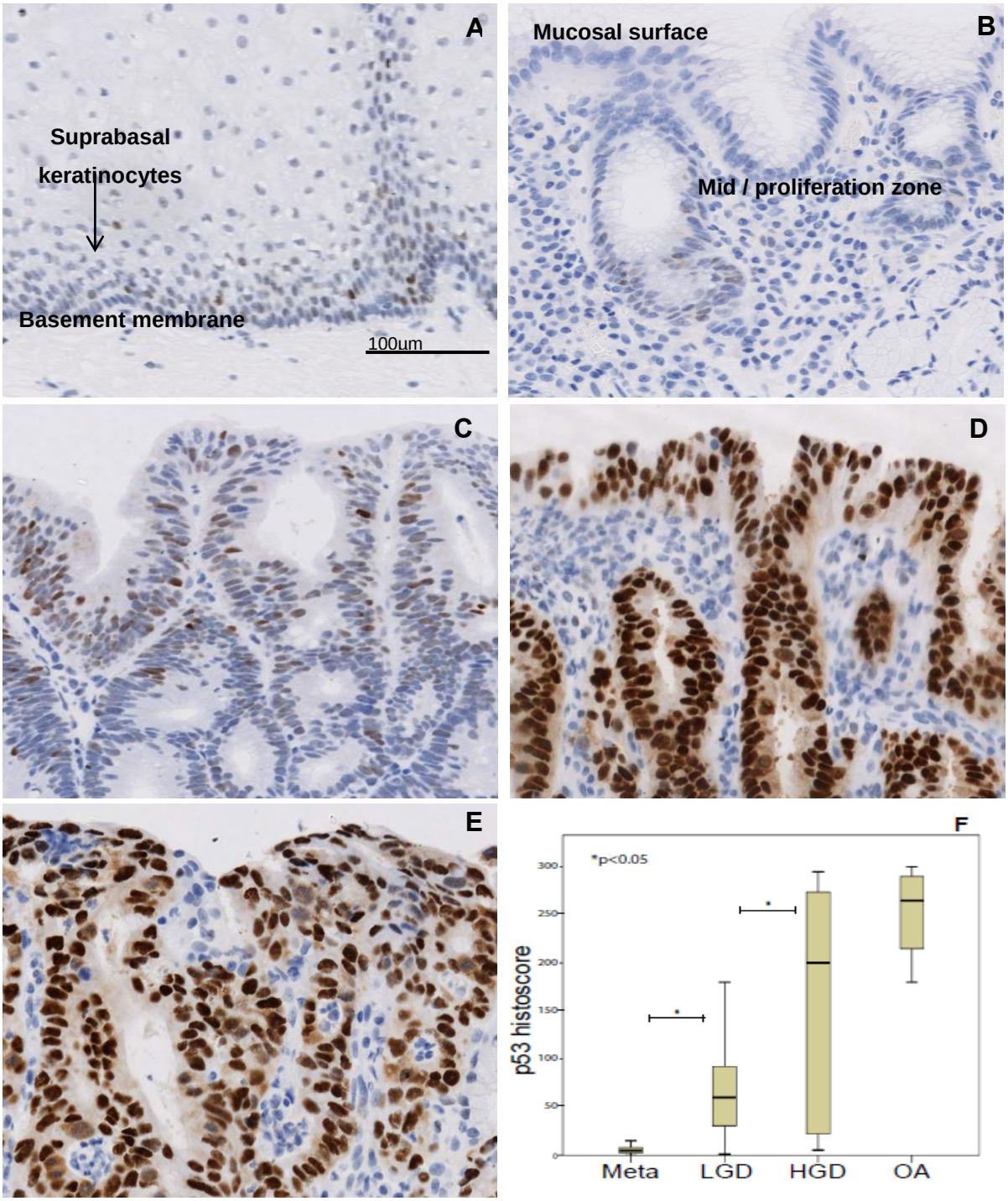
(A) The normal stratified squamous lining of the oesophagus. (B) Typical area of Barrett's metaplasia displaying a glandular mucosa with goblet cells seen more clearly in (C) with magnification x20. Alcian blue/PAS stains mucins within goblet cells, aiding detection (D). In the presence of dysplasia there is nuclear atypia and increased proliferation (E-F). High grade dysplasia is represented by loss of normal tissue architecture and areas of necrosis (F).

9.4.2 The relationship between p53 and p21

In normal oesophageal squamous epithelium, p53 staining was observed in the nuclei of scattered suprabasal cells (Figure 9.3A). Metaplastic Barrett's mucosa showed numerous weakly positive cells in the mid zone (Figure 9.3B). A change in the staining pattern was associated with the transition from LGD to HGD, with weak to moderate intensity staining of clusters of cells in dysplastic glands in cases with LGD (Figure 9.3C), and more intense diffuse staining throughout HGD and carcinoma (Figure 9.3D-E). Overall the extent and intensity of p53 staining increased significantly with progression from Barrett's metaplasia to adenocarcinoma (Figure 9.3F).

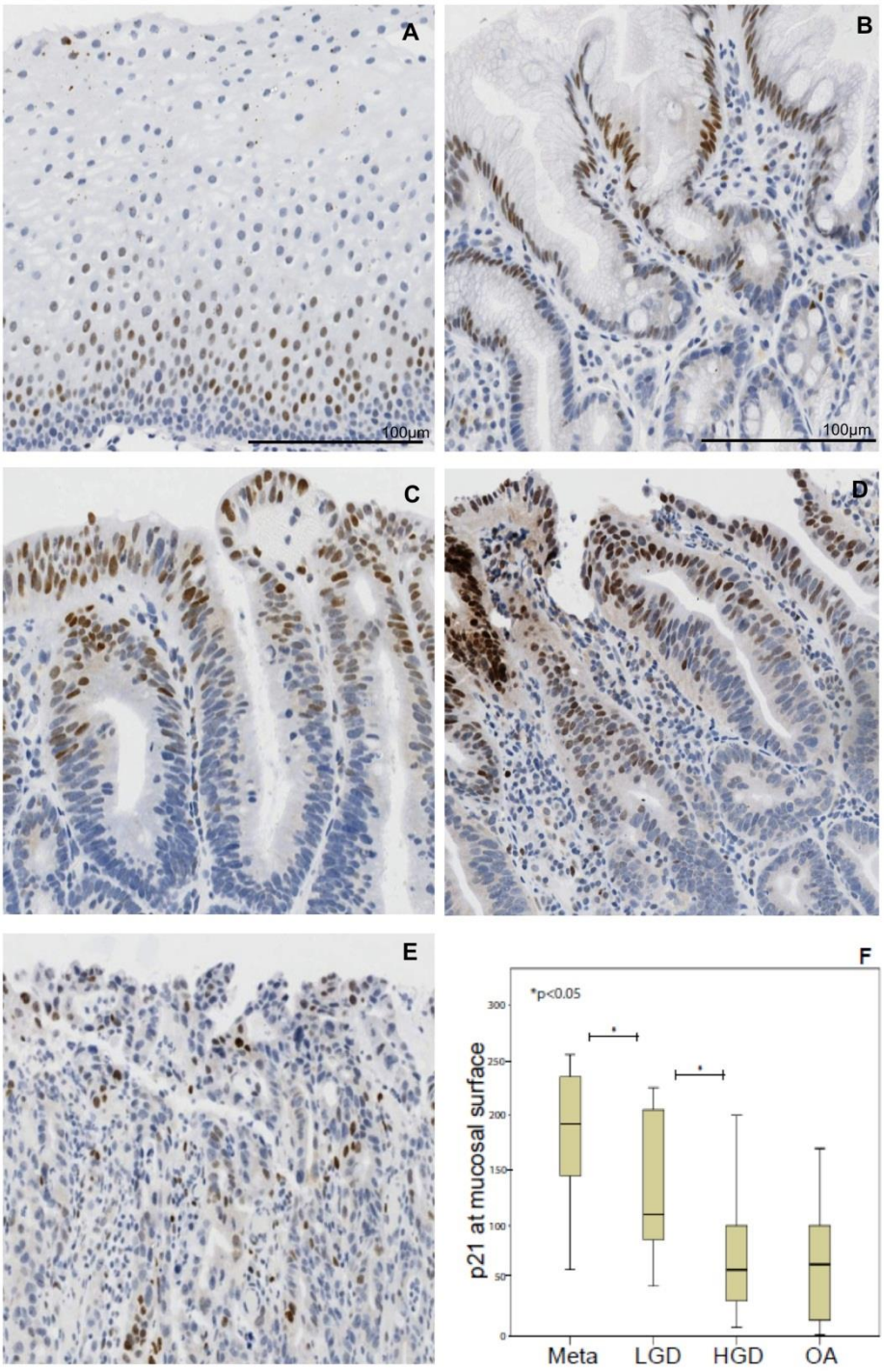
p21 is a tumour suppressor gene closely linked with p53 in controlling the cell cycle. In the squamous epithelium, p21 expression was limited to suprabasal cells with no surface expression (Figure 9.4A). In Barrett's metaplasia, p21 expression was strongly associated with the mucosal surface, with extension of expression to the middle and deeper zones in dysplasia (Figure 9.4B-D). There was a trend towards decreased surface p21 staining from metaplasia to cancer (Figure 9.4E-F). It would appear that a reciprocal relationship exists between p53 and p21: p53 staining increases with disease progression, while p21 staining decreases. Mutational inactivation of p53 is associated with its increased expression and failure to activate target genes, such as p21 (Goh, Coffill, & Lane 2011). Therefore these results are consistent with previous published reports associating mutational inactivation of p53 with progression of Barrett's metaplasia to adenocarcinoma (Reid, Prevo, Galipeau, Sanchez, Longton, Levine, Blount, & Rabinovitch 2001;Woodward, Klingler, Genco et al. 1998).

Figure 9.3 p53 expression in squamous tissue and Barrett's oesophagus



(A) Minimal p53 expression in normal squamous tissue, besides occasional positive cells in suprabasal layer. (B) Barrett's mucosa with occasional weakly positive cells in proliferative zones with no staining at the mucosal surface. (C) LGD with focal p53 expression extending to mucosal surface. (D) HGD displaying diffuse p53 expression in all areas and similar expression in cases with adenocarcinoma (E). (F) Boxplot of p53 histoscore by disease stage showing significant difference in expression. Magnification x20

Figure 9.4 p21 expression in squamous tissue, Barrett's metaplasia, dysplasia and adenocarcinoma



(A) Normal squamous epithelium maximally expressed p21 in suprabasal layer. (B) Barrett's metaplasia expressing p21 at the mucosal surface. (C) Low grade dysplasia with p21 expression at mucosal surface, migrating down to lower zones. (D) High grade dysplasia with reduced p21 expression at mucosal surface and in deeper glands. (E) Adenocarcinoma with reduced p21 expression at mucosal surface. (F) Boxplot quantitating expression of p21 at mucosal surface by disease stage.

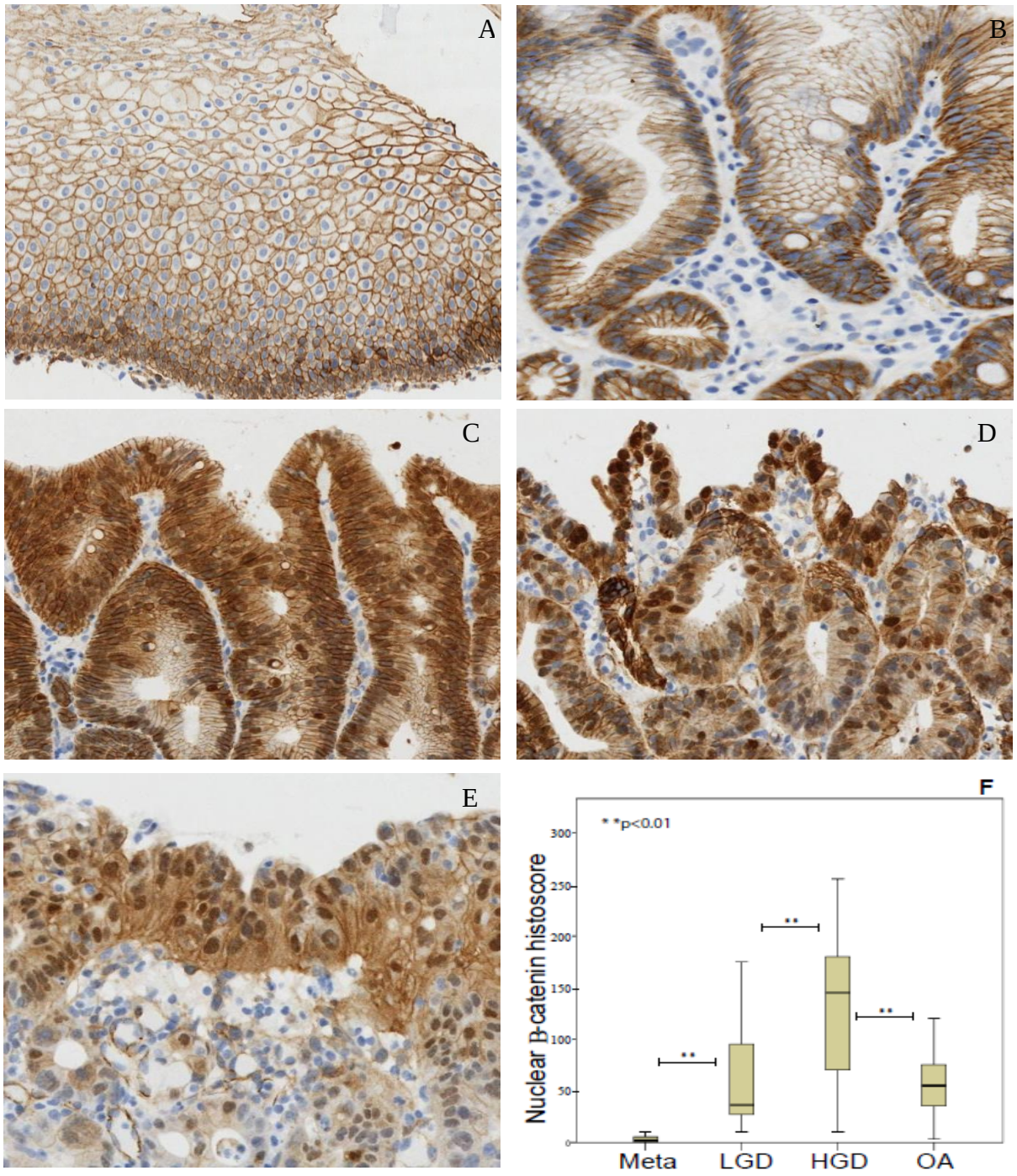
9.4.3 Expression of β-catenin in Barrett's oesophagus

On the basis of these findings, an attempt was made to identify other markers which were associated with Barrett's metaplasia. Previous studies have suggested a link between the presence of β-catenin and oesophageal adenocarcinoma, but few studies have assessed its role in patients with metaplasia and dysplasia, before the development of adenocarcinoma. Therefore an evaluation of the pattern of expression of β-catenin in Barrett's metaplasia and dysplasia was performed using immunohistochemistry.

Across the series from normal squamous epithelium to adenocarcinoma, three patterns of β-catenin expression were observed. In the normal squamous epithelium, β-catenin expression was predominantly membranous, with some cytoplasmic staining close to the basement membrane, and no definite nuclear accumulation (Figure 9.5A). A similar pattern was also found in Barrett's metaplasia (Figure 9.5B). However, increased cytoplasmic β-catenin was observed in LGD with some cells in focal dysplastic glands expressing nuclear β-catenin. Nuclear expression was most abundant in dysplasia, being present in 44% of LGD cases and 93% of HGD cases. Interestingly, nuclear β-catenin accumulation was most marked in HGD even compared with some cases of adenocarcinoma. The median nuclear β-catenin histoscore for Barrett's metaplasia was 2 (interquartile range [IQR] 0-5), compared with 36 (IQR 25-96) in LGD, 145 (IQR 60-180) in HGD and 55 (IQR 35-77) in adenocarcinoma. Some cases of adenocarcinoma expressed high levels of nuclear β-catenin, whereas others did not. The increase in cytoplasmic and nuclear β-catenin was associated with a marked reduction in membranous expression from metaplasia to carcinoma (Figure 9.5B-F).

β-catenin is a key player in the Wnt signalling pathway, and nuclear expression equates with activation of canonical Wnt signalling. Therefore it appears that based on β-catenin localisation, Wnt signalling is markedly upregulated in dysplasia, particularly HGD, compared with earlier stages of disease and the normal squamous epithelium.

Figure 9.5 β-catenin expression and localisation in Barrett's oesophagus



(A) Normal squamous epithelium expressing membranous β-catenin with minimal cytoplasmic and nuclear staining. (B) Barrett's metaplasia expressing membranous β-catenin with no nuclear staining. (C) LGD with focal areas of strong nuclear and cytoplasmic β-catenin staining. (D) HGD with obvious nuclear β-catenin expression and a marked reduction in membranous expression. (E) Oesophageal adenocarcinoma with focal areas of nuclear localisation and minimal membranous staining, while other areas show an overall reduction in β-catenin expression. (F) Boxplot quantitating nuclear β-catenin histoscore by disease stage. Magnification x20.

9.4.4 Expression of Wnt target genes

In order to understand the role of Wnt signalling in Barrett's metaplasia and disease progression, the expression of a panel of Wnt target genes was analysed. Since canonical Wnt signalling often promotes cell proliferation, Ki67 a marker of cell proliferation was initially stained.

In the normal squamous oesophagus, Ki67 was maximally expressed in the nuclei of suprabasal keratinocytes (Figure 9.6A). In Barrett's metaplasia, Ki67 was maximally expressed in a restricted mid-zone of proliferating cells (Figure 9.6B). In the presence of dysplasia, the proportion of Ki67-positive cells increased and the proliferative zone extended onto the mucosal surface (Figure 9.6C -D). Overall, Ki67 expression increased with progression from metaplasia to HGD and cancer (Figure 9.6B-F) with the highest proliferation scores were seen in HGD. The areas of increased nuclear β-catenin coincided with maximal areas of Ki67.

Cyclin D_1, a cell cycle proto-oncogene and Wnt target gene, was expressed in the suprabasal proliferative layer of the oesophageal squamous epithelium and in the proliferative zone in Barrett's metaplasia (Figure 9.7A-B). With increasing degrees of dysplasia, cyclin D_1 expression moved away from the middle proliferative zone to the mucosal surface (Figure 9.7C-D, F). The superficial cyclin D_1 expression in LGD and HGD recalls the expression of Ki67, and was associated with areas of nuclear β-catenin.

Sox9, an intestinal marker and target gene involved in the Wnt pathway, showed a similar pattern of staining to nuclear β-catenin, cyclin D1 and Ki67, with increased expression in HGD (Figure 9.8). There was a similar movement towards mucosal surface expression in LGD and HGD. Interestingly, Sox9 was also present in the normal squamous epithelium although it is most often seen in intestinal mucosae.

There was weakly detectable c-myc expression in the basal and suprabasal nuclei of the oesophageal squamous epithelium (Figure 9.9A). Barrett's metaplasia and LGD displayed weak heterogenous nuclear staining in some glands whereas other cases had no identifiable staining (Figure 9.9B-C). HGD was associated with scattered and mostly superficial areas of intense nuclear c-myc, with widespread staining found in adenocarcinoma (Figure 9.9D-E). Overall, c-myc expression was higher in dysplastic Barrett's epithelium than metaplasia (Figure 9.9F).

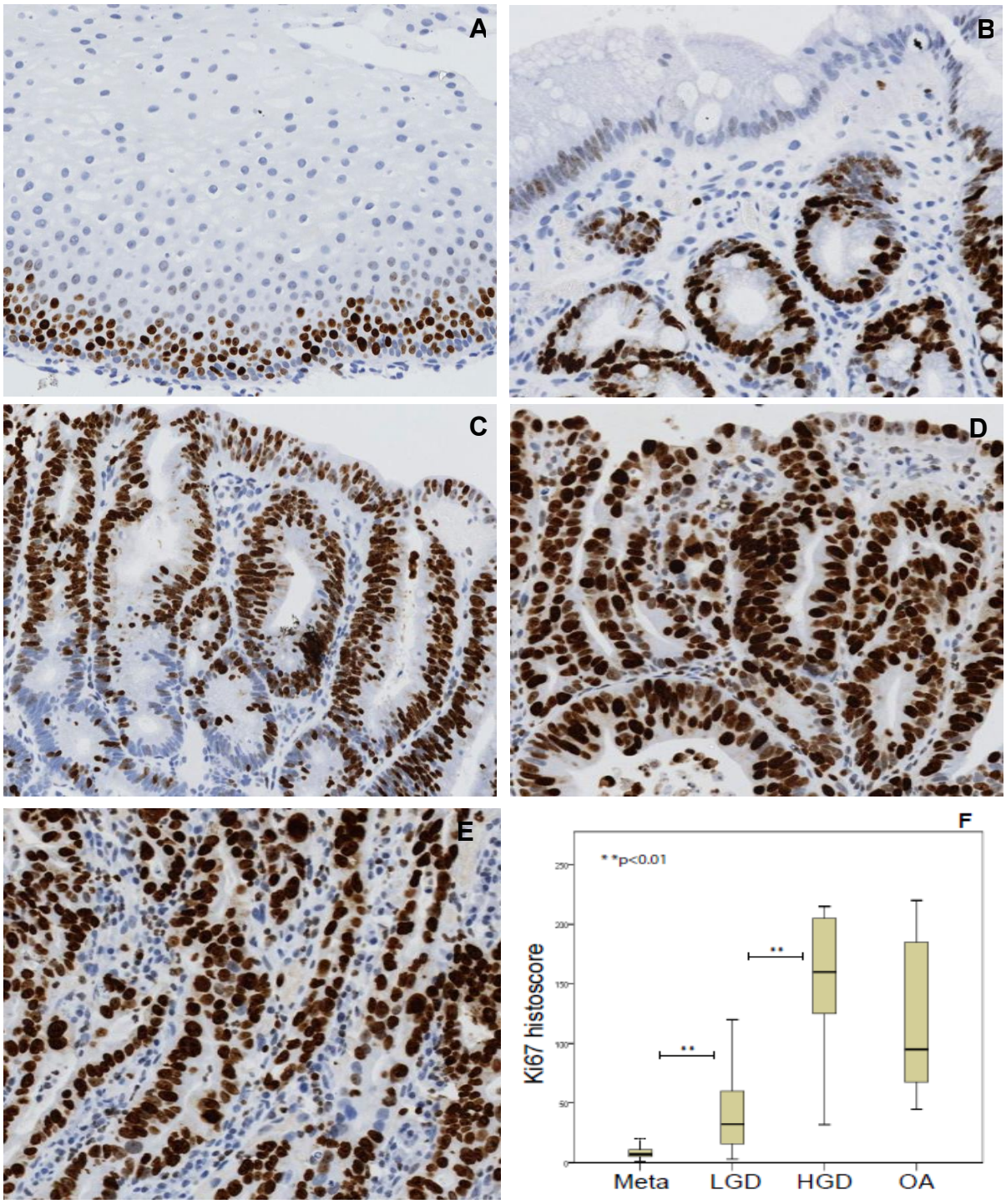
The expression of nuclear β-catenin and Wnt target genes increased with progressive dysplasia (particularly the transition from low grade to high grade dysplasia). These are summarised in Table 9.3 and displayed in the boxplots in each figure.

Table 9.3 p values for Wnt biomarkers in human oesophageal tissues

	Metaplasia vs LGD	LGD vs HGD	HGD vs Cancer
p53	<0.001	0.001	**0.142**
p21	0.006	0.050	**0.153**
B catenin	<0.001	0.001	**<0.001**
Cyclin D1	0.786	<0.001	**0.001**
Sox9	<0.001	<0.001	**0.393**
c-myc	0.955	0.020	**0.009**
Ki67	0.002	<0.001	**0.135**

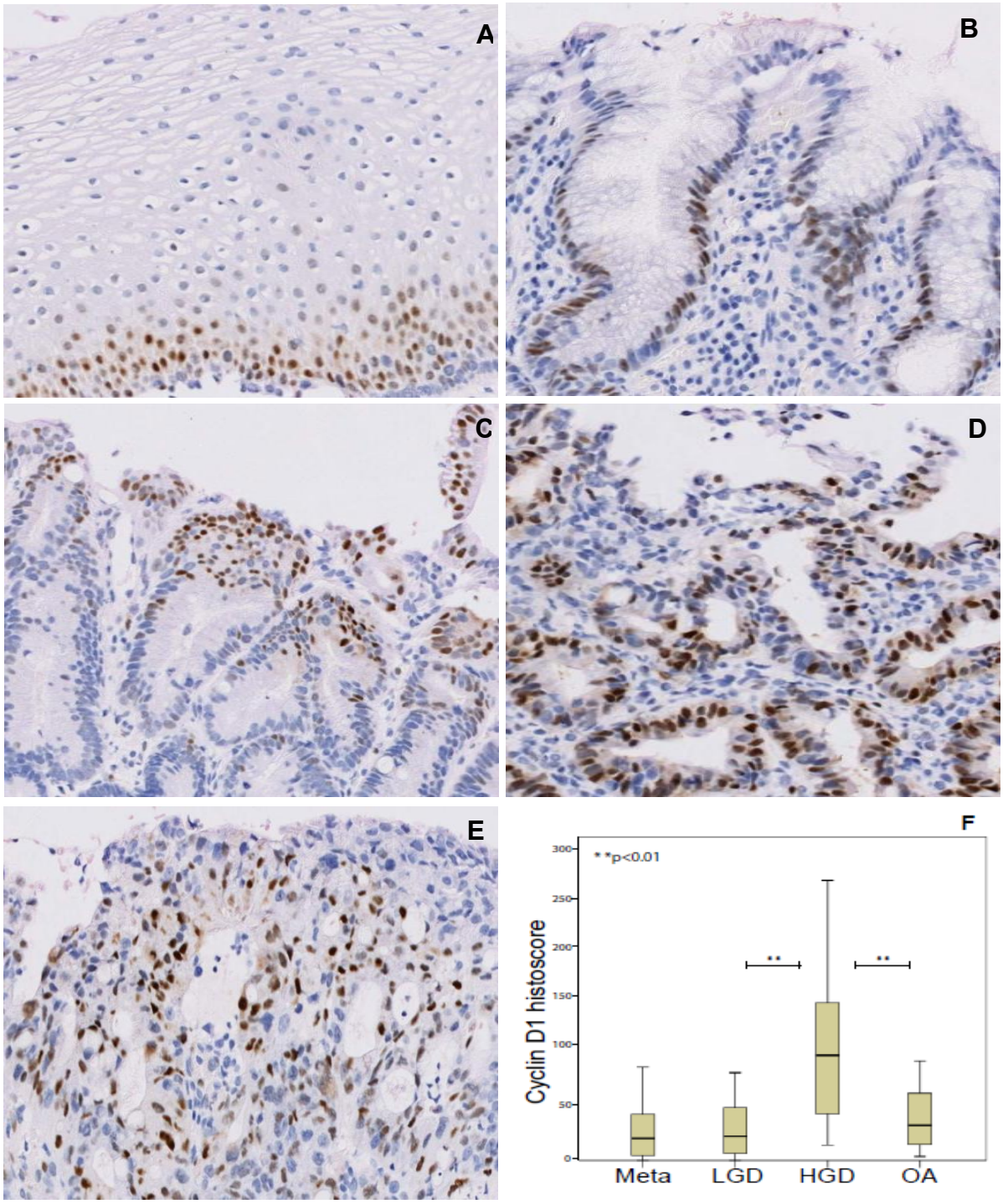
Difference in expression of biomarkers between metaplasia, LGD, HGD and adenocarcinoma. p value <0.05 significant (Chi square test)

Figure 9.6 Ki67 expression in squamous tissue and Barrett's oesophagus



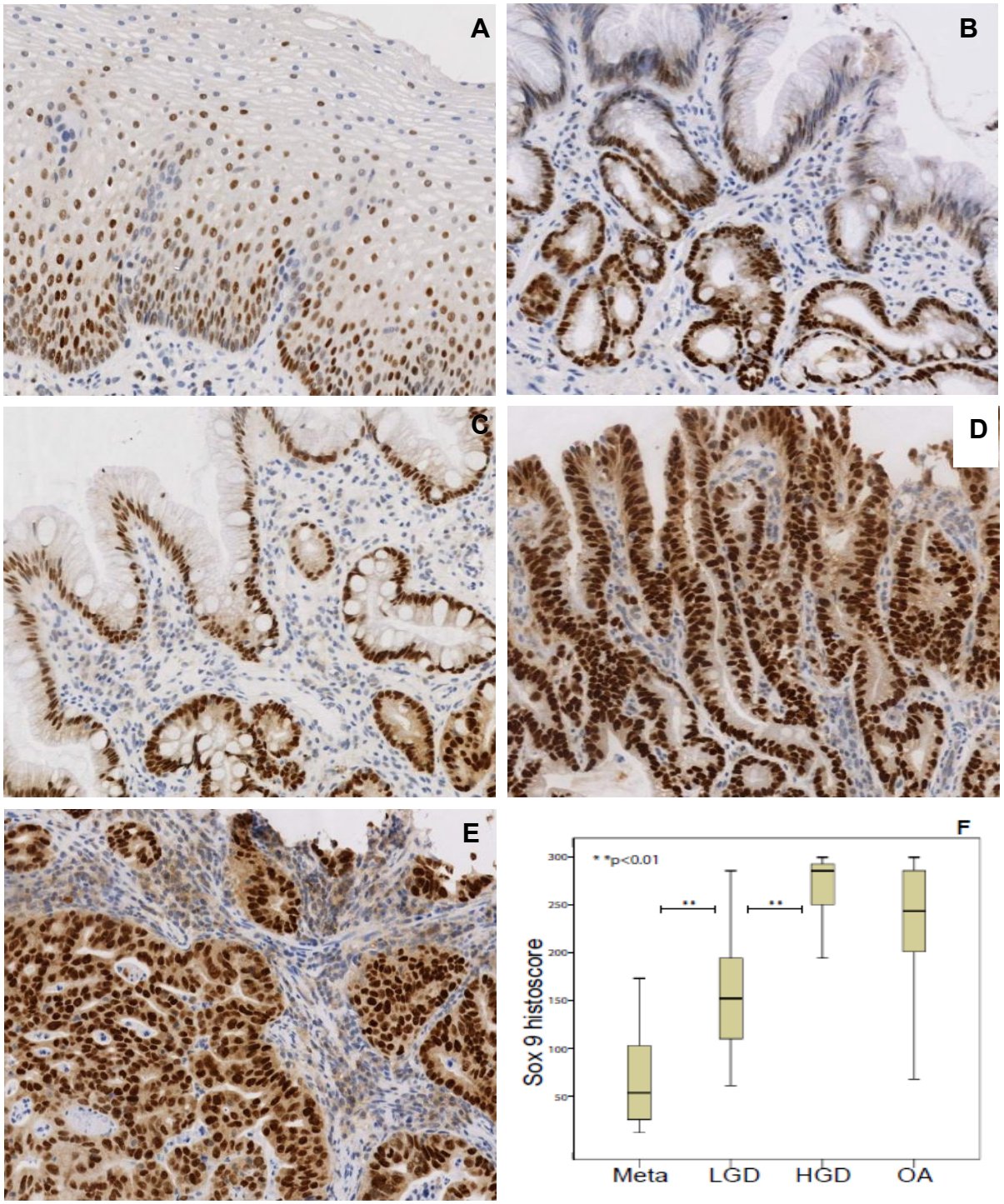
(A) Normal squamous epithelium with Ki67 positive nuclei in the suprabasal layers. (B) Barrett's metaplasia showing Ki67 expression in cells in the lower and middle crypts, with minimal expression on the mucosal surface. (C) LGD illustrating an increase in mucosal expression of Ki67. The extension of Ki67 positive cells onto the mucosal surface is highly characteristic of LGD. (D) HGD showing Ki67 expression throughout the mucosal epithelium. (E) Adenocarcinoma showing Ki67 expression throughout the tissue. Some sections had little Ki67 expression, particularly when necrosis was present. (F) Boxplot quantitation Ki67 expression by disease stage. Magnification x20.

Figure 9.7 Cyclin D1 expression in squamous oesophagus and Barrett's oesophagus



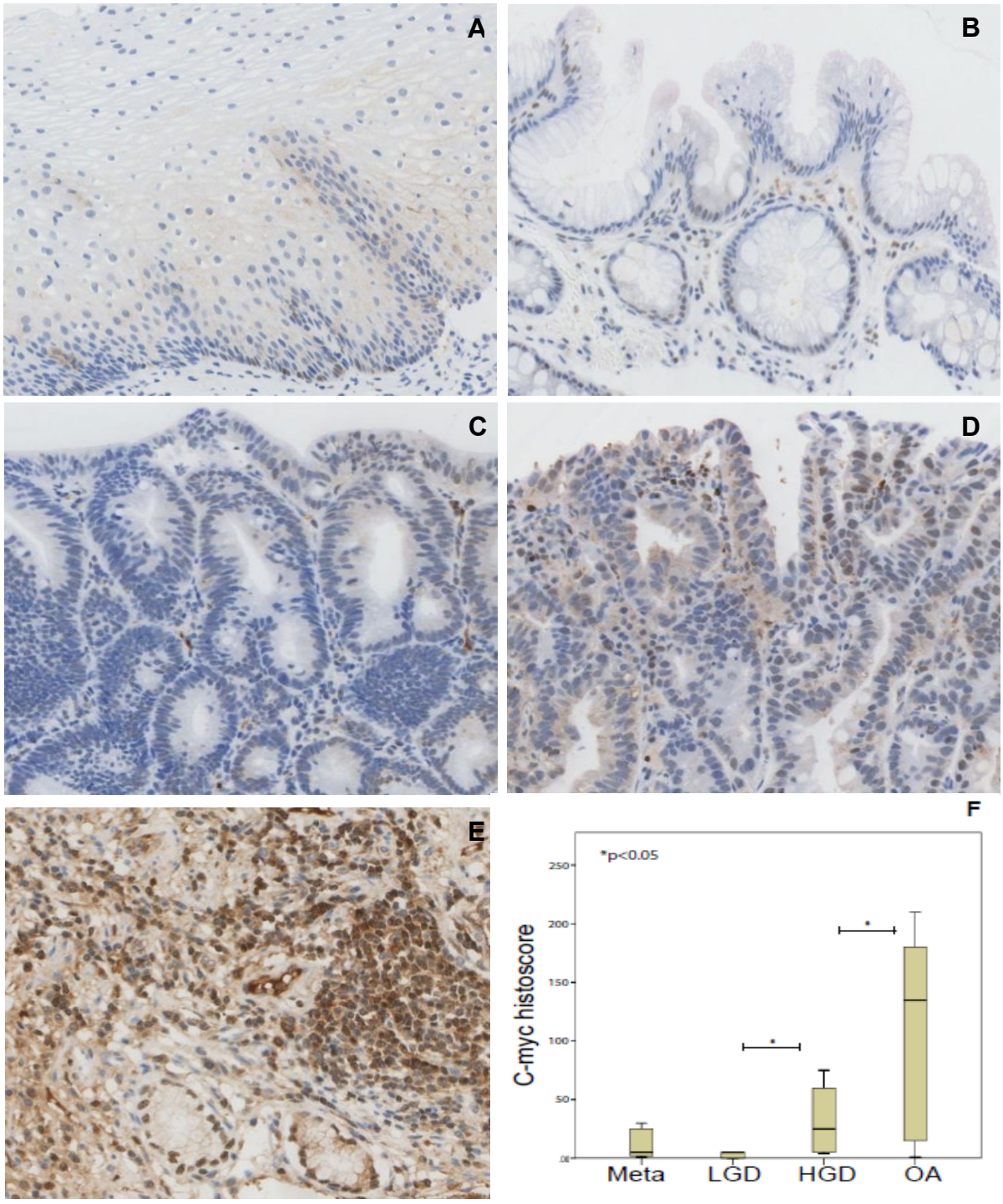
(A) Normal squamous epithelium showing cyclin D_1 positive cells in the proliferative suprabasal layer in a similar pattern as Ki67. (B) Cyclin D_1 expressed in the proliferative zone of Barrett's metaplasia. (C) Increased expression of cyclin D_1 in cells of the mucosal surface in LGD. (D) High levels of cyclin D_1 in HGD including at the mucosal surface and in the deeper proliferation zone. (E) Cyclin D_1 expression remains elevated in adenocarcinoma. (F) Boxplot quantitating cyclin D_1 expression by disease stage. Magnification x20.

Figure 9.8 Sox9 expression in squamous and Barrett's oesophagus



(A) Sox9 expression present in the suprabasal layer of the squamous epithelium. (B) In Barrett's metaplasia Sox9 was most highly expressed in the proliferation zone, resembling Ki67 and cyclin D_1 expression. (C) In LGD, Sox9 was upregulated with increased expression on the mucosal surface. (D) High levels of Sox9 expression in HGD. (E) High levels of Sox9 expression in adenocarcinoma. (F) Boxplot quantitating Sox9 expression by disease stage. Magnification x20.

Figure 9.9 C-myc expression in squamous oesophagus and Barrett's oesophagus



(A) Minimal c-myc expression in the basal layer of squamous oesophageal mucosa. (B) Occasional cells staining for c-myc in some glands, but no expression visible at the mucosal surface. (C) In LGD, c-myc expression is present in some dysplastic cells on the surface epithelium. (D) Focal areas of c-myc expression found in HGD. (E) Widespread c-myc expression in oesophageal adenocarcinoma. (F) Boxplot quantitating c-myc expression by disease stage. Magnification x20.

9.5 Discussion

This study presents data supporting the hypothesis that upregulation of canonical Wnt signalling is associated with the neoplastic progression of Barrett's oesophagus. Cell proliferation, as measured by Ki67 staining showed a similar pattern, consistent with the notion that activated Wnt signalling contributes, at least in part, to dysregulated cell proliferation and dysplasia.

It is generally accepted that the progression of Barrett's oesophagus to dysplasia and carcinoma is associated with increasing p53 expression, mainly due to the accumulation of mutant p53 (Moskaluk, Heitmiller, Zahurak et al. 1996;Prasad, Bansal, Sharma, & Wang 2010;Reid, Prevo, Galipeau, Sanchez, Longton, Levine, Blount, & Rabinovitch 2001;Woodward, Klingler, Genco, & Wolfe 1998). The results of this study reflect this phenomenon, especially in HGD and OA. It seems that p53 inactivation may be a later event in the metaplasia-dysplasia sequence, instead of being a key factor in the initial metaplastic transformation from a squamous to glandular mucosa.

There was an interesting reciprocal relationship between p53 and p21 expression. With increasing dysplasia, p21 expression reduced and was associated in a shift from a mucosal expression in metaplasia to generalised expression in adenocarcinoma.

This paper has shown activation of canonical Wnt signalling, reflected by increased cytoplasmic and nuclear β-catenin and increased expression of Wnt target genes, is associated with progression from Barrett's metaplasia to dysplasia and oesophageal adenocarcinoma. Previous reports have linked Wnt activation to progression of Barrett's metaplasia to adenocarcinoma (Clement, Jablons, & Benhattar 2007;Clevers 2006)' (Bian, Osterheld, Bosman et al. 2000). However, these studies assessed β-catenin in patients with established adenocarcinoma and associated dysplasia. In contrast, this paper studied patients with Barrett's metaplasia and dysplasia who have not progressed to adenocarcinoma. Therefore, in terms of the ultimate goal of early prognostication and risk assessment, the most relevant patient population was analysed in this study. By focusing on dysplasia associated with adenocarcinoma, previous studies might have selected an unrepresentative subset of samples. Moreover, the conclusions from this study are drawn from an analysis of both β-catenin localization and expression of a panel of Wnt target genes, whereas previous studies examined only β-catenin localization. This increases confidence in the

conclusions regarding the status of Wnt signalling activity in the tissues. Finally, while Wnt activation has been previously broadly linked to progression of Barrett's metaplasia and adenocarcinoma (Bian, Osterheld, Bosman, Fontolliet, & Benhattar 2000;Clement, Braunschweig, Pasquier et al. 2006), we specifically found that activation of Wnt signalling is most strongly associated with the transition to HGD.

Wnt signalling may be activated by overexpression of Wnt ligands, downregulation of Wnt antagonists and/or mutation of key downstream signalling components, e.g. β-catenin, APC or axin. Unlike colon cancer, Barrett's oesophagus and adenocarcinoma are not associated with frequent mutation of the APC, β-catenin or axin genes (Choi, Heath, Heitmiller et al. 2000;Clement, Braunschweig, Pasquier, Bosman, & Benhattar 2006;Koppert, van der Velden, van der Wetering et al. 2004). However, Clement *et al* showed upregulation of ligand Wnt2 in dysplasia and adenocarcinoma (Clement, Braunschweig, Pasquier, Bosman, & Benhattar 2006). Also, Wnt antagonists, secreted frizzled receptor proteins (SFRPs) and Wnt inhibitory factor 1 (WIF-1) have been shown to be hypermethylated and silenced in cases of Barrett's metaplasia (as well as adenocarcinoma) (Clement, Braunschweig, Pasquier, Bosman, & Benhattar 2006;Zou, Molina, Harrington et al. 2005). Together, these results suggest that activation of Wnt signalling in Barrett's-associated dysplasia and adenocarcinoma is frequently achieved by upregulation and downregulation of Wnt ligands and antagonists (Clement, Guilleret, He et al. 2008). Although there was no marked activation of Wnt signalling in Barrett's metaplasia without dysplasia, epigenetic silencing of Wnt antagonists reported in some studies might yield low level activation of Wnt signalling and promote progression to dysplasia. Given the association between acid and bile reflux and Barrett's (Shaheen & Richter 2009), altered Wnt signalling presumably initially stems, directly or indirectly, from this reflux.

This study has shown that Wnt targets, Cyclin D_1, c-myc and Sox9, are upregulated especially in HGD. Bani-Hani *et al* previously found increased levels of cyclin D_1 expression in Barrett's oesophagus and suggested this is associated with higher risk of progression to adenocarcinoma (Bani-Hani, Martin, Hardie, Mapstone, Briggs, Forman, & Wild 2000). Consistent with this role in cell proliferation (Sherr 2000), there was good concordance between cyclin D_1 expression and cell proliferation, indicated by Ki67, in Barrett's metaplasia with and without dysplasia and adenocarcinoma. In normal squamous epithelium, cyclin D_1 and Ki67 were both

maximally expressed in cells lying in the proliferative transit-amplifying zone immediately above the basement membrane. Both cyclin D_1 and Ki67 increased in expression along the metaplasia-carcinoma sequence, with peak expression observed in HGD.

C-myc is a pleiotropic proto-oncogene broadly implicated in a variety of cancers, including gastric, colon, breast and lung cancer (Pelengaris, Khan, & Evan 2002). Consistent with results presented here, increased c-myc expression has previously been shown in adenocarcinoma, compared with normal squamous epithelium (Schmidt, Meurer, Volkweis et al. 2007;Stairs, Nakagawa, Klein-Szanto et al. 2008). However, there is a paucity of data in the literature regarding expression of c-myc in Barrett's oesophagus. Here I have shown that, like other Wnt targets, c-myc protein expression is already upregulated in HGD.

Recent work by Blache *et al* has shown that Sox9 is a downstream β-catenin target gene, playing a role in the normal development of mouse intestine (Foster, Dominguez-Steglich, Guioli et al. 1994)' (Blache, van de Wetering, Duluc, Domon, Berta, Freund, Clevers, & Jay 2004). In human tissues, we found that Sox9 was upregulated with disease progression, but with a tendency to highest expression in HGD. Underscoring the link between Sox9 and dysplasia, in the mouse model described in the previous chapter, Sox9 is not expressed in the normal mouse squamous oesophageal epithelium, but is expressed in the immature quasi-dysplastic cells harbouring nuclear β-catenin.

A lower level of canonical Wnt signalling is one determinant of the anterior gut (oesophagus and stomach), compared to the posterior gut (small intestine and colon), where the importance of activated Wnt signalling is well-established (Gregorieff, Grosschedt, & Clevers 2004). Thus, it has been reasonable to hypothesize that abnormal activation of Wnt signalling in the anterior gut contributes to Barrett's metaplasia (together with other factors, such as exposure to excess acid and bile). While Wnt-induced expression of Sox9, CK8 and CLDN3 in the mouse oesophagus supports this notion to some extent, on balance data indicate that activated Wnt is not a key driver of metaplasia in either human or mouse. Instead, these results support a frequent and causative role for activation of Wnt signalling in oesophageal dysplasia.

9.6 Conclusion

In conclusion, the results from this study link activated Wnt signalling to HGD, and to a lesser extent to LGD. This raises the important possibility that the subset of patients with LGD and activated Wnt signalling carry a higher risk of progression to HGD and subsequent adenocarcinoma. Based on these results, it will be important to test the predictive power of activated Wnt signalling in a cohort of patients with LGD.

Chapter 10

Conclusions and Future Directions

The West of Scotland has one of the highest incidences of Barrett's oesophagus and adenocarcinoma in the world. Barrett's oesophagus is a common premalignant condition which has the potential to be diagnosed and treated at an early stage before invasive cancer develops.

This thesis has highlighted the important clinical issues surrounding Barrett's oesophagus and has focused on specific topics such as the clinical identification of dysplasia and risk of malignant progression, reinforcing the high risk nature of the Scottish Barrett's population and furthering the knowledge of underlying molecular abnormalities, in particular the Wnt signalling pathway.

The first hypothesis was proved to be true – patients with Barrett's oesophagus in the West of Scotland have high rates of progression to high grade dysplasia and oesophageal adenocarcinoma. The second hypothesis was disproved – the WavSTAT optical biopsy system was unable to correctly identify patients with Barrett's oesophagus or indeed dysplasia. Finally, I hypothesised the Wnt signalling pathway would be upregulated in patients with Barrett's oesophagus, however this was only partly proved. Wnt signalling is upregulated in patients with dysplasia, particularly high grade, but this thesis has failed to demonstrate upregulation of the Wnt pathway in metaplasia.

10.1 Molecular mechanisms

Despite being the focus of research for many years, the mechanisms underlying the initial metaplastic transformation of the oesophagus, from a squamous to a columnar mucosa remain unclear. The Wnt signalling pathway is known to play a crucial role in embryogenesis and the normal development of the gastrointestinal tract but its role in the progression of Barrett's metaplasia, dysplasia and adenocarcinoma has not been extensively investigated. This thesis presents the first mouse model to assess the role of Wnt signalling in mouse oesophagus. Although no phenotypic changes from a squamous to a columnar mucosa were noted, there was upregulation of expression of Wnt target genes in areas of dysplasia. One of the limitations of the Ah-Cre-ER β-catenin$^{\Delta ex3}$ mouse model related to the limited survival within

homozygous mice. These mice succumbed to other diseases, possibly before any overt phenotypic changes were noted. However, this mouse model has demonstrated that upregulated Wnt signalling contributes to abnormal maturation of cells within the epithelium and further mouse work should be carried out to further this work.

The second phase of the Wnt study assessed the role of Wnt signalling in human oesophageal biopsy tissue. Wnt signalling, as demonstrated by the presence of nuclear β catenin and downstream target genes, was upregulated in patients with dysplasia, particularly HGD but also noted in some cases of LGD. This study suggests that although Wnt signalling did not seem to play a major role in the initial metaplastic transformation of the squamous oesophagus, Wnt signalling is involved in the malignant progression of Barrett's oesophagus.

These results are novel and exciting but in order to understand the role of Wnt signalling and assess its use as a clinical biomarker of disease progression, further studies are required. Firstly, the results should be corroborated in a larger scale study. Ideally this would be a prospective study, but in the first instance a retrospective cohort with follow up data may be the most appropriate means of assessing Wnt signalling as a marker for progression. A tissue microarray of oesophageal tissue should be constructed providing more tissue for histological assessment. Perhaps the most interesting group of patients are those with low grade dysplasia, as some patients progress to high grade dysplasia while others do not. In an ideal world, a biomarker should be able to predict those with metaplasia at risk of progression, but in the meantime, if we were able to predict those with low grade dysplasia who are at risk of progression, these patients could be offered intensive surveillance, or endoscopic treatments such as RFA to eradicate the "at risk" epithelium. The ideal biomarker for Barrett's oesophagus will not appear overnight, but the Wnt pathway would be an interesting one to explore. Therefore, a tissue microarray of all low grade patients with Barrett's oesophagus in Glasgow is under construction to compare the expression of Wnt target genes in those who progress and those who do not progress to HGD or cancer.

This study has largely been an observational study using immunohistochemistry as a means of assessing Wnt expression. However there are various novel gene technologies available, and one method would be gene profiling of Barrett's tissue using next generation sequencing technology. This technology is in its infancy, but

would allow researchers to create a genetic profile in patients with Barrett's oesophagus, comparing any differences in those with metaplasia alone, versus those who progress.

The importance of understanding the molecular abnormalities associated with Barrett's oesophagus cannot be overemphasised. Effective and safe endoscopic therapies are now available, particularly for those with known dysplasia, and I would predict that in the future the key to the management of Barrett's oesophagus, and the ultimate reduction in the incidence of dysplasia and adenocarcinoma lie in the accurate identification of patients at risk of progression, and offering them early treatment. Furthermore, patients at no risk of progression could be reassured and discharged for surveillance.

10.2 Scotland is an "at risk" population

Within my cohort of patients with Barrett's oesophagus in Glasgow, 1 in 10 patients died from Barrett's associated oesophageal cancer. This is much higher than reported rates in the literature. Mortality from all-cause and cancer-specific deaths is higher in patients with Barrett's oesophagus living in deprived areas, with patients four times more likely to die than those in affluent areas. The annual risk of progression to HGD and adenocarcinoma were 0.2% and 0.4% respectively, rates which are similar to those quoted in recent European studies (0.2-0.4%) (de Jonge, van Blankenstein, Looman, Casparie, Meijer, & Kuipers 2010;Hvid-Jensen, Pedersen, Drewes, Sorensen, & Funch-Jensen 2011). However, patients with LGD at baseline endoscopy have greater risk of progression to HGD and neoplasia, with 40% patients progressing to HGD/adenocarcinoma within 10 years. The West of Scotland is clearly an "at risk" population and deprivation may play a role in disease progression, along with other environmental and genetic factors.

This study highlights the premalignant nature of Barrett's oesophagus in our population with two particularly important risk factors for disease progression – low grade dysplasia and deprivation. The presence of LGD is a clear risk factor to progression, and research to determine factors of progression, and improvement in the diagnosis and treatment of LGD is warranted. Barrett's oesophagus, and particularly dysplasia can be treated with endoscopic intervention (endoscopic resection and radiofrequency ablation) and specialist centres now have access to excellent endoscopic facilities allowing appropriate and timely intervention.

Therefore, we must not underestimate the premalignant nature of Barrett's oesophagus in the West of Scotland.

In order to tease out individual factors which may account for this high risk population and the role of deprivation, further work is required. A prospective database of all patients diagnosed with Barrett's oesophagus in the West of Scotland should be introduced. This will require careful planning of personnel and resources including a dedicated IT support team, administrators to ensure accurate data collection and clinicians and pathologists capable of performing surveillance, and reporting the histological findings. Prospective data collection of patient, endoscopic, histological and molecular factors would lead to an improved understanding of the disease. The database would also be an excellent audit resource assessing the efficacy of endoscopic therapies and outcome measures, and resource planning.

10.3 Improving the diagnosis of dysplasia

Dysplasia remains the current gold standard marker of disease progression, yet it carries its own difficulties. Improvements in high resolution endoscopes and image adjuncts such as NBI have improved the clinical assessment of Barrett's mucosa and areas of dysplasia or intramucosal cancer but histology is still necessary for the definitive diagnosis of dysplasia. The optical biopsy forceps (WavSTAT® Optical Biopsy system) designed by SpectraScience is an exciting and novel technology. The preliminary results of the first algorithm reported in this thesis are disappointing with no correlation between the optical and physical biopsy result. However a revised algorithm is underway by SpectraScience and the results are awaited with interest. The technology has the potential to allow the clinician to diagnose the presence of dysplasia depending on the autofluorescence of the oesophageal mucosa and may allow targeted biopsies of the dysplastic area.

One limitation of the current optical biopsy system is the small surface area which can be sampled in one reading, but this system could have a particular role in assessing the residual mucosa of patients post endoscopic therapies. Any suspect areas could then be effectively targeted, reducing the overall time and discomfort of the procedure, and reducing the workload in the histopathology laboratory. As a general surveillance tool, the optical biopsy system is limited and trimodal imaging modalities may be more successful.

Capsule sponge cytology is a newer technique in screening patients with Barrett's oesophagus, eliminating the need for endoscopic assessment. The attraction of this technique is that it can be performed in an outpatient setting, is associated with minimal patient discomfort, yet provides the pathologist with adequate tissue which can be analysed by cytology. At present, a study assessing the efficacy of the capsule sponge in obtaining cytology from Barrett's patients, and its ability in diagnosing the presence of dysplasia is underway in Glasgow. If the results are promising, this technique has the potential to replace current surveillance endoscopies in patients with metaplasia.

10.4 Conclusion

Barrett's oesophagus is a condition which continues to baffle clinicians, but further research must continue to ensure optimal treatment (or reassurance) for all patients in the West of Scotland. This thesis has highlighted the importance of a comprehensive surveillance programme in our "high risk" population - an ideal niche for future chemopreventative and molecular studies.

References

Cancer Research UK. 1-1-2008.
Ref Type: Online Source

Abela, J.E., Going, J.J., Mackenzie, J.F., McKernan, M., O'Mahoney, S., & Stuart, R.C. 2008. Systematic four-quadrant biopsy detects Barrett's dysplasia in more patients than nonsystematic biopsy. *American Journal of Gastroenterology*, 103, 850-855

Adams, P.D. & Enders, G.H. 2008. Wnt-signaling and senescence. *Cancer Biology and Therapy*, 7, (11) 1706-1711

Adler, R.H. 1963. The lower esophagus lined by columar epithelium: its association with hiatal hernia, ulcer, stricture and tumour. *Journal of Thoracic and Cardiovascular Surgery*, 45, 13

Alikhan, M., Rex, D., Khan, A., Rahmani, E., Cummings, O., & Ulbright, T.M. 1999. Variable pathologic interpretation of columnar lined esophagus by general pathologists in community practice. *Gastrointestinal Endoscopy*, 50, 23-26

Allison, P.R. & Johnstone, A.S. 1953. The oesophagus lined with gastric mucous membrane. *Thorax*, 8, 87-101

Anandasabapathy, S., Jhamb, J., & Davila, M. 2007. Clinical and endoscopic factors predict higher pathologic grades of Barrett dysplasia. *Cancer*, 109, 668-674

Armstrong, R.A., Blalock, J.B., & Carrera, G.M. 1959. Adenocarinoma of the middle third of the esophagus arising from ectopic gastric mucosa. *Journal of Thoracic Surgery*, 37, 398

AUGIS, BSG, & NCASP 2010, *National Oesophagogastric Cancer Audit 2010*.

Bailey, T., Biddlestone, L., Shepherd, N.A., Barr, H., Warner, P., & Jankowski, J.A. 1998. Altered cadherin and catenin complexes in the Barrett's esophagus-dysplasia-adenocarcinoma sequence. *American Journal of Pathology*, 152, (1) 135-144

Bani-Hani, K., Martin, I.G., Hardie, L.J., Mapstone, N., Briggs, J.A., Forman, D., & Wild, C.P. 2000. Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *Journal of National Cancer Institute*, 92, (16) 1316-1321

Barrett, M.T., Sanchez, C.A., Prevo, L.J., Wong, D.J., Galipeau, P.C., Paulson, T.G., Rabinovitch, P.S., & Reid, B.J. 1999. Evolution of neoplastic cell lineages in Barrett oesophagus. *Nature Genetics*, 22, (1) 106-109

Barrett, N.R. 1950. Chronic peptic ulcer of the oesophagus and "oesophagitis". *British Journal of Surgery*, 38, 172-182

Barrett, N.R. 1957. The lower esophagus lined by columnar epithelium. *Surgery*, 41, (6) 881-894

Berenson, M.M., Riddell, R.H., & Skinner, D.B. 1978. Malignant transformation of esophaeal columnar epithelium. *Cancer*, 41, 554-561

Bergmann, J.J. & Tytgat, G.N.J. 2005. New developments in the endoscopic surveillance of Barrett's oesophagus. *Gut*, 54, (Supplement 1) i34-i42

Bian, Y., Osterheld, M., Bosman, F.T., Fontolliet, C., & Benhattar, J. 2000. Nuclear accumulation of beta-Catenin is a common and early event during neoplastic progression of Barrett esophagus. *American Journal of Clinical Pathology*, 114, 583-590

Bird-Leiberman, E.L., Dunn, J.M., Coleman, H.G., Lao-Sirieix, P., Oukrif, D., Moore, C.E., Varghese, S., Johnston, B.T., Arthur, K., McManus, D.T., Novelli, M.R., O'Donovan, M., Cardwell, C.R., Lovat, L.B., Murray, L.J., & Fitzgerald, R.C. 2012. Population-based study reveals new risk-stratification biomarker panel for Barrett's oesophagus. *Gastroenterology*, 143, (4) 927-935

Bird-Leiberman, E.L., Neves, A.A., Lao-Sirieix, P., O'Donovan, M., Novelli, M.R., Lovat, L.B., Eng, W.S., Mahal, L.K., Brindle, K.M., & Fitzgerald, R.C. 2012. Molecular imaging using fluorescent lectins permits rapid endoscopic identification of dysplasia in Barrett's esophagus. *Nature Medicine*, 18, (2) 315-321

Blache, P., van de Wetering, M., Duluc, I., Domon, C., Berta, P., Freund, J., Clevers, H., & Jay, P. 2004. SOX9 is an intestine crypt transcription factor, is regulated by the

Wnt pathway, and represses the CDX2 and MUC2 genes. *Journal of Cell Biology*, 166, (1) 37-47

Bollschweiler, E., Wolfgarten, E., Gutschow, C., & Holscher, A.H. 2001. Demographic variations in the rising inidence of esophageal adenocarcinoma in white males. *Cancer*, 92, 549-555

Boyer, J., Laugier, R., Chemali, M., Arpurt, J.P., Boustiere, C., Canard, J.M., Dalbies, P.A., Gay, G., Escourrou, J., Napoleon, B., Palazzo, L., Ponchon, T., Richard-Mollard, B., Sautereau, D., Tucat, G., & Vedrenne, B. 2007. French Society of Digestive Endoscopy SFED guideline: monitoring of patients with Barrett's esophagus. *Endoscopy*, 39, 840-842

Bremner, C.G., Lynch, V.P., & Ellis, F.H. 1970. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. *Surgery*, 68, 209-216

Brewster, D.H., Fraser, L.A., McKinney, P.A., & Black, R.J. 2000. Socioeconomic status and risk of adenocarcinoma of the oesophagus and cancer of the gastric cardia in Scotland. *British Journal of Cancer*, 83, (3) 387-390

Brown, I.S., Whiteman, D.C., & Lauwers, G.Y. 2010. Foveolar type dysplasia in Barrett esophagus. *Modern Pathology*, 23, 834-843

Buttar, N.S., Wang, K.K., & Sebo, T.J. 2012. Extent of high grade dysplasia in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology* (120) 1630-1639

Canto, M.I. 2005. Chromoendoscopy and magnifying endoscopy for Barrett's esophagus. *Surgical Oncology Clinics of North America*, 18, 487-502

Canto, M.I., Setrakian, S., Willis, J., Chak, A., Petras, R., Powe, N.R., & Sivak, M.V.J. 2000. Methylene blue directed biposies improve detection of intestinal metaplasaia and dysplasia in Barrett's oesophagus. *Gastointestinal Endoscopy*, 51, 560-568

Carstairs, V. & Morris, R. Deprivation and Health in Scotland. 1991. Aberdeen, University Press.
Ref Type: Pamphlet

Casson, A.G., Zheng, Z., Evans, S.C., Geldenhuys, L., van Zanten, S.V., Veugelers, P.J., Porter, G.A., & Guernsey, D.L. 2005. Cyclin D1 polymorphism (G870A) nad risk for esophageal adenocarcinoma. *Cancer*, 104, 730-739

Caygill, C.P.J., Watson, A., Reed, P.I., & Hill, M.J. 2003. Characteristics and regional variations of patients with Barrett's oesophagus in the UK. *European Journal of Gastroenterology and Hepatology*, 15, (11) 1217-1222

Chandrasoma, P.T. 1997. Pathophysiology of Barrett's esophagus. *Seminars in Thoracic and Cardiovascular Surgery*, 9, (3) 270-278

Chandrasoma, P.T., Der, R., & Ma, Y. 2000. Histology of the gastro-esophageal junction: an autopsy study. *American Journal of Surgical Pathology*, 24, 402-409

Chandrasoma, P.T., Wijetunge, S., DeMeester, S.R., Ma, Y., Hagen, J.A., Zamis, L., & DeMeester, T.R. 2012. Columnar-lined esophagus without intestinal metaplasia has no proven risk of adenocarcinoma. *American Journal of Surgical Pathology*, 36, (1) 1-7

Chetty, R., Serra, S., & Asa, S.L. 2008. Loss of membrane localization and aberrant nuclear E-cadherin expression correlates with invasion in pancreatic endocrine tumours. *American Journal of Surgical Pathology*, 32, (3) 413-419

Choi, Y.W., Heath, E.I., Heitmiller, R., Forastiere, A.A., & Wu, T.T. 2000. Mutations in beta-catenin and APC genes are uncommon in esophageal and esophagogastric junction adenocarcinomas. *Modern Pathology*, 13, (10) 1055-1059

Clark, G.W., Ireland, A.P., & Chandrasoma, P.T. 1994. Inflammation and metaplasia in the transitional epithelium of the gastroesophageal junction: a new marker for gastroesophageal reflux disease. *Gastroenterology*, 106, A63

Clement, G., Braunschweig, R., Pasquier, N., Bosman, F.T., & Benhattar, J. 2006. Alterations of the Wnt signalling pathway during the neoplastic progression of Barrett's esophagus. *Oncogene*, 25, 3084-3092

Clement, G., Guilleret, I., He, B., Yagui-Beltran, A., Lin, Y., You, L., Xu, Z., Shi, Y., Okamoto, J., Benhattar, J., & Jablons, D.M. 2008. Epigenetic alteration of the Wnt inhibitory factor-1 promoter occurs early in the carcinogenesis of Barrett's esophagus. *Cancer Science*, 99, (1) 46-53

Clement, G., Jablons, D.M., & Benhattar, J. 2007. Targeting the Wnt signaling pathway to treat Barrett's esophagus. *Expert Opinion in Therapy and Targets*, 11, (3) 375-389

Clevers, H. 2006. Wnt/beta-catenin signaling in development and disease. *Cell*, 127, (3) 469-480

Clinical Resource and Audit Group (CRAG). Scottish Audit of Gastric and Oesophageal Cancer. 2005. Edinburgh.
Ref Type: Online Source

Cobb, M.J., Hwang, J.H., Upton, M.P., Chen, Y., Oelschlager, B.K., Wood, D.E., Kimmey, M.B., & Li, X. 2010. Imaging of subsquamous Barrett's epithelium with ultrahigh-resolution optical coherence tomography: a histologic correlation study. *Gastrointestinal Endoscopy*, 71, 223-230

Coggi, G., Bosari, S., Roncalli, M., Graziani, D., Bossi, P., Viale, G., Buffa, R., Ferrero, S., Piazza, M., Blandamura, S., Segalin, A., Bonavina, L., & Peracchia, A. 1997. p53 accumulation and p53 gene mutation in oesophageal carcinoma. A molecular and immunohistochemical study with clinicopathologic correlations. *Cancer*, 79, 425-432

Coleman, M. P., Babb, P., Damiecki, P., Grosclaude, P., Honjo, S., & Jones, J. 1999, *Cancer Survival Trends in England and Wales, 1971-1995: Deprivation and NHS Region.*, The Stationery Office, London.

Collard, J.M. 2002. High grade dysplasia in Barrett's oesophagus. The case for oesophagectomy. *Chest and Surgical Clinics of North America*, 12, 77-92

Colleypriest, B.J., Palmer, R.M., Ward, S.G., & Tosh, D. 2009. Cdx genes, inflammation and the pathogenesis of Barrett's metaplasia. *Trends in Molecular Medicine*, 15, (7) 313-322

Colleypriest, B.J., Ward, S.G., & Tosh, D. 2009. How does inflammation cause Barrett's metaplasia? *Current Opinion in Pharmacology*, 9, 721-726

Colotta, F., Allavena, P., Sica, A., Garlanda, C., & Mantovani, A. 2009. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*, 30, 1073-1081

Conio, M., Filiberti, R., & Blanchi, S. 2002. Risk factors for Barrett's esophagus: a case-control study. *International Journal of Cancer*, 97, 225-229

Cook, M.B., Shaheen, N.J., Anderson, L.A., Giffen, C., Chow, W.H., Vaughan, T.L., Whiteman, D.C., & Corley, D.A. 2012. Cigarette smoking increases risk of Barrett's esophagus: an analysis of the Barrett's and Esophageal Adenocarcinoma Consortium. *Gastroenterology*, 142, (4) 744-753

Coppola, D., Nasir, N.A., & Turner, L. 2010. Genesis of Barrett's Neoplasia: Current Concepts. *Mechanisms of Oncogenesis, Cancer Growth and Progression*

Corley, D.A., Levin, T.R., & Habel, L.A. 2002. Surveillance and survival in Barrett's adenocarcinomas: a population based study. *Gastroenterology*, 122, (3) 633-640

Correa, P., Piazuelo, M.B., & Wilson, K.T. 2010. Pathology of gastric intestinal metaplasia: clinical implications. *American Journal of Gastroenterology*, 105, (3) 493-498

Cothren, R.M., Richards-Kortum, R., Sivak, M.V., van Dam, J., Petras, R., & Fitzmaurice, M. 1990. Gastrointestinal tissue diagnosis by laser induced fluorescence spectroscopy at endoscopy. *Gastrointestinal Endoscopy*, 36, 105-111

Csendes, A., Smok, G., & Burdiles, P. 2003. Prevalence of intestinal metaplasia according to the length of the specialised columnar epithelium lining the distal esophagus in patients with gastroesophageal reflux. *Diseases of the Esophagus*, 16, 24-28

Curvers, W.L., Singh, R., Song, L.W., Wolfsen, H.C., Ragunath, K., Wang, K., Wallace, M.B., Fockens, P., & Bergmanm, J.J.G.H.M. 2008. Endoscopic tri-modal imaging for detection of early neoplasia in Barrett's oesophagus: a multi-centre feasibility study using high-resolution endoscopy, autofluorescence imaging and narrow band imaging incorporated in one endoscopy system. *Gut*, 57, 167-172

Curvers, W.L., ten Kate, F.J., Krishnadath, K.K., Vissers, K.J., Elzer, B., Baak, L.C., Bohmer, C., Mallant-Hent, R.C., van Oijen, A., Naber, A.H., Scholten, P., Busch, O.R., Blaauwgeers, H.G., Meijer, G.A., & Bergmann, J.J. 2010. Low grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *American Journal of Gastroenterology*, 105, (7) 1523-1530

Curvers, W.L., van Vilsteren, F.G., Baak, L.C., Bohmer, C., Mallant-Hent, R.C., Naber, A.H., van Oijen, A., Ponsioen, C.Y., Scholten, P., Schenk, E., Schoon, E., Seldenrijk, C.A., Meijer, G.A., ten Kate, F.J., & Bergmann, J.J. 2011. Endoscopic trimodal imaging versus standard video endoscopy for detection of early Barrett's neoplasia: a multicenter, randomised, crossover study in general practice. *Gastrointestinal Endoscopy*, 73, (2) 195-203

DaCosta, R.S., Wilson, C.B., & Marcon, N.E. 2007. Fluorescence and spectral imaging. *The Scientific World Journal*, 7, 2045-2071

de Jonge, P.J., van Blankenstein, M., Looman, C.W.N., Casparie, M.K., Meijer, G.A., & Kuipers, E.J. 2010. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut*, 59, 1030-1036

DeMeester, S.R. & DeMeester, T.R. 2000. Columnar mucosa and intestinal metaplasia of the esophagus. *Annals of Surgery*, 231, (3) 303-321

Dent, J. 2011. Barrett's esophagus: a historical perspective, an update on core practicalities and predictions on future evolutions of management. *Journal of Gastroenterology and Hepatology*, 26, (Supplement 1) 11-30

Derakshan, M.H., Liptrot, S., Paul, J., Brown, I.L., Morrison, D., & McColl, K.E. 2009. Oesophageal and gastric intestinal-type adenocarcinomas show the same male predominance due to a 17 year delayed development in females. *Gut*, 58, (1) 16-23

Dorer, R. & Odze, R.D. 2006. AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, ulcerative colitis and Crohn's disease. *American Journal of Surgical Pathology*, 30, (7) 871-877

Doucas, H., Garcea, G., Neal, C.P., Manson, M.M., & Berry, D.P. 2005. Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance. *European Journal of Cancer*, 41, 365-379

Dulai, G.S., Guha, S., Khan, K.L., Gornbein, J., & Weinstein, W.M. 2002. Preoperative prevalence of Barrett's esophagus in esophageal adenocarcinoma: a systematic review. *Gastroenterology*, 122, 26-33

Dvorak, K., Payne, C.M., & Chavarria, M. 2007. Bile acids in combination with low pH induce oxidative stress and oxidative DNA damage: relevance to the pathogenesis of Barrett's oesophagus. *Gut*, 56, 763-771

Edelstein, Z.R., Farrow, D.C., Bronner, M.P., Rosen, S.N., & Vaughan, T.L. 2007. Central adiposity and risk of Barrett's esophagus. *Gastroenterology*, 133, 403-411

Ellis, F.H. & Loda, M. 2004. p27 and Barrett's esophagus: a review. *Diseases of the Esophagus*, 17, 113-117

Ellis, F.H., Xu, X., & Kulke, M.H. 2001. Malignant transformation of the oesophageal mucosa is enhanced in p27 knockout mice. *Journal of Thoracic and Cardiovascular Surgery*, 122, 809-814

Farrow, D.C. & Vaughan, T.L. 1996. Determinants of survival following the diagnosis of esophageal adenocarcinoma (United States). *Cancer Causes and Control*, 7, (3) 322-327

Fitzgerald, R.C., Saeed, I.T., Khoo, D., Farthing, M.J., & Burnham, W.R. 2001. Rigorous surveillance protocol increases detection of curable cancers associated with Barrett's oesophagus. *Digestive Diseases and Sciences*, 46, (9) 1892-1898

Flejou, J.-F. 2005. Barrett's oesophagus: from metaplasia to dysplasia and cancer. *Gut*, 54, (Supplement 1) i6-i12

Fleskens, S.J.H.M., Takes, R.P., Otte-Holler, I., van Doesburg, L., Smeets, A., Speel, E.M., Slootweg, P.J., & van der Laak, J.A.W.M. 2010. Simultaneous assessment of DNA ploidy and biomarker expression in paraffin-embedded tissue sections. *Histopathology*, 57, 14-26

Ford, A.C., Forman, D., Reynolds, P.D., Cooper, B.T., & Moayyedi, P. 2005. Ethnicity, gender and socioeconomic status as risk factors for esophagitis and Barrett's esophagus. *American Journal of Epidemiology*, 162, (5) 454-460

Foster, J.M., Dominguez-Steglich, M.A., Guioli, S., Kwok, C., Weller, P.A., Stevanovi, M., Weissenbach, J., Mansour, S., Young, I.D., & Goodfellow, P.N. 1994. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature*, 372, 525-530

Galipeau, P.C., Li, X., Blount, P.L., Maley, C.C., Sanchez, C.A., Odze, R.D., Ayub, K., Rabinovitch, P.S., Vaughan, T.L., & Reid, B.J. 2007. NSAIDs modulate CDKN2A, TP53 and DNA content risk for progression to esophageal adenocarcinoma. *Public Library of Science (PLoS One)*, 4, (2) 67

Gatenby, P.A.C., Ramus, J.R., Caygill, C.P.J., Shepherd, N.A., & Watson, A. 2008. Relevance of the detection of intestinal metaplasia in non-dysplastic columnar-lined oesophagus. *Scandinavian Journal of Gastroenterology*, 43, (5) 524-530

Georgakoudi, I., Jacobson, B.C., van Dam, J., Backman, V., Wallace, M.B., Muller, M.G., Zhang, Q., Badizadegan, K., Sun, D., Thomas, G.A., Perelman, L.T., & Feld, M.S. 2001. Fluorescence, reflectance and light scattering spectroscopy for evaluating dysplasia in patients with Barrett's oesophagus. *Gastroenterology*, 120, 1620-1629

Giles, R.H., van Es, J.H., & Clevers, H. 2003. Caught up in a Wnt storm: Wnt signalling in cancer. *Biochemica et Biophysica Acta*, 1653, 1-24

Goh, A.M., Coffill, C.R., & Lane, D.P. 2011. The role of mutant p53 in human cancer. *Journal of Pathology*, 223, (2) 116-126

Goldblum, J.R. 2003. Barrett's oesophagus and Barrett's-related dysplasia. *Modern Pathology*, 16, 316-324

Goldblum, J.R. 2010. Controversies in the diagnosis of Barrett esophagus and Barrett-related dysplasia: one pathologist perspective. *Archives of Pathology and Laboratory Medicine*, 134, 1479-1484

Gono, K., Obi, T., Yamaguchi, M., Ohyama, N., Machida, H., Sano, Y., Yoshida, S., Hamamoto, Y., & Endo, T. 2004. Appearance of enhanced tissue features in narrow-band endoscopic imaging. *Journal of Biomedical Optics*, 9, (3) 568-577

Gonzalez, S., Yu, W.M., Smith, M.S., Slack, K.N., Rotterdam, H., Abrams, J.A., & Lightdale, C.J. 2010. Randomised comparison of 3 different sized biopsy forceps for quality of sampling in Barrett's esophagus. *Gastointestinal Endoscopy*, 72, (5) 935-940

Gregorieff, A., Grosschedt, R., & Clevers, H. 2004. Hindgut defects and transformation of the gastrointestinal tract in $Tcf4^{-/-}$/$Tcf1^{-/-}$embryos. *The EMBO Journal*, 23, (8) 1825-1833

Haggitt, R.C. 1994. Barrett's esophagus, dysplasia and adenocarcinoma. *Human Pathology*, 25, 982-993

Haggitt, R.C., Tryselaar, J., Ellis, F.H., & Colcher, H. 1978. Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. *American Journal of Clinical Pathology*, 70, (1) 1-5

Hahn, H.P., Blount, P.L., Ayub, K., Das, M., Souza, R.F., Spechler, S.J., & Odze, R.D. 2009. Intestinal differentiation in metaplastic, nongoblet columnar epithelium in the esophagus. *American Journal of Surgical Pathology*, 33, (7) 1006-1015

Hamilton, S.R. & Smith, R.R. 1987. The relationship between columnar epithelial dysplasia and invasive adenocarcinoma arising in Barrett's esophagus. *American Journal of Clinical Pathology*, 87, (3) 301-312

Hammeeteman, W., Tytgat, N.J., & Houthoff, H.J. 1989. Barrett's esophagus: development of dysplasia and adenocarcinoma. *Gastroenterology*, 96, 1249-1256

Hanahan, D. & Weinberg, R. 2011. Hallmarks of Cancer: The Next Generation. *Cell*, 144, (5) 646-674

Hanlon, P., Lawder, R.S., Buchanan, D., Redpath, A., Walsh, D., Wood, R., Bain, M., Brewster, D.H., & Chalmers, J. 2005. Why is mortality higher in Scotland than in England & Wales? Decreasing influence of socioeconomic deprivation between 1981 and 2001 supports the existence of a 'Scottish Effect'. *Journal of Public Health*, 27, (2) 199-204

Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M., & Taketo, M.M. 1999. Intestinal polyposis in mice with a dominant stable mutation of the ß-catenin gene. *The EMBO Journal*, 18, (21) 5931-5942

Haringsma, J. & Tytgat, G.N.J. 1999. Fluorescence and autofluorescence. *Baillieres Best Practice and Research in Clinical Gastroenterology*, 13, 1-10

Harrison, R., Perry, I., Haddadin, W., McDonald, S., Bryan, R., Abrams, K., Sampliner, R.E., Talley, N.J., Moayyedi, P., & Jankowski, J.A. 2007. Detection of intestinal metaplasia in Barrett's esophagus: an observational comparator study suggests the need for a minimum of eight biopsies. *American Journal of Gastroenterology*, 102, (6) 1154-1161

Hawe, A., Payne, W.S., Weiland, L.H., & Fontana, R.S. 1973. Adenocarcinoma in the columnar epithelial lined lower (Barrett) oesophagus). *Thorax*, 28, 511-514

Herrero, L.A., Weusten, B.L.A.M., & Bergmann, J.J. 2010. Autofluorescence and narrow band imaging in Barrett's esophagus. *Gastroenterology Clinics of North America*, 39, (4) 747-758

Hirota W, K., Zuckerman, M.J., Adler, D.G., Davila, R.E., Egan, J., Leighton, J.A., Qureshi, W.A., Rajan, E., Fanelli, R., Wheeler-Harbaugh, J., Baron, T.H., & Faigel, D.O. 2006. ASGE guideline: the role of endoscopy in the surveillance of premalignant conditions of the upper GI tract. *Gastointestinal Endoscopy*, 63, (4) 570-580

Holcombe, R.F., Marsh, J.L., & Waterman, M.L. 2002. Expression of Wnt ligands and Frizzled receptors in colonic mucosa and in colon carcinoma. *Journal of Clinical Pathology*, 55, 220-226

Hole, D.J. & McArdle, C.S. 2002. Impact of socioeconomic deprivation on outcome after surgery for colorectal cancer. *British Journal of Surgery*, 89, (5) 586-590

Holmes, R.S. & Vaughan, T.L. 2007. Epidemiology and pathogenesis of esophageal cancer. *Seminars in Radiotherapy and Oncology*, 17, 1-9

Horwhat, J.D., Maydonovitch, C., & Ramos, F. 2008. A randomised comparison of methylene-blue directed biopsy versus conventional four-quadrant biopsy for the detection of intestinal metaplasia and dysplasia in patients with long segment Barrett's oesophagus. *American Journal of Gastroenterology*, 103, (3) 546-554

Hoschuetzky, H., Aberle, H., & Kemler, R. 1994. Beta-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. *Journal of Cell Biology*, 127, 1375-1380

Hume, D. & Womersley, J. 1985. Analysis of death rates in the population aged 60 years and over of Greater Glasgow by postcode sector of residence. *Journal of Epidemiology and Community Health*, 39, 357-363

Hur, C., Choi, S.E., Rubenstein, J.H., Kong, C.Y., Nishioka, N.S., Provenzale, D.T., & Inadomi, J.M. 2012. The cost effectiveness of radiofrequency ablation for Barrett's Oesophagus. *Gastroenterology*, 143, (3) 567-575

Hvid-Jensen, F., Pedersen, L., Drewes, A.M., Sorensen, H.T., & Funch-Jensen, P. 2011. Incidence of adenocarcinoma among patients with Barrett's esophagus. *The New England Journal of Medicine*, 365, (15) 1375-1383

ISD Scotland. Cancer in Scotland. ISD Scotland, Edinburgh . 2012.
Ref Type: Online Source

Isenberg, G., Sivak, M.V.J., Chak, A., Wong, R.C.K., Willis, J., Wolf, B., Rowland, D.Y., Das, A., & Rollins, A. 2005. Accuracy of endoscopic optical coherence tomography in the detection of dysplasia in Barrett's esophagus: a prospective, double-blinded study. *Gastrointestinal Endoscopy*, 62, (6) 825-831

Jamieson, G.G., Mathew, G., Ludemann, R., Wayman, J., Myers, J.C., & Devitt, P.G. 2004. Postoperative mortality following oesophagectomy and problems in reporting its rate. *British Journal of Surgery*, 91, 943-947

Jankowski, J.A. & Barr, H. 2006. Improving surveillance for Barrett's oesophagus: AspECT and BOSS trials provide an evidence base. *British Medical Journal*, 332, 1512

Jankowski, J.A., Barr, H., Wang, K., & Delaney, B. 2010. Diagnosis and management of Barrett's oesophagus. *British Medical Journal*, 341, c4551

Jankowski, J.A., Harrison, R.F., Perry, I., Balkwill, F., & Tselepsis, C. 2000. Barrett's metaplasia. *The Lancet*, 356, 2079-2085

Jankowski, J.A., Provenzale, D., & Moayyedi, P. 2002. Oesophageal adenocarcinoma arising from Barrett's metaplasia has regional variations in the west. *Gastroenterology*, 122, 588-595

Jankowski, J.A., Wright, N.A., Meltzer, S.J., Triadafilopoulos, G., Geboes, K., Casson, A.G., Kerr, D., & Young, L.S. 1999. Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *American Journal of Pathology*, 154, (4) 965-973

Jansson, C., Nordenstedt, H., Johansson, S., Wallander, M.A., Johnsen, R., Hveem, K., & Lagergren, J. 2007. Relation between gastroesophageal reflux symptoms and socioeconomic factors: a population-based study (the HUNT study). *Clinical Gastroenterology and Hepatology*, 5, (9) 1029-1034

Kapadia, C.R., Cutruzzola, F.W., O'Brien, K.M., Stetz, M., Enriquez, R., & Deckelbaum, L. 1990. Laser-induced fluorescence spectroscopy of human colonic mucosa - detection of adenomatous transformation. *Gastroenterology*, 99, 150-157

Kara, M.A., Ennahachi, M., Fockens, P., ten Kate, F.J., & Bergmann, J.J. 2006. Detection and classification of mucosal vascular patterns in Barrett's esophagus by using narrow band imaging. *Gastointestinal Endoscopy*, 64, 200-205

Kara, M.A., Peters, F.P., Rosmolen, W.D., Krishnadath, K.K., ten Kate, F.J., Fockens, P., & Berger, J.M. 2005. High resolution endoscopy plus chromoendsocopy or narrow band imaging in Barrett's oesophagus: a prospective randomised crossover study. *Endoscopy*, 37, (10) 929-936

Kara, M.A., Peters, F.P., ten Kate, F.J.W., van Deventer, S.J., Fockens, P., & Bergmanm, J.J.G.H.M. 2005. Endoscopic video autofluorescence imaging may improve the detection of early neoplasia in patients with Barrett's esophagus. *Gastrointestinal Endoscopy*, 61, (6) 679-685

Kariv, R., Plesec, T.P., & Goldblum, J.R. 2009. The Seattle protocol does not more reliably predict the detection of cancer at the time of oesophagectomy than a less intensive surveillance protocol. *Clinical Gastroenterology and Hepatology*, 7, (6) 653-689

Kelty, C.J., Gough, M.D., van Wyk, Q., Stephenson, T.J., & Ackroyd, R. 2007. Barrett's oesophagus: intestinal metaplasia is not essential for cancer risk. *Scandinavian Journal of Gastroenterology*, 42, 1271-1274

Kemp, R., Ireland, H., Clayton, E., Houghton, C., Howard, L., & Winton, D.J. 2004. Elimination of background recombination: somatic induction of Cre by combined transcriptional regulation and hormone binding affinity. *Nucleic Acids Research*, 32, (11) e92

Kerkhof, M., Kusters, J.G., van Dekken, H., Kuipers, E.J., & Siersema, P.D. 2007. Biomarkers for risk stratification of neoplastic progression in Barrett esophagus. *Cellular Oncology*, 29, 507-517

Keswani, R.N., Noffsinger, A., Waxman, I., & Bissonnette, M. 2006. Clinical use of p53 in Barrett's esophagus. *Cancer Epidemiology, Biomarkers and Prevention*, 15, (7) 1243-1249

Kim, S.L., Waring, J.P., Spechler, S.J., Sampliner, R.E., Doos, W.G., Krol, W.F., & Williford, W.O. 1994. Diagnostic inconsistencies in Barrett's esophagus. *Gastroenterology*, 107, (4) 945-949

Kim, T., Grobmyer, S.R., Smith, R., Ben-David, K., Ang, D., Vogel, S.B., & Hochwald, S.N. 2011. Esophageal cancer - the five year survivors. *Journal of Surgical Oncology*, 103, 179-183

Kirkegaard, T., Edwards, J., Tovey, S., McGlynn, L.M., Krishna, S.N., & Mukherjee, R. 2006. Observer variation in immunohistochemical analysis of protein expression; time for a change? *Histopathology*, 48, 787-794

Kong, J., Crissey, M.A., Stairs, D.B., Sepulveda, A.R., & Lynch, J.P. 2011. Cox2 and B-catenin/T-cell factor signaling intestinalise human esophageal keratinocytes when cultured under organotypic conditions. *Neoplasia*, 13, (9) 792-805

Koppert, L.B., van der Velden, A.W., van der Wetering, M., Abbou, M., van der Ouweland, A.M., Tilanus, H.W., Wijnhoven, B.P.L., & Dinjens, W.N.M. 2004. Frequent loss of the AXIN1 locus but absence of AXIN1 gene mutations in adenocarcinomas of the gastro-oesophageal junction with nuclear beta-catenin expression. *British Journal of Cancer*, 90, (4) 892-899

Koppert, L.B., Wijnhoven, B.P.L., van Dekken, H., Tilanus, H.W., & Dinjens, W.N.M. 2005. The molecular biology of esophageal adenocarcinoma. *Journal of Surgical Oncology*, 92, 169-190

Krishnadath, K.K., Tilanus, H.W., van Blankenstein, M., Hop, W.C.J., Kremers, E.D., Dinjens, W.N.M., & Bosman, F.T. 1997. Reduced expression of the cadherin-catenin complex in oesophageal adenocarcinoma correlates with poor prognosis. *Journal of Pathology*, 182, 331-338

Kubo, A., Levin, T.R., Block, G., Rumore, G., Quesenberry, C.P., Buffler, P., & Corley, D.A. 2009. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. *Gastroenterology*, 136, (3) 806-815

Kuramochi, H., Vallbohmer, D., Uchida, K., & et al 2004. Quantitative tissue specific analysis of cyclooxygenase gene expression in the pathogenesis of Barrett's adenocarcinoma. *Journal of Gastrointestinal Surgery*, 8, 1007-1017

Kyrgidis, A., Kountouras, J., Zavos, C., & Chatzopoulos, D. 2005. New molecular concepts of Barrett's esophagus: clinical implications and biomarkers. *Journal of Surgical Research*, 125, 189-212

Lambert, R., Rey, J.F., & Sankaranarayanan, R. 2003. Magnification and chromoscopy with the acetic acid test. *Endoscopy*, 35, 437-445

Lao-Sirieix, P. & Fitzgerald, R.C. 2010. Role of the micro-environment in Barrett's carcinogenesis. *Biochemical Society Transactions*, 38, 327-330

Lao-Sirieix, P., Lovat, L., & Fitzgerald, R.C. 2007. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. *Clinical Cancer Research*, 13, (2) 659-665

Li, X.D., Boppart, S.A., van Dam, J., Mashimo, H., Mutinga, M., Drexler, W., Klein, M., Pitris, C., Krinsky, M.L., Brezinski, M.E., & Fujimoto, J.G. 2000. Optical coherence tomography: advanced technology for the endoscopic imaging of Barrett's esophagus. *Endoscopy*, 32, (12) 921-930

Liu, L., Hofstetter, W.L., Rashid, A., Swisher, S.G., Correa, A.M., Ajani, J.A., Hamilton, S.R., & Wu, T. 2005. Significance of the depth of tumour invasion and lymph node metastasis in superficially invasive (T1) esophageal adenocarcinoma. *American Journal of Surgical Pathology*, 29, (8) 1079-1085

Liu, W., Hahn, H., Odze, R.D., & Goyal, R.K. 2009. Metaplastic esophageal columnar epithelium without goblet cells shows DNA content abnormalities similar to goblet cell-containing epithelium. *American Journal of Gastroenterology*, 104, (4) 816-824

Longcroft-Wheaton, G., Duku, M., Mead, R., Poller, D., & Bhandari, P. 2010. Acetic acid spray is an effective tool for the endoscopic detection of neoplasia in patients with Barrett's oesophagus. *Clinical Gastroenterology and Hepatology*, 8, (10) 843-847

Lord, R.V.N., Wickramasinghe, K., & Johansson, J. 2004. Cardiac mucosa in the remnant oesophagus after oesopahgectomy is an acquired epithlium with Barrett's like features. *Surgery*, 146, 633-640

Lortat-Jacob, J., Maillard, J.N., Richard, C.A., Fekete, F., Huguier, M., & Conte-Marti, J. 1968. Primary esophageal adenocarcinoma: report of 16 cases. *Surgery*, 64, (3) 535-543

Lovat, L., Johnson, K., Mackenzie, G.D., Clark, B.R., Novelli, M.R., Davies, S., O'Donovan, M., Selvasekar, C., Thorpe, S.M., Pickard, D., Fitzgerald, R.C., Fearn, T., Bigio, I., & Bown, S.G. 2006. Elastic scattering spectroscopy accurately detects high grade dysplasia and cancer in Barrett's oesophagus. *Gut*, 55, 1078-1083

Lu, S. & Wang, T.D. 2008. In vivo cancer biomarkers of esophageal neoplasia. *Cancer Biomarkers*, 4, 341-350

Maas, L.C., Katz, L.A., & Pascale, J.F. 1974. Adenocarcinoma of the esophagus arising in metaplastic (Barrett's) epithelium. *Gastrointestinal Endoscopy*, 21, 73-74

Macleod, U., Ross, S., Fallowfield, L., & Watt, G.C. 2004. Anxiety and support in breast cancer: is this different for affluent and deprived women? *British Journal of Cancer*, 91, 879-883

Maley, C.C. 2007. Multistage carcinogenesis in Barrett's esophagus. *Cancer Letters*, 245, 22-32

Maley, C.C., Galipeau, P.C., Finley, J.C., Wongsurawat, V.J., Li, X., Sanchez, C.A., Paulson, T.G., Blount, P.L., Risques, R.A., Rabinovitch, P.S., & Reid, B.J. 2006. Genetic clonal diversity predicts progression to oesophageal adenocarcinoma. *Nature Genetics*, 38, 468-473

Maley, C.C., Galipeau, P.C., & Li, X. 2004. Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selceted in Barrett's esophagus. *Cancer Research*, 64, 3414-3427

Manjunath, S. & Jankowski, J.A. 2000. Interaction of environmental factors in oesophageal carcinogenesis. *Journal of Royal College of Surgeons Edinburgh*, 45, 260-262

Mannath, J., Subramanian, V., Hawkey, C.J., & Ragunath, K. 2010. Narrow band imaging for characterisation of high grade dysplasia and specialized intestinal metaplasia in Barrett's oesophagus: a meta-analysis. *Endoscopy*, 42, (5) 351-359

McCorkle, R.C. & Blades, B. 1955. Adenocarcinoma of the esophagus arising from aberrant gastric mucosa. *American Surgeon*, 21, 781

McKinney, A., Sharp, L., Macfarlane, G.J., & Muir, C.S. 1995. Oesophageal and gastric cancer in Scotland 1960-90. *British Journal of Cancer*, 71, (2) 411-415

Moersch, R., Ellis, F., & McDonald, J.R. 1959. Pathologic changes occurring in severe reflux esophagitis. *Surgical Gynaecology and Obstetrics*, 108, 476-484

Moll, R., Franke, W.W., & Schiller, D.L. 1982. The catalogue of human cytokeratins: patterns of expression in normal epithelia, tumors and cultures cells. *Cell*, 31, 11-24

Montgomery, E., Bronner, M.P., & Greenson, J.K. 2002. Are ulcers a marker for invasive carcinoma in Barrett's esophagus? Data from a diagnostic variability study with clinical follow-up. *American Journal of Gastroenterology*, 97, 27-31

Montgomery, E., Goldblum, J.R., & Greenson, J.K. 2001. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: a follow up study based on 138 cases from a diagostic variability study. *Human Pathology*, 32, 379-388

Morales, C.P., Souza, R.F., & Spechler, S.J. 2002. Hallmarks of cancer progression in Barrett's oesophagus. *The Lancet*, 360, 1587-1589

Morris, C.D., Armstron, G.R., Bigley, G., & et al 2001. Cyclooxygenase-2 expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. *American Journal of Gastroenterology*, 96, 990-996

Morson, B.C. & Belcher, J.R. 1952. Adenocarcinoma of the esophagus and ectopic gastric mucosa. *British Journal of Cancer*, 6, 127-130

Moskaluk, C.A., Heitmiller, R., Zahurak, M., Schwab, D., Sidransky, D., & Hamilton, S.R. 1996. p53 and $p21^{WAF1/CIP1/SDI1}$ gene products in Barrett esophagus and adenocarcinoma of the esophagus and esophagogastric junction. *Human Pathology*, 27, 1211-1220

Moyes, L.H. & Going, J.J. 2011. Still waiting for predictive biomarkers in Barrett's oesophagus. *Journal of Clinical Pathology*, 64, 742-750

Munro, A.J. & Bentley, A.H.M. 2004. Deprivation, comorbidity and survival in a cohort of patients with colorectal cancer. *European Journal of Cancer Care*, 13, 254-262

Murray, L., Sedo, A., Scott, A., McManus, D.T., Sloan, J., Hardie, L.J., Forman, D., & Wild, C.P. 2006. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut*, 55, 1390-1397

Murray, L., Watson, P., Johnston, B., Sloan, J., Mainie, I.M., & Gavin, A. 2003. Risk of adenocarcinom in Barrett's oesophagus; population based study. *British Medical Journal*, 327, 534-535

Naef, A.P., Savary, M., & Ozzello, L. 1975. Columnar-lined lower esophagus:an acquired lesion with malignant predisposition. *The Journal of Thoracic and Cardiovascular Surgery*, 70, (5) 826-834

Nair, K.S., Naidoo, R., & Chetty, R. 2005. Expression of cell adhesion molecules in oesophageal carcinoma and its prognostic value. *Journal of Clinical Pathology*, 58, 343-351

Ngamruengphong, S., Sharma, P., & Das, M. 2009. Diagnostic yield of methylene blue chromoendoscopy for detecting specialised intestinal metaplasia and dysplasia in Barrett's Oesophagus: a meta-analysis. *Gastrointestinal Endoscopy*, 69, 1021-1028

Nguyen, D.M., Richardson, P., & El-Serag, H.B. 2010. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology*, 137, (7) 2260-2266

NHS National Services Scotland. ISD Scotland Cancer Statistics. 2012.
Ref Type: Online Source

Nilsson, J., Skobe, V., Johansson, J., Willen, R., & Johnsson, F. 2000. Screening for oesophageal adenocarcinoma: an evaluation of a surveillance program for columnar metaplasia of the oesophagus. *Scandinavian Journal of Gastroenterology*, 35, 10-16

Noffsinger, A. 2008. Defining cancer risk in Barrett's esophagus: a pathologist's perspective. *Gastrointestinal Cancer Research*, 2, (6) 308-310

Nowell, P.C. 1976. The clonal evolution of tumour cell populations. *Science*, 194, 23-28

O'Riordan, J.M., Abdel-Latif, M.M., Ravi, N., McNamara, D., Byrne, P.J., McDonald, G.S.A., Keeling, P.W.N., Kelleher, D., & Reynolds, J.V. 2005. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *American Journal of Gastroenterology*, 100, 1257-1264

Oberg, S., Johannson, J., & Wenner, J. 2001. Endoscopic surveillance of columnar lined esophagus: frequency of intestinal metaplasia detection and impact of antireflux surgery. *Annals of Surgery*, 234, 619-626

Oberg, S., Peters, J.H., & DeMeester, T.R. 2000. Determinants of intestinal metaplasia within the columnar lined esophagus. *Archives of Surgery*, 135, 651-656

Odze, R.D. 2006. Diagnosis and grading of dysplasia in Barrett's oesophagus. *Journal of Clinical Pathology*, 59, (10) 1029-1038

Olliver, J.R., Wild, C.P., Sahay, P., Dexter, S., & Hardie, L.J. 2003. Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus. *Lancet*, 362, 373-374

Osterheld, M., Bian, Y., Bosman, F.T., Benhattar, J., & Fontolliet, C. 2002. beta-Catenin expresion and its association with prognostic factors in adenocarcinoma developed in Barrett's esophagus. *American Journal of Clinical Pathology*, 117, 451-456

Panjehpour, M., Overholt, B.F., Schmidhammer, J.L., Parris, C., Buckley, P.F., & Vo-Dinh, T. 1995. Spectroscopic diagnosis of esophageal cancer: new classification model, improved measurement system. *Gastrointestinal Endoscopy*, 41, (6) 577-581

Panjehpour, M., Overholt, B.F., Vo-Dinh, T., & Coppola, D. 2012. The effect of reactive atypia/inflammation on the laser-induced fluorescence diagnosis of non-dysplastic Barrett's esophagus. *Lasers in Surgery and Medicine*, 44, (5) 390-396

Panjehpour, M., Overholt, B.F., Vo-Dinh, T., Haggitt, R.C., Edwards, D.H., & Buckley, P.F. 1996. Endoscopic fluorescence detection of high grade dysplasia in Barrett's esophagus. *Gastroenterology*, 111, 93-101

Paraf, F., Flejou, J.-F., Pignon, J., Fekete, F., & Potet, F. 1995. Surgical pathology of adenocarcinoma arising in Barrett's esophagus: analysis of 67 cases. *American Journal of Surgical Pathology*, 19, (2) 183-191

Paull, A., Trier, J.S., Dalton, M.D., Camp, R.C., Loeb, P., & Goyal, R.K. 1976. The histologic spectrum of Barrett's esophagus. *New England Journal of Medicine*, 295, 476-480

Paulson, T.G., Galipeau, P.C., Xu, L., Kissel, H.D., Li, X., Blount, P.L., Sanchez, C.A., Odze, R.D., & Reid, B.J. 2008. p16 mutation spectrum in the premalignant condition Barrett's esophagus. *Public Library of Science (PLoS One)*, 3, (11) 3809

Pelengaris, S., Khan, M., & Evan, G. 2002. c-MYC: more than just a matter of life and death. *Nature Reviews Cancer*, 2, (10) 764-776

Pepe, M.S., Etzioni, R., & Feng, Z. 2001. Phases of biomarker developemtn for early detection of cancer. *Journal of National Cancer Institute*, 93, 1054-1061

Pera, M. & Pera, M. 2002. Experimental Barrett's esophagus and the origin of intestinal metaplasia. *Chest and Surgical Clinics of North America*, 12, 25-37

Pinto, D. & Clevers, H. 2005. Wnt, stem cells and cancer in the intestine. *Biology of the Cell*, 97, 185-196

Playford, R.J. 2005. New British Society of Gastroenterology (NSG) guidelines for the diagnosis and management of Barrett's oesophagus. *Gut*, 55, 442-443

Prach, A.T., MacDonald, T.A., Hopwood, D.A., & Johnston, D.A. 1997. Increasing Barrett's oesophagus: education, enthusiasm or epidemiology? *Lancet*, 350, 933

Prasad, G.A., Bansal, A., Sharma, P., & Wang, K.H. 2010. Predictor of progression in Barrett's esophagus: current knowledge and future directions. *American Journal of Gastroenterology*, 105, 1490-1502

Preston, S.L. & Jankowski, J.A. 2006. Drinking from the fountain of promise: biomarkers in the surveillance of Barrett's oesophagus - the glass is half full! *Gut*, 55, 1377-1379

Qualman, S.J., Murray, R.D., McClung, H.J., & Lucan, J. 1990. Intestinal metaplasia is age related in Barrett's esophagus. *Archives of Pathology and Laboratory Medicine*, 114, 1236-1240

Ramirez, A.J., Westcombe, A.M., & Burgess, C.C. 1999. Factors predicting delayed presentation of symptomatic breast cancer: a systematic review. *The Lancet*, 353, 1127-1131

Reid, B.J., Blount, P.L., Feng, G., & Levine, D.S. 2000. Optimising endoscopic biopsy detection of early cancers in Barrett's high grade dysplasia. *American Journal of Gastroenterology*, 95, 3089-3096

Reid, B.J., Blount, P.L., Rubin, C.E., Levine, D.S., Haggitt, R.C., & Rabinovitch, P.S. 1992. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance in a cohort. *Gastroenterology*, 102, (4) 1212-1219

Reid, B.J., Levine, D.S., Longton, G., Blount, P.L., & Rabinovitch, P.S. 2000. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low and high risk patient subsets. *American Journal of Gastroenterology*, 95, (7) 1669-1676

Reid, B.J., Li, X., Galipeau, P.C., & Vaughan, T.L. 2010. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nature Reviews*, 10, 87-101

Reid, B.J., Prevo, L.J., Galipeau, P.C., Sanchez, C.A., Longton, G., Levine, D.S., Blount, P.L., & Rabinovitch, P.S. 2001. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *American Journal of Gastroenterology*, 96, (10) 2839-2848

Reid, B.J. & Weinstein, W.M. 1987. Barrett's esophagus and adenocarcinoma. *Annual Review of Medicine*, 38, 477-492

Rex, D.K., Cummings, O.W., & Shaw, M. 2003. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. *Gastroenterology*, 125, 1670-1677

Reya, T. & Clevers, H. 2005. Wnt signalling in stem cells and cancer. *Nature*, 434, 843-850

Rhee, C.S., Sen, M., & Lu, D.S. 2002. Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. *Oncogene*, 21, 6598-6605

Riddell, R.H. & Odze, R.D. 2009. Definition of Barrett's esophagus: Time for a rethink - is intestinal metaplasia dead? *American Journal of Gastroenterology*, 104, (10) 2588-2594

Rogler, G., Brand, K., Vogi, D., & et al 1998. Nuclear factor κB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology*, 115, 357-369

Ronkainen J, Aro, P., Storskrubb, T., Johansson, S.F., Lind, T., Bolling-Sternevald, E., Vieth, M., Stolte, M., Talley, N.J., & Agreus, L. 2005. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology*, 129, 1828-1831

Rosenberg, J.C., Budev, H., Edwards, D.H., Singal, S., Steiger, Z., & Sundareson, A.S. 1985. Analysis of adenocarcinoma in Barrett's esophagus utilising a staging system. *Cancer*, 55, (6) 1353-1360

Ross, A., Kinney, T., Larghi, A., & et al 2005. Complete circumferential endoscopic mucosal resection as a treatment for early oesophageal carcinoma or Barrett's oesophagus with high grade dysplasia. *Gastointestinal Endoscopy*, 61, AB95

Roxburgh, C.S. & McMillan, D.C. 2010. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. *Future Oncology*, 6, 149-163

Rucker-Schmidt, R.L., Sanchez, C.A., Blount, P.L., Ayub, K., Li, X., Rabinovitch, P.S., Reid, B.J., & Odze, R.D. 2009. Nonadenomatous dysplasia in Barrett

esophagus: a clinical, pathologic and DNA content flow cytometric study. *American Journal of Surgical Pathology*, 33, 886-893

Ruol, A., Parenti, A., Zaninotto, G., Merigliano, S., Costantini, M., Cagol, M., Alfieri, R., Bonavina, L., Peracchia, A., & Ancona, E. 2000. Intestinal metaplasia is the probable common precurson of adenocarcinoma in Barrett esophagus and adenocarcinoma of the gastric cardia. *Cancer*, 88, (11) 2520-2528

Salahshor, S., Naidoo, R., Serra, S., Shih, W., Tsao, M., Chetty, R., & Woodgett, J.R. 2008. Frequent accumulation of nuclear E-cadherin and alterations in the Wnt signalling pathway in esophageal squamous cell carcinomas. *Modern Pathology*, 21, 271-281

Satake, S., Semba, S., Matsuda, Y., Usami, Y., Chiba, H., Sawada, N., Kasuga, M., & Yokozaki, H. 2008. Cdx2 transcription factor regulates claudin-3 and claudin-4 expression during intestinal differentiation of gastric carcinoma. *Pathology International*, 58, (3) 156-163

Sauvaget, C., Fayette, J.M., Muwonge, R., Wesley, R., & Sankaranarayanan, R. 2011. Accuracy of visual inspection with acetic acid for cervical cancer screening. *International Journal of Gynaecology and Obstetrics*, 113, (1) 14-24

Schlemper, R.J., Riddell, R.H., & Kato, Y. 2000. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut*, 47, 251-255

Schmidt, M.K., Meurer, L., Volkweis, B.S., Edelweiss, M.I., Schirmer, C.C., Kruel, C.D.P., & Gurski, R.R. 2007. c-Myc overexpression is strongly associated with metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *Diseases of the Esophagus*, 20, 212-216

Schnell, T.G., Sontag, S.J., Chejfec, G., Aranha, G., Metz, A., O'Connell, S., Seidel, U.J., & Sonnerberg, A. 2001. Long-term nonsurgical management of Barrett's esophagus with high grade dysplasia. *Gastroenterology*, 120, (7) 1607-1619

Schuhmacher, C., Becker, I., & Oswald, S. 1999. Loss of immunohistological E cadherin expression in colon cancer is not due to structural gene alterations. *Virchows Archives*, 434, 489-495

Segditsas, S. & Tomlinson, I. 2006. Colorectal cancer and genetic alterations in the Wnt pathway. *Oncogene*, 25, 7531-7537

Shackleford, G.M., MacArthur, C.A., Kwan, H.C., & Varmus, H.E. 1993. Mouse mammary tumor virus infection accelerated mammary carcinogenesis in Wnt-1 transgenic mice by insertional activation of int-2/Fgf-3 and hst/Fgf4. *Proceedings of the National Academy of Sciences*, 90, 740-744

Shaheen, N.J., Crosby, M.A., & Bozymski, E.M. 2000. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology*, 119, 333-338

Shaheen, N.J. & Richter, J.F. 2009. Barrett's oesophagus. *Lancet*, 373, 850-861

Sharma, P., Dent, J., & Armstrong, D. 2006. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C&M criteria. *Gastroenterology*, 131, 1392-1399

Sharma, P., Hawes, R.H., Bansal, A., Gupta, N., Curvers, W.L., Rastogi, A., Singh, M., Hall, M., Mathur, S.C., Wani, S., Hoffman, B., Gaddam, S., Fockens, P., & Bergmann, J.J. 2013. Standard endoscopy with random biopsies versus narrow band imaging targeted biopsies in Barrett's oesophagus: a prospective, international, randomised controlled trial. *Gut*, 62, 15-21

Sharma, P., Weston, A.P., Topalovski, M., & et al 2003. Magnification chromoendoscopy for the detection of intestinal metaplasia and dysplasia in Barrett's oesophagus. *Gut*, 52, 24-27

Sherr, C.J. 2000. The Pezcoller lecture: cancer cell cycles revisited. *Cancer Research*, 60, (14) 3689-3695

Shi, X.Y., Bhagwandeen, B., & Leong, A.S. 2008. p16, cyclin D1, Ki67 and AMACR as markers for dysplasia in Barrett esophagus. *Applied Immunohistochemistry and Molecular Morphology*, 2008, (16) 5-447

Shirvani, V., Ouatu-lascar, R., & Kaur, B. 2000. Cyclooxygenase 2 expression in Barrett's oesophagus and adenocarcinoma: Ex vivo induction by bile sals and acid exposure. *Gastroenterology*, 118, 487-496

Sikkema, M., de Jonge, P.J., & Steyerberg, E.W. 2010. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology*, 8, 235-244

Sikkema, M., Kerkhof, M., Steyerberg, E.W., Kusters, J.G., van Strien, P.M.H., Looman, C.W.N., van Dekken, H., Siersema, P.D., & Kuipers, E.J. 2009. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case control study. *American Journal of Gastroenterology*, 104, (11) 2673-2680

Singerland, J. & Pagano, M. 2000. Regulation of cdk inhibitor p27 and its deregulation in cancer. *Journal of Cell Physiology*, 183, 10-17

Singh, R., Anagnostopoulos, G.K., Yao, K., Karageorgiou, H., Fortun, P.J., Shonde, A., Garsed, K., Kaye, P.V., Hawkey, C.J., & Ragunath, K. 2008. Narrow-band imaging with magnification in Barrett's esophagus: validation of a simple grading system of mucosal morphology patterns against histology. *Endoscopy*, 40, (6) 457-463

Singh, R., Mei, S., & Sethi, S. 2011. Advanced endoscopic imaging in Barrett's oesophagus: a review on current practice. *World Journal of Gastroenterology*, 17, (38) 4271-4276

Singh, S.P., Lipman, J., Goldman, H., Ellis, F.H., Alzenman, L., Canhi, G., Signoretti, S., Chiaur, D.S., Pagano, M., & Loda, M. 1998. Loss or altered subcecllular localisation of p27 in Barrett's associated adenocarcinoma. *Cancer Research*, 58, 1730-1735

Sjogren, R.W. & Johnson, L.F. 1983. Barrett's esophagus: a review. *The American Journal of Medicine*, 74, 313-321

Skacel, M., Petras, R.E., Gramlich, T.L., Sigel, J.E., Richter, J.E., & Goldblum, J.R. 2000. The diagnosis of low grade dysplasia in Barrett's esophagus and its implications for disease progression. *American Journal of Gastroenterology*, 95, 3383-3387

Skinner, D.B., Walther, B.C., Riddell, R.H., Schmidt, H., Iascone, C., & DeMeester, T.R. 1983. Barrett's esophagus: comparison of benign and malignant cases. *Annals of Surgery*, 198, (4) 554-565

Smith, R.R.L., Hamilton, S.R., Boitnott, J.K., & Rogers, E.L. 1984. The spectrum of carcinoma arising in Barrett's esophagus. *American Journal of Surgical Pathology*, 8, 563-573

Sodhani, P., Gupta, S., Prakash, S., & Singh, V. 1999. Columnar and metaplastic cells in vault smears. *Cytopathology*, 10, 122-126

Souza, R.F., Krishnan, K., & Spechler, S.J. 2008. Acid, bile and CDX: the ABCs of making Barrett's metaplasia. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 295, 211-218

Souza, R.F., Morales, C.P., & Spechler, S.J. 2001. Review article: a conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. *Alimentary Pharmacology and Therapeutics*, 15, 1087-1100

Spechler S.J 2011. Barrett's esophagus surveillance: when, how often, does it work? *Gastroenterology Clinics of North America*, 21, (1) 9-24

Spechler SJ 2005. Dysplasia in Barrett's esophagus: limitations of current management strategies. *American Journal of Gastroenterology*, 100, 927-935

Spechler SJ 2011. Dysplasia in Barrett's esophagus: limitations of current management strategies. *American Journal of Gastroenterology*, 100, 927-935

Spechler SJ, Robbins, A.H., Rubins, H.B., Vincent, M.E., & Heeren, T. 1984. Adenocarcinoma and Barrett's esophagus: an overrated risk? *Gastroenterology*, 87, 927-933

Spechler, S.J. 2002. Barrett's esophagus. *New England Journal of Medicine*, 346, (11) 836-842

Spechler, S.J., Fitzgerald, R.C., Prasad, G.A., & Wang, K.H. 2010. History, Molecular mechanisms and endoscopic treatment of Barrett's esophagus. *Gastroenterology*, 138, 854-869

Spechler, S.J. 2001. Disputing dysplasia. *Gastroenterology*, 120, (7) 1864-1868 available from: PM:11375967

Srivastava, A., Hornick, J.L., Li, X., Blount, P.L., Sanchez, C.A., Cowan, D.S., Ayub, K., Maley, C.C., Reid, B.J., & Odze, R.D. 2007. Extent for low-grade dysplasia is a risk factor for the development of esophageal adenocarcinoma in Barrett's esophagus. *American Journal of Gastroenterology*, 102, 483-493

Stairs, D.S., Nakagawa, H., Klein-Szanto, A., Mitchell, S.D., Silberg, D.G., Tobias, J.W., Lynch, J.P., & Rustgi, A.K. 2008. Cdx1 and c-Myc foster the intiation of transdifferentiation of the normal esophageal squamous epithelium toward Barrett's esophagus. *Public Library of Science (PLoS One)*, 3, (10) 3534

Strum, M.B., Joshi, B.P., Lu, S., Piraka, C., Khondee, S., Elmunzer, B.J., Kwon, R.S., Beer, D.G., Appelman, H.D., Turgeon, D.K., & Wang, T.D. 2013. Targeted imaging of esophageal neoplasia with a fluorescently labeled peptide: first in human results. *Science Translational Medicine*, 5, (184) ra61

Takubo, K., Aida, J., Naomoto, Y., Sawabe, M., Arai, T., Shiraishi, H., Matsuura, M., Ell, C., May, A., Pech, O., Stolte, M., & Vieth, M. 2009. Cardiac rather than intestinal-type background in endoscopic resection specimens of minute Barrett adenocarcinom. *Human Pathology*, 40, 65-74

The AGA Institute Medical Position Panel 2011. American Gastroenterological Association Medical Position Statement on the management of Barrett's esophagus. *Gastroenterology*, 140, 1084-1091

The Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy 2006. ASGE guideline: the role of endoscopy in the surveillance of premalignant conditions of the upper GI tract. *Gastointestinal Endoscopy*, 63, (4) 570-580

Tileston, W. 1906. Peptic ulceration of the oesophagus. *American Journal of Medical Science*, 132, 240-265

Vakil, N., van Zanten, S.V., Kahrilas, P., Dent, J., Jones, R., & Global Consensus Group 2006. The Montreal definition and classification of gastroesophageal reflux

disease: a global evidence-based consensus. *American Journal of Gastroenterology*, 101, 1900-1920

Vieth, M. 2007. Low grade dysplasia in Barrett's oesophagus - an innocent bystander? *Endoscopy*, 39, 647-649

Vo-Dinh, T., Panjepour, M., Overholt, B.F., Farris, C., Buckley, P.F., & Sneed, R. 1995. In vivo cancer diagnosis of the esophagus using differential normalized fluorescence (DNF) indices. *Lasers in Surgery and Medicine*, 16, 41-47

von Holstein, C.S., Nilsson, A.M.K., Andersson-Engels, S., Willen, R., Walther, B., & Svanberg, K. 1996. Detection of adenocarcinoma in Barrett's oesophagus by means of laser induced fluorescence. *Gut*, 39, 711-716

Vousden, K.H. & Lane, D.P. 2007. p53 in health and disease. *Nat Rev Mol Cell Bio*, 8, (4) 275-283

Wallner, B., Syvan, A., Stenling, R., & Janunger, K.G. 2000. The esophageal Z-line appearance correlates to the prevalence of intestinal metaplasia. *Scandinavian Journal of Gastroenterology*, 35, 17-22

Walsh, D., Bendel, N., Jones, R., & Hanlon, P. 2010. It's not "just deprivation": why do equally deprived UK cities experience different health outcomes? *Public Health*, 124, (9) 487-495

Wang, K.K. & Sampliner, R.E. 2008. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *American Journal of Gastroenterology*, 103, 788-797

Wang, K.K., Wongkeesong, M., & Buttar, N.S. 2005. American Gastroenterological Association technical review on the role of the gastroenterologist in the management of esophageal carcinoma. *Gastroenterology*, 128, (5) 1471-1505

Wang, T.D. & van Dam, J. 2004. Optical biopsy: a new frontier in endoscopic detection and diagnosis. *Clinical Gastroenterology and Hepatology*, 2, (9) 744-753

Wang, X., Ouyang, H., Yamamoto, Y., Kumar, P.A., Wei, T.S., Dagher, R., Vincent, M., Lu, X., Bellizzi, A.M., Ho, K.Y., Crum, C.P., Xian, W., & McKeon, F. 2011.

Residual embryonic cells as precursors of a Barrett's like metaplasia. *Cell*, 145, (7) 1023-1035

Wani, S., Falk, G., Hall, M., Gaddam, S., Wang, A., Gupta, N., Singh, M., Singh, V., Chuang, K., Boolchand, V., Gavini, H., Kuczynski, J., Sud, P., Reddymasu, S., Bansal, A., Rastogi, A., Mathur, S.C., Young, P., Cash, B., Lieberman, D.A., Sampliner, R.E., & Sharma, P. 2011. Patients with nondysplastic Barrett's esophagus have low risk for developing dysplasia or esophageal adenocarcinoma. *Clinical Gastroenterology and Hepatology*, 9, (3) 220-227

Washington, K., Chiappori, A., Hamilton, K., Shyr, Y., Blanke, C., Johnson, D., Sawyers, J., & Beauchamp, D. 1998. Expression of beta-catenin, alpha-catenin and E-cadherin in Barrett's esophagus and esophageal adenocarcinomas. *Modern Pathology*, 11, (9) 805-813

Waxman, I. 2011. Advances in the diagnosis and management of Barrett's esophagus. *Gastrointestinal Endoscopy Clinics*, 21, (1) 1-188

Wei, W., Abnet, C.C., Lu, N., Roth, M.J., Wang, G., Dye, B.A., Dong, Z., Taylor, P.R., Albert, P., Qiao, Y., & Dawsey, S.M. 2005. Risk factors for oesophageal squamous dysplasia in adult inhabitants of a high risk region of China. *Gut*, 54, (6) 759-763

Weston, A.P., Sharma, P., Mathur, S.C., & et al 2004. Risk stratification of Barrett's esophagus: updated prospective multivariate analysis. *American Journal of Gastroenterology*, 99, 1657-1666

White, B.D., Chien, A.J., & Dawson, D.W. 2012. Dysregulation of Wnt/B-catenin signaling in gastrointestinal cancers. *Gastroenterology*, 142, (2) 219-232

Wicha, M.S., Liu, S., & Dontu, G. 2006. Cancer stem cells: an old idea - a paradigm shift. *Cancer Research*, 66, (4) 1883-1890

Wijnhoven, B.P.L., Tilanus, H.W., & Dinjens, W.N.M. 2000. Molecular biology of Barrett's adenocarcinoma. *Annals of Surgery*, 233, (3) 332-337

Wolfsen, H.C. 2009. New technologies for imaging of Barrett's esophagus. *Surgical Oncology Clinics of North America*, 18, 487-502

Woods, L.M., Rachet, B., & Coleman, M.P. 2006. Origins of socio-economic inequalities in cancer survival:a review. *Annals of Oncology*, 17, 5-19

Woodward, T.A., Klingler, P.J., Genco, P.V., & Wolfe, J.T. 1998. Possible p53-independent expression of $p21^{WAF/CIP1}$ in Barrett's esophagus. *Gastrointestinal Oncology*, A705,

Wright, T.A., Gray, M.R., & Morris, A. 1996. Cost effectiveness of detecting Barrett's cancer. *Gut*, 39, 574-579

www.cancerresearchuk.org. Cancerstats. 2006.
Ref Type: Online Source

Yamamoto, Y. & Gaynor, R.B. 2001. Therapeutic potential of inhibition of the NFκB pathway in the treatment of inflammation and cancer. *Journal of Clinical Investigation*, 107, 135-142

Younes, M., Ertan, A., Lechago, L.V., Somoano, J.R., & Lechago, J. 1997. p53 protein accumulation is a specific markers of malignant potential in Barrett's metaplasia. *Digestive Diseases and Sciences*, 42, (4) 697-701

Younes, M., Lebovitz, R.M., Lechago, L.V., & Lechago, J. 1993. p53 protein accumulation in Barrett's metaplasia, dysplasia and carcinoma: a follow up study. *Gastroenterology*, 105, 1637-1642

Zagari, R.M., Fuccio, L., Wallander, M.A., Johansson, S., Fiocca, R., Casanova, S., Farahmand, B.Y., Winchester, C.C., Roda, E., & Bazzoli, F. 2008. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. *Gut*, 57, (10) 1354-1359 available from: PM:18424568

Zagorowicz, E. & Jankowski, J.A. 2007. Molecular changes in the progression of Barrett's oesophagus. *Postgraduate Medicine Journal*, 83, 529-535

Zou, D., He, J., & Ma, X. 2011. Epidemiology of symptom-defined gastroesophageal reflux disease and reflux oesophagitis: the systematic investigation of gastrointestinal diseases in China (SILC). *Scandinavian Journal of Gastroenterology*, 46, (2) 133-141

Zou, H., Molina, J.R., Harrington, J.J., Osborn, N.K., Klatt, K.K., Romero, Y., Burgart, L.J., & Ahlquist, D.A. 2005. Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. *International Journal of Cancer*, 116, (4) 584-591