Investigating the natural history of Barrett’s oesophagus and its progression to oesophageal adenocarcinoma in the setting of improved assessment techniques and endoscopic therapy

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Submitted in total fulfilment of the requirements for the degree of

Doctor of Philosophy

August 2016
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Abstract

Barrett’s Oesophagus (BE) is a condition whereby the columnar cells that normally line the oesophagus are replaced by an intestinal metaplastic cell type. Over time, these new cells can become dysplastic and ultimately can develop into oesophageal adenocarcinoma (EAC). Dysplasia is generally graded by pathologists as either low or high grade dysplasia, though previously the terms mild, moderate and severe dysplasia, as part of a three tier system, were used.

The risk of malignant progression increases with worsening dysplasia. At present, we know that for high grade dysplasia (HGD) the risk of progression is very high and these patients must be treated to prevent the development of cancer. What is not clear from the current literature is how we should be managing low grade dysplasia (LGD) in BE, as malignant progression rates are variably reported and it is unclear whether we should be treating these patients aggressively or whether, in fact, surveillance only is appropriate.

Additionally, over the last five to ten years there have been significant advancements in the therapies used to treat and eradicate dysplastic BE endoscopically, most notably with the use of endoscopic resection of early neoplastic lesions and radiofrequency ablation of dysplastic BE. While these techniques have been shown to be safe and effective in trial populations, there are limited data on effectiveness in real-world cohorts. Furthermore, data have been lacking regarding the durability of outcomes following treatment.

This thesis involved a range of clinical studies that aimed to address the above issues. I studied a cohort of patients who were diagnosed over ten years ago with LGD within BE, and assessed the percentage of those that went on to develop high grade and cancerous lesions without intervention/treatment. The original histology slides from this group were reviewed by two expert gastroenterology pathologists to 1) confirm the original diagnosis of low grade dysplasia and, 2) also grade the dysplasia according to the three tier system. I have shown that while a consensus diagnosis of LGD does appear to confer an increased risk of malignant potential, it is primarily a diagnosis of moderate LGD that accounts for this risk increase. This finding suggests that in addition to BE patients with LGD undergoing expert histological review, consideration should be given to further risk stratifying this group as having mild or
moderate dysplasia, as this may influence a clinician’s decision to heighten surveillance or commence endoscopic therapy.

In the second study I evaluated whether a Specialised Barrett’s Unit was better able to detect and stage dysplastic Barrett’s Oesophagus when compared with community endoscopists. I found that assessment of these patients at a Barrett’s unit resulted in improved detection of mucosal abnormalities and cancers, that were often missed by community endoscopists. Endoscopic mucosal resection (EMR) of early cancers was a critical step in determining a patient’s appropriateness for endoscopic therapy. These results highlight the importance of adequate assessment by endoscopists with the appropriate training in Barrett’s assessment and technical ability to perform EMR of suspected cancers, prior to embarking on a definitive management course.

In a third clinical study, I assessed the safety, effectiveness and durability of combined endoscopic therapy (radiofrequency ablation and endoscopic mucosal resection) in one of Australia’s largest cohorts of patients with dysplastic Barrett’s oesophagus. This group had predominantly advanced histopathology and often complex Barrett’s segments. I found that in this real world cohort, the effectiveness and safety profile are similar to published data but that there was significant risk of recurrence of both Barrett’s and dysplasia. I demonstrated that recurrence appeared to occur more commonly in patients with advanced pre-treatment histology, and frequently at the gastroesophageal junction. This finding has important clinical implications and we advocate careful ongoing surveillance of this region, particularly in patients with prior advanced dysplasia or cancer.
Declaration

This thesis contains no material that has been previously submitted or accepted for the award of any other degree or diploma in any university or other institution. It comprises only my original work except where indicated in the preface and due acknowledgement has been made in the text to all other materials used. This thesis reflects work done during the period of candidature and the text is less than 100,000 words in length, excluding tables, figures, bibliographies and appendices.

Georgina Cameron, August 2016
Preface

The work presented in this thesis was carried out under the supervision of Dr Andrew Taylor, Head of Endoscopy, Department of Gastroenterology, St Vincent’s Hospital Melbourne (SVHM), and co-supervised by Associate Professor Paul Desmond, Department of Gastroenterology, SVHM.

The thesis includes three main results chapters, two of which have been submitted as papers and published, or accepted for publication by peer-reviewed journals. These are listed in detail as the first two publications under the Publications and Presentations section. All papers include a list of authors whose contributions are described in the next couple of paragraphs.

Inspired by a Dutch paper reporting significantly higher progression rates to cancer from low grade dysplasia than previously reported, Chapter 1 of this thesis attempted to recreate and expand on these previously published findings. Associate Professor Richard Williams (Head of Pathology, SVHM) and Dr Michael Christie (Pathology Department, Royal Melbourne Hospital (RMH)) oversaw all the Barrett’s pathology review for this chapter. Dr Stuart Galloway (Pathologist, SVHM) provided assistance in the initial search of the pathology database for patients who fit the criteria for inclusion in the study presented in Chapter 3. Yong Yu, facilitated the recutting of some slides that were used for review of patients studied in Chapter 3.

Chapters 4 and 5 were born from the analysis of the Barrett’s treatment cohort at SVHM and RMH. The SVHM and RMH Barrett’s databases were set-up in 2007/2008 in collaboration with Dr Andrew Taylor, A/Prof Paul Desmond and Prof Finlay Macrae. Dr Chatura Jayasekera, alongside Dr Taylor and Prof Macrae were integral in the recruitment of Barrett’s patients to be considered for what was then, novel endoscopic treatment of Barrett’s oesophagus. Alongside Dr Taylor, Dr Jayasekera maintained the database prospectively between 2010 and 2011 after which I continued this work at SVHM. Dr Francesco Amico was involved in populating the RMH database in 2014 along with Prof Macrae. These databases were the foundation of information used in Chapters 4 and 5. Jenny Peel from Microsoft Access provided technical support for these databases.
Dr Jayasekera collated the preliminary data presented in Chapter 4, comparing assessment of patients in the community versus a specialist Barrett’s Unit.

Endoscopic assessment, treatment and clinical management of the Barrett’s patient cohort presented in Chapters 4 and 5 were performed by Dr Taylor, Prof Macrae, Dr Robert Chen, Dr Jayasekera, and myself between 2008 and 2013. These procedures included gastroscopy with Barrett’s mapping, confocal endomicroscopy, endoscopic mucosal resection, radiofrequency ablation, and management of complications including strictures, bleeding and perforation. Dr Andrew Taylor was my principle supervisor in teaching me these endoscopic techniques.

Review of all pathology for Chapters 4 and 5 was again overseen by A/Prof Williams, with Dr Richard Norris, (Pathologist, SVHM) providing additional support.

Others have contributed to the work presented in this thesis as detailed below:

Robert Chilov assisted with data management and statistical analysis presented in Chapters 3, 4 and 5. Additional statistical support was provided by Dr Raymond Boston, Dr Edward Tsoi, Dr Thai Hong and Dr Darren Wong in the final analysis of Chapter 3.

I was fortunate to receive an Australian Postgraduate Award from the University of Melbourne while undertaking my research studies.
Acknowledgements

There are many people who have contributed to the work presented in this thesis, and I am very grateful for their involvement and support.

Firstly, I would like to thank my principal supervisor Dr Andrew Taylor and co-supervisor A/Prof Paul Desmond, for creating an endoscopy fellowship position that enabled me to further my clinical endoscopy skills, particularly in the field of Barrett’s assessment and treatment, while simultaneously conducting research in this area. I want to thank Andrew for his guidance on which direction to steer this research, and for his continued enthusiasm for the project, and encouragement when I encountered setbacks. I would like to thank Paul for his valuable insight into how to navigate the highs and lows of the research process, and importantly, maintaining my focus on completing this work. I feel incredibly lucky to have had such patient and supportive supervisors to lead me through this process and I am most grateful to them for this.

I would like to thank A/Prof Richard Williams for his significant contribution to this thesis, in reviewing an immense amount of Barrett’s pathology. Without his enduring commitment to research in this area, this thesis would not have been possible. I would like to also thank Dr Michael Christie, who also reviewed endless pathology slides, and was so generous with his time while completing his own research studies.

I also want to thank Dr Chat Jayasekera for collecting much of the preliminary clinical data for the Barrett’s database at St Vincent’s Hospital as this provided the foundations for much of the work presented in this thesis. Thanks also go to Prof Finlay Macrae for his initial involvement in helping to shape the direction of this thesis. His contribution the setting up the database at Royal Melbourne Hospital and ongoing management of this group of patients was also integral to much of the clinical results presented in Chapters 4 and 5.

I gratefully acknowledge the funding support I received from the Australian Postgraduate Award through the University of Melbourne while completing my studies.

To my fellow research fellow and friend Dr Ola Niewiadomski, thank you for being a sounding board for the trials and tribulations of juggling the research process and motherhood. I am very thankful that you have been several steps ahead of me along this path, and have helped
me navigate my way towards what sometimes seemed like an impossible finish line. Thank you to many of the other fellows, past and present, for providing often opportune assistance with various hurdles as they arose; statistics, formatting, proof reading to name just a few, and being a welcome distraction, during the research years.

I would like to thank both my parents and parents-in-law for their encouragement while working and studying, and particularly for the many hours of babysitting, without which I could not have completed these studies.

To my husband Rob, I am so thankful for your love and support. I cannot say how much I appreciate the innumerable hours you spent helping with statistical analysis, educating me on navigating the intricacies of Microsoft Excel and introducing me to the joys of Pivot Tables. I am looking forward to spending more quality time with you and our boys. To my son Nicholas, it has been so wonderful to see you grow into such a funny, bright, and talkative little boy. You have provided often hilarious relief from the research process and I am very proud of you. To my gorgeous, happy “baby James”, your due date in August was a true motivator to finish this work! Thank you for arriving a week late, allowing me the extra time to finally submit my thesis and properly enjoy my time with you.
Publications and Presentations arising from this Thesis

Journal Publications:

*Cameron GR*, Jayasekera CS, Williams R, Macrae FA, Desmond PV, Taylor AC. Detection and staging of esophageal cancers within Barrett’s esophagus is improved by assessment in specialized Barrett’s units. *Gastrointestinal Endoscopy*. 2014 Dec; 80 (6):971-83. DOI: 10.1016/j.gie.2014.03.051


Abstract Publications and Oral Presentations:


Oral presentation at Australian Gastroenterology Week (AGW) Adelaide, 2012


Oral Presentation at Digestive Diseases Week (DDW) Orlando 2013
Cameron GR, Jayasekera CS, Amico F, Williams R, Macrae FA, Desmond PV, Taylor AC. Radiofrequency ablation combined with Endoscopic Mucosal Resection is safe, effective and durable in the majority of patients for eradicating dysplastic Barrett’s oesophagus- Updated outcomes for the largest reported Australian experience with RFA. Journal of Gastroenterology and Hepatology 28 (s2): 35-51. October 2013.

Oral presentation at Australian Gastroenterology Week (AGW) Melbourne, 2013

Cameron GR, Jayasekera CS, Amico F, Williams R, Macrae FA, Desmond PV, Taylor AC. Radiofrequency ablation combined with Endoscopic Mucosal Resection is safe, effective and durable in the majority of patients for eradicating dysplastic Barrett’s oesophagus- Updated outcomes for the largest reported Australian experience with RFA. United European Gastroenterology Journal; 2013: 1 (s1) A40.

Oral presentation at United European Gastroenterology Week (UEGW) Berlin 2013

Cameron GR, Jayasekera CS, Amico F, Williams R, Macrae FA, Desmond PV, Taylor AC. Radiofrequency ablation combined with Endoscopic Mucosal Resection is safe, effective and durable in the majority of patients for eradicating dysplastic Barrett’s oesophagus- Updated outcomes for the largest reported Australian experience with RFA.

Best Moderated Oral Poster, St Vincent’s Hospital Melbourne Research Week 2013

Mahindra P, Cameron G, Desmond P, Williams, R, Taylor A. Rapid Progression from LGD to HGD Barrett’s- Myth or reality. GI Endoscopy 2015; 81, Issue 5, AB500 - AB501. DOI: 10.1016/j.gie.2015.03.98
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Chapter 1: Introduction, Literature Review and Aims of Research

1.1 Introduction

Barrett’s oesophagus (BE) is a condition where the squamous lining of the distal oesophagus is replaced with columnar epithelium, resulting in proximal displacement of the squamocolumnar junction. This is due to chronic inflammation and tissue injury secondary to reflux of gastric contents into the distal oesophagus. Over time, with ongoing tissue inflammation, the new epithelium can develop dysplasia (thought to progress through the steps of low grade dysplasia then high grade dysplasia) and ultimately lead to the development of oesophageal adenocarcinoma (EAC), a cancer that left untreated has a poor long-term survival.

In the last ten years there have been significant developments, not only our ability to detect and monitor BE, but also in our management of dysplastic BE. With development of newer endoscopic therapies, in cases which would have been previously managed surgically, we now have the potential to offer curative endoscopic therapy, even for early oesophageal cancers, thus reducing patient morbidity and mortality. The long-term outcomes of these treatments are still being assessed and it is important to see how controlled trial data applies in real world cohorts. It is also becoming apparent that adherence to best practice guidelines in managing dysplastic BE by community gastroenterologists is low and therefore the assessment and management of patients with dysplastic BE is likely improved when performed by endoscopists with expertise in this field.

While it is widely accepted that patients with HGD and neoplastic Barrett’s receive definitive treatment, the question of whether to treat patients with LGD is more controversial as the natural history of LGD and its risk for progression to adenocarcinoma is poorly understood. This is in part due to considerable variation existing between pathologists when grading dysplasia within Barrett’s which has likely impacted the reported data of progression rates for patients with documented LGD.
1.2 Overview of Barrett’s Oesophagus

1.2.1 History of Barrett’s Oesophagus

The eponymous term “Barrett’s oesophagus” was named after prominent cardiothoracic surgeon and journal editor Norman Rupert Barrett in the late 1950s. As described above, the term is used to describe the columnar lined mucosa of the distal oesophagus caused by acid reflux. This phenomenon had actually been described around 50 years earlier by a pathologist Wilder Tileston who presented a case series of three patients with peptic ulcers of the distal oesophagus secondary to reflux.8 Barrett himself had in fact believed that the columnar lined mucosa was part of the proximal stomach, fixed to a shortened oesophagus within the chest.9 His idea was challenged by Allison and Johnstone in 1953, who gave multiple histological reasons for this area representing the distal oesophagus and not the stomach. These included that unlike the stomach, the columnar-lined organ lacked a peritoneal covering, harboured squamous epithelial islands, and had submucosal glands and a muscularis propria typical of the oesophagus.8, 10 Perhaps it was because they were challenging the editor of the journal they had submitted to, that the authors suggested that the ulcers seen in the columnar lined oesophagus should be referred to as Barrett’s Ulcers. Barrett eventually accepted Allison and Johnstone’s arguments and, in a report published in 1957, suggested that the condition should be called “lower oesophagus lined by columnar epithelium”11 and the eponymous term Barrett’s oesophagus was born.

Three types of distal oesophageal columnar mucosa were described in 1976: a gastric fundic-type epithelium, a junctional (cardia-type) epithelium, and an intestinal-type metaplasia (or “specialized columnar epithelium”), that contained prominent goblet cells. It is the latter, intestinal metaplasia, that is generally accepted as being required for the diagnosis of Barrett’s oesophagus although this remains contentious.12

1.2.2 Definition of Barrett’s Oesophagus (Endoscopic and Histologic)

In the United States, Barrett’s oesophagus is defined as the proximal displacement of the squamocolumnar junction (SCJ) by at least 1cm above the gastroesophageal junction (GOJ), with the additional histological finding of intestinal metaplasia (IM).1, 3, 13 Though there is general agreement regarding the endoscopic features defining BE, there is not the same consensus with regard to histological definition. The view held by those in the US and many
other countries worldwide is that intestinal metaplasia, defined as columnar epithelium containing goblet cells, must be present.\textsuperscript{14} This is in contrast, however, to the British Society of Gastroenterology’s guidelines from 2006 and again in 2014 that state that columnar metaplasia without goblet cells is adequate for the diagnosis of BE.\textsuperscript{15, 4} In these latter guidelines, they do however state that while goblet cells are not required for the diagnosis of Barrett’s, their absence should be taken into account when deciding on the clinical management.

The requirement for specialised intestinal metaplasia to be present within the columnar lined epithelium (CLE) for the diagnosis of Barrett’s continues to be a point of debate between the Americans and the British. This is due to the apparent difference in risk for developing EAC in CLE containing IM vs. non-IM CLE. A large population based study of 8522 patients with BE, defined as CLE of the oesophagus with or without specialized IM, who were followed for 7 years, demonstrated significantly higher EAC rates in CLE with IM compared with non-IM CLE at index biopsies (0.38\% vs. 0.07\% per year; hazard ratio (HR)=3.54; P<0.001).\textsuperscript{16} Not all studies have corroborated this finding, however. For example, a study with 712 patients found that those who had glandular mucosa on biopsy without intestinal metaplasia had a similar cancer risk to those with specialized IM.\textsuperscript{17} Though the DNA content abnormalities of metaplastic CLE with or without goblet cells is similar\textsuperscript{18} some studies have suggested that cancer most commonly occurs in CLE with IM. In a study comparing the DNA aberrations in IM and non-goblet cell metaplasia genomes, IM genomes had a much higher frequency of cancer-associated mutations than the latter type.\textsuperscript{19}

Despite findings that CLE with IM has purportedly higher risk of development of EAC, one argument put forward by the British for including non-IM CLE in the definition of Barrett’s is that there may be sampling error leading to incorrect classification of CLE as non-IM CLE. Certainly, it has been shown that the yield for detecting IM in BE was almost doubled when 8 biopsies were taken vs. 4 biopsies (67.9\% vs. 34.8\% yield). This was demonstrated in a retrospective analysis of biopsies taken at 296 endoscopies over a 4-year period for Barret’s segments of mean 4cm length. To obtain a 100\% yield for IM, a minimum of 16 biopsies was required.\textsuperscript{20}

The 2015 American guidelines do acknowledge the potential for sampling error, however continue to advocate for the definition of BE to include IM within CLE unless future studies
demonstrate a significantly higher risk of malignant progression in non-IM CLE patients. One of the reasons for this is the potential negative impact diagnosing patients may have (both from an insurance and quality of life perspective). In a study of 118 patients undergoing Barrett’s surveillance, two thirds overestimated their risk of developing cancer in one year (mean of 13.6% risk of EAC per year), and one third overestimated their lifetime risk.

1.2.1.1 Endoscopic evaluation

Segments of columnar mucosa <1cm in length are classified as specialised IM of the oesophagogastric junction and not BE. It has been classified as a separate entity because this subset of patients has not demonstrated an increase in the development of dysplasia or EAC in long term follow-up of cohort studies when compared with patients who have >1cm segments. A population cohort study of 487 patients (401 with BE and 86 with IM at the GOJ) were followed for a median 7-8 years. Subjects with BE had a higher prevalence of advanced neoplasia than the latter group. Additionally, they had a cumulative risk of malignant progression of 7% at 10 years and increased risk of dying from EAC (mortality ration of 9.6) compared with those with IM at the GOJ, none of whom developed EAC.

The location of the GOJ is not always easy to identify, but is important when describing a BE segment length. Endoscopic localization of the gastroesophageal junction and measurement of z-line displacement is moderately reproducible, with mild to substantial variation in measured segment lengths between examinations. A study comparing the endoscopic and manometric location of the GOJ in 192 patients with repeat endoscopies 6 weeks apart found a 10% difference in the location at the second endoscopy by both modalities. These differences are likely related to change in position with respiration and peristalsis. A Japanese study of 84 endoscopists who were asked to identify the distal oesophagus concluded that using the proximal extent of the gastric folds was more accurate than identifying palisade vessels of the distal oesophagus (which could extend below the GOJ in the majority of patients).

Though studies have demonstrated an increased risk of developing EAC with increasing BE segment length, classification of BE into short (<3cm) or long (>3cm) segment is no longer recommended as it has little clinical relevance in that there is no evidence that a risk gradient may be demarcated at a particular segment length.
Use of the Prague Classification to assess the position of the GOJ, circumferential (C) length and maximal (M) length of the BE segment, has been shown to have high overall validity for the endoscopic assessment of visualized BE lengths, with overall reliability coefficients of 0.95 and 0.94 respectively. Reliability coefficients are also reportedly high for recognition of BE >1cm (0.72), location of GOJ (0.88) and location of diaphragmatic pinch (0.85), but not BE segments <1cm.  

1.2.1.2 Histology and biopsy protocol

As stated, endoscopic diagnosis of Barrett’s is not always straightforward or easily reproducible and often an endoscopist may consider a diagnosis of Barrett’s but await histological confirmation before assigning that label. A longitudinal cohort study of 107 patients with suspected BE endoscopically, but without histological confirmation of IM, found that 29% had IM confirmed at subsequent endoscopy 1-2 years later, therefore, repeat endoscopy should be considered in patients where BE is suspected endoscopically. An important message however was that the majority (71%) did not have IM detected in two years of follow-up, and this finding supports withholding a BE diagnosis for individuals with only suspected CLE.  

IM of the cardia is common (reported in around 20% of patients having routine upper endoscopy), however, taking biopsies of a normal or slightly irregular GOJ is not routinely recommended, as IM of the cardia does not equate to an increased association with BE compared with controls. IM at the GOJ has been shown to be associated with H. pylori but not EAC.  

IM has been shown to be unevenly distributed in a BE segment. Among patients with long segments of oesophageal columnar metaplasia, over 20% may not have intestinal metaplasia detected on a single set of endoscopic biopsies due to sampling error or interim development of intestinal metaplasia after the first examination. As previously mentioned, the yield for detecting IM in one study was almost doubled when 8 biopsies were taken in a BE segment vs. 4 biopsies (67.9% yield vs 34.8%). To maximise detection of IM and dysplasia it is recommended that the Seattle protocol, which involves taking 4 quadrant biopsies every 1-2cm of BE and targeted biopsies of any visible mucosal abnormality using jumbo biopsy forceps be used. It has been shown previously that rigorous biopsy protocols improve
the chances of detecting dysplasia and curable cancers. Despite this, some studies have shown that adherence to Seattle protocol by community endoscopists is as low as 27%.\textsuperscript{5, 38}

1.2.1.3 Summary of Recommendations based on evidence provided above:\textsuperscript{3}:

1. A diagnosis of BE should be made when there is \( \geq 1 \) cm columnar lined mucosa extending above the GOJ, with biopsy confirming IM
2. The GOJ should not routinely be biopsied if the z-line is normal or slightly irregular
3. The Prague classification should be used to describe the circumferential (C) and maximal (M) extent of the BE segment
4. The location of the diaphragmatic pinch, gastric folds and squamocolumnar junction should be described
5. At least 8 biopsies should be taken in in patients with suspected BE, or in short segment Barrett’s (1-2 cm), at least 4 biopsies for each circumferential segment of Barrett’s and 1 biopsy per cm tongue of BE
6. If Barrett’s is suspected endoscopically but not confirmed histologically, consider repeating endoscopy in 1-2 years

1.3 Epidemiology of Barrett’s Oesophagus

1.3.1 Prevalence

Two large epidemiological studies estimate the prevalence of Barrett’s in the population to be between 0.4% and 1.6%. The first study, from Sweden, surveyed a random sample of the adult population in 2 municipalities (n=3000; 74% response rate) regarding gastrointestinal symptoms. It found a 1.6% prevalence of BE in a random subsample of responders (n=1000) who underwent upper endoscopy.\textsuperscript{39} A second large study analysing both clinical and diagnoses found a prevalence of 0.4%.\textsuperscript{40}

Certain other studies have described a substantially higher prevalence of BE, but these results are likely confounded by the risk factor profiles of the study subjects, and not reflective of the broader population. In a study of almost one thousand patients undergoing colonoscopy, with gastroscopy added, BE was found in 6.8%. Importantly however, this patient population mainly comprised older, white males who are representative of the highest risk group.\textsuperscript{41} Another study of 110 asymptomatic patients who were undergoing sigmoidoscopy for CRC
screening reported a 25% BE prevalence. Again, this cohort were predominantly male, veterans and aged over 50. A Swedish prospective cohort study (Johansson et al) that performed endoscopy on 769 patients (mean age 53 years, 43% men) found a Barrett’s prevalence of 4% (predominantly in women (69%)) and overall IM prevalence of 14% (increasing by 8% per year of age). Certainly, in patients with chronic gastroesophageal reflux disease (GORD) the prevalence of Barrett’s oesophagus increases to between 5-15%.

1.3.2 Incidence
The incidence of BE is rising, and this rise does not appear to be due to the greater number of gastroscopies performed in the population. One prospective cohort study showed BE detection doubled from 20/1000 endoscopies to 40/1000 endoscopies over a 5-year period. A prevalence study from Australia showed that the endoscopic frequency of BE increased from 3 to 19 per one thousand endoscopies (p<0.001) when comparing years 1990 and 2002.

1.3.3 Incidence of Oesophageal Adenocarcinoma (EAC)
As the incidence of Barrett’s has been rising, so too has the incidence of EAC, with rates increasing since the 1970s. A 1998 SEER study showed the incidence of EAC among white males rose >350% since the mid-seventies. A later 2013 SEER analysis showed that while EAC incidence and incidence-based mortality continues to increase in the United States, the overall rate increase appears to be slowing since the mid-1990s. The average yearly percentage increase in incidence was around 6% for men and women. Despite the incidence increase, since the late-1990s, the incidence-based mortality for early stage cancers has levelled off noticeably.

In a study of over 11,000 patients with BE, followed for a median of 5 years, it was found that when compared with the risk in the general population, the relative risk of EAC among patients with BE was 11.3 and the annual risk of EAC was 0.12%. Where LGD was detected at the first gastroscopy the EAC incidence rate was 5.1 cases per 1000 person-years, compared with 1 case per 1000 person-years where no dysplasia was detected.
1.4 Pathology of Barrett’s Oesophagus

As previously stated, Barrett’s oesophagus is a condition where the normal squamous mucosa of the distal oesophagus is replaced with columnar epithelium containing intestinal metaplasia.\(^{27}\) This is considered to be the first step in the progression to oesophageal adenocarcinoma (EAC). Neoplastic progression from non-dysplastic Barrett’s Oesophagus (NDBE) to EAC is considered to be a multistep process where patients progress through stages of low grade dysplasia (LGD) to high grade dysplasia (HGD) before developing adenocarcinoma. Data that support this come from studies of oesophagectomy specimens containing adenocarcinoma which have adjacent areas with varying degrees of dysplasia and intestinal metaplasia. The presence of the latter is again supportive of the idea that IM is required for malignant progression.\(^{49-53}\)

This section details the histology of Barrett’s oesophagus, from non-dysplastic through the increasing grades of dysplasia and neoplasia, as well as its pathogenesis.

1.4.1 Columnar-lined Epithelium

The distal oesophagus can harbour three types of columnar epithelium – cardia type, fundic type and intestinal metaplasia.\(^{12}\)

1.4.1.1 Cardia and Fundic type

Below the gastro-oesophageal junction (GOJ) is the gastric cardia, a small part of the stomach where the mucosa consists of loosely packed mucous glands and very few functional gastric cells.\(^{54}\) The mucosal surface area of the cardia tends to increase over time, and within this area, there is considerable variation in cell types seen, primarily influenced by factors such as acid exposure.\(^{55}\) H. pylori infection can lead to development of intestinal metaplasia (IM) of the cardia and this is considered a columnar to columnar metaplastic reaction. This is important to differentiate from IM of the distal oesophagus associated with BE (which represents a squamous to columnar cell transition) as the risk of dysplastic or neoplastic progression with reflux-associated IM of the oesophagus is higher than for patients with H.pylori-associated IM of the cardia.\(^{56}\) Patients with IM of the cardia are not currently recommended to undergo ongoing endoscopic surveillance.
1.4.1.2 Intestinal Metaplasia

Intestinal metaplasia (IM) denotes the presence of goblet cells within columnar epithelium.\footnote{57} First described in 1951\footnote{58} goblet cells, described due to their shape, are epithelial cells containing acid mucin-filled cytoplasm\footnote{59} that are interspersed among columnar cells. They often exhibit a slightly more basophilic (blue) cytoplasm than the adjacent columnar cells.

Hematoxylin-eosin (HE) staining generally enables good visualisation of goblet cells (Figure 1.1a), however specialised histochemical stains are superior in demarcating true goblet cells from columnar cells and pseudogoblet cells. Goblet cells contain mucins (sialomucins and sulphated mucins) which stain positively with alcian blue at pH 2.5. This makes them distinct from adjacent columnar cells, which contain neutral mucins and are negative on alcian blue staining (Figure 1.1b).\footnote{60} An alcian blue stain performed in conjunction with a periodic-acid–Schiff (PAS) stain, can also be used to highlight the contrast between the alcian-blue positive goblet cells and strongly PAS-positive columnar cells (Figure 1.1c).\footnote{57}

Pseudogoblet cells are gastric foveolar cells that may be confused with goblet cells as they may stain weakly with alcian blue due to the presence of some mucin within the cell.\footnote{57}

A diagnosis of Barrett’s oesophagus has significant clinical implications for patients, both in terms of cancer risk and need for ongoing endoscopic surveillance, therefore it is important that Barrett’s associated IM is correctly identified. One study found that 38% of cases of gastric metaplasia (without IM) were called Barrett’s oesophagus by community based pathologists, which was mainly due to the presence of pseudogoblet cells which were mistaken for goblet cells.\footnote{61} The American Gastroenterological Association suggest that specialised histochemical stains may me a useful adjunct to improve diagnostic certainty, when there are only few goblet cells or numerous pseudogoblet cells present, to increase diagnostic certainty, that these stains are not routinely required to diagnose IM.\footnote{27} Importantly, IM of the GOJ without endoscopic evidence of BE, is relatively common (reported between 9% and 36% of patients),\footnote{62-64} however, the diagnosis of Barrett’s should only be made when there is both histological and endoscopic evidence to support the diagnosis.\footnote{1}
1.4.2 The Revised Vienna Classification and Dysplasia Subtypes

The revised Vienna Classification for gastrointestinal mucosal neoplasia was created in an attempt to standardise histologic terminology into biologically similar groupings with scores of 1-5 depending on the presence or absence of dysplasia or malignancy. The aim was to improve consensus diagnoses of degree of dysplasia/neoplasia between pathologists worldwide.65

1.4.2.1 Negative for Dysplasia

In non-dysplastic Barrett’s mucosa, the glands are simple, round or tubular shapes, and are separated by intervening lamina propria. There is no architectural complexity (e.g. irregularly shaped glands, glandular crowding, back-to-back or cribriform glands). The epithelial cells
maintain their polarity, with nuclei oriented toward the basal membrane. Basal mitotic activity may be seen (as noted below), but mitotic figures are not seen at the mucosal surface, and abnormal mitotic figures are not seen. The epithelial cells exhibit maturation with decreased nuclear/cytoplasmic ratios as they progress from the base of the crypts toward the luminal surface.\textsuperscript{57}

\textbf{Figure 1.2} \textsuperscript{66} (a) Intestinalised epithelium in Barrett’s oesophagus (BE) with mild regenerative changes. Nuclei are regularly shaped, and there is relatively low nuclear/cytoplasmic (N/C) ratio of the cells. (b) Marked regenerative change (tufting of epithelial cells, low N/C ratio, prominent nucleoli, preservation of cell polarity), close to squamocolumnar junction seen at high-power.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image1.png}
\caption{Intestinalised epithelium in Barrett's oesophagus (BE) with mild regenerative changes. (a) Nuclei are regularly shaped, and there is a relatively low nuclear/cytoplasmic (N/C) ratio of the cells. (b) Marked regenerative change (tufting of epithelial cells, low N/C ratio, prominent nucleoli, preservation of cell polarity), close to squamocolumnar junction seen at high-power.}
\end{figure}

\subsection*{1.4.2.2 Indefinite for Dysplasia}
This category is used when there is blurring between the morphological characteristics of true dysplasia and regenerative/inflammatory atypia.\textsuperscript{57} It is important to understand that this diagnosis does not lie between NDBE and LGD but may, in fact, mean that the patient has features suspicious for but not diagnostic of HGD. Factors leading to the diagnosis of indefinite for dysplasia may be technical (such as poor staining, cross cutting or loss of the surface epithelium), or due to severe active inflammation or ulceration leading to marked atypia. Diagnosing dysplasia in the presence of inflammation is should be done so judiciously, and instead, indefinite for dysplasia is often the more appropriate label. \textsuperscript{59} These patients are
recommended to have at least 6 weeks of acid suppression and then undergo repeat endoscopy and biopsy.\(^3\) Features that favour the diagnosis of dysplasia are the presence of an abrupt transition from normal to atypical epithelium, in addition to nuclear polymorphism, atypical mitoses and loss of nuclear polarity.\(^6\) A feature that favours regenerative change over dysplasia is that of “surface maturation” where the cytological atypia of cells seen in the deeper glandular mucosa (nuclear enlargement, slight hyperchromasia and stratification, and increased mitotic activity). These features are not diagnostic for dysplasia without surface involvement and indefinite for dysplasia is the appropriate diagnosis in this instance.\(^5,9\) Figure 1.3 shows an area considered indefinite for dysplasia.

**Figure 1.3** An area thought to be indefinite for dysplasia, seen at high-powered view. Although the crypts show some features of LGD, however as there is no surface epithelium present, evaluation of surface maturation is not possible.\(^6\)

#### 1.4.2.3 Low Grade Dysplasia

In LGD, the glandular/crypt architecture is generally preserved and the diagnosis is based on cytological atypia (slight enlarged and hyperchromatic elongated nuclei (pencil shaped), with faint nucleoli). There is typically reduced goblet cell density in areas of dysplasia, and less mucin present within dysplastic cells. Increased mitoses are seen, increased N/C ratio, possible slight loss of cell polarity, and absence of surface maturation. As noted previously, there is often an abrupt transition from dysplastic to non-dysplastic epithelium.\(^6\) Figure 1.4a/b.
Figure 1.4 (a) Barrett’s with LGD (medium-power view) showing pencil-shaped, hyperchromatic and stratified nuclei. (b) On high-power view the nuclei show clumped chromatin and faint small nucleoli.  

1.4.2.4 High Grade Dysplasia

In HGD, one sees greater cytological abnormalities and architectural distortion than in LGD, but the cut-off between the two grades is somewhat arbitrary and therefore differentiating the two can be difficult. Important features helpful in distinguishing LGD from HGD include loss of cell polarity, particularly at higher levels of the crypts and surface epithelium, full thickness nuclear stratification, atypical mitoses or substantial architectural distortion in the area of HGD. Cytologically, goblet cells are absent or reduced in number and there is significant mucin depletion. Cells with HGD show nuclear pleomorphism, increased N/C ratio, atypical mitoses, loss of cell polarity and full thickness nuclear stratification. Nucleoli, when present are often large and irregularly shaped (Figure 1.5b).
Figure 1.5 (a) HGD with crowded crypts and severe nuclear stratification and mild architectural distortion. At high-power (b), the nuclei have prominent nucleoli and a significant loss of cell polarity. The NC ratio of HGD is markedly increased when compared with LGD. 

1.4.2.5 Intramucosal Cancer (IMC) and Submucosal Cancer (SMC)

IMC is diagnosed when a single or small collection of neoplastic cells pass through the basement membrane to infiltrate the lamina propria. (Figure 1.6a). These cells may also be seen within the muscularis mucosa but the submucosa is never breached. Histological recognition of lamina propria invasion may be difficult because of the lack of objective criteria. Recognition of particular patterns used by GI pathologists to identify lamina propria invasion have poor to moderate agreement (0.21-0.47 kappa value).
Submucosal cancer (SMC) is where there is unequivocal invasion of the submucosa or deeper structures, often accompanied and characterised by a desmoplastic response in the tissue stroma to invasive tumour cords/acini.4

1.4.3 Pathogenesis of Barrett’s Oesophagus

The process by which the squamous mucosa of the distal oesophagus transforms to columnar metaplasia and the subsequent progression to dysplasia/neoplasia, is not completely understood.8 This chapter aims to summarise the current understanding of how genetic and epigenetic factors influence this process.

1.4.3.1 Development of Metaplasia (from squamous mucosa)

When one adult cell type is replaced with another, it is termed metaplastic change, and this transformation is required before a cell might further undergo dysplastic or neoplastic change.70 The precursor cell from which the intestinal metaplasia of Barrett’s oesophagus develops has not yet been identified. It has been proposed that acid reflux might induce alterations in the expression of key developmental transcription factors, causing mature oesophageal squamous cells to change into columnar cells (transdifferentiation) or causing immature oesophageal progenitor cells to undergo columnar rather than squamous differentiation (transcommitment).71-73 In a rat model of reflux oesophagitis, metaplasia developed from bone marrow stem cells that had entered the bloodstream and settled in the reflux-damaged oesophagus.74 Studies in mouse models have suggested that metaplasia might result from stem cells that have migrated upward from the gastric cardia75 or from
proximal expansion of embryonic-type cells at the gastroesophageal junction.\textsuperscript{76} It is not clear which of these processes contribute to the pathogenesis of Barrett’s oesophagus in humans.

The Barrett’s metaplastic lining is more resistant to damage than the squamous epithelium that it has replaced. A study looking at the thickness of BE compared with normal squamous mucosa in 200 patients found Barrett’s columnar epithelium is only minimally thicker (mean 0.50 mm) than normal squamous epithelium (0.49 mm).\textsuperscript{77} This difference does not appear to be clinically significant and does not influence choice nor efficacy of treatment.

**Role of acid and bile reflux**

Barrett’s metaplasia arises as a consequence of chronic reflux of gastric contents (containing acid, bile salts) and subsequent reflux oesophagitis. It is thought that oesophageal metaplasia may result from the induction of transcription factors (CDX1, CDX2, SOX9) or via activating developmental signalling pathways that determine an intestinal phenotype.\textsuperscript{73} Both animal and human models have demonstrated that bile from the duodenum may play an important role in the development of BE. One study found that columnar lined epithelium developed in the distal oesophagus of rats in whom the duodenum had been directly joined to the oesophagus (i.e. without exposure to stomach acid).\textsuperscript{78} Another study demonstrated that bile acids may induce oxidative DNA stress, another potential contributor to Barrett’s development.\textsuperscript{79}

Barrett’s metaplasia is thought to result from an increase in expression or activation of CDX1 and CDX2 transcription factors in the squamous cells as a result of acid and or bile exposure. This has been demonstrated in animal and ex-vivo human models.\textsuperscript{80-91} CDX1 and CDX2 are a type of homeotic gene which are determine the type of gastrointestinal cell type that arises from the endoderm.\textsuperscript{92}

**Stimulation of Developmental Signaling Pathways by GORD**

The currently proposed pathogenic pathway to Barrett’s metaplasia is that GORD induces the expression of CDX genes via morphogenetic factors such as Bone Morphogenic Protein 4 (BMP-4),\textsuperscript{93, 94} a protein involved in regulation of cellular differentiation, migration and proliferation. The evidence for this includes one animal study, where rats exposed to acid reflux had increased BMP-4 expression compared with the normal oesophageal squamous epithelium.\textsuperscript{95} Another ex-vivo human study showed gene pattern changes occurring in
squamous tissue that had been exposed to BMP-4, similar to gene patterns seen in Barrett’s mucosa.\textsuperscript{95}

Finally, the Hedgehog signaling pathway likely plays a role in oesophageal metaplasia. In a mouse model of reflux oesophagitis and BE, Sonic hedgehog expression was found in the Barrett’s metaplasia as well as in oesophageal squamous cells prior to the development of intestinal metaplasia, but not in normal adult oesophageal epithelium.\textsuperscript{72}

In summary, it appears that it is immune factors, chronic inflammation with resultant oxidative stress, and increased CDX transcription factors that influence the development of BE and its dysplastic progression,\textsuperscript{96, 97} though their relative roles remain incompletely understood.

1.4.4 Development of Barrett’s neoplasia

1.4.4.2 Genetic alterations

While there are many genetic variations implicated as factors in the development of Barrett’s neoplasia, none have been singled out to be crucial,\textsuperscript{96} therefore the usefulness of molecular biomarkers in clinical practice is somewhat limited.

Oncogene overactivity

Cyclin D1, A, E

Cyclin D1, A and E are subtypes of the cyclin family of oncogenes which are regulators of cell cycle progression. Increased expression of Cyclin D1, A and E has been found in Barrett’s tissue (both non-dysplastic and dysplastic) compared with normal squamous mucosa, in several studies,\textsuperscript{98-100} with the Cyclin A oncogene seemingly the most important. One study demonstrated a 7.6-fold increased risk of malignant progression for NDBE biopsies with cyclin A present compared with biopsies with absent cyclin A expression.\textsuperscript{99}

In a prospective study of 307 patients with BE, 12 developed EAC. Case-controlled analysis demonstrated a statistically significant increase in risk of malignant progression in patients whose recruitment biopsies stained positive for cyclin D1 (OR=6.85) suggesting its possible usefulness as a biomarker for progression risk.\textsuperscript{101}
Apoptosis avoidance

Apoptosis avoidance appears to be mediated by increased expression of COX-2, (via its prostaglandin production), with one study demonstrating substantially higher expression of COX-2 protein in Barrett’s metaplastic/dysplastic/neoplastic tissue, compared with (normal) squamous mucosa. This increased expression was inducible by acid and bile exposure. COX-2 inhibitors have been proposed as a chemoprevention strategy, based on this evidence (See Chapter 1.12).

Chromosomal abnormalities

Abnormal chromosomal cell content (aneuploidy) is implicated in Barrett’s metaplastic and neoplastic progression with increased risk of EAC among patients with BE (RR risk of 4.4 for tetraploidy, 11 for aneuploidy, and 20 when both chromosomal abnormalities are present). In a case controlled study of 27 patients with BE who progressed to HGD/EAC and 27 who did not, it was found that aneuploidy, strong Ki67 overexpression and moderate p53 overexpression were associated with an increased risk of malignant potential (HRs of 3.5, 5.2 and 6.5 respectively), irrespective of histological diagnosis. On further multivariate analysis, when LGD was present, aneuploidy was no longer a predictive risk factor.

Generalised DNA damage is also frequently seen in BE. This was demonstrated in one study where not only was DNA damage higher in BE mucosa when compared with normal oesophageal or gastric mucosa (P<0.001), but that the higher levels of DNA damage correlated with a higher risk for neoplastic progression compared with lower levels (OR=9.4; P=0.044). Microsatellite instability (seen in the development of colorectal cancer) may have a minimal role in the pathogenesis of BE.

Key growth regulatory pathways that contribute to neoplastic progression of Barrett’s

Tumour suppressor gene inactivation results in the uninhibited progression from G1 to S phase in the cell cycle and therefore uncontrolled cell proliferation. It is a critical step in the development of all cancers and can occur as a result of a nucleotide base excision or deletion, the deletion of a chromosomal region containing the gene (loss of heterozygosity), or promoter hypermethylation.
P16/Rb pathway

Rb is a protein that controls the major point of regulation in the cell cycle, and is critically important in regulating cell proliferation. P16 regulates the synthesis of proteins that control Rb function. Inactivation of p16 (via hypermethylation or loss of heterozygosity) allows cells to pass unhindered from G1 into S phase of the cell cycle and is, in fact, the earliest and most common genetic alteration found in NDBE. P16 abnormalities appear early on in the development of neoplasia within BE. For example, studies have reported p16 inactivation in up to almost 90% of biopsy specimens from patients with NDBE, with inactivation occurring most commonly through hypermethylation of its promoter (an epigenetic event). Loss of heterozygosity also occurs commonly. A study of EAC-containing oesophagectomy specimens found hypermethylation of the p16 promoter in 43% of the normal epithelium, 77% of associated Barrett's mucosa, and 85% of EACs.

The Rb protein may itself be a target for inactivation in the latter stages of Barrett’s carcinogenesis but not in NDBE.

P53 pathway

P53 is another tumour suppressor gene that also plays a role in cell apoptosis. When the p53 pathway is disrupted, cells are able to replicate without constraint, and to resist apoptosis. Abnormal p53 expression has been detected (with immunohistochemical staining) in NDBE and in increasing levels with progression to dysplasia/neoplasia. P53 abnormalities are usually associated with cancer development in BE but have been demonstrated to occur in high frequency in NDBE also; 50% to 90% of samples in one study. Flow cytometry is the best method for assessing p53 abnormalities. Loss of heterozygosity (LOH) and mutation are the main cause of p53 abnormalities, with the former the best marker for progression to HGD/EAC from NDBE. Cells with loss of P53 expression often display aneuploidy or tetraploidy.

The prevalence of p53 LOH detected by flow cytometry at baseline in BE patients, was demonstrated in one large prospective study, to increase from 6% in NDBE to 57% in HGD (p<0.001). P53 LOH conferred an increased risk of malignant progression (RR=16; p<0.001). In one case controlled study, patients who developed EAC had an odds ratio of 11.7 for having increased staining of non-functional p53 on biopsy. However, there was low sensitivity for an
initial biopsy showing increased staining (32%) and therefore the use of staining for non-functional p53 as a biomarker for progression is probably limited.\textsuperscript{122}

\textbf{Ras Pathway}

The Ras pathway is an intracellular signaling cascade that is activated when growth factors bind to cell surface receptors.\textsuperscript{123} Mutations in Ras are seen in the majority of human tumours and result in constant stimulation of the Ras pathway without the need for growth factor activation.\textsuperscript{124} Expression of oncogenic K-Ras or H-Ras has been frequently detected in dysplastic Barrett’s and EAC but not NDBE.\textsuperscript{125, 126} It is thought to be the resultant increase in epidermal growth factor receptor (EGFR) and its ligand, transforming growth factor alpha (TGF-\(\alpha\)) that account for increased activation of the mitogenic Ras pathway in early Barrett’s carcinogenesis.\textsuperscript{127}

\textbf{Telomerase-dependent senescence pathway}

Telomerase is the enzyme that synthesizes and maintains telomeres, which are long stretches of DNA located at the ends of chromosomes. Most normal cells, including squamous oesophageal cells lack telomerase, and with each cell division, telomere length is lost until eventually the cell leaves the cell cycle permanently. This progressive loss of telomeres is an intrinsic mechanism of cells that limits their proliferative capacity and it is termed senescence.\textsuperscript{128} NDBE biopsy specimens express low levels of telomerase which increase as the degree of dysplasia increases.\textsuperscript{129} Oesophageal adenocarcinomas also express high levels of telomerase.\textsuperscript{130}

\textbf{Cancer-related inflammation}

NF-\(\kappa\)B and STAT3 are among the key transcription factors known to mediate both inflammation and tumorigenesis. NF\(\kappa\)B activation increases as Barrett’s metaplasia progresses though advancing stages of dysplasia, which suggests that an inflammatory response might be promoting carcinogenesis.\textsuperscript{131} NF-\(\kappa\)B expression is found in 13% of biopsies of reflux-exposed squamous epithelium exposed to reflux, compared with up to 60% of Barrett’s metaplastic epithelium and up to 80% of EACs.\textsuperscript{131, 132} Similarly, expression of STAT3 in Barrett’s also increases with the severity of dysplasia, strengthening a possible a link between the inflammatory response and EAC development.\textsuperscript{79} STAT3 proteins have been found to regulate the expression of matrix metalloproteinases (MMPs), enzymes that can
degrade extracellular matrix and contribute to tumour invasion and metastasis.\textsuperscript{133} Certain MMP expression has been found in both NDBE and EAC.\textsuperscript{134-136}

1.4.5 Summary of the Role of Biomarkers

To date, no single biomarker has been identified that can adequately identify patients most at risk of neoplastic progression of Barrett’s, therefore panels of biomarkers have been developed with this aim in mind. One study of around 250 patients with Barrett’s were assessed with 3 biomarkers from oesophageal biopsies at baseline for p53 LOH, p16 LOH and aneuploidy. A combination of all three abnormalities had a cancer incidence of 80\% at 6 years compared with 20\% for one abnormality and 36\% for two abnormalities at 10 years.\textsuperscript{137} Currently however, no individual biomarkers or combination of markers are ready for use in clinical practice and will need to become validated in large prospective cohorts. These studies will prove challenging given the low overall progression of BE to HGD/EAC.\textsuperscript{3}

1.5 Pathologist Variability

It has been long recognised that there is inter-and intra-observer variability in the diagnosis of GI tract dysplasia.\textsuperscript{138} This relates to differentiating between regenerative change and LGD, LGD and HGD, and HGD and intramucosal cancer. In the case of definitive dysplasia, this is because these divisions involve unnatural cut-offs along a histological and biological continuum.\textsuperscript{68}

1.5.1 Interobserver Variability

Inter-observer variability is especially noticeable when comparing expert and non-expert GI pathologists,\textsuperscript{139} and is most pronounced when diagnosing NDBE, indefinite for dysplasia and LGD. In a study of 793 patients with BE who had their histology reviewed by general and expert pathologists there was considerable inter-observer variability when diagnosing NDBE or Indefinite/LGD in BE between non-experts and experts, but interestingly, also between expert pathologists, with kappa values of 0.24 and 0.27, respectively.\textsuperscript{7}

There is reasonable inter-observer agreement among GI pathologists for the extremes of dysplasia (i.e. NDBE or HGD/EAC). In one study, interobserver agreement was substantial for
HGD/EAC (κ=0.65) and moderate to substantial for NDBE (κ=0.58), but only fair for LGD (κ=0.32), and slight for IND (κ=0.15). In another study of 101 cases of BE, there were 61 cases with IND for dysplasia. There was fair overall agreement for the diagnosis of dysplasia (κ=0.35) but it was poor for IND for dysplasia (κ=0.18).

### 1.5.2 Double Reporting

Double reporting improves prediction of progression to EAC. Studies have shown that the prediction of progression of oesophageal dysplasia is improved if at least two GI pathologists agree on the diagnosis and increases further when a greater number of pathologist concur with the diagnosis. A pivotal study from 2010 demonstrated that the majority of community diagnoses of LGD in 147 patients were subsequently downstaged by two GI pathologists to NDBE or IND (85%). Where there was consensus agreement with the original diagnosis of LGD, there was a significantly higher risk of malignant progression. The cumulative risk for progression to HGD or EAC was 85.0% in 109.1 months compared with 4.6% in 107.4 months for patients downstaged to NDBE (P<0.0001). Additionally, the incidence rate of HGD/EAC was 13.4% per patient-year for patients with consensus LGD compared with 0.49% for those with NDBE.

Further work by the same group examined 239 additional patients with LGD diagnosed in the community who had biopsies reviewed by at least two GI pathologists and 73% of the cases were downgraded to indefinite for dysplasia or NDBE. For those with consensus LGD the incidence rate of HGD/EAC was 9.1% per patient-year, compared with 0.6% and 0.9% per patient-year for consensus NDBE and IND respectively.

Studies have suggested that community based pathologists have difficulty interpreting both NDBE and dysplasia. It has previously been recommended that in difficult cases, a second opinion should be sought from an expert pathologist experienced in BE. The Royal College of Pathology now recommends “double reporting” of diagnoses of HGD and this has been agreed upon in consensus statements by Barrett’s international experts. The British guidelines and American guidelines have extended this recommendation to include all grades of dysplasia.
1.5.3 Aids to Histological Diagnosis and Role of Biomarkers

As previously discussed in Chapter 1.4.4.2, the role of molecular markers (such as chromosomal/DNA abnormalities or gene mutations for example), in identifying patients at increased risk for malignant progression of BE has been studied.\textsuperscript{143-147} These genetic abnormalities in EAC driver genes generally occur exceptionally early in disease development (at the NDBE stage).\textsuperscript{148}

1.5.3.1 P53 staining

Of all the potential experimental biomarkers, the one with greatest body of evidence and which can also be applied in the routine clinical setting is immunohistochemistry for nuclear p53. P53 positivity rate in BE dysplasia is variably reported (between 50 and 89\%) in the literature.\textsuperscript{149, 150} Despite this, when p53 is positive it can improve inter-observer agreement for reporting dysplasia\textsuperscript{68} and can be a strong predictor for progression to HGD/EAC (odds ratio is quoted between 3 and 8 in varying studies).\textsuperscript{101, 105, 122, 151, 152}

One study demonstrated a correlation between malignant progression (from LGD to HGD/EAC) and p53 positivity (p=0.017), and for either p53 positivity or consensus diagnosis of LGD among three GI pathologists (p=0.014). P53 staining was 88\% sensitive and 75\% specific for progression from LGD to HGD/EAC with improved sensitivity (to 100\%) when adding a consensus LGD diagnosis between the three pathologists.\textsuperscript{153}

In a further study where five pathologists independently graded the histology of around 150 patients with NDBE and dysplastic Barrett’s, inter-observer agreement markedly improved following p53 immunohistochemistry. Additionally, when analysing progression risk according to consensus diagnoses, progression correlated with p53 positivity.\textsuperscript{68}

Loss of p53 expression has also been shown to be associated with progression to HGD/EAC. In one case controlled study of 720 patients with BE, while P53 overexpression was associated with an increased risk of neoplastic progression, the risk was even higher with loss of p53 expression (adjusted relative risks of 5.6 and 14.0 respectively). The positive predictive value (PPV) for neoplastic progression was 15\% for a histological diagnosis of LGD increasing to 33\% when LGD was present with coexisting aberrant p53 expression.\textsuperscript{151}
1.6 Risk Factors for Development of Barrett’s Oesophagus

Risk factors for the development of BE include chronic (>5 years) reflux symptoms, increasing age (>50 years), male gender, tobacco, central obesity and Caucasian race.³

In a population study of 822 men undergoing colorectal cancer screening, 9% were found to have BE. BE was associated with weekly reflux (OR=2.33), age (OR per 10 years=1.53), waist-to-hip ratio (OR per 0.10=1.44) and pack years of cigarette use (OR per 10 pack-years). A model based on reflux, age, abdominal obesity, and cigarette use more accurately classified the presence of Barrett’s oesophagus than did a model based on GORD alone.¹⁵⁴

1.6.1 Gastroesophageal Reflux Disease (GORD)

GORD is defined by either chronic reflux symptoms or the presence of mucosal inflammation secondary to gastric content reflux into the oesophagus.¹⁵⁵ This definition was updated at an international consensus conference in 2006. The ‘Montreal definition’ proposed the definition of GORD was ‘a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications’.¹⁵⁶

The prevalence of GORD has been estimated at between 10-20% of people (with weekly symptoms).¹⁵⁷ GORD has been shown in recent population studies to be increasing in prevalence, both in the United States and worldwide.¹⁵⁸,¹⁵⁹

A recent systematic review of the global burden of GORD (defined by either experiencing typical symptoms at least once weekly, or the Montreal definition) estimates prevalence rates of 18–28% in North America, 9–26% in Europe, 2.5–8% in East Asia, 9–33% in the Middle East, 23.0% in South America, and 12% in Australia. Furthermore, when comparing studies performed after 1995 to those performed prior, the prevalence rates are around 50% higher in the USA, Europe and East Asia.¹⁵⁹

In patients with GORD, there is an associated 10-15% risk of developing BE ⁴³ compared with 1-2% in the general population.³⁹,¹⁶⁰ An Italian cohort study surveyed 1033 individuals from two Italian towns and found the prevalence of reflux symptoms was 44%; (24% with frequent symptoms). The prevalence of oesophagitis and BE in this population were 11.8% and 1.3%, respectively.¹⁶⁰
1.6.2 Duration of GORD symptoms

GORD symptom duration has been shown to be a risk factor for the development of BE. A prospective, observational, community-based cohort study examined the duration of reflux symptoms and risk for developing BE. Of 701 patients with reflux symptoms, 11% had probable Barrett’s. When comparing patients who described reflux symptoms for less than 1 year, the odds ratio for BE was 3.0 for patients with symptoms for 1-5 years and 6.4 for patients with greater than 10 years of symptoms (p<0.001).161

1.6.3 Increasing age (>50)

A retrospective study using the Clinical Outcomes Research Database showed the incidence of BE in white men with GORD undergoing endoscopy was 2.1% in the third decade of life, rising to 9.3% in the sixth decade.162 A plateau was seen from the sixth to eight decades.

Additionally, in a cross-sectional Veterans Affairs study of 683 patients undergoing elective gastroscopy, it was found that an early age of onset (<30 years old) of frequent (>1x weekly) reflux symptoms correlated with the highest risk of BE (OR=15.1), and risk increased linearly with earlier age at onset of symptoms (P-trend=0.001). This association was independent of cumulative GORD symptom duration. People with early onset reflux symptoms who reported ever using proton pump inhibitors (PPI) were at especially high risk of BE (OR=31.1).163

1.6.4 Male gender

Male gender is both a risk factor for development of BE and EAC. A systematic review and meta-analysis of the sex ratio for Barrett’s oesophagus and showed an overall pooled male/female ratio of 2:1.164

1.6.4 Family history

Family history has been demonstrated to be an independent risk factor for development of BE as well as EAC. A cohort study found that BE was 5 times more common and patients who had a first or second degree relative with Barrett’s than controls who did not (24% versus 5% p<0.005). Family history was found to be strongly associated with BE (OR=12).165

In a further study by the same lead author, endoscopic screening was performed on relatives of patients with either familial BE or apparent isolated BE. Barrett’s was identified in 21%. Barrett’s (NDBE, dysplastic BE or EAC) was identified significantly more often in the
siblings and children of familial BE subjects, compared with patients with apparent isolated Barrett’s oesophagus (p≤0.05). Endoscopic screening of relatives of familial BE probands identified a multigenerational familial BE pedigree consistent with an autosomally dominant inherited trait.\textsuperscript{166}

Several recent studies have described single-nucleotide polymorphisms (SNPs) on gene loci that may predispose to developing BE. A model-free linkage analysis of 21 concordant-affected sibling pairs with BE/EAC and 11 discordant sibling pairs found that three major genes, MSR1, ASCC1, and CTHRC1 were associated with BE/EAC (all P<.001).\textsuperscript{167}

A genome-wide association study estimated the variance and genetic correlation between GORD, BE, and EAC considering all single nucleotide polymorphisms (SNPs) simultaneously. The genetic correlation between BE and EAC was found to be high. There was found to be extensive polygenic overlap between EAC and BE, suggesting that much of the genetic basis for oesophageal adenocarcinoma lies in the development of BE, rather than progression from BE to EAC.\textsuperscript{168} Three novel genome-wide significant loci for oesophageal adenocarcinoma and Barrett’s oesophagus combined were found.\textsuperscript{169}

Another genome-wide association study showed that common variants at the MHC locus and at chromosome 16q24.1 predispose to BE. Evidence was also found that SNP alleles predisposing to obesity also increase the risk of BE.\textsuperscript{170}

### 1.6.5 Caucasian race

Caucasian race has consistently been shown to be a risk for development of BE. An observational study of 4205 people diagnosed with Barrett’s oesophagus, found the annual incidence in 2006 per race-specific member-years was highest among non-Hispanic whites (39/100 000), followed by Hispanics (22/100 000), Asians (16/100 000), and blacks (6/100 000). They concluded that the demographic distributions of BE were comparable to those for EAC (and differed significantly by race, age and sex) and that therefore, demographic differences in EAC risk may arise partly from the risk of having BE, rather than from differing progression risk from BE to EAC.\textsuperscript{171}

A single centre retrospective cross-sectional analysis of 2100 patients who underwent upper endoscopy during a 1-year period found that whites had a significantly higher prevalence of BE than Hispanics (6.1% vs 1.7%, P=0.0002) and blacks (6.1% vs 1.6%, P=0.004). Black race and
Hispanic ethnicity were factors associated with decreased risk of BE (OR=0.34 and 0.38 respectively).\textsuperscript{172}

1.6.6 Tobacco usage

A recent meta-analysis of 39 studies and 7069 Barrett’s patients demonstrated that tobacco use is a risk factor for its development. Having ever smoked was associated with an increased risk of BE compared with non-GORD controls (OR=1.44) but not GORD controls, suggestive that the increased risk of Barrett’s associated with using tobacco may be mediated via increasing GORD. A greater number of pack-years smoked was associated with a greater risk of BE.\textsuperscript{173}

1.6.7 Central adiposity

Obesity is an independent risk factor for BE however it is the pattern of obesity that is relevant. A meta-analysis of 40 articles reporting on the effect of central adiposity on the development of oesophagitis, BE and EAC found that central adiposity, independent of overall body fat content (measured by body mass index (BMI)), was associated with oesophageal inflammation (OR 1.87), BE (1.88), and EAC (OR=2.51). Its effects were mediated by reflux-dependent and reflux-independent mechanisms.\textsuperscript{174}

Central obesity is a risk factor for Barrett’s in both men and women. An analysis of four case-control studies looked at 1102 cases (316 women, 786 men) and 1400 population controls (436 women, 964 men). People in the highest versus lowest quartiles of waist circumference (independent of BMI) had approximately 125% and 275% increases in the odds of BE among men and women, respectively (OR 2.24 and 3.75).\textsuperscript{175}

Overall body fat content has not been associated with BE risk. In a case-control study of 70 BE cases, no association was found between BMI, fat mass and BE, but waist-hip ratio was significantly associated with increased risk of BE (OR=1.45).\textsuperscript{176}

1.6.8 Other potential factors associated with the development of Barrett’s Oesophagus

1.6.9.1 Metabolic syndrome

In a population based control study of around 300 patients (one third each with BE, GORD, or without GORD), the metabolic syndrome was shown to be correlated with a twofold increased
risk of BE, with or without reflux symptoms (OR=2.00 and 1.90 respectively). The association was independent of smoking, alcohol consumption and BMI.\(^\text{177}\)

### 1.6.9.2 Type 2 Diabetes Mellitus

A large population-based case-controlled study demonstrated a 49% increased risk of BE in patients with T2DM, independent of obesity (measured by BMI in this case), smoking and GORD, with a stronger association seen in women than men.\(^\text{178}\)

### 1.6.9.3 Obstructive sleep apnoea (OSA)

A retrospective study using a multiple variable logistic model demonstrated that patients with OSA had an 80% increased risk (OR=1.8) of having BE, independent of age, sex, BMI, reflux symptoms and smoking. This association was thought to potentially be mediated through BMI and GORD independent mechanisms.\(^\text{179}\)

### 1.6.10 Factors not associated with the development of Barrett’s Oesophagus

#### 1.6.10.1 Alcohol

Alcohol consumption has not been shown to be a risk factor for development of BE. An Irish population study found that while drinking alcohol in early adulthood may result in the development of reflux oesophagitis, more recent consumption does not appear to be associated with an increased risk of oesophagitis, BE or EAC. In fact, it suggested that wine consumption may reduce the risk of these three oesophageal disorders.\(^\text{180}\)

A Californian case-control study of 320 BE patients, 316 reflux patients and 316 population controls found that total alcohol use was not significantly associated with the risk of BE. In fact, there was an inverse association for wine drinkers compared to non-drinkers (>7 glasses wine per week versus none: OR=0.44). This study also suggested that higher education status was significantly inversely associated with the risk of BE; individuals who completed ≥4 years of college had half the risk of developing BE (OR=0.47) compared to those who had not completed high school. Compared to population controls, patients with BE had lower levels of education.\(^\text{181}\)

Another multivariate logistic regression study of 258 patients with BE who were compared with 453 colonoscopy controls and 1145 gastroscopy controls, found that moderate alcohol consumption (14 to <28 drinks/week) actually conferred a lower risk of BE (OR=0.39).
Interestingly, in this study, no measure of smoking exposure (e.g. intensity, duration, age of onset, pack-years, cessation) was associated with risk of BE.  

1.6.10.2 Helicobacter pylori

Some studies have demonstrated a decreased risk of BE in patients with H. pylori. In a case control study of 633 men undergoing upper GI endoscopy, it was found that H. pylori infection was inversely associated with BE (OR 0.53) (most pronounced for the cagA+ strain (OR=0.36)) and a trend toward inverse association for erosive oesophagitis (OR=0.63, or 0.47 for cagA+ strain). In another case control study of around 200 cases and 400 controls, the overall OR for association between H. pylori and BE was 0.55. A stronger inverse association existed for patients with corpus atrophy or who used anti-secretory drugs ≥1 time per week (OR=0.28). The converse was also found to be true, with no inverse association in patients without these factors (OR: 1.32). This suggests that the decreased risk for BE in patients with H. pylori appears to arise in settings that would likely lower gastric acidity.

1.7 Risk Factors for Progression to Oesophageal Adenocarcinoma

The development of Barrett’s metaplasia is the first step in the development of dysplasia and ultimately adenocarcinoma. Studies have long demonstrated a much higher risk for patients with BE developing EAC over that of the general population (RR=30-40), however, importantly, the absolute overall risk is low (0-3%) in most studies. EAC incidence continues to rise, with poor 5-year survival rates. For EAC with regional or distant disease, 5-year survival is less than 20%. No studies to date have suggested any geographic variation in cancer progression.

The known risk factors for the development of neoplasia within BE include advancing age, increasing length of Barrett’s segment, central obesity, tobacco usage, lack of NSAID use, lack of PPI use, lack of statin use. These factors are summarised in this chapter.

1.7.1 Length of Segment

There is a correlation between increasing Barrett’s length and risk of developing EAC and the current guidelines recommend the use of the Prague Classification in describing segment length, as has been outlined previously.
segment Barrett’s (which had an arbitrary cut off at 3cm) is no longer thought to be useful and are not recommended, given neoplastic progression can be seen in all lengths of Barrett’s oesophagus.\textsuperscript{27,188}

There are multiple data to support this. In a multicentre study of 309 patients with BE that did use the 3cm length descriptor (5 with EAC, 11 HGD, 29 LGD), it was found that patients with BE length $\geq$3cm and had a significantly greater prevalence of dysplasia compared to length <3cm (23% vs 9%; $P=0.0001$). Importantly, the risk of dysplasia increased by 14% per cm of increased length.\textsuperscript{189} Additionally, in a prospective evaluation of 550 patients with BE, the length of BE segment was found to be an independent predictor of progression $p=0.012$.\textsuperscript{190} In another study of 1175 NDBE patients, when compared with non-progressors, patients who progressed to HGD/EAC had longer BE segments (6.1 versus 3.5 cm; $P<0.001$) with logistic regression analysis showing a 28% increase in risk of HGD/EAC for every 1cm increase in BE length ($P=0.01$). There was a non-significant trend towards patients with BE segment $\leq$3cm taking longer to develop HGD/EAC than those with lengths $>$4cm (6 versus 4 years).\textsuperscript{191} In a meta-analysis of 42 studies, 9 of which included short segment Barrett’s, a non-significant trend was found between cancer risk and length of segment.\textsuperscript{187}

### 1.7.2 Degree of Dysplasia

#### 1.7.2.1 Intestinal metaplasia

A large Norwegian study of 712 patients comparing patients with columnar mucosa of the distal oesophagus with or without IM, found no difference in the development of EAC between the two groups at a median of 12 years’ follow-up. The EAC rate was 0.34% per year (IM 0.37%, no-IM 0.30%; $p=N/S$). The major limitation of the study however, was its retrospective design and the limited number of biopsies taken which may have underdiagnosed IM in the group with columnar epithelium.\textsuperscript{17} While this finding supports the position of the British Society of Gastroenterology who do not require intestinal metaplasia to diagnose Barrett’s oesophagus it remains widely accepted that the presence of IM is required for neoplastic progression of BE and is the first step in the evolution to oesophageal adenocarcinoma. Progression from NDBE to EAC is considered to be a multistep process where patients progress through stages of LGD to HGD before developing adenocarcinoma.
Recent data suggests lower rates of progression to HGD/EAC from NDBE than previously believed. A 2012 meta-analysis of 57 studies and 11,434 patients demonstrated the pooled annual incidence of EAC was 0.33% (i.e. the incidence of EAC arising in NDBE is around 1 in 300 patients per year). The incidence of EAC in short-segment BE was less than 1 in 500 patients per year. This is lower than had previously been reported. An earlier meta-analysis found a 0.5% per year rate of progression from NDBE to EAC, with a similar rate echoed in a large multicentre cohort study.

The most important risk factor for malignant progression of Barrett’s oesophagus is the degree of dysplasia. In oesophagectomy studies, up to 50% of patients with adenocarcinoma have varying degrees of dysplasia surrounding. In clinical practice, it is the presence and degree of dysplasia that remains the most useful indicator for identifying patients at increased risk of EAC and ultimately affects the recommended surveillance protocol.

1.7.2.2 Low grade dysplasia

The presence of LGD is known to increases the risk of neoplastic progression over time when compared to NDBE, however the magnitude of this risk remains debateable with inconsistent data in the literature from different cohort studies. While some studies have demonstrated regression rates as high as 65-75% from LGD back to NDBE at later endoscopy, recently published studies highlighted these results may be confounded by the fact that community based pathologists may overcall the diagnosis of LGD.

In one study of 147 patients with an initial diagnosis of LGD by a community based pathologist, following expert review by two GI pathologists, 85% were down-staged to IM or indefinite for dysplasia. These patients had a risk of progression to HGD/EAC of 0.49% per year compared to those confirmed to have true LGD (defined as both pathologists agreeing with the original diagnosis), where the risk of progression was far greater at 13.4% per year. Of those with “true LGD” there was a cumulative risk of progressing to HGD or EAC of 85.0% in 109.1 months compared with 4.6% in 107.4 months for patients down-staged to NDBE (P<0.0001). Further cohort studies have also described a higher rate of neoplastic progression when LGD is confirmed by expert and multiple pathologists.
In a 2009 systematic literature review, the authors found a 0.6%-1.6% per year rate of progression of LGD to cancer. A later 2014 meta-analysis examining 24 studies with 2694 patients found the pooled annual incidence of EAC was 0.5% for EAC alone and 1.7% for HGD/EAC combined.

1.7.2.3 High grade dysplasia

HGD is a well-recognized risk factor for progression to cancer and is an accepted trigger for definitive treatment of the entire Barrett’s segment due to this concern. In fact, prior to 2008, the published guidelines recommended that patients with HGD be routinely considered for oesophagectomy. This was in part due to the high prevalence of EAC found in patients with HGD who underwent surgery. Prevalence rates vary in the literature, with older surgical series reporting the risk of concomitant adenocarcinoma in patients with HGD approaching 40%. More recently, a meta-analysis of 23 studies of patients undergoing oesophagectomy for BE and HGD reported a 12.7% incidence of invasive adenocarcinoma. Importantly, in the absence of visible lesions in BE, the prevalence of EAC in patients who had oesophagectomy was only 3%.

Quoted progression rates of HGD to EAC vary in the literature, mainly reflecting different study sample sizes and designs. A 2008 meta-analysis of 4 studies and 236 patients, reported the weighted annual incidence of developing EAC from HGD was 6.6%. In the AIM-dysplasia trial, however, where 127 patients with dysplasia were randomized to ablation therapy, compared with surveillance, a 19% yearly progression rate to EAC in the HGD arm was reported.

The extent of HGD within the BE segment is also a risk factor for progression. A study of 67 patients examined whether focal HGD (cytologic/architectural changes of HGD limited to ≤5 crypts) conferred a lower malignant potential than diffuse HGD (>5 crypts involved in a single biopsy) or multifocal HGD (involving >1 biopsy). One and three year cancer-free survival was significantly higher for focal compared with diffuse HGD (93% and 86% vs. 62% and 44%; P<0.001) On multivariate analysis, diffuse HGD had a 3.7-fold increase in the risk of EAC compared with focal HGD (P=0.02), and multifocal HGD was associated with an increased risk of EAC at 12 months versus focal HGD.
1.7.3 Gastroesophageal Reflux Disease (GORD)

There is evidence for acid and bile reflux as pathogenic factors in the development of both Barrett’s and Barrett’s neoplasia (as previously discussed in Chapter 1.4.3.1). Certainly, symptomatic reflux has been shown to be a risk factor for development of EAC. Three large case-control studies demonstrated a positive association between reflux symptoms and risk of EAC, with more prolonged and severe symptoms accentuating this risk. One population study from Sweden, demonstrated a 7.7 odds ratio for developing EAC in patients with recurrent reflux symptoms compared with those without recurrent symptoms. The risk increased, the more frequent, severe, and longer lasting the reflux symptoms. For those with long-standing and severe reflux symptoms, the odds ratio for developing EAC was 43.5 (compared with 4.0 for adenocarcinoma of the cardia). Importantly however, 40% of those with EAC reported no previous GORD symptoms. Another case-controlled study reported an adjusted odds ratio of 5.5 for patients with EAC having daily reflux. The third study reported an at least two-fold increased risk of EAC in those with symptomatic reflux.

Due to the low incidence of EAC and high prevalence of patients with symptomatic reflux, the overall risk of EAC in an individual with GORD is low. There have been no randomized trial data demonstrating screening endoscopy for those with GORD results in decreased EAC incidence or increased life expectancy. This is discussed in more detail in Chapter 1.8.

1.7.4 Biomarkers

Biomarkers have the potential to predict cancer progression risk in patients with BE (as discussed in Chapters 1.4.4.2, 1.4.5 and 1.5.3.) however none have been validated in large clinical studies.

1.7.5 Patient Demographics

1.7.5.1 Increased age

Increasing age has been shown to be a risk for development of EAC. A multicentre study of 309 patients with Barrett’s oesophagus (5 with EAC, 11 HGD, 29 LGD) found that the risk of dysplasia increased by 3.3% per year of age. The Danish Cancer Registry and Surveillance, Epidemiology and End Results (SEER) program showed the risk of EAC increases with age, peaking at 75-79 years. Interestingly, a birth cohort effect has been described where a
40% increase in incidence for each five year increase in the date of birth was noted, with increased incidence among patients aged 45-65.\textsuperscript{216}

The EAC incidence of men under 50 years old with reflux symptoms is very low (1.0/100,000 for those at 35 years), but the incidence in older men with weekly GORD symptoms is substantial (for instance, at the age of 70 years, incidence was 60.8/100,000 person-years).\textsuperscript{218}

1.7.5.2 Male gender
The risk of developing EAC from Barrett’s is significantly higher in men. In a SEER database study, women comprised only 12\% of all EACs (i.e. Males had an approximate 7-fold risk of EAC). Additionally, where the incidence of EAC in older men with weekly GORD symptoms is substantial (60.8/100,000 person-years at 70 years), the incidence of EAC in women with GORD is very low 3.9/100,000 person years at 60 years).\textsuperscript{218} These studies have been supported by other data showing men have a 6-8-fold risk of EAC than women.\textsuperscript{216, 217}

1.7.5.3 Caucasian race
The risk of developing EAC is higher for those of Caucasian background than other ethnic groups. A large retrospective study of cases of oesophageal adenocarcinoma and squamous cell carcinoma found that Caucasians predominantly develop EAC over SCC compared with black patients who predominantly developed SCC (92\% of their oesophageal cancers).\textsuperscript{219} One SEER database analysis between 1973-1998 showed an increase rate of EAC in Caucasians and Hispanics but not in blacks.\textsuperscript{220} A later SEER database analysis from 1992 to 1998 found that the rate of EAC for Caucasian males was 4.2 per 100,000 per year, which was twice that of Hispanics and four times higher than those of blacks, Asians/Pacific Islanders, and Native Americans (p<0.01).\textsuperscript{221}

1.7.5.4 Family history
As discussed in Chapter 1.6.7, familial clustering of Barrett’s is reported in about 7\% of individuals with BE or EAC.\textsuperscript{222} A positive family history of BE or EAC is associated with an increased risk of BE,\textsuperscript{165, 222} and up to 28\% of first-degree relatives of patients with Barrett’s with HGD/EAC also have BE. Studies on familial aggregation have implicated genetic factors in the development of BE,\textsuperscript{165} and a recent genome-wide study has identified the first two loci associated with the disease.\textsuperscript{170} This is discussed in more detail in the Chapter concerning risk factors for developing BE (1.6.7).
In an analysis of the Swedish cancer registry the Standard incidence ratio for adenocarcinoma was 4.05 when a parent was diagnosed with SCC and 3.52 for any oesophageal cancer.223

1.7.6 Modifiable Risk Factors

1.7.6.1 Obesity

Several meta-analyses have found an association with increased weight (whether BMI or central adiposity) and development of EAC. The mechanism by which obesity may impact the development of EAC is not entirely clear but may occur through several pathways including increased reflux secondary to higher intra-abdominal pressure, or simply the molecular effects of adiposity.198

A meta-analysis of 9 studies looking at the association of BMI and GORD, including its complications (oesophagitis, Barrett’s oesophagus, and EAC) found a positive association in 6 of 7 studies looking at EAC, finding the pooled odds ratios for EAC were 1.52 for BMI of 25-30, and 2.78 for BMI>30.224 Another showed a BMI>25 was associated with an increased risk of EAC (OR for males was 2.2 and for females was 2.0). The risk increased with higher BMI (OR=1.8 for BMI 25-30 and 2.4 for BMI>30).225

An Australian case-control study also demonstrated a trend toward risk of EAC development and BMI increase, with the highest risks seen for BMI ≥40 (OR=6.1) compared with healthy BMI.226 In one German population study, however, while there was a strong association between an increased BMI and the progression from reflux disease to Barrett’s oesophagus, this association was not demonstrated in the progression from Barrett’s to cancer. Their results suggest that obesity mediates its risk for EAC primarily through the development of BE. This group did not assess central obesity or intra-abdominal fat as separate risk factors, however.227

One systematic review and meta-analysis proposed that the main increase in risk of EAC development related to GORD rather than obesity itself.228, however a meta-analysis of 40 articles reporting on the effect of central adiposity on oesophageal inflammation, BE and EAC found that central adiposity, independent of BMI, was associated with oesophageal inflammation (OR=1.87), Barrett’s oesophagus (OR=1.88), and neoplasia (OR=2.51). Its effects were mediated by reflux-dependent and reflux-independent mechanisms.174
1.7.6.2 Helicobacter pylori

In addition to being protective for the development of Barrett’s oesophagus, H. pylori (particularly the cagA+ strain) appears to be protective against EAC. In EAC patients there were significant inverse relationships with both the H. pylori prevalence (pooled OR=0.52; \( P<0.001 \)) and the prevalence of H. pylori cagA+ strain (pooled OR=0.51; \( P=0.006 \)). A similar relationship was demonstrated for patients with BE.\(^{229}\)

The protective effect of H. pylori is thought to be due to the decreased acid produced in patients with gastric atrophy,\(^{184}\) however the protective effects have also been seen in patients without gastric atrophy, suggesting another mechanism may be at play.\(^{230}\)

It is not recommended that H. pylori be left untreated to protect against the development of EAC, particularly as infection is a risk factor for peptic ulcer disease, and additionally, it is classified as a carcinogen and critical in the development of gastric cancer.

1.7.6.3 Alcohol

Alcohol consumption has not been shown to increase the risk of EAC development in BE in a couple of case controlled studies.\(^{231, 232}\) One Swedish study that included 189 cases of EAC found on multivariate analysis and logistic regression, there was no association between EAC development and alcohol use.\(^{231}\)

1.7.6.4 Smoking

There has been a proposed link between cigarette smoking and development of EAC. One previously mentioned Swedish case-controlled study demonstrated a weak or absent connection between tobacco smoking and the development of EAC\(^{231}\), however other studies have shown an association. In an Irish population study of 227 oesophageal adenocarcinoma patients (compared with similar number of BE patients and controls) EAC patients were more likely than controls to be ex- or current smokers, with odds ratios of 1.72 and 4.84 respectively.\(^{233}\) A more recent population study showed that any history of smoking was associated with a 2.6-fold increased risk in developing of HGD/EAC among patients with Barrett's oesophagus.\(^{227}\)

1.7.6.5 Diet

A couple of population based case-control studies have suggested a link between diet and development of EAC. One demonstrated a 15% increased risk of EAC in people with a low
fruit and vegetable intake. Another suggested that high saturated fat intake may increase the risk of EAC, whereas higher intake of fibre, vitamins and some carotenoids, may decrease the risk. One of the more recent studies showed that a high fruit and vegetable intake appeared to be protective with a dose-response effect (P(trend)=0.051), although the odds ratio among highest fruit and vegetable consumers were not significantly reduced in adjusted analysis (OR 0.60).

1.7.6.6 Medications

Non-steroidal anti-inflammatories (NSAIDS)
A meta-analysis undertaken among a total of 9 observational studies comprising 5446 participants (605 with HGD or EAC) found that COX inhibitor use in patients with Barrett’s was associated with a reduced risk of HGD/EAC (relative risk (RR)=0.64). Use of aspirin decreased the risk of HGD/EAC (RR=0.63) as did non-aspirin COX inhibitors (RR=0.50), with the chemopreventative effect seemingly independent of duration response.

These results are in line with a previous systematic review and meta-analysis of observational studies which showed a 33% reduction in the risk of developing oesophageal adenocarcinoma or squamous cell carcinoma in aspirin and NSAID users, with benefit greatest in those using aspirin (OR of 0.5).

Proton Pump Inhibitors (PPIs)
A meta-analysis based on 7 observational studies with 2813 BE patients (317 cases with HGD/EAC, 84.4% PPI users) demonstrated a 71% reduced risk of malignant progression with PPI users (OR=0.3) with a trend towards a dose-response relationship for PPI use >2-3 years. No significant effect was shown for H2A blockers.

Statins
In a meta-analysis of 5 observational studies looking at 2,125 patients with BE (312 with EAC) statin use was found to be associated with a 41% risk reduction in EAC (adjusted OR=0.6). The number needed to treat to prevent one case of EAC was 389.

Multivitamins
There is some evidence that multivitamin use may be protective in the development of EAC. One prospective cohort study of over 300 patients with Barrett’s demonstrated a significantly decreased risk of EAC in those patients who took ≥1 multivitamin tablet/day compared to
those not taking multivitamins (HR=0.38). Significant inverse associations were also observed between the use of supplemental vitamin C and E and the risk of EAC.\textsuperscript{240} There is not enough data however to support routine use of multivitamins in patients with BE.

1.7.3 Causes of Death in patients with BE

A meta-analysis of 19 studies and 7,930 patients reported most BE patients die of causes other than BE associated cancer. A pooled incidence rate of fatal EAC was 3/1000 person years (based on 88 deaths from EAC and 1271 from other causes. In 12 of those studies reporting cause-specific mortality, 7% of deaths were from EAC and 93% were from other causes; 25% cardiac, 20% pulmonary, 16% other malignancies.\textsuperscript{233}

1.8 Role of Screening at Risk Populations

The principles of a screening program are to identify an at risk population with a screening tool that is reliable, cost effective and acceptable to the patient. There should be effective treatment available for early stage disease detected by such a screening programme and evidence of improved patient outcomes with intervention.\textsuperscript{241} In the case of BE, the goal of a screening and surveillance program is to identify individuals at risk for progression to EAC, a malignancy that has been increasing in incidence since the 1970s.\textsuperscript{2, 47}. Because survival of patients diagnosed with EAC remains very poor (<20% 5 year survival)\textsuperscript{2}, it is hypothesised that screening for BE will lead to improved detection of dysplasia, earlier intervention for high risk patients, and ultimately a decreased incidence of EAC.\textsuperscript{242}.

Over 90% of EACs are diagnosed in patients without a prior diagnosis of BE, despite increased use of endoscopy,\textsuperscript{243} and notably, around 40% of EACs develop in people without significant GORD.\textsuperscript{196}. This is problematic in the case of screening for BE as screening only occurs for patients with chronic GORD, a large group of at risk patients will be missed. In contrast, if a screening program were rolled out to the wider population, it is unlikely to be cost effective. The 2004 AGA guidelines suggested that screening for BE and dysplasia did not lead to improved mortality from EAC, even in high risk groups.\textsuperscript{27} For example, while one study showed that prior upper endoscopy was associated with improving the stage of EAC at diagnosis, it did not alter long-term survival, meaning that the benefit of screening/surveillance in decreasing EAC-associated mortality could not be confirmed.\textsuperscript{244}
Additionally, many patients with BE die of causes other than oesophageal ADC. The hazard ratio for death in BE patients compared with the general population is 1.37, and less than 45% of this slight increase of risk is due to EAC.  

Despite these findings, endoscopic screening of populations at highest risk for BE (50-year-old men with GORD symptoms) followed by surveillance and/or intervention has been shown on more recent economic modelling studies to be cost effective ($10,000-50,000/QALY gained). The yield of performing a repeat endoscopy following an initial negative endoscopy for BE is low (2.3%). Risk factors for subsequent diagnosis of BE were male gender and oesophagitis. Subsequent studies have reported a BE prevalence for between 9 and 12% on repeat endoscopy following treatment of oesophagitis with PPIs. It is therefore recommended that for patients with severe oesophagitis, that repeat endoscopy be considered after a course of PPI therapy.

1.8.1 Screening Techniques

1.8.1.1 Conventional Endoscopy

Conventional endoscopy is considered the gold standard for screening and assessing BE and this technique is discussed in more detail in Chapter 1.9.

1.8.1.2 Transnasal endoscopy

Un-sedated transnasal endoscopy offers a viable alternative to conventional endoscopy for BE screening. It has been found to have comparable sensitivity and specificity for diagnosing Barrett’s (98% and 100%), and has been demonstrated to be feasible and safe for use in the community. It is associated with lower costs than conventional endoscopy as non-physician providers could be trained to perform the procedure. Discomfort to the patient and inability to intubate the nasopharynx in a small percentage of patients remain the limitations of this procedure.

1.8.1.3 Oesophageal video capsule endoscopy

While this technique is well-tolerated and a patient-preferred method for viewing the distal oesophagus, there is insufficient accuracy (sensitivity and specificity of 78% and 73% respectively) and therefore it is not recommended for Barrett’s screening.
1.8.1.4 Cytosponge
A gelatin-coated sponge attached to a string, expands to a sphere when swallowed and is able to collect oesophageal cytology samples when withdrawn. While safe and well-tolerated, sensitivity and specificity when combined with trefoil factor 3, a protein marker, is still only 73 and 94% for BE diagnosis. In an economic modelling study this method was found to be more cost-effective than no screening or sedated endoscopy.

1.8.2 Summary of Recommendations for BE screening
Below is a summary of recommendations regarding Barrett’s screening from the ACG 2015 guidelines:

1. Screening of general population is not recommended
2. Consider overall life expectancy of patient prior to screening
3. Consider screening for Barrett’s in men with chronic (>5 years) and/or frequent (weekly or more) symptoms of GORD and two or more risk factors for BE/EAC (i.e. age >50, Caucasian race, presence of central obesity (defined as waist hip ratio (WHR) >0.9 or waist circumference (WC) >102cm), current or past smoking history, and a confirmed family history of BE or EAC in a first degree relative).
4. Screening females is not recommended given the much lower risk of EAC than in males with chronic GORD. Consider however, if presence of multiple risk factors (as above but with WHR >0.8, WC > 88cm).
5. Consider unsedated transnasal endoscopy
6. If initial endoscopic evaluation is negative for BE, repeating endoscopy is not recommended unless LA grade B, C, or D oesophagitis is present, then repeat endoscopy after 8-12 weeks of PPI to ensure healing and exclude underlying BE.

1.9 Advanced Imaging Techniques for Assessment of Barrett’s Oesophagus
Advanced imaging techniques provide enhanced visualisation of mucosal and vascular patterns, resulting in improved detection of mucosal abnormalities that may harbour dysplasia or neoplasia. The current American and British guidelines recommend surveillance
of Barrett’s be performed with high definition white light endoscopy (HD-WLE), but use of advanced imaging techniques other than electronic chromoendoscopy is not recommended at this time. This chapter summarises the endoscopic techniques used to assess a Barrett’s segment.

1.9.1 White Light Endoscopy (Standard and High Definition)

Standard definition endoscopes are equipped with charge-coupled devices (CCDs) that produce an image resolution of up to 400,000 pixels. In a study of Caucasian patients with reflux symptoms, white light endoscopy with standard definition endoscopes was found to have a sensitivity of 82% and PPV of 32% for detecting BE. Subsequent advances in technology have resulted in smaller CCDs with higher number of pixels which can offer endoscopic images with resolution that ranges from 850,000 to over one million pixels. Those are classed as high-definition endoscopes.

Newer generation high-definition (HD) endoscopes have more recently been developed by all major manufacturers and can generate image and video resolution of over 2 million pixels. This allows better visualization of mucosal surface details and could, in theory, improve detection of early neoplastic lesions including those observed in BE.

1.9.2 Narrow Band Imaging (NBI)

NBI uses spectral narrow-band optical filters instead of the full spectrum of white light resulting in improved imaging of the superficial vascular and mucosal patterns. When NBI was compared with standard imaging techniques, one prospective, blinded, tandem endoscopy study of 65 patients showed an incremental diagnostic yield for dysplasia on a per-patient basis. Compared with SD-WLE, NBI-targeted biopsies detected dysplasia in more patients, detected higher grades of dysplasia (P<0.001), and required fewer biopsies to do so (mean 4.7 versus 8.5; P < 0.001). Two additional studies reported an increased dysplasia detection only in the per-biopsy analysis.

A meta-analysis of eight studies (446 patients and 2,194 lesions) found that NBI has a sensitivity and specificity of 96% and 94% respectively for the diagnosis of HGD, and 95% and 65% respectively for IM. Inter-observer agreement for the interpretation of NBI findings is only moderate. Although NBI was rated more highly than HD-WLE for imaging quality, this did not result in improved inter-observer agreement or increased yield for identifying early
neoplasia in BE. This applied to non-expert as well as expert endoscopists.\textsuperscript{267} Overall, while NBI performed by an expert endoscopist appears to increase the targeted yield of dysplasia, it seems that HD-WLE alone may be sufficient to maximise dysplasia detection on a per-patient basis.\textsuperscript{4}

A number of different classifications have been proposed to describe mucosal pits in non-dysplastic and dysplastic BE. Using NBI, a study of 51 BE patients showed the sensitivity, specificity, and PPV of an irregular/distorted mucosal pattern harbouring HGD were 100%, 98.7%, and 95.3%, respectively. Using a ridge/villous pattern for diagnosis of NDBE had a sensitivity, specificity, and PPV of 93.5%, 86.7%, and 94.7%, respectively. This study concluded that if biopsies were just taken of areas with irregular/distorted pattern, no patient with HGD would have been missed. Importantly, NBI could not distinguish NDBE from areas harbouring LGD.\textsuperscript{268} See Figure 1.7 for examples of mucosal patterns seen with NBI.\textsuperscript{265}

One study used magnified NBI images to image and biopsy randomly selected areas in 63 patients with BE. IM was characterized by either villous/gyrus-forming patterns, which were mostly regular with regular vascular patterns, or a flat mucosa with regular, normal-appearing long branching vessels. HGD was characterized by 1) irregular/disrupted mucosal patterns, 2) irregular vascular patterns, or 3) abnormal blood vessels. All areas harbouring HGD had at least 1 abnormality, and 85% had $\geq$2 abnormalities. The frequency of abnormalities showed a significant rise with increasing grades of dysplasia. For detection of HGD, magnified NBI images had a sensitivity, specificity, PPV and negative predictive value (NPV) of 94%, 76%, 64% and 98% respectively.\textsuperscript{269}

A further study supportive of these findings looked at the use of HD magnification endoscopy with NBI in visualising microstructural and microvascular patterns in 50 Barrett’s segments. Analysing 340 targeted mucosal points they demonstrated a high yield for detection of IM and HGD.\textsuperscript{270}
Figure 1.7: Examples of the different oesophageal surface patterns seen during NBI examination: (A) Circular mucosal pattern. (B) Ridged/villous mucosal pattern. (C) Absent mucosal pattern. (D) Irregular mucosal pattern. (E) Regular vascular pattern. (F) Irregular vascular pattern.

1.9.3 Chromoendoscopy

Chromoendoscopy uses dyes to stain the mucosa and provide greater detail of the mucosal patterns and vasculature, thereby aiding detection of subtle mucosal abnormalities that may harbour dysplasia or early neoplasia. It is difficult to compare the various chromoendoscopy techniques employed as there is little consistency in the method used, concentration of the stain, or classification of staining patterns.271

1.9.3.1 Methylene blue

Methylene blue (MB) dye is actively absorbed by columnar intestinal-type cells272 and it has been used to improve the yield of detection of IM in BE.273-275 Methylene blue solution (0.1-1.0%) selectively stains columnar epithelium with intestinal metaplasia (uptake in dysplastic tissue is variable). High sensitivity for IM (91%-98%) and variable specificity (43%-97%) has been reported.273, 274, 276-278 Staining characteristics associated with HGD/EAC are light to absent staining (p=0.01) and moderate to marked heterogeneity (p=0.01).273

Significant enrichment of IM in MB-targeted biopsy samples compared with random samples was found in an historical cohort. Sharma’s group concluded that
MB chromoendoscopy significantly increases the detection of IM and requires fewer biopsies in patients with suspected BE with greater than 1 cm of columnar-appearing mucosa. It does not appear to be beneficial in patients with irregular Z lines (<1 cm).279

A number of randomised and cohort studies have investigated the detection rate of IM and dysplasia with MB chromoendoscopy with conflicting results. Positive studies include a cohort of 86 patients that found increased detection of dysplasia (p=0.053) with MB leading to a significantly higher proportion of biopsy specimens containing intraepithelial neoplasia or early cancer, despite the smaller number of biopsies obtained per patient (80.9% versus 26.4%; P<0.005).280 In a cohort of 43 patients, though fewer biopsies were taken per patient using MB directed biopsies, dysplasia or cancer was diagnosed in significantly more patients (44% vs. 28%; P=0.03) than by random biopsy technique.276

Conversely, in a randomised cross-over study of 30 patients with a history of dysplastic Barrett’s assigned to either MB-directed biopsy (MBDB) or random biopsy before repeating the alternative technique within 6 months, found that overall, dysplasia was identified in 17 of 18 patients by random biopsy and in 9 of 18 by MBDB, showing MBDB to be significantly less sensitive in detecting dysplasia than random biopsy in BE (P=0.02).281 Two further randomised cross-over studies found that detection of IM and dysplasia was not significantly different from random four quadrant biopsies.282, 283 A further randomised cross-over trial of 57 patients found MBDB technique diagnosed significantly more specialized intestinal metaplasia compared to the random biopsy technique (75% versus 68%; P=0.032) on per-biopsy protocol but no significant differences in the diagnosis of dysplasia/neoplasia (MBDB 12%, random biopsy 10%).278

A recent meta-analysis found no incremental yield of detecting either IM or dysplasia using MB chromoendoscopy compared with standard endoscopy with random biopsies.284

Safety Considerations
Methylene blue has been shown to induce oxidative damage of DNA when photosensitised by white light. Increased DNA damage is seen in Barrett’s mucosa after chromoendoscopy using methylene blue, the effect requiring both the presence methylene blue and the white light emitted at time of endoscopy.285 Though MB-induced DNA changes have not been shown to be permanent, clinically significant, or increase cancer risk285, 286 the lack of evidence
for efficacy coupled with potential risk of DNA damage means that methylene blue for surveillance endoscopy for dysplasia cannot be recommended.\textsuperscript{287}

\textbf{1.9.3.2 Acetic acid (AA)}

The value of AA to improve diagnostic yield of surveillance endoscopy has been studied. AA induces intracellular protein denaturation with swelling of the mucosal surface and enhancement of the architecture. In a prospective study of 49 patients with BE, the combination of acetic acid and magnification endoscopy (enhanced magnification endoscopy (EME)) characterised 4 pit patterns: 1) round pits, 2) reticular, 3) villous, and 4) ridged. Villous and ridged patterns had an 87% sensitivity and 100% sensitivity for IM respectively.\textsuperscript{288}

Randomised cross-over studies have produced contradictory results on the diagnostic yield of this technique for diagnosis of IM. In a study of 31 patients with BE, magnifying endoscopy enabled the prediction of Barrett’s mucosa with 84\% accuracy (100\% sensitivity; 66\% specificity). AA–targeted biopsies obtained a significantly higher percentage of tissues containing specialised columnar epithelium compared to random biopsies (78\% versus 57\%). The number of biopsies required to detect IM was half that of random biopsies.\textsuperscript{289} Another randomised trial of 137 patients comparing the yield of performing EME (with targeted biopsies of type iii and iv mucosal patterns) to standard endoscopy, found no difference between the rates of detection of IM (P=1.0). The authors of this study therefore questioned the utility of this method in reducing sampling error to identify IM.\textsuperscript{290}

AA-enhanced magnification has been shown to have a higher dysplasia yield in surveillance of BE. In a non-specialist centre, 24\% of patients had a histological upgrade compared with previous random biopsies at an earlier conventional surveillance endoscopy.\textsuperscript{291} Additionally, AA-targeted biopsies were shown to have a sensitivity and specificity of 96.7\% and 66.5\% respectively for the diagnosis of HGD/EAC in a large single-centre prospective study of 701 BE patients (406 with prior HGD/IMC, 295 without). They concluded that advanced endoscopic imaging identifies the vast majority of BE patients with early neoplasias, and the additive effect of four quadrant biopsy was minimal. Therefore, in low- and high-risk patients, limiting endoscopic surveillance to guided biopsies is justified in specialised high-volume centres with permanent quality control (but not in the community setting).\textsuperscript{292}
A single-centre retrospective cohort study demonstrated significantly increased yield for detecting neoplasia compared with standard endoscopy with random biopsies (p=0.001), with sensitivity and specificity of 96% and 80%. Correlation was demonstrated between lesions that were predicted to be neoplasias by acetic acid and those diagnosed by histological analysis (r=0.98). This same group found that in high risk populations, there was a significant cost reduction in using AA-guided biopsies over Seattle protocol.

1.9.3.3 Indigo carmine (IC)
IC is a contrast agent that allows detailed inspection of the mucosal pattern in combination with magnification endoscopy. A study of 80 patients with suspected BE found three types of mucosal patterns after spraying indigo carmine and using magnification: 1) ridged/villous, 2) circular, and 3) irregular/distorted. Magnification chromoendoscopy with IC helped to visually identify areas with IM (97% with ridged/villous pattern) and HGD (100% with irregular distorted pattern) but not patients with LGD (which appeared similar to IM). A prospective multicentre study found that the ridged/villous pattern had a 71% sensitivity for IM, while irregular/distorted pattern had an 83% sensitivity/88% specificity for HGD/early cancer.

The limitation for IC is the need for magnification and narrow field of view. There is only one randomised trial evaluating IC chromoendoscopy for detection of dysplasia within Barrett’s but it failed to find an increased detection rate compared with HD-WLE.

1.9.4 Autofluorescence Imaging (AFI) and Endoscopic Trimodal Imaging (ETMI)
AFI exploits endogenous fluorophores excited by short wavelengths (ultraviolet or blue light) and has been studied in the context of BE assessment. Endogenous fluorophores include collagen, reduced nicotinamide adenine dinucleotide, elastin, flavin, aromatic amino acids, and porphyrins. When excited, fluorophores give off a fluorescent light (autofluorescence).

An early study of 35 patients with Barrett’s showed no significant benefit in detection of dysplasia using AFI over white light and random biopsies.

Initial cohort studies found that while AFI could improve the diagnostic yield of dysplasia compared with standard endoscopy, there was a corresponding high false positive rate. To overcome this, AFI has been incorporated into a high definition NBI endoscope with magnification (endoscopic trimodal imaging or ETMI). A randomised feasibility study showed
that the addition of AFI to HD endoscopes increased early neoplasia detection (on a per lesion and per patient basis) in patients with BE. The false positive rate of AFI was reduced from 81% to 26% after detailed inspection with NBI. This finding was not confirmed on subsequent multicentre randomised studies however, where ETMI only improved the diagnostic yield of dysplasia in the per-biopsy analysis.

1.9.5 Confocal endomicroscopy

Confocal endomicroscopy (CEM) provides real time in vivo microscopic level imaging (up to 1250-fold magnification and 250um depth) of the GI tract during endoscopy. The mucosal images seen are cross sectional as opposed to perpendicular (as is seen in histology sections). An exogenous fluorescent contrast agent is required for CEM as the natural fluorescence of the GI tract is inadequate. IV Fluorescein is the most commonly used agent. An initial study of 27 patients assessing colonic pathology in 390 locations assessed with CEM and subsequently correlated to biopsy findings found a high sensitivity, specificity and accuracy (97.4%, 99.4%, and 99.2% respectively) for predicting neoplastic changes.

The first study in BE of 63 patients with BE using fluorescein contrast-CEM found demonstrated high sensitivity, specificity and accuracy for both the detection of BE (98.1%, 94.1% and 96.8%) and neoplasia (92.9%, 98.4%, and 97.4% respectively). There was very good interobserver agreement (Kappa=0.83) for predicting the histological diagnosis. A Confocal Barrett’s Classification was developed, however as this was based on selected still high-quality images viewed following the procedure therefore it is not representative of true in vivo analysis.

A prospective, RCT with in vivo prediction of mucosal histopathology found that CEM with targeted mucosal biopsies increased the yield for neoplasia from 17% to 34%, with around 60% fewer biopsies needed to achieve a diagnosis (two thirds of patients did not require any biopsies).

In an international, multicentre, prospective randomised controlled trial (RCT), probe-based CEM was used in 101 BE patients undergoing surveillance or treatment of HGD/EAC. They found that when combined with HD-WLE, CEM significantly improved the ability to detect neoplasia in BE patients compared with HD-WLE alone.
CEM was used in 40 patients who were enrolled in a tertiary hospital study assessing accuracy of HD-WLE, NBI, and CEM in identifying HGD and IMC. CEM had high accuracy rates for detecting HGD/IMC but the benefit over HD-WLE/NBI was minimal. CEM resulted in finding one further point of HGD in a patient where HGD had already been detected by NBI but this did not impact on clinical outcome. 310

While the results of these studies are all positive, there are limitations for its use in the real world setting, such as the length of time required to perform the procedure, the need for an expert endoscopist with adequate experience in the technique which has a significant learning curve in using the equipment.

1.10 Surveillance of Barrett’s Oesophagus

Survival rate for invasive oesophageal adenocarcinoma (EAC) is very poor with <13% overall survival at 5 years. 311 The rationale for ongoing surveillance in patients with BE is to improve the chances of detecting at an early stage EAC and, thus improving survival. 13 If dysplasia and/or neoplasia can be detected at an early stage, then this is potentially a treatable and curable condition. Surveillance should detect cancer before submucosal invasion occurs, where the risk of lymph node metastases significantly increases and varies between 9 and 50% depending upon the depth of invasion. 312 In patients where there is nodal disease (often without any symptomatology to the patient), there is decreased survival. 313

1.10.1 Benefits of Surveillance

While there are no RCTs evaluating the efficacy of surveillance, several observational studies have shown that patients who have EAC detected while in a BE surveillance program, have it detected at an earlier stage and with significantly improved survival compared with those who are not undergoing surveillance. 314, 315 The potential for lead and length time bias in non-randomised studies is recognised. 316

One retrospective study assessed the impact of endoscopic surveillance on long-term survival in 80 patients undergoing resection of EAC (12 of whom had progressed to EAC from NDBE and 68 of whom were found to have BE at the time of EAC diagnosis). Median survival was significantly increased for patients in the surveillance group compared with the de novo cases
(109 vs. 12 months; P<0.001). After stratifying for the stage of EAC, surveillance was the only predictor of survival. \textsuperscript{317}

Importantly, nodal involvement is far less likely in surveyed patients compared with non-surveyed patients. In a study comparing patients presenting for the first time with EAC (n=54) with patients with EAC discovered during BE surveillance (n=16), surgical pathology showed that surveyed patients had significantly earlier stages of cancer detected (p=0.0001) and only one surveillance patient (6%) had nodal involvement compared with 34 (63%) first presenters (p=0.0001). Two year survival was significantly higher for the surveillance group (86% versus 43%; P=0.0029).\textsuperscript{318}

A large population-based cohort study from the Netherlands also demonstrated reduced two-year and five-year mortality in patients diagnosed with EAC while under surveillance (n=452) compared with patients not under surveillance (n=219), with adjusted hazard ratio of 0.79.\textsuperscript{319}

A further study of 716 EAC cases in Northern Ireland found that patients with a prior BE diagnosis (n=52), had a significantly lower tumour stage (44% versus 11% with stage 1 or 2 disease; p<0.001), higher percentage of low or intermediate grade tumours (46% versus 27%; p=0.011), and a higher rate of surgical resection (50% vs 26%; p<0.001). Those with previous BE diagnosis also had a significantly lower mortality (HR for death 0.39).\textsuperscript{320}

Not all studies have demonstrated that surveillance improves survival from EAC. In a retrospective case-controlled study during the years of 1995-2009, comparison of surveillance history was made between 38 cases of patients with known BE who died of EAC and 101 living BE patients under surveillance. Surveillance within 3 years was not associated with a decreased risk of death from EAC (adjusted odds ratio=0.99), and fatal cases were nearly as likely to have received surveillance (55.3%) as were controls (60.4%).\textsuperscript{321} Importantly, it was also noted that patients were more likely to have had dysplasia during surveillance, and 50% had advanced disease at diagnosis, suggesting that surveillance practices may have been inadequate.

1.10.2 Limitations of surveillance

Dysplasia may not be endoscopically visible and the distribution of dysplasia and cancer is highly variable. Therefore, even with a rigorous biopsy protocol, there is potential for
sampling error. Additionally, adherence to guidelines by practicing endoscopists is problematic and has been shown to worsen with increasing length of Barrett’s.\textsuperscript{5, 322}

1.10.3 Counselling patients prior to surveillance

The most recent American and British guidelines recommend that following a diagnosis of BE, discussion should occur regarding the low but significant cancer risk, recommended surveillance intervals to detect dysplasia and early stage tumours, and the therapeutic options available.\textsuperscript{3, 4}. It is important to educate patients as studies have suggested that patients both over- and under-estimate their cancer risk. In a questionnaire study of 92 respondents, 68% overestimated their 1-year risk of cancer (mean 13.6%) and 38% overestimated their lifetime risk.\textsuperscript{21}. Another questionnaire study of 169 respondents found 60% underestimated the overall risk of EAC development in BE and 69% underestimated their own risk.\textsuperscript{323}.

1.10.4 Surveillance technique

Advanced imaging techniques used for assessment and surveillance of Barrett’s segments have been discussed in some detail in Chapter 1.9, with further details below.

1.10.4.1 Use of High Definition Endoscopes over Standard Definition Systems

Optimal surveillance technique includes systematic visualisation of the mucosa using high definition white-light endoscopes (HD-WLE) in addition to narrow band imaging (NBI). HD-WLE has been shown to be superior to standard definition WLE for the detection of dysplastic lesions. In a retrospective cohort study of patients with NDBE undergoing routine surveillance, HD was superior to SD in targeted identification of all dysplastic lesions (OR=3.27) as well as overall dysplasia recognised on both random and target biopsies (OR=2.36).\textsuperscript{261}

1.10.4.2 Use of Narrow Band Imaging and other advanced imaging techniques

As already discussed in Chapter 1.9.2, NBI use has been shown to detect more areas of dysplasia than white light alone.\textsuperscript{263, 265} In a randomised trial comparing NBI to HD-WLE, both HD-WLE and NBI detected 92% patients with IM, but with few biopsies required per patient for NBI (3.6 vs 7.6, p<0.0001) and with NBI detecting a higher proportion of dysplasia (30% vs 21%, p=0.01).\textsuperscript{265} Detection of mucosal abnormalities harbouring HGD and EAC is also improved using HD-WLE and NBI.\textsuperscript{263, 269, 310}
A recent meta-analysis of fourteen studies including 843 patients found that the yield for detecting dysplasia or neoplasia was increased by 34% by using advanced imaging techniques (P<0.0001), with no significant difference between whether virtual chromoendoscopy or chromoendoscopy was used.\textsuperscript{324}

\subsection{1.10.4.3 Inspection time}
Increased mucosal inspection time has been shown to directly correlate with detection of HGD/EAC. In a study of 112 patients undergoing endoscopic surveillance by 11 individual endoscopists, patients with longer Barrett’s inspection times were more likely to have a suspicious lesion detected (P<0.001), have a greater number of lesions detected (P<0.0001), and more likely to be diagnosed with HGD/EAC (P=0.001). Additionally, endoscopists with average inspection time of more than one minute/centimetre of Barrett’s detected more patients with suspicious lesions (P=0.04), with direct correlation between average inspection time and detecting patients with HGD/EAC (rho=0.63, P=0.03). There was a trend towards higher HGD/EAC rate overall (P=0.06).\textsuperscript{325}

\subsection{1.10.4.4 Attention to the right hemisphere}
Early cancers appear to have a predilection to develop on the right hemisphere of the segment extending from the 12-6 o’clock position. In a study of 119 patients with HGD and EAC there was a significantly higher rate of HGD/EAC in the right hemisphere compared with the left (85% versus 15%; P=0.0001), and particularly between 12 and 3 o’clock (65%).\textsuperscript{326}

An Australian study of patients undergoing endoscopic resection of biopsy-proven HGD/EAC demonstrated that in Barrett’s segments ≤5cm, the 2 to 5 o’clock area accounted for around 50% of endoscopically visible lesions and associated neoplasia.\textsuperscript{327}

\subsection{1.10.4.5 Seattle protocol biopsies and targeting of visible mucosal abnormalities}
A systematic biopsy protocol detects more dysplasia and early cancer than performing \textit{ad hoc} random biopsies,\textsuperscript{37} and has been demonstrated to be safe. Eleven of 705 patients undergoing systematic biopsy protocols had adverse events (AEs), 5 requiring hospital admission. There were no deaths, perforations, aspiration, or oesophageal stricturing as a result of the procedures and all patients recovered from their AE.\textsuperscript{36}

In a cohort study comparing patients undergoing four-quadrant biopsy every 2cm (n=180) compared with non-systematic biopsies (n=182), the prevalence of LGD per patient was 18.9%
versus 1.6% (P<0.001) and prevalence of HGD was 2.8% versus 0% (P=0.03). The incidence of LGD was 2.2% versus 6.6% (NS) and that of HGD was 2.8% versus 0% (P=0.03).^328

Subtle mucosal abnormalities such as ulceration, erosion, plaque, nodule or stricture should also be sampled, even if the appearances are trivial, as there is an association with such lesions harbouring cancer.^329 In a cohort study of 69 patients referred to a specialised Barrett’s unit for management of dysplasia (18 with EAC at referral), there was an increased detection of mucosal abnormalities by the Barrett’s unit compared with that of the referring endoscopists (65 cases with VMA at Barrett’s unit vs. 29 at referral (p<0.001)). Increased VMA detection was associated with an additional 10 cases of EAC being found (56% increase cancer detection, p=0.036).^330

The addition of routine cytological sampling to endoscopic biopsies appears to add little to performing surveillance biopsies alone. In 530 patients undergoing BE endoscopic surveillance with paired biopsy and cytology found a higher dysplasia detection rate using histology rather than cytology (24.0% vs. 15.7%, respectively; P<0.0001).^331

1.10.4.6 EMR of mucosal abnormalities thought to harbour HGD/EAC
Mucosal abnormalities that are encountered during surveillance of BE patients with known dysplasia should be removed with EMR, as EMR will change the diagnosis in ~50% of patients when compared with performing biopsy alone as there is a larger tissue sample available for pathologist review. EMR also improves inter-observer agreement among pathologists. In a study of 150 EMRs of focal lesions, the histology was changed in 49% and resulted in 30% relevant change in clinical management.^332

In 47 patients undergoing EMR of visible mucosal abnormalities as part of their Barrett’s assessment, 51% had upstaging of their histology from baseline. Importantly, in 5 cases, EMR resulted in detection of endoscopically treatable intramucosal cancer and in 12 cases, EMR resulted in identifying submucosal cancer. These diagnoses impacted the subsequent clinical management in all cases.^330

1.10.4.7 Computer-assisted or wide-field “brush biopsy” tissue acquisition for increasing the yield of dysplasia
This area of research remains under investigation. 117 patients with dysplastic BE had forceps and brush-biopsy specimens taken of their Barrett’s segment. The yield of dysplasia detection
was increased by 42% with the addition of brush biopsy to forceps (overall yield of forceps alone was 25%). The number needed to test to detect one extra case of dysplasia was 9.4.\textsuperscript{334}

A large study of 1,266 patients enrolled in BE screening demonstrated that computer-assisted analysis an abrasive “brush-biopsy” can significantly improve detection BE and dysplasia for purposes of screening. Of 363 diagnosed with BE by forceps biopsy alone an extra 146 additional cases of BE were identified by adding brush biopsy. Brush biopsy addition increased overall BE detection by 40%. When the effect was analysed for patients with symptomatic reflux, brush biopsy addition increased BE yield by 71%. The addition of brush biopsy to forceps increase the detection of dysplasia by 88%.\textsuperscript{335}

### 1.10.6 Grading of dysplasia

It is recommended that dysplasia be described using the standard five-tier system summarised below (as previously described in Chapter 1.4.2 in great detail).\textsuperscript{138}:

1. Negative for dysplasia
2. Indefinite for dysplasia
3. LGD
4. HGD
5. Cancer

Caution should be exercised when making the diagnosis of dysplasia in the presence of inflammation and instead indefinite for dysplasia is a more appropriate label.\textsuperscript{59} Where erosive oesophagitis is present, the clinician should not perform surveillance biopsies but instead, surveillance endoscopy and mapping biopsies should be performed after patients have received 6 weeks of acid suppression.\textsuperscript{3} More detail around grading of dysplasia can be found in Chapter 1.4.2.
1.10.6 Surveillance Intervals

The current ACG guidelines\(^3\) recommend the following surveillance intervals depending on presence and level of dysplasia:

1. **NDBE**: surveillance at 3-5 years. ACG guidelines published in 2008 suggest if 2 endoscopies 12 months apart lack dysplasia surveillance can be at a frequency of 3 yearly.\(^1\)

2. **IND**: there is paucity of data. It is reasonable to use double dose PPI to decrease the ongoing inflammation. A retrospective study found that Indefinite for dysplasia was associated with a similar risk for progression to cancer as LGD.\(^201\)

   Recent data suggests an especially high risk of progression to higher grades of dysplasia within the first year of a diagnosis of indefinite for dysplasia, but a risk comparable to NDBE after the first year. Longer Barrett’s segment and multifocal indefinite for dysplasia were associated with neoplastic progression.\(^336\) This latter finding was also supported by a prospective study of 276 patients with NDBE, LGE and IND, who compared progression rates to HGD/EAC. They too found that patients with multifocal IND had similar rates of progression as those with LGD.\(^337\)

3. **LGD**: Diagnosis should first be confirmed by a second BE pathologist. Patients should receive aggressive PPI therapy to decrease any changes associated with regeneration or inflammation. A repeat endoscopy after optimisation of PPI may result in downgrading of LGD to NDBE. If LGD is found and the decision is made not to treat endoscopically, annual surveillance is recommended until two examinations in a row are negative for dysplasia. Following this, the surveillance intervals can return to that of NDBE. ACG guidelines recommend 4 quadrant biopsies every 1cm of BE.\(^3\) The British guidelines recommend 6 monthly surveillance until two consecutive scopes show NDBE, before reverting to NDBE surveillance.\(^4\) Though endoscopic ablation therapy in patients with LGD is associated with decreased rates of progression to EAC, this is not an indication on its own to treat and surveillance is an acceptable alternative. Treatment with ablative therapy is discussed in a separate section.
4. The presence of HGD is an indication for definitive management and should not be surveyed due to high risk of progression to cancer. These patients should have their diagnosis confirmed by a second GI pathologist.

1.11 Staging of dysplastic and neoplastic Barrett’s Oesophagus

In patients with EAC, depth of invasion determines the curative potential of endoscopic therapy. The quoted rates of lymph node (LN) metastases in patients with intramucosal cancer range from 0-7%, (2% being the median approximate). Once the submucosa is breeched the rate increases to >20% LN spread.\textsuperscript{338-342}

**Subclassification of T1a and T1b Oesophageal Adenocarcinoma.\textsuperscript{4}**

**T1a tumours**

- m1 Carcinoma in situ or with questionable invasion beyond basement membrane
- m2 Invasion into lamina propria
- m3 Invasion into muscularis mucosa

**T1b tumours**

- sm1 Invasion into upper third of submucosa (within 500um)
- sm2 Invasion into middle third of submucosa
- sm3 Invasion into lower third of submucosa

**T1a lesions:**

In contrast to other parts of the GI tract, the oesophagus has lymphatic vessels within the lamina propria as well as larger channels within the submucosa and muscularis propria. The important implication is that even intramucosal carcinoma has the potential to metastasize.\textsuperscript{343} Despite this, lesions confined to the mucosa have a very low rate of lymphatic involvement.\textsuperscript{344, 345} These lesions may be optimally treated with EMR followed by ablation to eradicate the remaining Barrett’s mucosa.

In a large retrospective study of 290 patients with oesophageal cancer (157 with EAC), 70/157 had IMC with no lymph node (LN) metastases found. 21% of patients with SMC had LN spread. These findings contrasted to the higher rates of LN spread seen in those patients with SCC (7.7% for IMC and 36.4% for SMC).\textsuperscript{342} This study followed from an earlier study of 71 patients.
with EAC that found that LN metastases were present in 0/38 patients with T1a tumours versus 10/56 (17.9%) patients with tumours invading the submucosa (pT1b).\textsuperscript{346}

In a 2010 retrospective study, an expert gastrointestinal pathologist retrospectively reviewed 54 T1 adenocarcinomas (from a pool of 258 oesophagectomy specimens) and classified them as IMC or SMC. The latter group were further subclassified as either SM1, SM2 or SM3 based on the depth of tumour invasion. Nodal metastases were present in 0%, 21%, 36% and 50% for those with IMC, SM1, SM2 and SM3 disease respectively. The differences were significant between intramucosal and submucosal tumours (p<0.0001), although not between the various subclassifications of submucosal tumours (p=0.503) Therefore any submucosal breach has high LN spread therefore not endoscopically resectable.\textsuperscript{344}

In a study of 114 patients with IMC (T1a m1-3) treated surgically (n=38) or endoscopically (EMR followed by APC of residual NDBE), No LN metastases were found in the oesophagectomy group, where a median of 29 lymph nodes were removed.\textsuperscript{345}

**T1b sm1 lesions:** Lesions with superficial submucosal invasion have conflicting data with respect to the likelihood of LN invasion. Consideration for surgery/chemoradiotherapy is appropriate in this group however for those at high risk of surgical complications, endoscopic therapy can be considered as an alternative with reported outcomes encouraging.\textsuperscript{347}

One analysis of 66 patients with low risk SM1 lesions (low risk being defined as polypoid or flat, good-to-moderate differentiation, without lymphovascular invasion) removed endoscopically found that of 61 patients assessed for remission, 87% achieved complete endoscopic remission (97% for tumours <2cm, 77% for tumours >2cm) with one patient developing a lymph node metastasis (1.9%). Importantly, the risk of developing LN metastases after endoscopic resection for SM1 EAC was lower than the risk of surgery.\textsuperscript{347}

In a study of 126 patients with EAC (75 with T1a, 51 with T1b), LN metastases were rare with T1a tumours (1.3%) and significantly increased with submucosal invasion (22%; p=0.0003). Division of the submucosa into thirds did not show a layer with a significantly decreased prevalence of node metastases in this cohort. Other risk factors for LN metastases were poor differentiation (P=0.0015), lymphovascular invasion (P<0.0001), and tumour size ≥2cm (P=0.01).\textsuperscript{348} Another study of 67 patients with T1b tumours (36 adenocarcinoma) who
underwent oesophagectomy found no correlation with the depth of submucosal invasion and LN involvement.\textsuperscript{349}

If considering endoscopic therapy for T1b sm1 tumours, those with well differentiated tumours without lymphovascular invasion have the best prognosis. Sepesi’s group (previously described) found that poor differentiation, lymphovascular invasion, and number of harvested lymph nodes were significantly correlated with nodal disease (p values of 0.024, 0.049, and 0.037 respectively.\textsuperscript{344}

**T1b sm2 and sm3**

Submucosal lesions involving the middle and deep thirds of the submucosa are associated with high rates of lymphatic involvement.\textsuperscript{344} In a study including 80 patients, 31 with sm1, 23 with sm2, and 26 with sm3 adenocarcinoma, superficial and deep submucosal invasion were associated with substantial rates of metastatic lymphadenopathy (12.9% and 20.4%, respectively).\textsuperscript{350} Another study found 36% LN involvement in tumours with deep submucosal disease invasion.\textsuperscript{351}

**1.11.1 Role of EMR in staging EAC**

The key determinant to assessing depth of invasion of EAC and thus the likely success of endoscopic ablative therapies is EMR (See section on EMR for technical aspects). It is the most accurate staging technique for Barrett’s related early neoplasia.\textsuperscript{352} Identification of mucosal abnormalities that may harbour advanced dysplasia or cancer, and removal using EMR, is a critical aspect of Barrett’s assessment and staging. It is imperative to remove intramucosal cancer (IMC) and exclude submucosal cancer (SMC) before commencing RFA as they are not reliably ablated by this technique.\textsuperscript{353-355}

In a study of 40 patients undergoing EMR, 24% of those with HGD were upstaged to IMC and 40% with IMC upstaged to SMC.\textsuperscript{356} In a study of patients with dysplastic BE being managed at a specialised Barrett’s unit, 47 patients underwent EMR during assessment for lesions suspicious for cancer. EMR resulted in upstaging of the histology from baseline in 24 of 47 patients (51%). Importantly, in 5 patients, EMR resulted in detection of endoscopically treatable early cancer (IMC). In a further 12 patients EMR resulted in identifying SMC and subsequent referral for surgery or chemoradiotherapy.\textsuperscript{330}
There is less pathologist inter-observer variability when reviewing EMR specimens, likely secondary to the amount of tissue reviewed compared with biopsy. A study comparing 251 EMR specimens with 269 biopsy samples showed that submucosa was present in the 88% of EMRs, compared with 1% of biopsy specimens (P<0.0001). 99% of biopsy specimens included lamina propria but only 58% included muscularis mucosa. Inter-observer agreement for LGD and HGD was significantly higher for EMR specimens than biopsy specimens (κ=0.33 vs 0.22, P<0.001 for LGD; κ=0.43 vs 0.35, P=.018 for HGD.\textsuperscript{357}

### 1.11.2 Role of Endoscopic Ultrasound (EUS) in staging EAC

Data demonstrate that between 15-25% of patients with superficial EAC will be under-staged with EUS; 4-12% are over-staged.\textsuperscript{356, 358, 359} Routine use of EUS prior to EMR is unwarranted, rather, EUS is important for detecting local and regional LN metastases rather than for T-staging as this information may exclude patients from definitive endoscopic therapy.\textsuperscript{343}

The sensitivity and specificity of EUS for determining true pathologic staging was shown to be poor for early EAC in a study of 107 patients with HGD/T1a and T1b lesions. Tumour depth was correctly staged by EUS in only 39% of T1a tumours and only 51% of T1b tumours. Importantly, for those lesions stages by EUS as T1a with tumour invading into but not through lamina propria, there were actually positive lymph nodes in 15% of pathologic specimens (2/13).\textsuperscript{360}

In 135 patients with HGD or IMC undergoing staging EUS, none had pathologic lymph nodes of metastases present. No endosonographic abnormalities were seen in any patient with non-nodular mucosa, therefore EUS did not impact clinical management. For patients with nodular neoplasia, resection of the nodule with histologic examination had greater utility than staging by EUS.\textsuperscript{361}

Another study demonstrating that there is little role for EUS in workup of early EAC due to poor sensitivity and specificity, was a retrospective cohort study of 131 patients with oesophageal neoplasia. In 80% of cases EUS findings were unremarkable, however in this group 24% were found after EMR to have either submucosal invasion, deep margins positive for cancer or lymphovascular invasion. In the remaining 20% of cases where EUS findings did raise the possibility of submucosal invasion or LN metastases, less than half had corroborative endoscopy findings.\textsuperscript{358}
A retrospective study of 179 patients had EUS prior to oesophagectomy for EAC (n=134) or squamous cell cancer (n=45). The overall accuracy of EUS in identifying the correct T stage was 74% with sensitivity and specificity for T1, T2 and T3 staging being 82% and 91%, 43% and 85%, and 83% and 86% respectively. EUS detected node positive disease accurately in 73%. T2 cancers in particular were over-staged.  

There may be a role for EUS in T1b (submucosal) lesions to assess for evidence of locoregional lymph node involvement. In a meta-analysis of 19 international studies with 1019 patients the pooled sensitivity, specificity, positive and negative likelihood ratio of EUS for T1b staging were 0.86, 0.86, 5.13, and 0.17 respectively. There was significant heterogeneity between studies however.  

1.11.3 Role of PET/CT in staging EAC  
The utility of positron emission tomography and computed tomography (PET/CT) in the setting of Barrett’s associated malignancy is to exclude distant disease and LN involvement in patients with T1b cancers, as if either were present, curative endoscopic therapy would not be possible and this would likely result in a patient being referred for surgery or chemo-radiotherapy. There is no yield in performing PET/CT for T1a lesions.  

1.12 Chemoprevention in Barrett’s Oesophagus  
1.12.1 Acid Suppression  
There is paucity of data with regard to chemoprevention in BE, partly because of the overall low risk of progression to neoplasia in BE and partly because we are now intervening earlier with treatment than we did previously (i.e. RFA for patients with LGD within BE). Despite this, the current guidelines recommend that patients with BE receive once-daily PPI therapy. Routine use of twice-daily dosing is not recommended unless required due to poor reflux control or oesophagitis. 

Many patients with BE are already prescribed PPI therapy for control of reflux symptoms, but for those without GORD in whom BE is found incidentally there is evidence that they too may benefit from PPI use. Several cohort studies suggest that BE subjects maintained on PPI
therapy have a decreased rate of progression to neoplasia than those on H2 receptor blockers only or no acid suppression.\textsuperscript{238, 364-367}

A multicentre prospective cohort study found that in 540 BE patients, 7\% progressed to HGD/EAC. Use of PPIs at the beginning of the study or during follow-up reduced the risk of neoplastic progression (HRs of 0.41 and 0.21 respectively).\textsuperscript{364}

An Australian cohort of 350 patients followed for a median 4.7 years found that patients who delayed using a PPI for \textgeq 2 years following BE diagnosis had 5.6-fold increased risk of developing LGD at any given time as those who used a PPI in the first year. Similar results were demonstrated for the risk HGD/EAC development.\textsuperscript{365} The same group also found that the use of PPIs prior to a diagnosis of Barrett’s also reduces the risk of HGD.\textsuperscript{368} Another cohort study of 236 patients in a multivariate analysis, the use of PPI after BE diagnosis was independently associated with reduced risk of dysplasia (HR of 0.25; P<0.0001).\textsuperscript{367}

\subsection*{1.12.2 Aspirin/NSAID use}

Aspirin or non-steroidal anti-inflammatories (NSAIDs) should not be routinely prescribed to patients with BE as a chemo-preventative agent.\textsuperscript{3} While there is some indirect evidence that aspirin may be useful for chemoprevention the side-effect profile is not benign with AEs such as GI or cerebral bleeding potentially catastrophic. Additionally, given more recent evidence demonstrating a significant risk reduction in cancer development in treating patients with LGD with endoscopic therapy, fewer patients in this category are likely to enter surveillance programs only (see Chapter 1.13 on endoscopic therapy).

The indirect evidence for aspirin use includes a meta-analysis of 2 cohort and 3 case-control studies that included 1813 cancer cases. A protective association (30\% reduction in OR) was found between any use of aspirin/NSAID and oesophageal cancer (adenocarcinoma or squamous cell), (OR=0.57). Both intermittent and frequent medication use were protective (OR of 0.82 and 0.54 respectively), with greater protection with more frequent use.\textsuperscript{237}

An Australian study of patients with oesophageal cancer (any type) compared with controls found that at least weekly use of aspirin or NSAIDS compared with never-users was associated with a reduced odds ratio for developing EAC, oesophagogastric junction ADC and oesophageal SCC, with recent use in the last 5 years associated with greatest risk reduction.\textsuperscript{369} Another epidemiological study found that when comparing 630 oesophageal and gastric
cancers to 695 case controls, the multivariate-adjusted odds ratios for EAC were reduced with use of aspirin/NSAIDs but only among patients with cyclin D1 positive tumours (OR=). A similar finding was reported for gastric tumours.\textsuperscript{370}

The results of a large, multicentre, RCT randomising patients with BE to aspirin or placebo are still awaited. Commencing in 2006, the aspirin esomeprazole chemoprevention trial (AspECT) randomised 2500 BE patients to esomeprazole with or without aspirin. In 2009, more than 85% of the participants had tolerated the medications at the initial intended doses, with dropout rate of 7%. Interim analysis was due in 2011 but has not yet been reported.\textsuperscript{371}

### 1.13 Endoscopic Therapy of Barrett’s oesophagus

Determining the appropriateness of patients to undergo endoscopic treatment of their Barrett’s oesophagus is a critical step in successful management. As such, patients need to be adequately assessed by experienced endoscopists who are additionally able to perform ablation therapy and EMR. Access to other staging procedures (such as EUS), expert GI pathologists, and experienced upper GI surgeons is also important. It is therefore suggested that patients are best treated in the tertiary setting.\textsuperscript{372}

**Summary of Recommendations for treating patients with dysplastic BE**\textsuperscript{3}

1. Patients with nodularity in the BE segment should undergo EMR of the nodular lesion(s) as the initial diagnostic (and therapeutic) step, with histologic assessment of the EMR specimen guiding further therapy. Patient with HGD/IMC should undergo ablation of the remaining Barrett’s.
2. Radiofrequency ablation (RFA) is the preferred ablative therapy for non-nodular dysplastic Barrett’s.
3. Where neoplasia is found at the deep margin of the EMR specimen, the patient should be referred for surgery, chemoradiotherapy or further resection (not ablative therapy).
4. There has been no demonstrated benefit of staging nodular BE with EUS or other imaging prior to performing EMR.
5. For those with NDBE, RFA should not be performed.
6. For those with flat, confirmed LGD/HGD, RFA is appropriate.
7. In patients with T1a EAC, endoscopic therapy is the preferred approach (EMR +/- RFA)
8. In patients with T1b EAC, endoscopic therapy may be appropriate in some cases; in those with superficial SM1 disease or those who are poor surgical candidates, however, consultation with surgical oncology multidisciplinary team should occur.

9. In patients with T1b disease, EUS may be beneficial in sampling lymph nodes given the increased prevalence of LN involvement in these patients.

1.13.1 Assessment prior to treatment

Identification of mucosal abnormalities that may harbour advanced dysplasia or cancer, and removal using EMR, is a critical aspect of Barrett’s assessment and staging. It is imperative to remove intramucosal cancer (IMC) and exclude submucosal cancer (SMC) before commencing RFA as they are not reliably ablated by this technique.353-355 (See section on depth of ablation of RFA, Chapter 1.13.4.1)

Identification of these often subtle lesions requires careful, systematic assessment of the Barrett’s segment. Detection of mucosal abnormalities harbouring HGD/EAC is shown to be improved using HD-WLE and NBI modalities.263, 269, 310, 330 (See Chapter 1.9 on advanced imaging modalities)

Where irregularity is detected, EMR or ESD should be performed, for both therapeutic benefit and staging of the lesion.347, 373 (See Chapter 1.11 on staging) EMR is generally adequate to ascertain the depth of invasion.

1.13.2 Endoscopic Mucosal Resection (EMR)

Identification of mucosal abnormalities that may harbour advanced dysplasia or cancer, and removal using EMR, is a critical aspect of Barrett’s assessment and staging. (See Chapter 1.11.1 on EMR Staging of EAC). It also provides a therapeutic option for treatment of dysplastic Barrett’s oesophagus.

1.13.2.1 Method

Two techniques exist for EMR. The cap and snare technique involves a submucosal injection of saline to lift the mucosa which is then sucked into a transparent cap, with a preloaded snare then deployed over the sucked up mucosa which is then able to be resected. In the duette system (multiband mucosectomy (MBM)), the mucosa is sucked into the cap that is preloaded with multiple rubber bands, and a band is deployed (analogous to the variceal
bANDING TECHNIQUE). The mucosa is then snared either over or under the applied band and resected. It is not routine to inject submucosally for this technique, however this should be considered when resecting near the GOJ due to the increased risk of perforation. This system allows multiple resections within a single intubation.

Two RCTs have compared the two techniques. One included 84 patients undergoing resection of Barrett’s neoplasia with either cap and snare technique, or MBM found that piecemeal EMR with MBM was faster and cheaper than with the cap. Despite the lack of submucosal lifting, MBM does not appear to be associated with more perforations. Although MBM results in slightly smaller specimens, this is not thought to be of clinical relevance because the depth of resections does not differ between both techniques. The other study compared 50 cap and snare resections and 50 MBM resections and found no significant difference in diameter or depth of resection.

Both techniques have similar success rates with a number of case series suggesting remission rates of IM between 76%-100% and of HGD/IMC between 86%-100%.352, 376-379

1.13.2.2 Therapeutic Utility of EMR, Efficacy

EMR is not only important for staging of dysplastic BE, but by removing the Barrett’s tissue and dysplasia, also provides a therapeutic option for its treatment. Ablative therapies especially radiofrequency ablation (RFA) ablate to a depth of 500um which correlates with the epithelium. If there is invasion into the lamina propria or to the muscularis mucosa RFA cannot reliably ablate to this depth and EMR is required to remove this tissue.

EMR should be considered that therapy of choice for dysplasia associated with visible lesions. Visible lesions contain the most advanced histological staging in surgical resection specimens and this is confirmed by experience with step-wise radical EMR of the entire Barrett’s segment.208, 372

While there are no randomized trials comparing EMR with other endoscopic therapies there are several large studies looking at efficacy and safety of EMR.

1.13.2.3 Efficacy

In a non-blinded, non-randomized study of 100 patients with low-risk EAC arising in BE treated with EMR alone, local remission was achieved in 99% after median 2 months and a maximum
of 3 resections. Recurrent or metachronous cancers were found in 11% during follow-up, all of which were able to be successfully retreated with EMR. The 5-year survival rate was 98%.\textsuperscript{380}

In a large multicentre study from Amsterdam, 169 patients with short segment BE (median 3cm) with HGD/IMC underwent stepwise EMR every 4-8 weeks, until complete radical circumferential resection of BE and neoplasia was achieved. Remission of neoplasia was achieved in 98% and IM in 85% of patients. After a median follow-up of 32 months, remission of neoplasia was sustained in 95% and IM in 81%.\textsuperscript{372}

In a study comparing surgical resection with EMR (with APC of remaining BE segment) for patients with early Barrett’s cancer, complete remission was achieved in 100% of surgical patients and 99% of EMR patients with one of the EMR patients dying prior to remission being achieved. There was an overall recurrence rate of 6.6% in the EMR group in a median of 4 years (one with local recurrence and four with metachronous tumours) but all were able to be successfully retreated endoscopically. Morbidity and 90 day mortality was significantly lower in the EMR group.\textsuperscript{345}

The presence of negative deep and lateral margins on EMR pathology correlate with absence of residual disease at the EMR site at oesophagectomy and thus can be considered curative. The presence of submucosal invasion of cancer cells on EMR specimens is associated with a high prevalence of residual disease at surgery (50%) and metastatic lymphadenopathy (31%).\textsuperscript{381}

EMR is not adequate as sole therapy for T1a or T1b EAC. A cohort study demonstrated that around 20% of patients treated with EMR who achieved complete resection of the primary lesion subsequently developed recurrent HGD or EAC in the remaining Barrett’s segment.\textsuperscript{373} Another demonstrated that after EMR for focal neoplasia, >80% of patients had HGD or LGD in the remaining Barrett’s epithelium.\textsuperscript{382} Therefore, all patients with successful resection of T1a EAC as well as T1b EAC selected for endoscopic therapy, should undergo ablation of the remaining segment as this significantly reduces their risk of recurrent neoplasia.\textsuperscript{212, 373, 383} This has been shown to be safe and effective for eradicating any remaining flat dysplasia.\textsuperscript{382, 384}

1.13.2.4 Safety

EMR carries a low but significant risk of complication.\textsuperscript{374, 385} EMR series from expert high-volume centres have shown a low rate of significant complications (<3%),\textsuperscript{373, 380, 385} but the
complication rate is significantly higher when performed by endoscopists with less experience (during first 20 EMR procedures).\textsuperscript{386}

**Strictures, perforation and bleeding**

Stricture formation is one of the most common complications, particularly when circumferential EMR is performed.\textsuperscript{387} In a previously mentioned study from Amsterdam with 169 patients undergoing stepwise radical circumferential EMR, almost 50% developed symptomatic stenosis. Additionally, one patient had progression of neoplasia during treatment and died of metastasised adenocarcinoma (0.6%). Acute, severe complications occurred in 1.2% of patients (bleeding, perforation).\textsuperscript{372}

Another smaller study of 39 patients undergoing stepwise circumferential resection of Barrett’s neoplasia followed by resection of the remaining Barrett’s segment found a symptomatic stricture rate of 26%. These were able to be treated with bougie dilatation.\textsuperscript{377} Another small study of 65 patients (94 procedures) undergoing EMR for HGD/IMC found acute bleeding occurred in 4 patients (6%), and strictures in 5 (7.5%). No perforations occurred.\textsuperscript{388} In another, where 144 resections were performed in 100 patients, all were performed without technical problems. Eleven minor complications (bleeding without decrease of Haemoglobin >2 g/dL; treated with injection therapy) occurred.\textsuperscript{380}

In a large study looking at intra-procedural and early (<30 day) complications, 243 MBM procedures with 1060 resections were performed in 170 patients. MBM was performed for focal lesions (n=113), for BE removal as part of a (stepwise) radical endoscopic resection protocol (n=117), and as escape treatment after RFA (n=13). The only acute complication was bleeding which occurred in 3% and was able to be endoscopically managed. No perforations occurred despite the absence of submucosal lifting. Early complications consisted of delayed bleeding (2%) that was able to be endoscopically managed, and stenosis, which occurred in 48% of patients treated under the stepwise radical resection protocol. No stenosis was seen for patients treated for focal lesions or in escape treatment.\textsuperscript{387}

1.13.3 Endoscopic Submucosal Dissection (ESD)

ESD is not routinely used in the management of dysplastic BE, so is mentioned here only briefly. It is primarily used for en bloc resection of gastric cancers and while it allows the most accurate histological assessment of depth and lateral margin clearance, it is technically
challenging, with long procedural time and does not offer significant advantage over EMR for EAC.\textsuperscript{389}

1.13.4 Radiofrequency ablation (RFA)

1.13.4.1 Dosimetry and Depth of Ablation

Barrett’s columnar epithelium has been shown to be minimally thicker than normal squamous epithelium (0.50 versus 0.49mm).\textsuperscript{77} While this difference is not clinically significant it does reinforce the fact that endoscopic therapies need to adequately ablate to a minimum of this depth.

A phased study in porcine and human models found that full thickness oesophageal epithelial removal was possible at 12J/cm\textsuperscript{2} when the RFA electrode had 100\% contact, but this extended to the muscularis mucosa only and not submucosa.\textsuperscript{354} In a study of 13 patients with EAC who underwent ablation prior to oesophagectomy, the efficacy of 3 energy densities (8, 10 or 12 J/cm\textsuperscript{2}) were assessed and comparisons also made between one versus two ablations. They found that ablation depth was related to energy delivered. Complete epithelial removal occurred where 10 J/cm\textsuperscript{2} and 12 J/cm\textsuperscript{2} energy was used, with maximum depth of injury to the muscularis mucosae. A second treatment did not significantly increase the depth of injury.\textsuperscript{353} Another study of 8 patients undergoing RFA at varying energy levels (minimum 10J/cm\textsuperscript{2} x 2; maximum 14J/cm\textsuperscript{2} x 4) prior to undergoing oesophagectomy demonstrated the maximum ablation depth increased as the combination of energy density and number of applications escalated, although no specimen demonstrated histologic evidence of ablation to the submucosa. Complete removal or ablation of all IM and HGD was achieved in 9 of 10 ablation locations. A single focus of HGD was found within the lamina propria at the edge of an ablation zone in one patient.\textsuperscript{355}

1.13.4.2 Technical aspects of Radiofrequency Ablation\textsuperscript{390}

The BarrX™ ablation system comprises four distinct ablation catheters. The two most frequently used are the BarrX™ 360 RFA balloon catheter, known henceforth as RFA\textsuperscript{360} (Figure 1.8) which is used for primary circumferential ablation, and the BarrX™ 90 RFA focal catheter, known henceforth as RFA\textsuperscript{90} (Figure 1.9), used for secondary focal RFA or primarily as treatment for shorter segments of BE. A BarrX™ 60 RFA focal catheter and BarrX™ ultralong RFA focal catheter offer alternatives to the RFA\textsuperscript{90} device.
Prior to circumferential RFA, a sizing catheter with a balloon at its distal end is inflated and calculates the mean inner oesophageal diameter for the length of the balloon to determine the appropriate size RFA\textsuperscript{360} balloon catheter to use.

Full details of The RFA\textsuperscript{360} and RFA\textsuperscript{90} ablation procedures are outlined in the Methodology chapter (Chapter 2.1.2.5).

**Post–treatment care\textsuperscript{390}**

Adequate acid suppressant therapy is very important after RFA, to minimize patient discomfort and to promote oesophageal healing and neosquamous epithelial growth. Patients should be prescribed high–dose PPIs while undergoing treatment. Additional H2–receptor blockers and sucralfate is sometimes prescribed for those with ongoing reflux symptoms of with persistent inflammation, though there is currently no evidence to support that this improves healing.

Patients usually have a liquid diet for the first 24 hours and over a few days return to soft and normal diet as their symptoms allow. Common symptoms following RFA include mild dysphagia/odynophagia, chest pain and sore throat which may persist for a few days. Simple analgesia with paracetamol and topical lignocaine viscous is usually adequate to control these symptoms. NSAIDs should be avoided where possible. In the event of severe chest pain or fevers (rare) patients may be admitted to hospital for observation and management.

**Figure 1.8 RFA\textsuperscript{360}**

**Figure 1.9 RFA\textsuperscript{90} ablation catheter**
1.13.4.3 Efficacy

The definition of successful endoscopic therapy is achieving complete remission of dysplasia (CRD), as well as IM (CRIM), in the tubular oesophagus, as demonstrated by taking 4 quadrant biopsies at the GOJ as well as for every centimeter for the extent of the original Barrett’s length. It has been suggested that routine biopsies of the cardia be performed as several case series reported recurrence of neoplasia in the cardia or GOJ following successful ablative therapy. It is acknowledged that there is potential for biopsy sampling error and some have advocated for two negative biopsy sessions to occur before stating that a patient has achieved CRIM/CRD.

The definition of failure of ablation therapy is somewhat contentious. One study showed that even in patients who underwent four RFA sessions without achieving CRIM, >50% eventually achieved this endpoint. This suggests that risk factors for poor responders may exist.

Non dysplastic Barrett’s Oesophagus (NDBE)

The AIM-2 trial (Effectiveness phase of the Ablation of Intestinal Metaplasia trial) demonstrated that 70% of patients with NDBE achieved CRIM at 12 months (after mean 1.5 ablation sessions of 10J/cm x 2). After further focal ablation therapy (mean 1.9 ablations) 98% of the remaining patients had achieved CRIM at 30 months. At 5 years’ follow-up, CRIM was demonstrated in 92%, biopsy depth was adequate to detect recurrence, and all failures (n=4) achieved to CRIM with single-session focal RFA.

Though efficacious, ablative therapy for NDBE is not recommended due to the low risk of progression to neoplasia (recent data suggesting lower rates of progression than previously thought) and low but not insignificant rate of complications. Additionally, ablation for NDBE has not been shown to be cost effective. (See Chapter 1.13.4.5 below). These patients should be surveilled only.

The clinician may embark on treatment in patients that they feel are at higher life-time risk of developing HGD/EAC (e.g. family history of EAC or long segment Barrett’s) but as yet, the evidence to support this is unclear. In a of 1175 NDBE patients (excluding patients who developed HGD or EAC within 1 year of their BE diagnosis), longer segments of Barrett’s were
shown to be associated with risk for progression. Patients who progressed to HGD/EAC had longer BE segments (6.1 vs 3.5 cm; P<0.001). Additionally, a 28% increase in progression risk was demonstrated for every 1cm increase in BE length (P=0.01).\textsuperscript{191} In a study of 713 patients with NDBE, at 4 years’ follow-up 3.4% developed HGD/EAC. The risk of developing HGD/EAC was mainly influenced by the presence of LGD, a ≥10-year duration of BE, increased Barrett’s length, and presence of oesophagitis.\textsuperscript{399}

**Low Grade Dysplasia (LGD)**

In a small single-centre study of 10 patients with confirmed LGD circumferential ablation was performed with focal ablation at 12 months as required. At 2 years, CRD and CRIM rates were 100% and 90% respectively, with no significant stricture or buried IM detected at this time.\textsuperscript{400}

Following this came the AIM-dysplasia trial, a multicentre, sham-controlled trial where 127 patients were randomised to RFA or sham procedure (2:1). In those with LGD receiving RFA treatment, CRD was achieved in 90.5% (compared with 22.7% in the sham-control group (P<0.001)).\textsuperscript{212}

More recent data demonstrates that in patients with confirmed LGD within BE, RFA treatment results in significant reduction in progression to HGD/EAC or EAC alone. In a 2014 RCT, 68 patients receive RFA (with 68 controls). RFA reduced the risk of progression to HGD/EAC by 25.0% (1.5% versus 26.5%; P<0.001) and the risk of progression EAC alone by 7.4% (1.5% versus 8.8%; P=0.03). For the RFA group, CRD was achieved in 92.6% CRIM in 88.2% (compared with CRD of 27.9% and CRIM of 0.0% for the control group (P<0.001). AEs occurred in 19% of patients receiving RFA (P<0.001); most commonly stricture, occurring in 11.8% patients receiving RFA. All strictures were treatable with (median of 1) dilatation.\textsuperscript{401}

**High Grade Dysplasia (HGD)**

In patients with HGD within BE, ablative therapy should be performed over surgery or surveillance due to its proven efficacy\textsuperscript{212} and favourable side-effect profile over surgery.\textsuperscript{402} (See Chapter 1.14.3.2 on Surgical Complications)

A multicenter study 142 patients undergoing circumferential RFA for HGD within (median 6cm) Barrett’s reported no serious adverse effects. At a median follow-up of 12 months CR-HGD was achieved in 90.2% of patients, CR-D in 80.4%, and CR-IM in 54.3%.\textsuperscript{403}
In the previously mentioned AIM-dysplasia trial, among patients with HGD, CRIM was achieved in 81.0% of those in the ablation group, compared with 19.0% of the control group (P<0.001). Overall, 77.4% of patients in the ablation group (with HGD and LGD) achieved CRIM compared with 2.3% of the control group (P<0.001). Additionally, patients in the treatment group had reduced malignant progression (3.6% vs. 16.3%, P=0.03) and fewer EACs (1.2% vs. 9.3%, P=0.045).^{212}

**HGD/LGD**

The follow-up of the AIM dysplasia trial was published in 2011. After 2 years, of 106 patients, CRD and CRIM were achieved in overall 95% and 93% (or those with LGD the figures were 98%/98%; for those with HGD, 93%/89% respectively). After 3 years, of 56 patients, CRD and CRIM was achieved in 98%/91%. Kaplan-Meier analysis showed that CRD remained in >85% of patients and CRIM in >75%, without maintenance RFA. Serious adverse events occurred in 3.4%, with rate of stricture 7.6%. The rate of EAC was 0.55% per patient-year or 1.37% per patient-year for HGD/EAC.^{393}

**1.13.4.4 Safety**

Safety of RFA is discussed at the end of this chapter.

**1.13.4.5 Cost effectiveness of RFA**

Cost analyses models have demonstrated that RFA treatment is cost-effective for treatment of HGD and LGD.\textsuperscript{404} Using a Markov model, in the analysis of a hypothetical group with HGD, comparison was made between treatment with initial RFA, or endoscopic surveillance followed by surgery if cancer was detected. Initial RFA was more effective than the latter approach. In analyzing the group with LGD, initial RFA followed by surveillance was more cost-effective than surveillance followed by RFA if HGD was detected. For patients with NDBE, endoscopic surveillance followed by RFA was more cost effective than initial treatment with RFA.\textsuperscript{398}

**1.13.5 Combination Endoscopic Therapy**

Combination endoscopic therapy (CET) generally refers to a combination of mechanical removal of Barrett’s with EMR and ablative therapy of the remaining Barrett’s segment (usually RFA). Mucosal ablation should generally be performed at least 2 months after initial EMR to allow the mucosal defect to heal.\textsuperscript{343}
In an early single centre study of eleven patients, six underwent EMR for visible mucosal abnormalities. Prior to ablation the most advanced pathological grade was LGD in 2 and HGD in 9 patients. Following a median of two RFA and two RFA, CRIM was achieved in all patients and persisted for a median 14 months’ follow-up after the last treatment. No AEs or strictures occurred, and no buried Barrett’s was detected in any of the 473 biopsies of neosquamous mucosa.

In a later multicenter European study, 16 patients with EAC, and 7 with HGD underwent EMR of visible lesions prior to RFA; Following RFA treatment, CRD was achieved in 95% and CRIM in 88% of patients (improving to 100% and 96% respectively following escape EMR in 2 patients with CRD). After an additional median follow-up of 22 months, no neoplasia recurred.

In a large retrospective US study of 244 receiving RFA for BE with dysplasia or IMC, 33% had EMR also. CRIM was achieved in 80% of patients, and CRD in 87%. Disease progression occurred in 4 patients. 23 patients (9.4%) had a treatment-related complication during 777 procedures (3%), including strictures (8.2%), post-procedural hemorrhages (1.6%), and hospitalizations (1.6%).

**1.13.6 Other Ablative Therapies**

Numerous case series on alternative endoscopic ablative techniques are reported however RFA appears to be the preferred therapy, given its body of data to support safety and efficacy. There is evidence for the use of photodynamic therapy (PDT) in the setting of BE with HGD. There is also some promising data on cryotherapy for achieving CRIM in patients with HGD. All ablation modalities improve eradication when compared with surveillance for HGD, but they should only be used as a primary treatment in the case of flat dysplasia. Alternative ablative therapies are summarised below.

**1.13.6.1 Photodynamic Therapy (PDT)**

In this technique, a photosensitiser drug (usually porfima sodium) is administered before the procedure where it selectively accumulates in the malignant oesophageal tissue. At endoscopy, light activates the photosensitiser drug, which leads to oxygen radical formation and tissue destruction.
Efficacy

Published trials have demonstrated the efficacy of PDT in eradicating Barrett’s dysplasia.\textsuperscript{412-417}

In a multicentre RCT, PDT with omeprazole was shown (at 5 years’ follow-up) to be significantly more effective than omeprazole alone in eliminating HGD within BE (77% versus 39%; P<0.0001). Progression to cancer was also significantly lower in the PDT group (15% versus 29%; P=0.27).\textsuperscript{412} There is less evidence with other photosensitisers such as 5-aminolevulinic acid (ALA). One study showed a complete response in 97% of HGD patients and in 100% of IMC patients treated with ALA-PDT after a median follow-up period of 37 months.\textsuperscript{416} A retrospective study from the MAYO clinic of 199 patients with HGD, found that PDT (n=129) was comparable to surgery (n=70) in terms of surgical mortality and long-term survival after a mean follow-up period of approximately 5 years.\textsuperscript{381} PDT was also shown to be cost effective when compared with oesophagectomy in patients with HGD.\textsuperscript{418}

Limitations

The relatively high rate of adverse side effects is the main downside to PDT use.\textsuperscript{406} Patients need to remain out of sunlight after the procedure due to photosensitivity. Additionally, symptomatic strictures are reported in up to 36% of patients.\textsuperscript{419} The risk for development of stricture increases with a history of prior EMR or oesophageal stenosis, and the number of PDT treatments received per session.\textsuperscript{420}

Another concern about PDT is development of sub-squamous BE glands that could harbour neoplastic potential, the rate of which was found to be 14% in a systematic review.\textsuperscript{421} One study comparing APC and PDT found buried BE in 21-24% of patients.\textsuperscript{422} Another study that examined histological and pathological specimens after PDT, found buried glands 51% of patients; 27% of these harboured dysplasia or carcinoma.\textsuperscript{423} Cases of adenocarcinoma arising from buried Barrett’s glands have also been reported.\textsuperscript{412, 424}

Given these limitations, PDT has fallen out of favour in recent years, and referral centres have adopted EMR and RFA as the preferred endoscopic treatment modalities for BE.\textsuperscript{425}

1.13.6.2 Cryoablation

Cryotherapy is based on spraying the targeted tissues with cycles of either liquid nitrogen or rapidly expanding carbon dioxide gas, via spraying catheters passed through the working
channel, causing rapid freezing and slow thawing that destroy tissues through immediate and delayed effects.\textsuperscript{426} Promising cohort data demonstrate high rates of CRIM and CRD, and a safety profile similar to other ablative techniques.\textsuperscript{407, 427, 428} In a single centre study with 32 patients, CR-HGD was 100%, and CRIM was 84% at 2-year follow-up. At last follow-up (range 24-57 months), CR-HGD was 97% and CRIM was 81%. No serious adverse events occurred. Stricture was seen in 3 patients (9%).\textsuperscript{408}

Multi-centre RCTs are required to confirm these results and establish longer-term durability. The optimal treatment protocol and direct comparative data with RFA will be needed before cryotherapy’s role is clear among the ablative techniques for BE.

1.13.6.3 Argon Plasma Coagulation (APC)

APC delivers a high frequency, monopolar current of ionized argon gas resulting in coagulation of the epithelium with 1-3mm depth of injury depending on the gas flow rate, generator settings (most studies use 40-90W), distance to the mucosa and duration of application.\textsuperscript{429}

APC has been studied principally for ablation of NDBE.\textsuperscript{430-432} CRIM rates for this subgroup range from 36%-97% in published RCT data.\textsuperscript{430, 431, 433-436} RCT data for patients with HGD exists in a study comparing APC (n=13) and PDT (n=13) where PDT was found to be more effective at eradicating dysplasia than APC but at higher cost.\textsuperscript{424}

In a more recent study of 63 patients undergoing APC (n=33) versus surveillance (n=30) a median number of 4+/-1.6 sessions were required to achieve CRIM with mean follow-up of over two years for both groups. APC treatment resulted in a significantly higher recurrence-free survival than surveillance alone (P=0.005).\textsuperscript{437}

In one large case series of 129 patients with NDBE or LGD, patients were randomly allocated to APC ablation or surveillance. APC reduced the extent of the BE by 95% in the majority of ablated patients, both in the short and long term, however the authors found that progression to HGD still occurred in one patient in the APC group, suggesting surveillance is still required long term.\textsuperscript{438}

Given the high relapse rates observed, the concern regarding buried glands and a low benefit risk in this patients, APC is no longer used as ablation treatment, and is reserved as a “touch up” therapy to treat areas of IM left behind by other ablative modalities. Other techniques
like multipolar electrocoagulation and laser therapies have also been replaced by other ablation modalities.\textsuperscript{425}

### 1.13.6.4 Multipolar electrocoagulation

Multipolar electrocoagulation (MPEC) was one of the earliest ablative methods studied for the treatment of Barrett oesophagus. A probe transmitting electrical energy directly to the contacted tissue causes increased temperature and resultant tissue destruction. The depth of burn is dependent on the probe pressure, duration of contact and the generator settings.\textsuperscript{429} Studies have demonstrated the ability of MPEC to eradicate NDBE.\textsuperscript{430, 439} One large multicentre case series of 58 patients with IM, demonstrated a CRIM rate of 78\% at 6 months but no longer term results are available.\textsuperscript{439} An RCT comparing MPEC with APC found that complete reversal of BE was achieved and could be maintained in approximately 70\% of patients, irrespective of the technique.\textsuperscript{430}

MPEC is time consuming and therefore not realistic to use this method to treat long segments of BE, and it has now largely been replaced by other ablative methods.\textsuperscript{425}

### 1.13.6.5 Lasers

The data involving laser therapy, where an intense light beam is used is destroy the epithelium, is limited to small cases series. Lasers used include the Neodymium: yttrium aluminium garnet laser, which produces an injury depth of 3-4mm, and the Potassium phosphate laser, which produces a more superficial injury (depth \(\sim\)1mm).\textsuperscript{411}

Complications of laser therapy include retrosternal pain, dysphagia, odynophagia, nausea, vomiting, fever, epigastric pain, sore throat, headache, oesophageal strictures, bleeding and oesophageal perforation. Short-term success rates for the treatment of BE with use of laser therapy have been variable. For example, in a case series of 31 patients, BE was eradicated in 21 patients after 5-7 treatments. There were eight patients with recurrent IM, seven of whom were re-ablated successfully. One patient developed EAC and required oesophagectomy. One perforation, one GI bleed and one stricture were reported.\textsuperscript{440} The rate of residual BE in patients after this treatment ranges from 0\% to 85\%, and rates of buried glands range from 0\% to 90\%.\textsuperscript{441-443}
1.13.7 Complications of endoscopic therapy

The main complications of endoscopic therapy have been addressed within each modality however they are summarised below.

1.13.7.1 Strictures

EMR has the highest rate of stricture formation, particularly when circumferential EMR is performed\textsuperscript{387}, with symptomatic stricture rates as high as 50%.\textsuperscript{372} Reported stricture rates vary from 12.5% to 88%, which is dependent on EMR extent and number of sessions.\textsuperscript{444-446} As a rule, the greater the extent of resection, the higher the chance of stricture formation. Most EMR strictures are able to be treated successfully with endoscopic dilatation.\textsuperscript{446}

PDT has a significant symptomatic stricture rate of around 35%\textsuperscript{419} with the risk for development of stricture increasing with a history of prior EMR or oesophageal stenosis, and the number of PDT treatments received per session.\textsuperscript{420} Strictures typically develop within a month of PDT. In a study of 116 patients undergoing PDT, factors that influenced stricture formation were found to be length of BE, multiple PDT treatments and presence of IMC.\textsuperscript{447}

RFA has stricture rates of <6% and these tend not to be clinically significant (i.e. Do not tend to require intervention).\textsuperscript{212}

Strictures occurred in 2/13 (15%) of patients undergoing APC of their entire Barrett’s segment in a randomised control trial of 26 patients comparing APC to PDT.\textsuperscript{424}

1.13.7.2 Bleeding

For EMR, bleeding occurring at time of the procedure is not uncommon (~10%),\textsuperscript{380} but significant bleeding requiring re-endoscopy or blood transfusion is much lower at around 1%.\textsuperscript{372, 373, 377, 388} For ablation techniques, variable bleeding rates (0-14%) have been reported.\textsuperscript{410}

1.13.7.3 Perforation

For EMR, the perforation rates are generally <1%.\textsuperscript{372, 380, 387, 388} For the other endoscopic techniques cases of perforation are reported but not common.\textsuperscript{410}
1.13.7.4 Minor Complications

Minor complications such as transient chest discomfort and odynophagia secondary to local tissue injury are commonly described but to varying degrees. \(^{410}\) PDT has also been associated with photosensitivity 0-69\%. \(^{406, 422}\)

1.13.7.5 Buried Barrett’s Oesophagus

A concern with regard to endoscopic ablation is the development of buried glands under the neosquamous epithelium, also termed “buried Barrett’s”. It is defined as specialized columnar epithelium covered by a layer of squamous epithelium with no communication with the surface. \(^{394}\)

When the ablation procedure does not destroy all of the metaplastic epithelium, then the partially ablated mucosa may heal with an overlying layer of neosquamous epithelium that buries metaplastic glands in the lamina propria, becoming undetectable to endoscopic surveillance. The significance of buried glands is unknown, and whether they might be present or not before ablation, but the development of adenocarcinoma under squamous epithelium has been reported, \(^{448}\) and specifically, cases of EAC arising from buried Barrett’s glands has also been reported. \(^{412, 424}\)

Patients who have had PDT appear to be most at risk for developing buried Barrett’s. One previously mentioned study that examined histological and pathological specimens after PDT, found buried glands 51\% of patients; 27\% of these harboured dysplasia or carcinoma. \(^{423}\) A recent systematic review evaluated the evidence of buried metaplasia before and after ablation therapies. The baseline prevalence ranged from 0\% to 28\%; in patients after PDT treatment it was 14.2\%; and after RFA in only 0.9\%. \(^{421}\) The true incidence and risk of progression to neoplasia might be underestimated. Besides the inherent sampling error from biopsy protocols, it is unclear whether the size and depth of biopsy specimens may be insufficient to identify buried glands. \(^{449, 450}\) A recent study using tridimensional optical coherence tomography (3D-OCT) found buried glands in 72\% of patients (13/18) before and in 6\% of patients (10/16) after initial achievement of CRIM with RFA therapy (79). \(^{451}\) Further studies are needed to resolve this issue.
1.13.7.6 Progression of Disease
As discussed in the individual treatment sections above, many smaller studies have demonstrated cases of EAC development while patients are undergoing endoscopic eradication therapy.\textsuperscript{345, 373, 438} In a meta-analysis and systematic review of 2307 patients undergoing endoscopic ablative therapy for NDBE, LGD and HGD, 43 cases were identified of patients developing EAC following treatment; 37 with prior HGD, 2 with prior LGD and 4 with prior NDBE. This corresponded to a risk of EAC development of 6.1%, 0.8% and 0.3% for HGD, LGD, and NDBE respectively following ablation therapy.\textsuperscript{452} For patients undergoing endoscopic eradication therapy for their BE, surveillance should continue, particularly in high risks groups (i.e. those with prior advance dysplasia/neoplasia).

1.13.7.7 Risk of recurrence after endoscopic therapy
Rates of recurrence of IM and dysplasia following successful eradication of BE are variably reported in the literature.\textsuperscript{453} In one study of 592 patients who had documented CRIM at two consecutive endoscopies following RFA treatment showed a 33% recurrence rate of IM at two years (22% of which was dysplastic i.e. 7.3% dysplasia recurrence in the whole cohort). No predictors of recurrence were identified in this study, either endoscopic or demographic.\textsuperscript{392}

In one large retrospective analysis that sought to identify predictors of recurrence, of 1634 patients achieving CRIM, there was a 20% recurrence rate at 2.4 years’ follow-up. This was primarily recurrence of IM (86%), but included LGD (5.7%), HGD (4.5%) and IMC (3.9%). Risk factors for recurrence, on multivariate analysis, included, older age, longer BE segments and non-Caucasian race, and on bivariate analysis also included more advanced pre-treatment histology.\textsuperscript{454} In a single centre retrospective analysis of patients achieving CRIM with RFA, demonstrated a 25% IM recurrence and 8.5% dysplasia recurrence at 1 year.\textsuperscript{455}

In contrast to both these studies, a systematic review and meta-analysis of prospective and retrospective studies of RFA durability found much lower pooled recurrence rates following RFA (0.9% for dysplasia and 13% for IM following an average 1.5 years’ follow-up. IM recurrence rates ranges from 8-21%.\textsuperscript{456} A later meta-analysis of 41 studies and 4443 patients reported pooled incidence rates (IR) of recurrent IM, dysplasia, and HGD/EAC after radiofrequency ablation of 9.5%, 2.0%, and 1.2% per patient-year respectively. When all types of endoscopic therapies were included, pooled IRs of recurrent IM, dysplasia, and HGD/EAC
were 7.1%, 1.3%, and 0.8% per patient-year, respectively, with increasing age and BE length predictive of recurrence.\textsuperscript{457}

Hence, continued surveillance after CRIM is imperative. Additional studies with long-term follow-up are needed.

### 1.14 Surgical Therapy

Less than ten years ago, surgical oesophagectomy was the standard treatment for patients with early EAC and HGD Barrett’s oesophagus. Published guidelines from 2008 recommended that patients with HGD be routinely considered for oesophagectomy.\textsuperscript{1} This was in part due to the high prevalence of EAC found in patients with HGD who underwent surgery.\textsuperscript{205} Prevalence rates vary in the literature, with older surgical series reporting the risk of concomitant adenocarcinoma in patients with HGD approaching 40%.\textsuperscript{205} A meta-analysis of 23 studies of patients undergoing oesophagectomy for BE and HGD reported a 12.7% incidence of invasive adenocarcinoma. Importantly, the invasive EAC rate was 11% in those with visible lesions compared with 3% for those with no visible lesion.\textsuperscript{208}

This last point is important, because these studies were published prior to the routine use of high definition endoscopes and advanced imaging techniques which are better able to identify subtle mucosal abnormalities that may harbour neoplasia. A study of 41 patients with pre-operative HGD and 19 with pre-operative IMC found an overall rate of submucosal invasive carcinoma of 6.7% (5% for HGD, 11% for IMC). Thoise with SMC had visible lesions at endoscopy. This suggests that if if patients have been adequately assessed and staged, then patients HGD and early EAC, particularly where no endoscopically visible lesions are seen, can be treated endoscopically.\textsuperscript{210}

#### 1.14.1 Role of surgery in the context of newer endoscopic techniques.

As has been discussed in the endoscopic therapy section, there is now strong evidence for the use of EMR and RFA ablation in the treatment of patients with LGD, HGD and early cancers. Oesophagectomy remains the treatment of choice for fit candidates with T1b sm2-3 disease, either alone or in combination with radiation and/or chemotherapy.\textsuperscript{3} It is still often considered in appropriate patients who have T1a or T1b sm1 disease with poor prognostic
factors such as poor tumour differentiation or lymphovascular invasion. Surgery must include complete longitudinal resection of the Barrett’s segment and lymphadenectomy for T1b tumours because of the significant risk of LN involvement.

1.14.2 Type of operation

1.14.2.1 Transthoracic Approach (Ivor Lewis Oesophagectomy)

The Ivor Lewis procedure consists of a combination of laparotomy and mobilisation of the stomach, and thoracotomy (usually right sided), resection of the oesophagus and oesophagogastric anastomosis. This allows the creation of an anastomosis between stomach and oesophagus either in the left or right pleural space.

1.14.2.2 Transhiatal Oesophagectomy

Only allows anastomosis between stomach and oesophagus at the level of the neck. This approach avoids the need for thoracotomy, with low in-hospital mortality and length of stay reported in one high-volume centre study. In a RCT, the transhiatal approach was associated with less perioperative morbidity than the transthoracic approach, with no significant difference in perioperative mortality or long-term overall survival. It was noted that a subgroup of patients with 1-8 LNs involved had a better survival in the transthoracic group suggesting that this approach may be preferable in patients with significant risk of lymphadenopathy.

1.14.2.3 Minimally invasive laparoscopic or thorascopic oesophagectomy

There are no RCTs comparing minimally invasive oesophagectomy (MIO) with open surgery although there is a trial underway in France.

A series of 222 patients undergoing MIO (including 47 with HGD only), found the procedure to be safe, with a 30 mortality of 1.4% and shorter hospital stay (7 days). There remains a high complication rate including a leak rate of 11.7%. A UK series found that there were fewer pulmonary complications in patients undergoing MIO compared with open Ivor Lewis oesophagectomy (8% vs 23%). This was supported in another study showing similar reduction in pulmonary complications in the first few months following MIO compared with open oesophagectomy.

Other surgeries include Vagal-Sparing Oesophagectomy for HGD and Merendino Segmental Oesophagectomy but are not discussed in detail here.
1.14.3 Surgical outcomes

1.14.3.1 Effectiveness

A number of centres have published their outcomes following oesophagectomy for Barrett’s with HGD. Overall cancer free survival is predominantly affected by the presence of cancer within the resected specimen. For HGD and T1 EAC, case series suggest that the 5-year survival rates range between 80-90% and 3-year survival is greater than 90%.402, 465, 466

Barrett’s recurrence can occur following oesophagectomy. In one study assessing recurrence of BE/EAC in 45 patients who underwent curative oesophagectomy, 18% had recurrent Barrett’s metaplasia or neoplasia thought due to metachronous disease.467 In another study of 48 patients undergoing oesophagectomy, 50% were found to have columnar metaplasia and 25% intestinal metaplasia on biopsies taken 1-2cm above the anastomosis at surveillance endoscopy. The prevalence of BE appeared to increase over time following oesophagectomy, likely related to the time of chronic acid and bile exposure.468 These studies suggest that surveillance gastroscopies are still required following oesophagectomy with ablation of recurrent Barrett’s if it occurs.469

In a previously quoted 2011 study from Pech et al, that compared oesophagectomy to EMR (with APC of residual NDBE) in 114 patients treated for T1a m1-3 intramucosal cancers, it was demonstrated that complete remission was achieved in all surgical patients with long-term remission over a median follow-up of 3.7 years, of 100%.345 Surgery was associated with significantly higher morbidity and mortality however, as discussed in the section below.

1.14.3.2 Adverse Events

Oesophagectomy has been associated with a mortality and morbidity of up to 5% and 30% respectively.470-472 Operative morbidity and mortality correlates to the volume of procedures performed per year by both the hospital centre and the surgeon, with patient outcomes demonstrated to be better in high volume centres and with increased surgeon experience. One multicentre study from the US, demonstrated that the volume of oesophagectomies (high volume >5/year, low volume <5/year) was an independent risk factor for operative mortality and that high volume centres showed a tendency towards decreased complications and length of stay.473 Another study found that the observed associations between hospital volume and operative mortality are largely mediated by surgeon volume.474
Much of the morbidity and mortality is due to anastomotic failure/leak. A meta-analysis of 7500 oesophagectomy patients found that transthoracic oesophagectomy had a 50% relative risk reduction in leak rates compared with the transhiatal approach. This technique however was associated with higher early pulmonary morbidity and mortality rates, with overall 5-year survival similar between techniques. A large prospective randomised study of 83 patients, however, found no difference in leak rates and survival between the two surgical approaches.

In pooling results from several studies, the weighted average for morbidity was 33%, mortality 1.4%, and anastomotic leak 2.8%, with a median follow up between 12 to 59 months.

In the Pech et al 2011 study that compared oesophagectomy to EMR with or without APC, 32% of the surgical group had major post-operative complications compared with 0% in the endoscopically treated group (P<0.001). The 90-day mortality rate was 2.6% (1/38) for the surgical group and 0% for the EMR group, which was not statistically significant (P=0.333). The overall recurrence rate in the endoscopic group was 6.6% (though all were able to be subsequently managed endoscopically).

1.14.4 Quality of life

Oesophagectomy has a negative impact on QOL however the adverse effects lessen in patients surviving >2 years. There is some evidence that QOL is improved with MIO over open surgery, at least in the short term.

1.14.5 Anti-reflux surgery

While there have been studies looking at the relative benefits of anti-reflux surgery in the prevention of development of neoplasia within Barrett’s, there is only weak data to suggest this may be beneficial. Therefore, indications for anti-reflux surgery should be the same for BE patients as for patients with GORD without BE.

1.15 Surveillance following Eradication of Barrett’s Oesophagus

Following successful eradication of dysplasia and IM within Barrett’s, patients should continue to have surveillance to detect recurrence of IM or dysplasia at the GOJ or within the neosquamous mucosa. The neosquamous mucosa appears to be more permeable than
normal squamous epithelium, but does not appear to harbour genetic abnormalities, and appears biologically stable.

Some cohort studies demonstrate recurrence of IM/dysplasia of >20% at 2-3 years. The majority of recurrences that have been reported are non-dysplastic, however up to a quarter may be dysplastic or even neoplastic.

Surveillance intervals and biopsy protocol recommendations following eradication of Barrett’s are based on expert opinion and on intervals based on published cohort studies.

1. For those with prior IMC or HGD, surveillance should be performed every three months for 1 year then every 6/12 for 1 year then annually thereafter.
2. For those with prior LGD, surveillance is recommended every 6/12 for 1 year then annually thereafter.
3. Surveillance should be performed with HD WLE/NBI with 4 quadrant biopsies taken at the GOJ and for every 1cm of prior Barrett’s length.
4. If recurrent IM or dysplasia is detected, this should be treated as per guidelines previously listed.
5. After complete remission of intestinal metaplasia (CRIM) is achieved, the goal of anti-reflux medication is to control reflux symptoms adequately and/or the presence of oesophagitis.

1.15.1 Recurrence of intestinal metaplasia/dysplasia following CRIM

1.15.1.1 Site

There is inconsistency in the current literature regarding IM recurrence, partly due to lack of consensus in its definition. Recurrent or residual IM typically occurs in distinct patterns; as visible islands in the tubular oesophagus, buried IM under neosquamous mucosa, and at the GOJ. One study demonstrated that the GOJ was the most common site for recurrent IM post-ablation (71%). The clinical significance of IM at the GOJ is not well established and several clinical trials assessing RFA efficacy did not routinely biopsy the GOJ at follow-up.
1.15.1.2 Rates of recurrence

A US Multicentre Consortium whose definition included isolated IM of the cardia/GOJ found that, among patients achieving CR-IM by 24 months, 20% developed recurrent IM within a year; 33% after 2 years. IM of the cardia/GOJ accounted for almost half of the recurrences reported.\(^392\) In another study, recurrent IM was described in 26% of 53 patients treated successfully with RFA. None relapsed with dysplasia though this cohort consisted of predominantly NDBE to being with.\(^489\) This figure was supported by another study where there was 26% cumulative IM recurrence in 47 patients treated successfully with RFA.\(^455\) This group had predominantly HGD pre-treatment, though also included NDBE and IMC. Four (9%) had recurrent dysplasia at the neosquamocolumnar junction with none detected endoscopically.

In a study of 54 patients achieving CRIM, biopsies were obtained from the GOJ (median 20) which was focally ablated at least once. Recurrent focal IM was found in 19 patients (35%), however, this finding was not reproduced at subsequent endoscopies in all but two patients (median 5 follow-up endoscopies), with no increase in IM incidence at the GOJ over time equating to a sustained remission of 93%. The findings were suggestive that focal IM at the GOJ post-RFA is not related to residual or recurrent BE.\(^487\).

In contrast, where IM of the GOJ was not considered recurrence, reported recurrence rates are much lower. Of 198 patients achieving CR-IM in a UK study, only 9% had recurrent IM by end of follow-up; 47% of those however, had recurrent dysplasia.\(^488\)

Recurrence rates in cohorts treated with EMR alone\(^485\) versus combination EMR/ablation\(^490\) have reported comparable recurrence rates. Recurrence rates also appear to be similar across different ablation methods for dysplastic BE, such as cryotherapy\(^408\) and PDT.\(^491\)

1.15.1.3 Significance of IM at the GOJ

Whether IM at the GOJ represents true recurrence, persistent IM in an area not initially ablated, or cardia intestinal metaplasia is uncertain. In two studies where biopsies were routinely taken just below the neosquamocolumnar junction post-RFA, one found persistent IM in only 4% of 54 patients and no dysplasia\(^487\); the other found recurrent dysplasia in 9% of 47 patients, with all dysplasia located at the GOJ.\(^455\) With IM thought to precede dysplasia,
this suggests that IM at the GOJ may not be a benign entity and it follows that surveillance endoscopy should include biopsies at the GOJ.

1.15.1.4 Subsquamous (“buried”) Barrett’s
The prevalence of buried Barrett’s following ablation is variably reported. A systematic review suggests that the occurrence of buried Barrett’s is lower following RFA (0.9%), than after PDT (14.2%). A higher prevalence has been suggested, particularly at the GOJ, by a study using optical coherence tomography. Importantly, buried IM can predispose to subsquamous neoplasia.

One study suggested that standard biopsies obtained at post-eradication surveillance endoscopy may not be of sufficient depth to detect buried Barrett’s as the majority of the biopsies (>60%) did not contain lamina propria, however this finding has not been substantiated in other studies.

1.15.1.5 Risk factors for recurrence
Some studies suggest that risk factors for recurrence of IM/dysplasia following CRIM include older age, longer BE segment, presence of hiatus hernia and more advanced pre-ablation histology. Other observational studies suggest that uncontrolled reflux may be a risk factor for post-ablation recurrence.

1.15.1.6 Treatment of recurrent IM/dysplasia
In the majority of cases where recurrent IM or dysplasia is detected, this is able to be managed endoscopically with further ablation or EMR, though cases of invasive EAC have been reported.

1.16 Who should treat Barrett’s Oesophagus?
Expert opinion suggests that a number of core competencies are required before performing ablation therapy, which is only one component of management of these patients. Endoscopists wishing to perform ablation therapy should also be experienced in assessment of Barrett’s oesophagus, use of advanced imaging techniques to detect advanced dysplasia/neoplasia, and performance of EMR. Current British guidelines recommend that
endoscopists performing endoscopic therapy for dysplastic Barrett’s should have performed a minimum of 30 supervised RFA cases and 30 EMR cases.\textsuperscript{4}

Despite this guideline there is little data surrounding the learning curve for these techniques. For RFA, a single endoscopic case series demonstrated no difference in eradication of IM, complications, or procedure time for the first 25\% of cases vs. latter 75\% of cases.\textsuperscript{498} In a multicentre study however, among 7 different endoscopists, the rates of CRIM achieved ranged from 62-88\% with positive correlation found between both patient volume and RFA volume.\textsuperscript{499}

EMR competency is necessary as it is often required pre-ablation as part of the staging process, and may also be required during the ablation treatment pathway, as progression of disease can occur during this time.\textsuperscript{212, 333, 486, 500} EMR carries a low but significant risk of complication.\textsuperscript{374, 385} EMR series from expert high-volume centres have shown a low rate of significant complications (<3\%),\textsuperscript{373, 380, 385} but the complication rate has been shown in at least one study to be significantly higher when performed by endoscopists with less experience (during first 20 EMR procedures).\textsuperscript{386} Another multicentre Dutch study that examined a structured training program for EMR found no difference in complication rates, completeness of resection, or time per resection for the first 10 vs. second ten resections (30\% used the multiband techniques).\textsuperscript{386} Endoscopists should have the ability to recognise and manage both immediate and delayed complications, most notably bleeding, perforation and stricture.

Ideally, RFA and EMR should be performed in a tertiary referral hospital for management of oesophageal disease, with access to both the best endoscopic equipment to facilitate endoscopic management of dysplastic BE with RFA and EMR, as well as access to surgeons who specialise in oesophageal surgery.
1.17 Conclusion

The above review of the literature serves as a background to this thesis. It demonstrates the relatively high prevalence of Barrett’s in the general population and risk factors for progression to EAC, a cancer that left untreated has a poor long term survival, but with early detection and endoscopic management is often curable. It highlights the significant technological advancements that have been made over the last decade in improving our assessment and management of patients with dysplastic Barrett’s but also highlights the importance of these tools being used by experienced endoscopists and not necessarily in the wider endoscopy community. It also demonstrates the uncertainly regarding the natural history of early Barrett’s dysplasia, and while guidelines exist with regard to managing these patients, questions remain as to whether these patients can be better risk stratified and whether close observation, rather than treatment is a more cost effective strategy in the long term.
1.18 Aims of this research

The aims of this research include:

1) Prospective recruitment of patients with dysplastic Barrett’s oesophagus for endoscopic assessment and management at a tertiary referral centre.

2) To assess the effectiveness, safety and durability of endoscopic management in patients referred with dysplastic and early neoplastic Barrett’s oesophagus, over a five-year period, in the real world setting.

3) To assess whether assessment at a specialised Barrett’s unit improves detection and staging of patients referred with dysplastic Barrett’s when compared with assessment in the community setting.

4) In a retrospective cohort of patients diagnosed with low grade dysplasia (LGD) within Barrett’s, assess the degree of agreement on the original diagnosis with two expert GI pathologists and whether this impacted the risk for progression to EAC.

5) In this same cohort, reassess whether LGD could be reassigned as mild or moderate dysplasia and assess whether this affected progression rates to high grade dysplasia or cancer.
Chapter 2: Methodology

This chapter expands on the study design, materials and methods used in chapters 4 and 5 of this thesis, both of which are presented as published papers in PDF format. Methodology for Chapter 3 is already described in detail within Chapter 3 itself.

2.1 Chapter 4: Detection and Staging of Oesophageal Cancers within Barrett’s Oesophagus is Improved by Assessment in Specialized Barrett’s Units

2.1.1 Synopsis

This was a prospective cohort study set at a single tertiary hospital Barrett’s Unit where patients with dysplastic Barrett’s oesophagus were referred for assessment and definitive management. Assessment was performed with high definition white light endoscopy (HD-WLE), narrow band imaging (NBI), Seattle protocol and targeted biopsies in addition to endoscopic mucosal resection (EMR) where lesions were thought to harbour neoplasia. We aimed to compare the mucosal lesion and cancer detection rates within dysplastic BE in the community compared with a Specialized Barrett’s unit in addition to assessing the impact of endoscopic mucosal resection (EMR) on disease staging and management.

2.1.2 Study Design, Methods and Materials

2.1.2.1 Referrals

Consecutive patients referred to St Vincent’s Hospital Melbourne from November 2008 to September 2011 for management of dysplastic Barrett’s were prospectively entered into a central Microsoft Access database, specifically made for Barrett’s patients. Further details of this database are described in Chapter 2.2.2.2 and shown in Appendix 5. Patient demographics, most advanced histology at and prior to referral, and referral endoscopy details were recorded. These details, where provided, included the Barrett’s extent, use of NBI, presence and size of hiatus hernia and description of any mucosal abnormality.

Referrals by community endoscopists to the tertiary centre were triggered by the discovery of any level of dysplasia on biopsy, whether this be low grade, high grade or early cancer, as at the time of this study, only two tertiary hospitals in the state were providing endoscopic
management of dysplastic Barrett’s with RFA. In some cases, the referring endoscopist may have suspected early neoplasia, particularly if they had made reference to a mucosal abnormality of concern. In other cases, where no mucosal abnormalities were described, it is assumed that the discovery of dysplasia or neoplasia was fortuitous.

2.1.2.2 Referral endoscopy details
Comprehensive referral endoscopy details were retrospectively collected from the endoscopist, general practitioner, endoscopy centre, or hospital medical records, with patient consent obtained as required. Details of Seattle protocol adherence, use of Prague classification, and documentation of mucosal abnormalities were able to be obtained from the endoscopy and histology reports. Clinical patient details such as PPI use were obtained from the clinical referral letter. Details of use of HD-WLE and NBI and type of sedation used required further information from the endoscopy centres (see example of letter requesting this information attached in Appendix 6).

2.1.2.3 Referral histology details
Referral histopathology was retrospectively obtained for the patient cohort and reviewed by an expert GI pathologist (RW). Where downgrading or upgrading of the histological diagnosis occurred, the reviewed result was used as the baseline histology to compare subsequent histological findings following full endoscopic assessment. Where the referral histology was unavailable for review the original histological grade was used as the baseline.

2.1.2.4 Assessment and Management at the Specialized Barrett’s Unit
Systematic assessment using HD-WLE then NBI was performed by two experienced endoscopists (AT, CJ) using Olympus H-180 endoscopes. Barrett’s extent was documented according to Prague classification (Appendix 1.4) Any mucosal abnormalities seen were described according to size, position (centimetres from mouth, o’clock position), Paris Classification (Appendix 1.3), and mucosal pattern (irregularity/loss). These details were recorded in a Microsoft Excel Spreadsheet (separate to the database) and was maintained by the PI.

Mapping biopsies were taken according to Seattle Protocol (Appendix 1.5) with targeted biopsies of any mucosal abnormalities. Biopsies were labelled according to level and o’clock position in the neutral scope position to facilitate more accurate location at subsequent
endoscopy. Areas thought to harbour HGD or cancer, based on appearance characteristics or through biopsy confirmation were removed with EMR via the duet and snare technique (See Section on EMR technique (1.13.2.1) for further details). Initially, such lesions were biopsied first, with EMR performed several weeks later. In later cases, EMR, where required, was often performed at initial assessment. Biopsies were assessed for presence of IM and grade of dysplasia using the revised Vienna classification (IM without dysplasia (Non-dysplastic (ND)BE), indefinite for dysplasia (IND), LGD, HGD or cancer). EMR specimens were evaluated for infiltration depth, vertical resection margins, tumour differentiation or grade of dysplasia, and lymphatic/vascular invasion.

The most advanced histology after assessment was recorded with patients’ subsequent management dependent on this outcome. Those with SMC were referred for oesophagectomy or chemoradiotherapy as endoscopic therapy was not considered definitive. In those with IMC or nodular HGD, EMR was performed until we were confident that no cancer remained prior to commencing RFA. For those with flat dysplastic Barrett’s, RFA was performed at approximately 3 monthly intervals until remission of dysplasia and intestinal metaplasia (IM) was achieved.

2.1.2.5 Technical aspects of the RFA\textsuperscript{360} and RFA\textsuperscript{90} ablation procedures

As mentioned in Chapter 1.13.4.2, the BarrX™ ablation system comprises four distinct ablation catheters. The two most frequently used are the BarrX™ 360 RFA balloon catheter, known henceforth as RFA\textsuperscript{360} (Figure 1.8) which is used for primary circumferential ablation, and the BarrX™ 90 RFA focal catheter (20mm x 13mm), known henceforth as RFA\textsuperscript{90} (Figure 1.9), used for secondary focal RFA or primarily as treatment for shorter segments of BE. A BarrX™ 60 RFA focal catheter and BarrX™ ultralong RFA focal catheter offer alternatives to the RFA\textsuperscript{90} device.

Prior to circumferential RFA, a sizing catheter with a 4cm long noncompliant balloon at its distal end is inflated and calculates the mean inner oesophageal diameter for the length of the balloon.

The RFA\textsuperscript{360} ablation catheter also holds a balloon (available in five outer diameters (22, 25, 28, 31 and 34mm) at its distal end, with a 3cm long bipolar electrode on its outer surface. After inflation and activation via foot switch, RF energy is delivered to the electrode. As
previously mentioned, dosimetry studies have shown that for circumferential ablation two applications of RF energy at 10 or 12 J/cm² and 40W/cm² are the most effective regimens to ablate the full thickness of the epithelium.\textsuperscript{353-355}

Stepwise circumferential and focal ablation of a BE segment typically starts with a circumferential ablation procedure using the RFA\textsuperscript{360} balloon, following the steps below:

1. Record the length of the Barrett’s segment to be treated noting the level (cm from the mouth) of the top of the gastric folds and top of the circumferential and maximal segments of Barrett’s. N-acetyl cysteine (1%) is often flushed over the oesophageal wall to remove excess mucous, to enable better contact between the electrode and mucosal surface. Introduce a guide wire into the stomach under vision then remove the endoscope.

2. Size the inner oesophageal diameter. The sizing balloon catheter is passed over the wire and positioned 5cm above the most proximal extent of Barrett’s (i.e. the distal balloon will be positioned 1cm above the Barrett’s segment. Balloon inflation is activated with a foot pedal and the diameter calculated. The balloon is advanced in 2cm increments and the step repeated until the balloon in no longer restrained, indicating its position within hernia or stomach. Generally, sizing is performed blind, however if there is concern about focal stricturing, the procedure can be visualized concurrently with the endoscope positioned just above the balloon on inflation.

3. Select the appropriate size RFA\textsuperscript{360} ablation catheter. The recommendation is to use a catheter smaller than the smallest measured diameter calculated by the sizing balloon, though the endoscopist may elect in certain cases to use a larger diameter device in special circumstances, under endoscopic vision (for example, above and below a focal narrowing).

4. First ablation. The RFA\textsuperscript{360} catheter is introduced over the wire, followed by the endoscope. Under vision, the proximal margin of the electrode is placed 1cm above the most proximal extent of the Barrett’s segment. The balloon is inflated and electrode activated via a foot switch. Moving distally the balloon is repositioned, allowing a small overlap of 5-10mm with the previous ablation zone, until the entire Barrett’s segment has been ablated.

5. Remove slough. The catheter is removed and electrode cleaned with wet gauze. A soft cap is fitter to the tip of the endoscope and used to slough off the coagulum from the ablated area. Removal of the coagulum has been shown to increase the efficacy of the first ablation session from 90% surface regression to 95%.\textsuperscript{405, 503}

Patients return for endoscopic review and repeat RFA treatment ideally between 8-12 weeks. This may be a second RFA if there is still a significant residual circumferential extent of Barrett’s, or focal RFA which is performed as outlined below:

1. Introduce the RFA catheter. The RFA electrode is fitted over the tip of the endoscope and positioned at the 12 o’clock position in the video image. The endoscope is gently advanced, never forced, under vision into the upper oesophagus, and may be aided by jaw thrust provided by a nursing assistant.

2. First ablation. Position the Barrett’s mucosa to be treated at the 12 o’clock position in the endoscopic image. Deflecting the tip of the scope (and hence catheter) upward brings the electrode in close contact with the mucosa. A double application of 12J energy is applied before moving to the next segment to be treated. Ablation of the entire Z–line with the RFA device is recommended, to ensure eradication of intestinal metaplasia at the GOJ.

3. Remove slough. This can be performed with a soft cap (as described in step 5 above) or with the leading edge of the electrode. Remove coagulum from electrode surface with wet gauze.

4. Second ablation. Repeat steps 1 and 2 with the aim to repeat double ablation over all the previously ablated areas.

This process is repeated every 2-3 months, until remission of BE is achieved endoscopically and histologically. The majority of patients will need one circumferential ablation session and 1-2 focal ablation sessions to achieve CRD and CRIM.

Post-ablation care of the patient is discussed in Chapter 1.13.4.2.

2.1.2.6 Statistical analysis and ethics approval

For the patient cohort, comparison was made between the rate of detection of mucosal abnormalities and cases of cancer in the community setting, compared with the Specialised Barrett’s Unit. Testing for significance of increase in detection of mucosal abnormalities and EAC at a Barrett’s unit was performed using Chi square/Fisher’s exact test at a single tail significance level of 5%. Factors contributing to improved detection rate at the specialised unit were also compared (time for assessment, use of HD-WLE/NBI, adherence to Seattle
Protocol). We assessed the role of EMR in staging the most advanced histopathology and how this impacted on management outcomes for this group.

This study was approved by the hospital’s Human Research Ethics Committee.

2.1.2.7 Study limitations

It is important to note that this study was not a randomised comparison study, in that patients with Barrett’s were not randomised to assessment by either a community or specialist endoscopist. Additionally, where the community endoscopists were often screening patients for the first time, the specialist endoscopists had prior knowledge of previous endoscopy and histopathological findings; arguably an advantage when endoscopically assessing the patient, and thus, potentially a limitation of this study. A more rigorous study design would have blinded both the community and specialist endoscopists to any prior endoscopy/histopathology result. This study is, however, likely to more accurately reflect real world endoscopy practice, highlighting both the limitations or difficulties faced by community endoscopists and the advantages of assessment at a tertiary referral centre.

2.2 Chapter 5: Recurrent Intestinal Metaplasia at the Gastroesophageal Junction following endoscopic eradication of dysplastic Barrett’s oesophagus may not be benign

2.2.1 Synopsis

This was a prospective cohort study performed at two tertiary hospitals (St Vincent’s Hospital Melbourne (SVHM) and Royal Melbourne Hospital (RMH)) of patients referred for assessment and management of dysplastic Barrett’s oesophagus. We aimed to determine effectiveness, safety and durability of RFA with or without EMR for dysplastic BE patients in a real world cohort. Primary reported outcomes were rates of achieving complete remission of dysplasia (CR-D) and intestinal metaplasia (CR-IM), rates of adverse outcomes, and durability of remission.
2.2.2 Study Design, Methods and Materials

2.2.2.1 Patient Cohort
The patient cohort comprised patients with dysplastic Barrett’s oesophagus who were referred to St Vincent’s Hospital Melbourne and The Royal Melbourne Hospital between 2008 and September 2013.

2.2.2.2 Barrett’s database
A Barrett’s database was established in 2008 using the Microsoft Access Program. Comprehensive patient data for each referred patient was prospectively entered into the database. At referral this included patient demographics, referrer and referral endoscopy details, prior treatment and pre-assessment histology (Appendix 5.1). Detailed reports of any subsequent endoscopies performed (including assessment endoscopy, RFA procedures, EMR procedures and follow-up endoscopy procedures) were also recorded prospectively in this database along with histological outcomes (such as complete remission of IM or dysplasia) and documentation of any complications (See Appendices 5.2, 5.3 and 5.4).

Referral histology was reviewed by an expert gastrointestinal pathologist (RW) and any changes to the original diagnosis was recorded on the referral/home page. Plans for subsequent management were also details on this page.

Information regarding any staging procedures (EUS/CT/PET) was recorded on a separate page in the patient’s database (Appendix 5.5)

2.2.2.3 Assessment Endoscopy
This was performed as previously outlined in the methods section of the Chapter 5 PDF (See Chapter 5.2) and as described above in Chapter 2.1.2.4.

2.2.2.4 Management
Patients’ subsequent management depended on the most advanced histology after assessment and suitability for CET. Those with submucosal cancer (SMC) were referred for oesophagectomy or chemoradiotherapy as endoscopic therapy was not considered definitively curative. In those with IMC or nodular HGD, EMR was performed until we were confident no cancer remained prior to commencing RFA.
In the treatment group, RFA was performed at 3 month intervals, unless delay occurred due to patient illness/social reasons. RFA was performed using the BARRX™ HALO system as previously described in the literature \(^{390}\) (See 1.13.4.2 for technique of RFA). The HALO\(^{360}\) or HALO\(^{90}\) ablation catheters were used at the discretion of the endoscopist (AT, FM, CJ, GC) depending on case specifics. Typically, HALO\(^{360}\) was used initially, unless the segment was patchy or significant narrowing existed from prior EMR scarring. Where HALO\(^{360}\) was used, the GEJ was overlapped and treated with the balloon. Where HALO\(^{90}\) was employed the GEJ was focally ablated. In most cases, the oesophagus was flushed with N-acetyl-cysteine pre-treatment. Patients received double-dose proton pump inhibitor and topical anaesthetic post-procedure. Interval EMR was performed where suspicious lesions were detected at subsequent endoscopy.

2.2.2.5 Defining and documenting adverse outcomes

EMR and RFA adverse events (AE) were defined as events requiring surgery, unplanned hospital admission, bleeding requiring transfusion or unplanned endoscopic procedure. For patients living remotely, admission for observation was arranged when minor intra-procedural bleeding occurred. These patients were included in AE rates. AEs were documented against the endoscopic procedure for which it occurred.

2.2.2.6 Follow-up

Follow-up evaluation commenced at the first post-treatment endoscopy confirming CR-IM (no endoscopic Barrett’s and no IM histologically, including at the GEJ). Recurrence was defined as IM or dysplasia identified after achieving CR-IM. IM at the GEJ but not within the cardia was considered recurrence. Surveillance endoscopies were performed at 3, 6 and 12 months, then annually for those with prior IMC/HGD, and 6 and 12 months, then annually for those with prior LGD. Surveillance biopsies were taken in four quadrants every 2cm commencing just distal to the neo-squamocolumnar junction at the GEJ, extending proximally for the original maximal (M) length of the segment.

As discussed in the published paper and its introduction (Chapter 5.2 and 5.1 respectively), recurrence rates of IM following endoscopic eradication of Barrett’s are variable in the literature, relating to both differences in definitions of recurrence, and also the location of
where surveillance biopsies are taken, particularly distal oesophageal biopsies. For instance, if surveillance biopsies are taken across the new squamous–columnar junction they could include some normal cardia with intestinal metaplasia and could be reported by the pathologist as residual or recurrent Barrett’s. Our endoscopists endeavoured to take surveillance biopsies just distal to the neo-squamocolumnar junction and just above, with a view to reporting recurrence/residual Barrett’s as the presence of IM in biopsies above the junction, but not within the cardia. We appreciate, however, that precise biopsy sampling in this location can be technically difficult and as such recurrence rates at the GOJ may therefore be impacted.

Where possible, patients were followed in the tertiary centre for at least one year after achieving CR-IM however in some cases, due to social circumstances or distance from the hospital, patients were discharged to their referring endoscopist. Advice was given regarding surveillance intervals and biopsy protocol. Subsequent endoscopy/histology reports were obtained for database entry. Patients with recurrent IM or dysplasia were re-referred to the tertiary centre regarding further management.

2.2.2.8 Statistical analysis and Ethics Approval

Absolute remission rates and Kaplan-Meier estimates with 95% confidence intervals for achieving CR-D and CR-IM at 1, 2 and 3 years were calculated. IM/dysplasia recurrence rates (absolute, and Kaplan-Meier estimates) were calculated for those achieving CR-IM. Testing for statistical significance was performed using Chi square/Fisher’s exact test at a single tail significance level of 5%.

This study was approved by the hospitals’ Human Research Ethics Committees.
Chapter 3: Classifying low grade dysplasia in Barrett’s oesophagus as mild or moderate dysplasia may better predict the risk of malignant progression.

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3.1 Abstract

Background and Aims: Reported malignant progression rates for low grade dysplasia (LGD) in Barrett’s oesophagus (BE) vary widely. Expert histological review of LGD is advised, but limited data are available on its clinical value. This retrospective cohort study aimed to determine the value of classifying LGD as either mild or moderate dysplasia by investigating the incidence rates of high grade dysplasia (HGD) and oesophageal adenocarcinoma (ADC) after expert histological review of LGD.

Methods: Histopathology reports of all patients diagnosed with LGD between 1995 and 2002 in a single tertiary hospital were reviewed by two expert pathologists. This panel diagnosis according to a two-tier and three-tier grading system of dysplasia was subsequently compared with the histological outcome during endoscopic follow-up.

Results: 116 LGD patients (69% men; mean age 61.5 years ±12.5) were included. Following histological review and consensus diagnosis, 88% were downstaged to non-dysplastic BE (NDBE) or indefinite for dysplasia (IND). In 12% the initial LGD diagnosis was confirmed. Endoscopic follow-up was performed in all patients with a median follow-up of 53 months (IQR= 29-75). The incidence rate of HGD/ADC per patient-year for the whole cohort was 1.8%. After consensus diagnosis was achieved, for confirmed LGD (n=14), the risk of HGD/ADC was 6.8% per patient-year. When patients with confirmed LGD were further classified into mild
(n=12) or moderate (n=2) dysplasia, the risk of malignant progression was 4.0% and 21.1% per patient-year respectively. Patients downstaged to NDBE or IND had a malignant progression risk of 1.4% and 0% per patient-year, respectively. Kaplan-Meier analysis showed that the cumulative risk for developing HGD/ADC for patients with a consensus diagnosis of LGD was 72.5% in 105.8 months. This was significantly higher than patients with a consensus diagnosis of NDBE (32.3% in 187.2 months; p=0.003). When LGD was classified as mild (n=12) or moderate (n=2) dysplasia the cumulative risk for developing HGD/ADC was 55.6% in 105.8 months and 100% in 95 months respectively. Moderate dysplasia had a significantly higher cumulative risk for malignant progression compared to NDBE (p<0.0001), whereas the diagnosis of mild dysplasia did not (p=0.121).

**Conclusions:** Confirmed LGD in BE confers an increased risk of malignant progression. When further stratified, a consensus of moderate dysplasia appears primarily responsible for this increased risk. While the vast majority of patients with community diagnosis of LGD will be downstaged after expert review and have a lower progression risk, the risk is not negligible and these patients warrant continued follow-up. BE patients with LGD should undergo expert histological review of the diagnosis for adequate risk stratification with consideration given to further risk stratifying those with confirmed LGD as mild or moderate dysplasia, particularly in cases where double reporting is not possible.

**Keywords:** Barrett’s oesophagus, low grade dysplasia, neoplasia, oesophageal adenocarcinoma

**Abbreviations:** Adenocarcinoma (ADC), Barrett’s oesophagus (BE), confidence interval (CI), high grade dysplasia (HGD), low grade dysplasia (LGD), non-dysplastic Barrett’s oesophagus (NDBE)
3.2 Introduction

Reported malignant progression rates for low grade dysplasia (LGD) in Barrett’s oesophagus (BE) vary widely in the literature.\textsuperscript{140, 195, 198-200} This is an important issue clinically, as there remains controversy as to whether these patients should be managed aggressively, aiming to eradicate dysplasia and Barrett’s, or whether surveillance alone may be appropriate, and even whether surveillance intervals could be reduced. Expert histological review of LGD is advised, but limited data are available on its clinical value.

A pivotal study by Curvers et al\textsuperscript{140} found that the majority of cases of LGD diagnosed by community pathologists were downgraded to non-dysplastic BE (NDBE) or indefinite for dysplasia (IND) and that those who were downgraded had a very low rate of progression to high grade dysplasia or adenocarcinoma (HGD/ADC). When there was a consensus diagnosis of LGD, the risk for progression to HGD/ADC was significant. These findings were supported in a follow-up study by Duits et al\textsuperscript{141}

It is well established in the literature that there is considerable inter- and intra-observer variability in the diagnosis of GI tract dysplasia\textsuperscript{138}, particularly when diagnosing NDBE, IND and LGD, even among expert pathologists.\textsuperscript{7} This is because divisions between no dysplasia and degrees of dysplasia involve unnatural cut-offs along a histological and biological continuum.\textsuperscript{68}

Double reporting improves prediction of progression to oesophageal ADC. Studies have shown that the prediction of progression of oesophageal dysplasia is improved if at least two GI pathologists agree on the diagnosis and increases further when a greater number of pathologist concur with the diagnosis.\textsuperscript{140} In their study, a diagnosis of LGD was made in 147 patients. After pathology review, 85% of the patients were downstaged to NDBE or IND. In only 15% of the patients was the initial diagnosis LGD. Endoscopic follow-up was carried out in 83.6% of patients, with a mean follow-up of 51.1 months. For patients with a consensus diagnosis of LGD, the cumulative risk of progressing to HGD/ADC was 85.0% in 109.1 months compared with 4.6% in 107.4 months for patients downstaged to NDBE (P<0.0001). The incidence rate of HGD/ADC was 13.4% per patient per year for patients in whom the diagnosis of LGD was confirmed. For patients downstaged to NDBE, the corresponding incidence rate was 0.49%.
Further work by the same group examined 239 additional patients with LGD diagnosed in the community who had biopsies reviewed by at least two GI pathologists and 73% of the cases were downgraded to IND or NDBE. For confirmed LGD, the risk of HGD/ADC was 9.1% per patient-year. Patients downstaged to NDBE or IND had a malignant progression risk of 0.6% and 0.9% per patient-year, respectively.

The findings from these two studies suggest that if LGD were classified as mild (where the distinction between no dysplasia and presence of dysplasia is more blurred) and moderate (where the diagnosis is more obvious, and a consensus for LGD more easily achieved) that this may also better predict progression to HGD/ADC even without a double reporting (i.e. at an individual pathologist level).

Studies have suggested that community based pathologists have difficulty in the interpretation of both NDBE and dysplasia. It has previously been recommended that in difficult cases, a second opinion should be sought from an expert pathologist experienced in BE. The Royal College of Pathology now recommends “double reporting” of diagnoses of HGD and this has been agreed upon by consensus statements by Barrett’s international experts. The British guidelines and American guidelines have extended this recommendation to include all grades of dysplasia.

3.3 Aims

This retrospective cohort study aimed to confirm previous findings by Curvers et al and Duits et al that a consensus diagnosis of LGD from expert pathologists conferred a higher risk for progression to HGD/ADC.

It also aimed to determine the value of classifying LGD as either mild or moderate dysplasia by investigating the incidence rates of HGD and ADC after expert histological review of LGD.

3.4 Methods

Setting and Source population

In a single tertiary hospital setting (St Vincent’s Hospital Melbourne (SVHM)), all patients with a diagnosis of LGD within Barrett’s oesophagus detected between January 1995 and December 2002 as documented on the central hospital database were identified.
Database search

The St Vincent’s Pathology Database was searched between the years of 1995 and 2002 for pathology reports containing:

1. Any report with oesophagus in the summary or coded as oesophagus (i.e. all oesophagus); AND within that group
2. Only cases coded with 73330 (Barrett’s oesophagus (this was coded at pathologist discretion and may or may not have used the British definition)); AND
3. Having the word dysplasia in the summary or have been coded as dysplasia.
4. The specific search term (to enable future replication) was: “mcode:73330 (tcode:62 OR summary:oesophagus) (summary:dysplasia OR tcode:74*)”

This search term aimed to find episodes of patients with LGD within Barrett’s oesophagus. This search had the potential to miss episodes if Barrett’s oesophagus or dysplasia were not mentioned in the summary or if the pathologist had not assigned the codes for Barrett’s oesophagus or dysplasia.

Study population

The database search yielded 1316 patients with 5041 linked histology reports. After manual exclusion of non-gastroscopy episodes there were 3978 associated episodes. These reports were read by the principal investigator, as were any earlier or later episode reports pertaining to those patients that fell outside the dates of 1995-2002. Patients were included or excluded from analysis base on the criteria below:

Inclusion criteria for the study:

Inclusion criteria for the study were as follows:

i) Patients met the standardized definition of Barrett’s oesophagus, that is, presence of columnar-lined mucosa in the distal oesophagus of any length at endoscopy and presence of LGD/mild/moderate dysplasia on histology

ii) follow-up histology at least 1 year after the time of initial diagnosis of LGD

Exclusion Criteria:

Exclusion criteria for the study were as follows:
i) Patients where LGD was first detected after 2002

ii) Patients with HGD or ADC diagnosed prior to the diagnosis of LGD

iii) Patients diagnosed with HGD or ADC less than one year following the detection of LGD, given this may have represented missed HGD/ADC at earlier endoscopy rather than represent true progression.

iv) Patients with fewer than two gastroscopies with oesophageal histology recorded.

v) Patients with only two oesophageal histology episodes recorded, where episodes were less than 12 months apart

Clinical follow-up
For all patients with an initial diagnosis of LGD (regardless of their consensus diagnosis after expert pathology review), pathology results for all subsequent follow-up procedures were also retrieved from the database to ascertain whether progression to HGD/ADC had occurred.

Where the patient had a St Vincent’s Hospital UR number, clinical follow-up was obtained from their medical records (pathology, emergency attendances, hospital admissions and discharge or death summaries)

Once the cohort was established, these patients were additionally searched for in the Victorian Cancer Registry (VCR) to determine if any additional patients had received a diagnosis of oesophageal ADC. As there is mandatory reporting of cases of cancer by pathology companies to the VCR, this aimed to maximise our detection rate of those in our cohort with malignant progression.

Study Endpoints
The primary endpoints were the development of HGD or ADC during endoscopic follow-up. If the patient developed HGD or ADC, this diagnosis was confirmed by the two expert pathologists according to the criteria detailed below. A secondary endpoint was the inter-observer agreement between the pathologists.

Sorting the data prior to pathologist review
For the patients eligible to be included in the cohort (n=122 initially), for each patient, episodes that were to be reviewed included the initial episode of LGD, in addition to the first and last recorded episode for that patient and any episode documenting dysplasia. Interval episodes with NDBE were not included for review unless they were the first or last episode
for that patient. As such, the reviewing pathologists reviewed a mixture of episodes that had been originally reported as LGD, NDBE, HGD and ADC. They were blinded to all original diagnoses.

**Retrieval of slides from archives and de-identification process**

The relevant patient episodes were retrieved by the principal investigator from the SVHM archives, equating to 994 slides. Each slide was given a unique identification number that was affixed manually to each slide on retrieval. A master-list of these identification numbers was kept by the PI only. Each number enabled the principal investigator to track which patient, episode and slide number was being referenced and additionally, allowed sorting of the collected data by patient and episode number.

In earlier episodes (77 episodes between 1995 and 2000), the plastic slide cover was lifting off the base of the glass. Following initial review of a handful of these slides by the reviewing pathologists it was felt that there was too much artefact to properly assess the slides and the decision was made to recut these. These slides were used in place of the original for the pathologist reviews.

Slides were placed in groups of approximately 50, in order of the original laboratory number (i.e. in chronological order), meaning that each group contained episodes from differing patients. Episodes were always complete within a group (i.e. they were never split across batches).

A master list was kept by the PI to enable tracking of each episode and to which batch each episode belonged (Figure 3.1).
Pathologist Panel and Histological Review

The pathologist panel for this study consisted of two pathologists (RW and MC) both with extensive experience in Barrett’s related dysplasia.

Pathologist Review

Both pathologists were required to review all retrieved H&E stained slides of paraffin embedded biopsy specimens. Pathologists were blinded to the patient identifier, original histological diagnosis and any previous review results. Cases were reviewed independently by each pathologist and where disagreement occurred, a dedicated meeting was held to reach consensus diagnosis. Additionally, where progression to HGD/ADC occurred, the pathologists had a consensus meeting to confirm the diagnosis.

One batch of slides (approximately 50 slides) were sent to each pathologist on a rotating basis. A corresponding Microsoft Excel spreadsheet was sent also, for the pathologist to record their review results. Each sheet contained the slide laboratory number and unique identifier to enable the PI to link this data to the specific patient at a later date, without identifying the patient to the reviewer.

The de-identified slides for the study cohort were reviewed independently by each gastroenterology pathology expert (RW and MC) and re-stratified according to the two tier
Vienna Classification (Non-dysplastic, LGD, HGD, ADC) [Appendix 1.6] older three tier system (Non-dysplastic, mild dysplasia, moderate dysplasia, severe dysplasia, ADC) [Appendix 1.7] by the most advanced identifiable histology. While broadly following the Vienna Classification, ultimately, the decision to assign any grade of dysplasia was left to each individual pathologist. In turn, the decision to classify LGD as mild or moderate dysplasia was also the decision of the individual pathologist, based on their overall impression of the architectural and cytological changes rather than requiring strict histological features to be present in order to assign a category. This was because it was recognised that LGD dysplasia occurs in a continuum from NDBE to HGD with even expert GI pathologists disagreeing on where the cut-off from one level of dysplasia to the next should be.

The highest histologic grade for each slide was recorded on a standardised excel spreadsheet with a drop down box to choose from. See sample spreadsheet below (Figure 3.2). The standardised definition of Barrett’s Oesophagus was used [Appendix 1.2].

In addition to recording the most advanced histology (according to two tier and three tier system), the pathologists also recorded the total number of biopsies in episode, number of biopsies with dysplasia, slide number with worst histology, whether the dysplasia, if present, was focal (LGD diagnosed at one level of the Barrett's segment) vs multifocal (LGD diagnosed at > 1 level), presence of inflammation (in epithelium or lamina propria). Space for free comment was also given.
Drop down boxes included

<table>
<thead>
<tr>
<th>Cardia</th>
<th>Cardia</th>
<th>Focal</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous</td>
<td>Squamous</td>
<td>Multifocal</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>NDBE</td>
<td>NDBE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IND</td>
<td>IND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGD</td>
<td>Mild</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGD</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMC/ADC</td>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>IMC/ADC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These worksheets were returned to the PI with the data entered into a master list to enable comparison of the two pathologists’ reviews.

In the beginning, two test batches were reviewed by the pathologists. These were then reviewed with the pathologists together in order to achieve a general consensus regarding what was considered NDBE, Indefinite for dysplasia, LGD, HGD and IMC/ADC

**Collating the pathologists’ results**

Following each batch review by the individual pathologist, their completed spreadsheets were stored both in an excel file, specific to the pathologist, and additionally, collated in a combined master workbook (excel), with each combined batch result stored on a separate tab (Figure 3.3).
Figure 3.3 Example of Master workbook sheet, containing both pathologists’ individual reviews of each slide.

At the conclusion of the 21 batch reviews, these results were further summarised in an additional tab to summarise all results into one spreadsheet (Figure 4).

Figure 3.4. Summary of all patient episode reviews (example section)
Determining pathologist agreement

A scoring system was used, where points were allocated for each incremental level of dysplasia recorded by each pathologist in order to later calculate pathologist agreement or disagreement.

Points allocated for each level of dysplasia:

- NDBE = 0
- IND = 0.5
- LGD Mild = 2
- LGD Mod = 3
- HGD/Severe = 5
- IMC/ADC = 6

Therefore, the sum of points for each potential outcome was as follows:

- NDBE/NDBE = 0
- NDBE/IND = 0.5
- IND/IND = 1
- LGD Mild/NDBE = 2
- LGD Mild/IND = 2.5
- LGD Mod/NDBE = 3
- LGD Mod/IND = 3.5
- LGD Mild/LGD Mild = 4
- LGD Mild/LGD Mod = 5
- LGD Mod/LGD Mod = 6
- HGD/NDBE = 6.5
- LGD Mild/HGD = 7
- LGD Mod/HGD = 8
- HGD/HGD = 10
- HGD/IMC = 11
- IMC/IMC = 12
**Statistical analysis**

For statistical calculations, both the Statistics and Data Package (STATA/SE 14.1, StataCorp LP Statistics/Data Analysis StataCorp, Texas) and Microsoft Excel XLSTAT 2016 were used.

Mean and standard deviation were used for continuous variables with a normal distribution. Median and inter-quartile range were used for continuous variables with a skewed distribution.

For statistical analysis of continuous variables that were normally distributed a student T test was performed. For statistical analysis of continuous variables with a skewed distribution a non-parametric test was used (Mann Whitney Test). For statistical analysis of non-continuous variables, Chi-square or Fisher’s exact test were used. All reported p values were two-tailed and p values of <0.05 were considered statistically significant.

The end point for Kaplan-Meier survival analysis was a diagnosis of HGD or ADC during follow-up. Kaplan-Meier analysis was used to estimate the cumulative risk of progression to HGD/ADC. Time to progression was calculated as the interval between the date of first episode of LGD and date of biopsies documenting HGD/ADC. Follow-up data were censored at the time of first event (progression to HGD/ADC) or last endoscopic follow-up. Differences in cumulative risk of progression to HGD or ADC for the different consensus diagnoses were compared using the log-rank statistic.

Interobserver agreement between pathologist 1 and pathologist 2 (prior to a consensus diagnosis being reached) was assessed using Cohen’s (weighted) $k$. The strength of agreement was categorised as follows: 0.00-0.20, poor; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, good; and 0.81-1.00, very good.

**Ethical consideration**

This study was approved by the medical ethics review board of St Vincent’s Hospital. Subsequent ethics approval was sought and granted by the Victorian Cancer Council in order to obtain information on the included cohort as to whether any further patients had progressed to HDG/ADC of whom we were previously unaware.
3.5 Results

Original Database search

The St Vincent’s Hospital pathology database was searched for cases of LGD diagnosed between 1995 and 2002. This yielded 1316 patients with 5041 reports. After excluding non-gastroscopy episodes that left 3978 reports. Following review of all histology reports by the principal investigator, using the exclusion and inclusion criteria, 122 patients were eventually included for analysis.

After further review, a further 5 were excluded; two were found to have a diagnosis of HGD prior to 1995, one was found to have cancer diagnosed prior to 1995, following VCR database search, and finally, after retrieval of slides from the archives took place, two patients’ slides were not available for review and these patients were subsequently excluded from analysis. See Figure 3.5 below.
Figure 3.5: Flowchart of patients included in this study. ADC, adenocarcinoma; HGD, high grade dysplasia; LGD, low grade dysplasia; VCR, Victorian Cancer Registry

Search of Pathology database yielded 1316 patients with diagnosis of oesophageal dysplasia between 1995 and 2002

Exclusions via database information:
- patients with fewer than 2 gastroscopies with oesophageal biopsies
- patients without oesophageal biopsies at least 1 year after the diagnosis of LGD
- patients with HGD/ADC documented prior to LGD diagnosis
- patients progressing to HGD/ADC within 1 year of LGD diagnosis

122 patients

5 patients further excluded:
- VCR found to have ADC prior to LGD (n=1)
- Found to have HGD prior to LGD (n=2)
- Slides not available for review (n=2)

Cohort for Analysis
n=117
Cohort for analysis
There were 117 patients (81 (69%) male) included for analysis. The mean age when LGD was first detected was 61.5 years (SD 12.5). Eighty-four percent (98/117) were prevalent cases with LGD being their first diagnosis in database; 16% (19/117) were incident cases where NDBE preceded the first LGD diagnosis. Each patient had a median of 4 gastroscopies (range 2-17; IQR = 3-7). The median number of follow-up gastroscopies after the first LGD diagnosis, including up to the time of progression where progression occurred was 2 (1-10; IQR = 1-2). The median number of biopsies per endoscopy was 5 (1-80; IQR = 3-8).

Consensus Diagnosis
On a per-patient basis (n=117), the following consensus diagnosis was made: IMC in 1 patient (0.9%), LGD in 14, (11.9 %), IND in 15, (12.8%) and NDBE in 87 (74.3%). The patient with a consensus diagnosis of IMC was excluded from further analysis.

Follow-up and Progression to HGD/ADC
The median follow-up in months for the cohort (from first LGD to last endoscopy/or first RFA treatment/or progression to HGD/ADC) was 54.9 months (29.6-76.4).

Eleven of 116 (9.5%) patients progressed to HGD (n=6) or ADC (n=5). The incidence rate per patient-year for the inception cohort was 1.8% (95% CI 1.0-3.2). The median time to progress to HGD/ADC where it occurred (n=11) was 34.4 months (IQR= 23-94.2); median follow-up for those who did not progress was 56.4 months (12-187.2; IQR 30.8-74.6). The length of follow-up in months between those who progressed and those who did not was not significantly different (p=0.3835). The median time to progress from first LGD to HGD where it occurred (n=8) was 31.8 months (13.1- 95; IQR 24.2-71.8); median time to progress from LGD to ADC where it occurred (n=5) was 64.6 (IQR 18.7, 133.4)

Incidence of HGD/ADC per patient-year following consensus diagnosis
As stated above, the incidence rate of HGD/ADC per patient-year for the entire inception cohort (n=116) was 1.8% (95% CI 1.0-3.2). After consensus diagnosis was achieved, for confirmed LGD (n=14), the incidence rate of HGD/ADC was 6.8% (95% CI 2.5-18.0) per patient-year, which was higher (though not significantly; p=0.196) than for those with a consensus diagnosis of NDBE (n=87) with an incidence rate of 1.4% (95% CI 0.68-3.0) per patient-year. For those with a consensus diagnosis of IND (n=15) the progression risk was 0% per patient-
When patients with confirmed LGD were further classified into mild (n=12) or moderate (n=2) dysplasia the risk of malignant progression was 4.0% (95% CI 1.0-16.1) and 21.1% (95% CI 5.3-84.4) per patient-year respectively, the latter being significantly higher than the NDBE incidence rate (p=0.040). See Table 3.1 below.

**Table 3.1. Patient follow-up characteristics of the inception cohort and consensus diagnosis categories**

<table>
<thead>
<tr>
<th>All patients (n=116)</th>
<th>Consensus Diagnosis Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 tier</strong></td>
<td>LGD (n=14)</td>
</tr>
<tr>
<td>Mean age with standard deviation</td>
<td>Mild (n=12) Mod (n=2)</td>
</tr>
<tr>
<td>Mean age with standard deviation</td>
<td>61.4 (12.5)</td>
</tr>
<tr>
<td>Median follow-up endoscopies with IQR</td>
<td>2 (1-2)</td>
</tr>
<tr>
<td>Median follow-up in months with IQR</td>
<td>54.9 (29.6-76.4)</td>
</tr>
<tr>
<td>Patient-years of follow-up</td>
<td>612.5</td>
</tr>
<tr>
<td>Number and proportion of patients progressing to HGD/ADC</td>
<td>11 (9.5%)</td>
</tr>
<tr>
<td>HGD/ADC incidence per patient-year (with 95% CI)</td>
<td>1.8% (1.0-3.2)</td>
</tr>
<tr>
<td>2-year cumulative risk of HGD/ADC (95% CI)</td>
<td>2.8% (0.9-8.6)</td>
</tr>
<tr>
<td>5-year cumulative risk of HGD/ADC (95% CI)</td>
<td>6.1% (2.8-13.4)</td>
</tr>
<tr>
<td>8-year cumulative risk of HGD/ADC (95% CI)</td>
<td>15.1% (7.1-31.1)</td>
</tr>
<tr>
<td>10-year cumulative risk of HGD/ADC (95% CI)</td>
<td>15.1% (7.1-31.1)</td>
</tr>
<tr>
<td>15-year cumulative risk of HGD/ADC (95% CI)</td>
<td>34.7% (16-75.2)</td>
</tr>
</tbody>
</table>

ADC, adenocarcinoma; CI, confidence interval; HGD, high grade dysplasia; IND, indefinite for dysplasia; IQR, interquartile range; LGD, low grade dysplasia; Mild, mild dysplasia; Mod, moderate dysplasia; NDBE, Non-dysplastic Barrett’s oesophagus
Among the patients who were downstaged to NDBE or IND after consensus diagnosis, 7 of the 102 patients (6.8%) progressed to HGD/ADC with median time to progression of 34.4 months (IQR 21.9-99.0). In 2 of 7 patients (29%) LGD was diagnosed and confirmed by the expert pathology panel during the interval between the downstaged referral LGD diagnosis and progression to HGD/ADC.

Cumulative risk of developing HGD/ADC following consensus diagnosis
Kaplan-Meier statistics showed that the cumulative risk for developing HGD/ADC of the whole inception cohort was of 34.7% (95% CI 14.9-67.3) in 187.2 months (Figure 3.6). Patients with a consensus diagnosis of LGD (n=14) had a cumulative risk for developing HGD/ADC of 72.5% (95% CI 30.7-98.9) in 105.8 months. This was significantly higher compared with patients with a consensus diagnosis of NDBE (n=87) (32.3% (95% CI 10.9-73.3) in 187.2 months; p=0.003). For those with a consensus diagnosis of IND (n=15) the cumulative risk for developing HGD/ADC was 0% in 173.8 months.

For patients with a consensus diagnosis of LGD, when this was further stratified into either mild (n=12) or moderate (n=2) dysplasia the cumulative risk for developing HGD/ADC was 55.6% (95% CI 13.4-100.0) in 105.8 months and 100% (95% CI 8.9-100) in 95 months respectively (Figure 3.7). When categorized as mild dysplasia the cumulative risk was not significantly higher when compared with a consensus diagnosis of IND (p=0.186) or NDBE (p=0.121), but when categorised as moderate dysplasia the risk was significantly higher than for NDBE (p<0.0001). See summary in Table 3.1 above.
Figure 3.6. Kaplan-Meier curve with cumulative risk of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and patients with a consensus diagnosis of low grade dysplasia (LGD), indefinite for dysplasia (IND), or non-dysplastic Barrett’s oesophagus (NDBE).
Figure 3.7. Kaplan-Meier curve with cumulative risk of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and patients with a consensus diagnosis of mild dysplasia (Mild), moderate dysplasia (Mod) indefinite for dysplasia (IND), or non-dysplastic Barrett’s oesophagus (NDBE).
Individual Pathologist Review

117 patients had their pathology episodes reviewed independently by both pathologists, prior to a consensus diagnosis being reached. The episodes included were the first episode of LGD, any other episode with dysplasia, and first and last gastroscopy. The median number of episodes reviewed for each patient was 3 (2-11; IQR= 2-4) with a total of 394 episodes reviewed. The median number of slides per episode was 1 (1-62; IQR= 1-2). A total of 859 slides were reviewed for the cohort included for analysis. The median number of biopsies per slide was 5 (1-80; IQR = 3-8).

Individual Pathologist Agreement with the original diagnosis of LGD

Pathologist 1 agreed with the original diagnosis of LGD for the inception cohort in 25% (95% CI 17.4-33.9). Pathologist 2 agreed with the original diagnosis in 6% of cases (95% CI 2.5-12).

Agreement between pathologists in inception cohort prior to a consensus diagnosis being reached

In 77 of 117 cases (67.5%) both pathologists initially agreed after the first reading with the following diagnoses:

- NDBE in 75 cases,
- LGD in 3 cases,
- IMC in 1 case (this case was excluded from the subsequent cohort analysis)

In 38 cases (32.5%) both pathologists disagreed with each other:

- In 8 cases there was disagreement between NDBE and IND,
- In 5 cases there was disagreement between IND and LGD;
- and in 25 cases there was disagreement between NDBE and LGD.

This resulted in an unweighted K- value of 0.14 (95% CI 0.006-0.317) for the two tier system and 0.098 (95% CI 0.003-0.184) for the three tier system. The degree of agreement was best for the extremes of the spectrum (For IMC the Cohen’s kappa value was 1.00 (excellent) and for NDBE it was 0.20 (still poor) and worst for the mid-spectrum diagnoses (k-value for IND was -0.056, for Mild dysplasia was -0.034 and for LGD was 0.078).
The Cohen’s (weighted) K-value was slightly improved but still in the “poor” category at 0.185 (95% CI 0.004-0.324) for the two tier system and 0.195 (95% CI 0.044-0.422) for the three tier system. When the expert pathology reviews were dichotomised, counting LGD or above as “high risk” and NDBE/IND as “low risk”, the proportion of complete agreement was 74.4% with kappa value of 0.115.

Agreement between pathologists for all LGD episodes reviewed, prior to a consensus being reached.

When all LGD episodes were included for this analysis, the findings were similar; in 148 (60.7%) of 244 total LGD episodes reviewed both pathologists initially agreed after the first reading with the following diagnoses:

- NDBE in 138,
- LGD in 9,
- IMC in 1.

In 96 cases (39.7%) the pathologists disagreed with each other:

- in 25 there was disagreement between NDBE and IND;
- in 11 there was disagreement between IND and LGD (all mild);
- in 58 (52 Mild/6 mod) between LGD and NDBE; and,
- in 2 cases, disagreement between LGD (both moderate) and HGD.

This resulted in an unweighted K- value of 0.089 (95% CI -0.042-0.165) and weighted kappa of 0.153 (95% CI 0.073-0.304) for the two tier system and an unweighted K-value of 0.066 (95% CI 0.057-0.165) and weighted kappa of 0.177 (95% CI 0.060-0.307) for the three tier system. Consensus Meeting results

At the time of consensus meeting, for a consensus to be reached, Pathologist 1 tended to downgrade his initial responses (downgraded 19, upgraded 4, remained the same in 17). Pathologist 2 tended to upgrade his initial responses (upgraded 21, downgraded 6, remained the same in 13). In more detail, P1 changed his opinion to meet P2 in 13 cases (3 upgraded from NDBE to IND; 2 downgraded from IND to NDBE and 8 downgraded from mild to NDBE). P2 changed his opinion to meet P1 in 17 cases (1 upgraded from NDBE to IND; 7 upgraded from NDBE to LGD; 2 upgraded from IND to LGD; 2 upgraded from Mild to Mod; 2 downgraded...
from Mild to IND; 1 downgraded from Mild to NDBE; 2 downgraded from IND to NDBE). In 10 cases, P1 and P2 agreed to meet in the middle (9 downgraded by P1 and upgraded by P2 (7 Mild/NDBE changed to consensus IND; 2 NDBE/Mod changed to consensus Mild) and 1 upgraded P1 and downgraded P2 and (NDBE/Mild changed to consensus IND).

Incidence rate per patient-year and cumulative risk of HGD/ADC at an individual pathologist level according to two tier and three tier system.

Pathologist 1

Of 117 cases, pathologist 1 agreed with the diagnosis of LGD in 29 cases, corresponding with an agreement rate of 25% (95% CI 17.4-33.9). Those patients were further sub-classified as having mild dysplasia (n=25) or moderate dysplasia (n=4). The remainder of the 117 cases were classified as IND (n=5), NDBE (n=82) and IMC in one case. The patient classified as IMC was excluded from further analysis.

As previously mentioned, the incidence rate of HGD/ADC per patient-year for the entire inception cohort (n=116) was 1.8% (95% CI 1.0-3.2). For pathologist 1, the incidence rate for progression to HGD/ADC per patient-year was 1.5% (95% CI 0.7-3.2) for NDBE (n=82), 0% for IND (n=5), and 3.1% (95% CI 1.2-8.3) for LGD (n=29). When LGD was further classified as either mild dysplasia (n=25) or moderate dysplasia (n=4) the incidence rates were 1.7% (95% CI 0.4-6.1) and 15.0% (95% CI 3.8-60.0) respectively. See Table 3.2 below.

For pathologist 1, Kaplan-Meier analysis showed that for patients categorised as LGD (n=29) the cumulative risk for developing HGD/ADC was 39.2% (95% CI 38.0-40.3) in 146.4 months (Figure 3.8). This was not significantly higher when compared with patients with a consensus diagnosis of NDBE (n=82) who had a cumulative risk of progression of 33.8% (32.8-34.9) in 187.2 months; p=0.184. Nor was it significantly higher when compared with those with IND (n=5) with a cumulative risk of progression of 0% in 164.2 months; p=0.382.

For patients categorised as LGD by Pathologist 1, when this was further stratified into either mild (n=25) or moderate (n=4) dysplasia the cumulative risk for developing HGD/ADC was 24.2% (23.1-25.3) in 146.4 months and 100% (95% CI 100-100) in 95.0 months respectively (Figure 3.9). Again, when categorized as mild dysplasia the cumulative risk was not significantly higher when compared with a consensus diagnosis of IND (p=0.52) or NDBE
(p=0.75), but when categorised as moderate dysplasia the risk was significantly higher than for NDBE (p=0.0005).

Figure 3.8. Kaplan-Meier curve for Pathologist 1, with cumulative risk of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and patients with a diagnosis of low grade dysplasia (LGD), indefinite for dysplasia (IND), or non-dysplastic Barrett’s oesophagus (NDBE).
Figure 3.9. Kaplan-Meier curve for Pathologist 1, with cumulative risk of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and patients with a diagnosis of mild dysplasia (Mild), moderate dysplasia (Mod) indefinite for dysplasia (IND), or non-dysplastic Barrett’s oesophagus (NDBE).

Pathologist 2

Of 117 cases, pathologist 2 agreed with the diagnosis of LGD in 7 cases corresponding to a 6% agreement rate (95% CI 2.5-12). In all 7 cases the patients were classified as mild dysplasia according to the three-tier grading system. The remainder of the 117 cases were classified as IND (n=8), NDBE (n=101) and IMC in one case. The patient classified as IMC was excluded from further analysis.

For pathologist 2, the incidence rate for progression to HGD/ADC per patient-year was 1.3% (95% CI 0.6-2.8) for those classified as NDBE (n=101), 2.2% (95% CI 0.3-15.7) for those classified as IND (n=8) and 8.2% (95% CI 2.6-25.4) for those classified as LGD/Mild (n=7). See table 3.2 below.
For pathologist 2, Kaplan-Meier analysis showed that the cumulative risk for developing HGD/ADC of patients categorised as LGD (n=7) was 100% (95% CI 100-100) in 95 months (Figure 3.10). This was significantly higher when compared with patients with a consensus diagnosis of NDBE (n=101) who had a cumulative risk of progression of 32% (31-33) in 187.2 months; p=0.0009. It was not significantly higher when compared with those with IND (n=8) with a cumulative risk of progression of 12.5% (11.8-13.3) in 173.8 months; p=0.238. The cumulative risks for NDBE and IND were also not significantly different (p=0.718).

Figure 3.10. Kaplan-Meier curve for Pathologist 2, with cumulative risk of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and patients with a diagnosis of low grade dysplasia (LGD), indefinite for dysplasia (IND), or non-dysplastic Barrett’s oesophagus (NDBE). Pathologist 2 classified all patients with LGD as mild dysplasia (Mild), thus they share the same curve on the graph.
Table 3.2. Incidence rates and cumulative progression rates of HGD/ADC according to histological category as assigned by individual pathologists.

<table>
<thead>
<tr>
<th>Category - two tier - three tier</th>
<th>NDBE</th>
<th>IND</th>
<th>LGD</th>
<th>Mild</th>
<th>Moderate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathologist 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients in category (Total = 116)</td>
<td>82</td>
<td>5</td>
<td>29</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Patient-years of follow-up</td>
<td>454.1</td>
<td>29.5</td>
<td>128.9</td>
<td>115.6</td>
<td>13.3</td>
</tr>
<tr>
<td>Incidence rates (%) of HGD/ADC per patient-year with 95% CI</td>
<td>1.5% (0.7-3.2)</td>
<td>0%</td>
<td>3.1% (1.2-8.3)</td>
<td>1.7% (0.4-6.1)</td>
<td>15% (3.8-60.0)</td>
</tr>
<tr>
<td>Cumulative progression rate (%) to HGD/ADC with 95% CI</td>
<td>33.8% (32.8-34.9) in 187.2 months</td>
<td>0% in 164.2 months</td>
<td>39.2% (38.0-40.3) in 146.4 months</td>
<td>24.2% (23.1-25.3) in 146.4 months</td>
<td>100% (100-100) in 95.0 months</td>
</tr>
<tr>
<td>Pathologist 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients in category (Total = 116)</td>
<td>101</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Patient-years of follow-up</td>
<td>530.8</td>
<td>45.1</td>
<td>36.6</td>
<td>36.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Incidence rates (%) of HGD/ADC per patient-year with 95% CI</td>
<td>1.3% (0.6-2.8)</td>
<td>2.2% (0.3-15.7)</td>
<td>8.2% (2.6-25.4)</td>
<td>8.2% (2.6-25.4)</td>
<td>N/A</td>
</tr>
<tr>
<td>Cumulative progression rate (%) to HGD/ADC with 95% CI</td>
<td>32% (31-33) in 187.2 months</td>
<td>12.5% (11.8-13.3) in 173.8 months</td>
<td>100% (100-100) in 95 months</td>
<td>100% (100-100) in 95 months</td>
<td>N/A</td>
</tr>
</tbody>
</table>

ADC, adenocarcinoma; CI, confidence interval; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; Mild, mild dysplasia; Mod, moderate dysplasia; NDBE, Non-dysplastic Barrett’s oesophagus
Incidence rate per patient-year of HGD/ADC depending on concordance between original diagnosis and Pathologist 1 and 2, but prior to a consensus diagnosis being reached

As previously stated, the incidence rate of HGD/ADC per patient-year for the entire inception cohort (n=116) was 1.8% (95% CI 1.0-3.2). Prior to a consensus diagnosis meeting, the incidence rates were analysed according to whether both pathologists downgraded the LGD diagnosis, if one agreed or if both agreed. When both pathologists downgraded to NDBE (n=73) or NDDBE/IND (n=8), the incidence rates per patient-year were 1.5% (95% CI 0.7-3.2) and 1.9% (95% CI 0.3-13.4) respectively. When one pathologist agreed with the diagnosis (n=30) the progression rate was in fact lower than the inception cohort at 0.8% (95% CI 0.1-5.4). When both pathologists agreed with LGD (n=3) the incidence rate per patient-year rose to 17.3% (95% CI 5.6-53.9). See Table 3.3.

Table 3.3. Patient follow-up characteristics of the inception cohort and diagnosis categories according to the level of agreement of both pathologists (i.e. prior to consensus diagnosis being reached).

<table>
<thead>
<tr>
<th>Inception cohort with LGD</th>
<th>Downgraded to NDBE</th>
<th>Downgraded to NDDBE/IND</th>
<th>One agreed with LGD</th>
<th>Both agreed with LGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>75</td>
<td>8</td>
<td>30</td>
<td>3</td>
</tr>
</tbody>
</table>

| Median follow-up endoscopies (IQR) | 2 (1-2) | 2 (1-2) | 2 (2-3) | 2 (1.3-7.5) | 2 (1.5-2) |
| Mean follow-up in months (with standard deviation) | 63.4 (44.1) | 65.8 (44.12) | 79.8 (62.9) | 52.4 (37.8) | Median 93.4 (IQR 56.1-94.2) |

| Patient-years of follow-up | 612.5 | 411.2 | 42.6 | 131 | 17.3 |
| Number (and %) of patients progressing to HGD/ADC | 11 (9.5%) | 6 (8%) | 1 (12.5%) | 1 (3.3%) | 3 (100%) |

| Incidence of HGD/ADC per patient-year with 95% CI | 1.8% (1.0-3.2) | 1.5% (0.7-3.2) | 1.9% (0.3-13.4) | 0.8% (0.1-5.4) | 17.3% (5.6-53.9) |
| Cumulative progression rate to HGD/ADC with 95% CI | 34.7% (16-75.2) | 38.4% (37.2-39.6) | 12.5% (11-13.3) | 4.3% (4.1-4.6) | 100% (100-100) |

In 187.2 months In 187.2 months In 173.8 months In 146.4 months In 95 months

ADC, adenocarcinoma; CI, confidence interval; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; Mild, mild dysplasia; Mod, moderate dysplasia; NDBE, Non-dysplastic Barrett’s oesophagus
Using Fisher’s exact test, when both pathologists agreed with diagnosis of LGD, the proportion of patients progressing was significantly higher when compared to when only one pathologist agreed (3/3 patients (100%) vs 1/30 patient (3.33%); p=0.001) or when compared to both downgrading (to NDBE or IND) (3/3 patients (100%) vs 7/83 patients (8.43%); p=0.001).

When only one pathologist agreed with the diagnosis of LGD, the proportion of patients progressing was actually slightly lower when compared to both pathologists downgrading (1 patient, 3.33%) vs 7 patients (8.43%) but this was not statistically significant (p=0.679).

Cumulative risk of HGD/ADC depending on degree of agreement between pathologists (prior to a consensus diagnosis being reached).

When the data was analysed prior to a consensus diagnosis being reached, Kaplan-Meier analysis showed that for patients where both pathologists, independent of each other, downgraded to NDBE (n=75) the cumulative risk for developing HGD/ADC was 38.4% (95% CI 37.2-39.6) in 187.2 months. Where one had called NDBE and the other IND (n=8) the cumulative risk for malignant progression was 12.5% (11.8-13.3) in 173.8 months. For those where only one pathologist agreed with the diagnosis of LGD and the other had downgraded to NDBE or IND (n=30) the cumulative risk for developing HGD/ADC was 4.3% (4.1-4.6) in 146.4 months. Where both agreed with the diagnosis of LGD (n=3) the cumulative risk was 100% (100-100) in 95 months. See Figure 3.11. This was significantly higher than for those where both categorised as NDBE (p<0.0001) and also for those where only one pathologist categorised as LGD (p=0.002). It was not significantly higher than the cumulative risk for those classified as NBDE/IND (p=0.058). There was no significant difference in the cumulative risks for progression for those where both pathologists downgraded to NDBE and those where one pathologist called LGD (p=0.662) or those with NBDE/IND (p=0.993).
Figure 3.11. Kaplan-Meier curve with cumulative risks of developing high grade dysplasia (HGD) or oesophageal adenocarcinoma (ADC) for the whole inception cohort and following independent pathologist review (i.e. prior to a consensus diagnosis being reached) patients categorised as 1) non-dysplastic Barrett’s oesophagus by both pathologists (NDBE/NDBE), 2) NDBE by one pathologist and indefinite for dysplasia by the other (NDBE/IND), 3) low grade dysplasia by one pathologist and IND or NDBE by the other (LGD/NDBE), or 4) both pathologists categorising as LGD (LGD/LGD).
Overall pathologist agreement with a diagnosis of LGD

394 pathology episodes were reviewed over the course of this study, of which 244 were LGD. Of the 244 LGD episodes, 164 (67%) were downgraded to NDBE/IND, in 67 cases (27.5%) one pathologist agreed with the diagnosis of LGD and the other downgraded to NDBE or IND, and in 9 cases (3.7%) both pathologists agreed with LGD diagnosis. In 3 cases (0.9%) the pathologists upgraded to HGD or ADC. These agreement rates are similar to when looking at just the initial LGD episodes (n=117), where 83 cases (71%) were downgraded to NDBE/IND, 30 cases (25.6%) where one pathologist agreed with LGD, 3 cases (2.6%) where both agreed with LGD, and in 1 cases (0.9%) where both upstaged to HGD/ADC. Weighted and unweighted kappa values for pathologist agreement have been previously discussed, but were overall poor (≤ 2.0).

A consensus LGD diagnosis was more likely in patients who progressed to HGD/ADC than non-progressors.

In the cohort for analysis (n=116) there were 230 episodes of LGD reviewed in total. Prior to a consensus diagnosis being reached, when comparing patients who progressed to HGD/ADC (n=11) with those that did not progress (n=105), on a per-patient basis, those who progressed were more likely to have had a prior episode of LGD where both pathologists agreed with the diagnosis (5 of 11 patients (45.5%) vs. 4 of 105 patients (3.8%); p=0.0001.

A diagnosis of moderate dysplasia (but not mild dysplasia) was statistically more likely in patients who progressed to HGD/ADC than non-progressors,

Patients who progressed were also more likely to have had a prior LGD episode classed as moderate dysplasia than those who did not progress (4 of 11 patients (36.4%) vs 4 of 105 patients (3.8%) p=0.0015. Patients who progressed appeared just as likely as those who did not progress to have had their worst prior LGD episode classified as mild (4 of 11 patients (36.4%) vs 35 of 105 patients (33.3%); p=0.886.
Original versus Recut slide review

In the older archived slides (between 1995 and 2000), the slide cover had lifted from the base, rendering the slide very difficult to review due to significant artefact. As such these slides had to be recut, with the recut slide being reviewed in place of the original.

394 episodes were reviewed within 22 batches with total of 859 slides (Note that the total number of slides reviewed by the pathologists was actually greater than 1000, but not all were ultimately included for analysis). Twelve batches were original slides (n=483) and ten batches were of recut slides (n=376). Of the 117 initial LGD, 18 were original slide reviews and 99 were recuts (98 if excluding the patient who was ultimately excluded from analysis due to being upstaged to ADC). 1/18 patients from the “original slide group” progressed to HGD/ADC (5.6%) and 10/98 patients from the “Recut slide group” progressed (10.2%). This was not statistically significant (p=0.54).

Did recutting the slides impact the results?

To assess whether recutting the slides may have impacted the individual pathologists’ review results, the results from all 394 pathology episodes were reviewed after being divided into two categories (those cases where original slides were reviewed and those where recut slides were reviewed).

For pathologist 1 and 2, there was no statistically significant difference in proportional agreement with the original diagnosis when reviewing original or recut slides, where the original diagnosis was adenocarcinoma, HGD or NDBE. Similarly, for the first episodes of LGD there was no statistically significant difference between proportional agreement between originals and recuts for pathologist 1 or 2 (p=0.733 and p=0.429 respectively). The only category where there was a statistically significant difference between proportional agreement with originals and recuts was for pathologist 2, when all episodes of LGD were analysed. (p=0.004). See Table 3.4.
Table 3.4. Pathologists’ proportional agreement with the original histological diagnosis where original or recut slides were reviewed and comparative significance.

<table>
<thead>
<tr>
<th>Original histology diagnosis</th>
<th>Total original</th>
<th>Total recut</th>
<th>Pathologist 1 (% agreement and comparative p-values)</th>
<th>Pathologist 2 (% agreement and comparative p-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original slides</td>
<td>Recut slides</td>
<td>P-Value</td>
<td>Original slides</td>
</tr>
<tr>
<td>ADC (n=6)</td>
<td>3</td>
<td>3</td>
<td>3 (100%)</td>
<td>N/S</td>
</tr>
<tr>
<td>HGD (n=11)</td>
<td>7</td>
<td>4</td>
<td>2 agree (28.6%) 5 downgrade (71.5%)</td>
<td>1 agree (25%) 3 downgrade (75%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 3 LGD (42.9%) - 2 NDBE (28.6%)</td>
<td>- 1 LGD (25%) - 2 IND (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/S</td>
<td>1 agree (14.3%) 6 downgrade (85.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 3 LGD (42.9%) - NDBE (42.9%)</td>
<td>- 1 LGD (25%) - 2 NDBE (50%)</td>
</tr>
<tr>
<td>LGD (n=244)</td>
<td>77</td>
<td>167</td>
<td>2 upgrade (1.2%) 38 upgrade (22.8%)</td>
<td>1 upgrade (1.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>127 downgrade (76%)</td>
<td>14 agree (18.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 6 IND (3.6%) - 121 NDBE (72.5%)</td>
<td>61 downgrade (79.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/S</td>
<td>- 9 IND (11.7%) - 53 NDBE (68.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 upgrade (0.6%)</td>
<td>10 agree (6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156 downgrade (93.4%)</td>
<td>- 13 IND (7.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 upgrade (85.6%)</td>
<td>- 143 NDBE</td>
</tr>
<tr>
<td>First episode LGD (n=117)</td>
<td>18</td>
<td>99</td>
<td>4 agreed (22.2%) 14 downgrade (77.8%)</td>
<td>1 upgraded (1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 2 IND (11.1%) - 12 NDBE (66.7%)</td>
<td>25 agree (25%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>73 downgrade (74%)</td>
<td>73 downgrade (74%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 3 IND (3%) - 70 NDBE (71%)</td>
<td>- 3 IND (3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/S</td>
<td>16 downgrade (88.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 1 IND (5.6%) - 15 NDBE (83.3%)</td>
<td>- 7 IND (7.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 upgrade (1%)</td>
<td>93 downgrade (94%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>93 downgrade (94%)</td>
<td>- 86 NDBE</td>
</tr>
<tr>
<td>NDBE (n=133)</td>
<td>89</td>
<td>44</td>
<td>5 upgrade (5.6%) 84 agree (94.4%)</td>
<td>1 upgrade (2.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43 agree (97.7%)</td>
<td>5 upgrade (5.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/S</td>
<td>84 agree (94.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 upgrade (2.3%)</td>
<td>43 agree (97.7%)</td>
</tr>
</tbody>
</table>

ADC, adenocarcinoma; CI, confidence interval; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; Mild, mild dysplasia; Mod, moderate dysplasia; NDBE, Non-dysplastic Barrett’s oesophagus; N/S, Non-significant

3.6 Discussion

This cohort study of 116 Barrett’s patients diagnosed with LGD by general pathologists in a tertiary hospital setting sought to confirm the finding of Curvers’ 2010 study (which was subsequently supported by their follow-up publication in 2015 by Duit et al while this study was proceeding) that demonstrated a consensus diagnosis of LGD accurately stratified risk of malignant progression to HGD/ADC. Additionally, this study sought to assess whether further classification of patients with confirmed LGD as having mild or moderate dysplasia better predicted the risk for progression. With a median follow-up time of 59 months (almost 5 years), two expert Barrett’s pathologists reviewed all episodes pertaining to the patient
cohort and reached a consensus diagnosis for the original LGD diagnosis according to a two tier and three tier system of grading dysplasia.

**Impact of consensus diagnosis of LGD on incidence and cumulative rates of malignant progression.**

Similar to the aforementioned studies, where 73-85% of patients were downgraded to NDBE or IND the vast majority of this patient cohort (88%) were downgraded to NDBE or IND after consensus diagnosis.\textsuperscript{140,141} This is despite the pathology being originally reviewed in a tertiary hospital setting and is possibly accounted for by the fact that the original reviews were performed by a variety of pathologists with differing Barrett’s expertise. The incidence rates for malignant progression for confirmed LGD was 6.8%, which while higher than for NDBE (1.4%) and IND (0%) was not as striking as that reported for Duits’ cohort (9.1%) and Curvers’ cohort (13.4%). Notable also, was that though patients with a consensus diagnosis of NDBE had a reduced incidence rate of progression compared with consensus LGD, it was not negligible at 1.4% and not dissimilar to the overall progression rate of the cohort (1.8% per patient-year). In our study, 7 patients who were downstaged to NDBE /IND progressed to HGD/ADC but two of these patients had an interval diagnosis of confirmed LGD prior to progression. When these patients were included in the LGD consensus category, the risk of progression to HGD/ADC for patients with NDBE dropped to 1.1% per patient-year.

Where a consensus of LGD was made the cumulative risk for developing HGD/ADC was 17.5% at 5 years and 72.5% at approximately 8 years (105.8 months). This was significantly higher than patients with patients with a consensus diagnosis of NDBE (32.3% in 187.2 months; \textit{p}=0.003) and for those with a consensus diagnosis of IND where the cumulative risk for developing HGD/ADC was 0% in approximately 15 years (173.8 months).

Importantly, in our study, we excluded patients for analysis who were found to have developed HGD or ADC within one year of the initial diagnosis of LGD as it was felt that these patients may have had prevalent disease at baseline. This differed from Curvers’ and Duits’ studies where these patients were included. Duit et al noted this in their discussion, accepting that the steep increase in cumulative risk in the first 6 months from confirmed LGD diagnosis may have been reflective of prevalent disease at baseline but as their KM curve still demonstrated a constant increase after 6 months, they felt there was still value in their conclusion that a consensus diagnosis of LGD was predictive of malignant progression.
Impact of three tier system over two tier system after consensus diagnosis

As stated above, for patients with a consensus diagnosis of LGD there was a 6.8% incidence of progression per patient-year. When these patients were further classified as mild or moderate dysplasia the risk of malignant progression was 4.0% and 21.1% per patient-year respectively. The cumulative risk of malignant progression for those with a consensus diagnosis of LGD was 75.2% at around 8 years’ follow-up; for mild dysplasia this was reduced to 55.6% (and not significantly higher than for consensus IND or NDBE) but for moderate dysplasia was 100%, significantly higher than for NDBE ($p<0.0001$). This result suggests that it may be the diagnosis of moderate dysplasia (within a consensus LGD group) that is primarily responsible for the significantly increased progression risk.

Impact of classifying LGD as mild or moderate on progression rates at the individual pathologist level:

At the individual pathologist level, for pathologist 1 at least, the three tier system also appeared to affect both incident and cumulative progression rates to HGD/ADC. When classified as mild dysplasia the incidence rate per patient-year of HGD/ADC was 1.7% compared with 1.5% for NDBE, 0% for IND and 3.1% for LGD, but when classified as moderate dysplasia it was 15%. The cumulative risk for progression for patients with mild dysplasia was 24.2% compared with 33.3% for NDBE, 0% for IND and 39.2% for LGD, with these differences not statistically significant, however the cumulative risk for moderate dysplasia was 100%, which was significantly higher than for NDBE ($p=0.0005$).

For pathologist 2, the three tier system of grading dysplasia did not appear to impact incidence or cumulative risk rates but this was likely due to having such a high threshold for diagnosing LGD in the first place. As such, when Pathologist 2 diagnosed LGD the incidence rate for HGD/ADC per patient-year was 8.2% and cumulative risk was 100%, already significantly higher than for those diagnosed with NDBE, therefore further stratifying into mild or moderate made no difference. This raises the question as to whether for Pathologist 2, the diagnosis of LGD was already on the moderate end of the spectrum.

These findings highlight the subjective nature in which the diagnosis of LGD is made (discussed in more detail below). It also suggests that weight would ideally be given to a diagnosis of LGD made by a pathologist with a higher threshold for calling dysplasia in the first place. It should not be ignored that in this study, as in the previous Duits’ and Curvers’ papers,
that at least one pathologist (whether in the community or a general pathologist in a tertiary hospital) had made an initial diagnosis of LGD. It follows that where two other pathologists did not agree, that this initial diagnosis, was at the very most, on the milder end of the spectrum for LGD and where both pathologists agreed it was likely to be on the moderate end of the spectrum.

It is important to note again, that in our study, the pathologists were reviewing not only the first episode of LGD for each patient in the cohort, but all episodes of dysplasia (whether LGD, HGD or ADC) and cases of NDBE (where these were the first or last episodes), and were blinded to the original diagnoses in all cases. This is a point of difference from the Curvers and Duits studies, where the reviewing pathologists were aware that all cases they were reviewing had been diagnosed as LGD and were blinded only to the clinical follow-up. This fact likely contributed to our much lower agreement rates with the original diagnoses.

**Pathologists agreement**

The proportion of complete agreement between pathologists 1 and 2 for the initial diagnosis of LGD was 67% but with poor interobserver agreement between pathologists as measured by k-values (unweighted K-values of 0.14 and 0.10 and weighted k-values 0.19 and 0.20 for the two and three tier systems in the initial cohort respectively). The interobserver variability was based predominantly on disagreement between the presence of either NDBE or LGD. These kappa values, while low, are not inconsistent with the literature published on interobserver agreement for LGD, even between expert GI pathologists. The proportion of complete agreement in this study was improved to 74% once dichotomized (with NDBE/IND considered low risk and LGD considered high risk) but with a paradoxically lower k-value of 0.12. Since the k-value is affected by prevalence of the investigated disease, this low kappa value could be explained by the asymmetrical distribution of cases in this study (low rate of true LGD). Therefore the interpretation of the k value in this situation is ambiguous.

There is currently no reliable solution to the problem of interobserver variability in diagnosing dysplasia in BE and in fact it has been shown in past studies that the effect of a consensus meeting does not bring much improvement in interobserver variability. Strategies such as computerised morphometry and p53 staining are likely to be the most useful adjuncts available to pathologists to reduce interobserver variability in diagnosing dysplasia.
Computerised morphometry, a technique whereby and H&E-stained histologic images are scanned and digitized using a computerized image analysis system and indices of size, shape, texture, symmetry and architectural distribution of the epithelial nuclei are measured has been shown to downgrade a similar number of LGD diagnoses as expert pathologists. It has been demonstrated to be a valid tool for determining the grade of dysplasia in BE and even aid in prediction of patients who may progress from HGD to invasive adenocarcinoma. Limitations of morphometry are that the expertise and equipment required is not readily available to most pathology departments, and additionally, morphometry provides no solutions to technical issues such as tangential cutting or severe inflammation. P53 immunohistochemistry and protein quantification has probably been the most widely used adjunct to dysplasia diagnosis, and of the generally available tools provides the best evidence thus far for correlation with outcome. In one study, P53 immunohistochemistry improved interobserver variation in dysplasia diagnosis and correlated strongly with progression. In less definite cases, p53 positivity, in an area of interest, correlated with likelihood of progression. Importantly, while overexpression of P53 has been shown to increase risk of neoplastic progression BE patients, the risk has been shown to be even higher with loss of p53 expression (relative risk of 5.6 vs. 14.0). It has also been shown that the histological diagnosis of LGD with concurrent aberrant p53 expression is a more powerful predictor of neoplastic progression than the histological diagnosis of LGD alone (positive predictive value of 33% vs. 15%). Routine use of p53 immunohistochemistry would likely be of significant assistance to pathologists in cases of suspected dysplasia within BE, and particularly useful in the tertiary hospital setting where these patients are managed. The addition of computerised morphometry for suspected or confirmed cases of Barrett’s dysplasia could also be considered for routine use in the tertiary setting in the future but would require education and training for those using it.

As previously mentioned, our results demonstrated that patients with a consensus diagnosis of NDBE had a reduced incidence rate of progression when compared with consensus LGD, but importantly, it was not negligible at 1.4% and not dissimilar to the overall progression rate of the cohort (1.8% per patient-year). Two of seven patients who did progress from NDBE, were found to have confirmed LGD at an intervening time, supporting the notion of a step wise progression from non-dysplastic to neoplastic Barrett’s and the need for interval
surveillance of all Barrett’s patients, even those at low risk for progression. Cases that may be downgraded by pathologists to NDBE or indefinite for dysplasia, include the histological finding of basal crypt dysplasia-like atypia (BCDA), in the presence of surface maturation. While surface maturation is a useful indicator in recognizing regenerative change, our pathologists feel that if significant nuclear atypia is present in the crypts the absence of surface involvement should not prevent a diagnosis of dysplasia, and there is evidence to support this. A study looking at the clinical importance of BCDA, found there was a high association with conventional dysplasia and/or adenocarcinoma and recommended its finding be recognised as a subtype of true dysplasia.510. A further study found that immunopositivity for p53 confined to the crypts showing nuclear atypia was good supportive evidence for a dysplasia diagnosis. Additionally, in many cases where surface involvement was present, there were also areas showing apparent maturation of similarly dysplastic crypts.68

Our data supports prior findings that a consensus diagnosis of LGD between expert pathologists does confer a greater risk for malignant progression but that it is the LGD that are on the upper end of the LGD spectrum that are predominantly responsible for this risk. In the absence of a consensus diagnosis being achievable (e.g. in the community setting) using a subdivision of mild or moderate dysplasia for those with LGD within BE may be a simple addition to a histology report that may alert the clinician to a patient’s higher risk for progression and need for closer surveillance or consideration of endoscopic therapy.

**Limitations of study**

A limitation of this study is that clinical data acquisition relied on retrospective access to the pathology database, hospital medical records and cancer registry records. It cannot be entirely excluded that some patients in the cohort may have had histology recorded at an earlier time, outside of the specified tertiary hospital. This would have impacted reported incidence and prevalence rates. Additionally, of 117 patients, 60 did not have clinical information at the hospital other than pathology records, therefore the extent of clinical follow-up in this patient cohort is likely an underestimate and it is possible that not all cases of progression to HGD/ADC were detected. We sought to minimise this possibility by
accessing the state cancer registry however appreciate that it is possible we may have missed cases of malignant progression if patients had clinical follow-up interstate.

The recutting of slides was required in around 45% of all reviewed episodes and in around 80% of the original LGD cases and this may have impacted the overall results as it is possible that a focus of dysplasia seen in the original slide was not seen in the recut slide. Recutting of slides is a common procedure among pathologists when further examining areas of interest or concern. Pathologist interpretation will be impacted by the quality of the cut of the slide and any artefact present (e.g. tangential cutting, thickness of the cut, vibration artefact).

Finally, while the division of consensus LGD into mild and moderate categories did impact progression rates in a statistically significant way, we accept that the overall numbers with a consensus diagnosis of LGD (and particularly moderate LGD) were low and that further studies with a larger cohort of patients with consensus LGD, categorised as mild or moderate, are required to support our findings.

3.7 Conclusion

Our study confirms the findings of previously published literature that confirmed LGD in BE confers an increased risk of malignant progression. When further stratified, a consensus of moderate dysplasia appears be primarily responsible for this increased risk. Additionally, at an individual pathologist level, stratifying LGD as either mild or moderate dysplasia may better predict the risk for progression from LGD to HGD/ADC. BE patients with LGD should undergo expert histological review of the diagnosis for adequate risk stratification with consideration given to further risk stratifying those with confirmed LGD as mild or moderate dysplasia, particularly in cases where double reporting is not possible. While the vast majority of patients with community diagnosis of LGD will be downstaged after expert review and have a lower progression risk, the risk is not negligible and these patients warrant continued endoscopic follow-up. Confirmation of these results in further studies will be useful in decision making and strategies regarding endoscopic therapy versus close surveillance for individuals with LGD in Barrett’s.
Chapter 4: Detection and staging of esophageal cancers within Barrett’s esophagus is improved by assessment in specialized Barrett’s units

4.1 Introduction and Summary

Significant technological and endoscopic advancements have been made over the last decade with respect to treating and eradicating dysplastic Barrett’s oesophagus (BE). Previously, where high grade dysplasia (HGD) was present this typically was an indication for oesophagectomy as not only were the rates of progression to cancer reportedly very high, surgical series demonstrated that these patients often had synchronous neoplastic lesions present at the time of surgery.\textsuperscript{205-208} We now have evidence that endoscopic mucosal resection (EMR) of early neoplastic lesions and eradication of the remaining Barrett’s segment with radiofrequency ablation (RFA) is just as effective as surgery for HGD/early cancer, and with significantly less morbidity and mortality.\textsuperscript{382, 397, 405}

It is imperative that thorough assessment of the Barrett’s segment is undertaken in order to identify lesions that may harbour HGD or early cancer before deciding that a patient is appropriate for endoscopic therapy.\textsuperscript{263, 269, 310} Furthermore, EMR is essential for accurate staging of early cancers and to remove cancer cells before embarking on a course of RFA treatment which is only effective in ablating flat dysplasia and not neoplasia.\textsuperscript{353-355}

This study aimed to find out whether assessment at a Specialised Barrett’s Unit was better able to detect and stage dysplastic Barrett’s Oesophagus when compared with community endoscopists. We found that Barrett’s unit assessment resulted in improved detection of mucosal abnormalities and cancers that were often missed by community endoscopists. This finding has important clinical implications as it suggests that endoscopic treatment of dysplastic BE should remain a subspecialty area, and we would strongly advocate that patients be referred by community endoscopists to Specialised Barrett’s Units for assessment and staging prior to embarking on a definitive management course.
Detection and staging of esophageal cancers within Barrett’s esophagus is improved by assessment in specialized Barrett’s units

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**Background:** Identification and resection of mucosal abnormalities are critical in managing dysplastic Barrett’s esophagus (BE) because these areas may harbor esophageal adenocarcinoma (EAC).

**Objectives:** To compare mucosal lesion and EAC detection rates in dysplastic BE in the community versus a BE unit and assess the impact of EMR on disease staging and management.

**Design:** Prospective cohort study.

**Setting:** Tertiary referral center.

**Patients:** Patients with dysplastic BE.

**Interventions:** Reassessment with high-definition white-light endoscopy (HD-WLE), narrow-band imaging (NBI), and Seattle protocol biopsies. EMR performed in lesions thought to harbor neoplasia. Review of referral histology and endoscopies.

**Main Outcome Measurements:** Mucosal lesion and EAC detection rates in a BE unit versus the community. Impact of EMR on management.

**Results:** Sixty-nine patients were referred (88% male; median age, 69 years). At referral, HD-WLE/NBI use was 57%/14%, and Seattle protocol adherence was 20%. Eighteen patients had intramucosal cancer. Lesions were detected in 65 patients in the BE unit versus 29 patients at referral (P < .001). EMR was performed in 47 patients. BE unit assessment confirmed EAC in all 18 patients and identified 10 additional patients (56% increased cancer detection, P = .036); all 10 had lesions identified in the BE unit (vs 3 identified at referral). EMR in these patients found submucosal cancer (n = 4) and intramucosal cancer (n = 6), resulting in esophagectomy (n = 4) and chemoradiotherapy (n = 1).

**Limitation:** Academic center.

**Conclusion:** BE assessment at a BE unit resulted in increased lesion and EAC detection. EMR of early cancers was critical in optimizing patient management. These data suggest that BE unit referral be considered in patients with dysplastic BE. (Gastrointest Endosc 2014;80:971-83.)

Until recently, surgical esophagectomy was the standard treatment for patients with early esophageal adenocarcinoma (EAC) and high-grade dysplasia (HGD) in Barrett’s esophagus (BE). This was associated with mortality and morbidity rates as high as 5% and 50%, respectively.1,3 A pivotal study that changed this approach to management was the AIM Dysplasia (Ablation of Intestinal Metaplasia Containing Dysplasia) trial, a sham-controlled study demonstrating that radiofrequency ablation (RFA) was highly effective for eradicating dysplastic BE.4 Subsequent studies have found similar efficacy rates, and it is now widely accepted that combined endoscopic therapy using EMR in conjunction with RFA is a viable alternative to esophagectomy.

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with RFA is a credible alternative to surgery for patients with early EAC and HGD in BE.3

Identification of mucosal abnormalities that may harbor advanced dysplasia or cancer, and removal by using EMR, is a critical aspect of BE assessment and staging. It is imperative to remove intramucosal cancer (IMC) and exclude submucosal cancer (SMC) before commencing RFA as they are not reliably ablated by this technique.4,5 Furthermore, if cancer cells are found to extend into the submucosal layer, lymph node spread is reportedly as high as 30%.6 These patients are usually referred for surgical resection or chemoradiotherapy.

Identification of these often subtle lesions requires careful, systematic assessment of the BE segment. Detection of mucosal abnormalities harboring HGD/EAC is shown to be improved by using high-definition white-light endoscopy (HD-WLE) and narrow-band imaging (NBI) modalities;4,7,8 however, in a recently published survey in the United States, these modalities were used by only one-third of practicing gastroenterologists when assessing BE.9,10 Additionally, studies show that rigorous endoscopic surveillance protocols result in significantly increased detection of HGD/EAC.11,12 Conversely, without comprehensive sampling, detection of HGD/EAC is significantly decreased.13,14 Despite this, adherence to the Seattle protocol by practicing community gastroenterologists is reportedly as low as 30% to 50%, and, notably, adherence rates vary inversely with the length of BE.15,16 It is therefore highly likely that detection of mucosal abnormalities and cancers will be lower in the community setting than in a specialized BE unit, where use of HD-WLE and NBI and adherence to a rigorous biopsy protocol are consistently used.

AIMS

In a cohort of patients with dysplastic BE identified in the community setting, we aimed to determine the additional detection rate of mucosal abnormalities and EACs identified in a BE unit. We aimed to compare endoscopy methods used in the community versus those used in a BE unit to see which factors contributed to overall lesion and cancer detection rates. We also aimed to assess the impact of EMR on histopathological staging and subsequent patient management.

METHODS

Referrals

Consecutive patients referred to St. Vincent’s Hospital Melbourne from November 2008 to September 2011 for management of dysplastic BE were prospectively entered into a central database. Patient demographic characteristics and the most advanced histology at and before referral and referral endoscopy details were recorded. These details,
remained before commencing RFA. For those with flat dysplastic BE, RFA was performed at approximately 3-month intervals until remission of dysplasia and intestinal metaplasia (IM) was achieved.

Referral endoscopy details
Comprehensive referral endoscopy details were retrospectively collected from the endoscopist, general practitioner, endoscopy center, or hospital medical records, with patient consent obtained as required. Details included Seattle protocol adherence, use of HD-WLE and NBI, use of the Prague Classification, and documentation of mucosal abnormalities.

Referral histology details
Referral histopathology was retrospectively obtained and reviewed by an expert GI pathologist (R.W.). Where downgrading or upgrading of the histological diagnosis occurred, the reviewed result was used as the baseline histology to compare subsequent histological findings after full endoscopic assessment. Where the referral histology was unavailable for review, the original histological grade was used as the baseline.

Analysis
Testing for significance of increase in detection of mucosal abnormalities and EAC at a BE unit was performed by using the χ² or Fisher exact test at a single-tail significance level of 5%. We assessed the role of EMR in staging the most advanced histopathology and how this affected management outcomes for this group.

This study was approved by the hospital’s Human Research Ethics Committee.

RESULTS

Patient details and histology at referral
Sixty-nine patients (61 male) were included in this study. Demographic and clinical details and the most advanced pathology at referral are summarized in Table 1.

The cases of 65 of 69 patients were reviewed by our GI pathologist (R.W.), with 4 unavailable for review. Fifty-five reviews resulted in agreement with the original histological grade. In 7 cases, there was downgrading (1 low-grade dysplasia [LGD] to IM, 6 HGD to LGD), and in 3 cases, there was upgrading (3 HGD to IM). As such, the most advanced histological grades at baseline were 18 IM, 23 HGD, 22 LGD, and 6 IM. After full assessment, which took into account the baseline pathology and results of assessment endoscopy with or without EMR, there was an overall upgrading of the most advanced histology with more than 75% having HGD (24), IM (15), or SMC (13), and 25% with LGD (15) or IM (2). Table 2 summarizes these findings.

Referral versus assessment endoscopy
There were 45 referring endoscopists (33 gastroenterologists referred 51 patients and 10 surgeons referred 18 patients) with the majority of endoscopies performed in the private setting (n = 41). BE surveillance was performed in 55 cases. In 32 of these surveillance cases, (performed by 25 endoscopists), no mucosal abnormality was described. Nine of these 32 cases (or 4 of 25 endoscopists) adhered to the Seattle protocol with a median number of biopsies per maximal length of BE in the remaining cases being 1 (range 0.75-2.8). The median time between referral endoscopy and BE unit assessment was 2.7 months (range 0.1-11.7).
TABLE 2. Comparison of most advanced histopathology at referral, review, and after full assessment

<table>
<thead>
<tr>
<th>Most advanced histopathology</th>
<th>At referral</th>
<th>External histology review</th>
<th>After referral histology</th>
<th>After full assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concordant</td>
<td>DG</td>
<td>US</td>
<td>Unavailable</td>
</tr>
<tr>
<td>IM (n = 5)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LGD (n = 17)</td>
<td>15</td>
<td>1</td>
<td>DG</td>
<td>IM to LGD</td>
</tr>
<tr>
<td>HGD (n = 32)</td>
<td>21</td>
<td>6</td>
<td>DG</td>
<td>US to LGD</td>
</tr>
<tr>
<td>IMC (n = 15)</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>IMC to IMC</td>
</tr>
<tr>
<td>SMC (n = 9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>55</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Total (n = 69)

DG: Downgrade; US: upgrade; IM, intestinal metaplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; IMC, intramucosal cancer; SMC, submucosal cancer.
*Where review of referral histology not available (4 cases), original histological grade used.
†Full assessment defined as assessment endoscopy with or without EMR and review of referral histology.

In the referral cohort, HD-WLE was used in 39 (57%), NBI use was documented in 10 (14%), and Seattle protocol was adhered to in 20% (n = 14). See Table 3 for full details.

Lesion detection at referral and assessment

Mucosal abnormalities were identified in 65 of 69 cases (94%) at BE unit assessment (median range 1-5) compared with 29 of 69 (42%) described at referral (P < .001). Lesion characteristics are summarized in Table 3; some were very subtle lesions of doubtful significance; however, all abnormal areas were targeted with biopsies to ensure they did not represent a dysplastic area.

In all patients, mucosal abnormalities were detected with a combination of HD-WLE and NBI. The use of CEM in 40 patients did not detect any additional patient with a lesion, although in 1 patient, CEM identified an additional focus of HGD not seen with HD-WLE/NBI. This did not affect the patient's subsequent management.

Characteristics of the predominant lesions seen in each case were as follows: 12 were nodules (11 Paris Classification 01A, 1 Paris Classification 01B-C), 51 were flat nodular lesions (24 Paris Classification 02A, 7 Paris Classification 02A+C, 3 were areas of depression (Paris Classification 02C), and 19 were flat areas with irregular mucosal pattern (Paris Classification 02B). The histologies of the predominant lesions after full assessment (obtained with biopsy or EMR) are listed in Table 3.

The median size of the predominant lesions was 10 mm (range 3-40 mm). Of 65 lesions, 43 had an irregular mucosal pattern, 11 had loss of the normal villous pattern, and 6 had a normal pattern (5 not stated). Inflammation was present at assessment in a small number of cases in which lesions were detected (8 of 65).

The role of EMR in BE assessment and staging

Forty-seven patients (68%) underwent EMR during assessment as it was thought that the predominant lesion detected may harbor early cancer. Staging and management outcomes are summarized in Table 4. In 50 patients, EMR was performed at a second procedure according to early protocol; in 17, EMR was performed at the initial endoscopy.

EMR resulted in upstaging of the histology from baseline in 24 of 47 patients (51%). Importantly, in 5 patients, EMR resulted in detection of endoscopically treatable early cancer (IMC). Of these, 4 went on to have endoscopic therapy with EMR with or without RFA, and 1 patient who was relatively young and fit elected to have esophagectomy. In a further 12 patients, EMR resulted in identifying SMC, leading to referral for esophagectomy (n = 9) or chemoradiotherapy (n = 2). One medically frail patient was treated with endoscopic therapy alone.

Detection of cancers at referral and assessment

Overall, 28 cases of cancer were detected in our cohort of 69 patients (13 SMC, 15 IMC [7/15 IMC invaded the muscularis mucosa]). Of these, 18 cancers were detected on biopsy at referral. Mucosal abnormalities were described in 16 of these 28 patients (57%) at referral, whereas all 28 patients had abnormalities detected at BE unit assessment (Table 5). Where an abnormality was described and targeted at referral (16 patients), 13 of the cancers were detected and 3 were missed (81% detection rate). Where no abnormality was detected (12 patients), 5 of the cancers were detected and 7 were missed (42% detection rate). The rate of cancer detection at referral was significantly

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**TABLE 3. Details of referral endoscopy versus assessment endoscopy at Barrett’s unit**

<table>
<thead>
<tr>
<th>Referral endoscopy details</th>
<th>Assessment endoscopy details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proceduralists</strong></td>
<td><strong>Proceduralists</strong></td>
</tr>
<tr>
<td>Gastroenterologists, n = 51</td>
<td>Gastroenterologist, n = 69</td>
</tr>
<tr>
<td>Surgeons, n = 18</td>
<td>Surgeons, n = 0</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>Sedation type</td>
</tr>
<tr>
<td>Anesthetist, n = 66</td>
<td>General anesthesia, n = 2</td>
</tr>
<tr>
<td></td>
<td>Anesthesiologist, n = 66</td>
</tr>
<tr>
<td></td>
<td>Propofol, n = 57</td>
</tr>
<tr>
<td></td>
<td>Without propofol, n = 3</td>
</tr>
<tr>
<td></td>
<td>Not stated, n = 7</td>
</tr>
<tr>
<td><strong>Endoscope used</strong></td>
<td><strong>Endoscope used</strong></td>
</tr>
<tr>
<td>High definition, n = 39</td>
<td>High definition, n = 69</td>
</tr>
<tr>
<td>Standard definition, n = 19</td>
<td>Standard definition, n = 0</td>
</tr>
<tr>
<td>Not stated, n = 11</td>
<td>Not stated, n = 0</td>
</tr>
<tr>
<td>Narrow-band imaging, n = 10</td>
<td>Narrow-band imaging, n = 69</td>
</tr>
<tr>
<td>White light only, n = 59</td>
<td>White light only, n = 0</td>
</tr>
<tr>
<td><strong>CEM use</strong></td>
<td><strong>CEM use</strong></td>
</tr>
<tr>
<td>Yes, n = 0</td>
<td>Yes, n = 40</td>
</tr>
<tr>
<td>No, n = 69</td>
<td>No, n = 29</td>
</tr>
<tr>
<td>Duration of procedure, min</td>
<td>Duration of procedure (all), min</td>
</tr>
<tr>
<td>Median, 10</td>
<td>Median, 68</td>
</tr>
<tr>
<td>Minimum, 5</td>
<td>Minimum, 19</td>
</tr>
<tr>
<td>Maximum, 60</td>
<td>Maximum, 160</td>
</tr>
<tr>
<td>Prague Classification used</td>
<td>Prague Classification not used</td>
</tr>
<tr>
<td>Yes, n = 9</td>
<td>Yes, n = 69</td>
</tr>
<tr>
<td>No, n = 60</td>
<td>Numerical, n = 49</td>
</tr>
<tr>
<td>Non-numerical, n = 6</td>
<td>0</td>
</tr>
<tr>
<td>No indication of length, n = 5</td>
<td></td>
</tr>
<tr>
<td>Presence of lesion at referral endoscopy</td>
<td></td>
</tr>
<tr>
<td>Description of lesion</td>
<td>Lesion seen at assessment</td>
</tr>
<tr>
<td>Yes, n = 29</td>
<td>Yes, n = 65</td>
</tr>
<tr>
<td>Nodularity, n = 4</td>
<td>Nodular, n = 3; HGD, n = 9; LGD, n = 7</td>
</tr>
<tr>
<td>Ulcer, n = 5</td>
<td>Ulcer, n = 5</td>
</tr>
<tr>
<td>Erosion, n = 2</td>
<td>Erosion, n = 2</td>
</tr>
<tr>
<td>Mucosal abnormality, n = 6</td>
<td>Mucosal abnormality, n = 6</td>
</tr>
<tr>
<td>Paris classification, n = 0</td>
<td>Paris classification, n = 0</td>
</tr>
</tbody>
</table>

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higher when an abnormality was targeted (13 of 16 vs 5 of 12, $P = .039$).

**Further details of the cancers not detected at referral**

Of the 28 cases of cancer in our cohort, BE unit assessment confirmed EAC in the original 18 cases found at referral and identified an additional 10 cases (4 SMC, 6 IMC), corresponding to a 56% increase in cancer detection ($P = .036$). In these 10 cases, the median time between referral endoscopy and BE unit assessment was 5.2 months (range: 0.9–10.1 months).

In 7 of these 10 cases, no mucosal abnormality had been described at referral endoscopy, and the worst histology on random biopsy specimens was LGD in 2 and HGD in 5. In 3, lesions were described, although biopsies revealed HGD as the most advanced histology. We looked at these 10 cases in more detail (Table 6). Mucosal abnormalities were detected in all cases at BE unit assessment, and after EMR of these lesions, 6 IMCs and 4 SMCs were found. Figures 1A–8B show lesions from eight of these cases (see Table 6 for corresponding case number and figures). Lesions detected at assessment but not referral ranged from subtle flat lesions (Paris Classification 02B) with irregular mucosal patterns best seen on NBI to small, slightly raised areas of nodularity (Paris seen 02A) to larger, more obvious nodules (Paris seen 01S).

**DISCUSSION**

This is a large single-center experience of assessment and management of patients with dysplastic BE. Three important conclusions can be inferred from our results. The first is that systematic assessment of BE segments by experienced endoscopists with access to HD-WLE and NBI results in increased detection rates of mucosal abnormalities. Second, this increased detection correlates with an increased cancer detection rate. Finally, EMR of mucosal lesions harboring cancer is critical in staging early cancers in BE, and ultimately this staging affects whether a patient can be managed endoscopically.

There is clear evidence in the medical literature that mucosal lesions harboring cancer in BE may be missed. Until 2008, the published guidelines recommended that patients with HGD be routinely considered for esophagectomy.25 This was in part attributed to the high prevalence
of EAC found in patients with HGD who underwent surgery.\textsuperscript{21} Prevalence rates vary in the medical literature, with older surgical series reporting the risk of concomitant adenocarcinoma in patients with HGD approaching 40%.\textsuperscript{22,23} More recently, a meta-analysis of 23 studies of patients undergoing esophagectomy for BE and HGD reported a 12.7% incidence of invasive adenocarcinoma.\textsuperscript{24}

Furthermore, in the absence of visible lesions in BE, the prevalence of EAC in patients who had undergone esophagectomy was 3%.\textsuperscript{25,26} Additional evidence that mucosal abnormalities and cancers may be missed was suggested in a recent case-control study that showed no survival benefit of performing BE surveillance. This included 8 patients who ultimately died of EACs, which were detected within 10 months of having had negative findings on surveillance endoscopy.\textsuperscript{27} These cases are unlikely to represent patients with rapidly progressive disease, but more likely their cancer was missed at endoscopy.

Our data provide direct evidence that lesions harboring cancer are often missed in the primary setting but can be identified by careful endoscopy at an expert center. The rate of upgrading from LGD or HGD to EAC in our cohort was 36% (25% from HGD, 11% from LGD), which echoes the rate of undiagnosed cancers in the surgical resection medical literature.\textsuperscript{21} Also of note, of 22 patients with LGD, 11 had focal lesions identified, and 7 of these were upgraded to HGD (n = 4), IMC (n = 2), or SMC (n = 1), suggesting that LGD is often associated with more serious

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### TABLE 6. Further details of the 10 cancers not detected at referral endoscopy

<table>
<thead>
<tr>
<th>Cancer found at assessment</th>
<th>Case (figure)</th>
<th>Pathology at referral</th>
<th>Lesion seen at referral</th>
<th>Paris Classification at assessment</th>
<th>Time (mo) between referral endoscopy/assessment</th>
<th>Description of lesion</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMC</td>
<td>1 (Fig. 1 A,B)</td>
<td>HGD</td>
<td>2.3</td>
<td>No</td>
<td>02A</td>
<td>30-mm flat nodule at 41-42 cm at the 8-12 o’clock position with loss of regular mucosal pattern</td>
<td>Esophagectomy</td>
</tr>
<tr>
<td>2 (Fig. 2 A,B)</td>
<td>HGD</td>
<td>7.3</td>
<td>No</td>
<td>02A</td>
<td>Chemoradiotherapy</td>
<td>20-mm area of nodularity at 40-41 cm 9-1 o’clock with irregular mucosal pattern</td>
<td>Chemoradiotherapy</td>
</tr>
<tr>
<td>3 (Fig. 3 A,B)</td>
<td>LGD</td>
<td>7.9</td>
<td>No</td>
<td>02C</td>
<td>Esophagectomy</td>
<td>25-mm flat nodular area at 32-34 cm at the 11-3 o’clock position with irregular mucosal pattern</td>
<td>Esophagectomy</td>
</tr>
<tr>
<td>4</td>
<td>HGD</td>
<td>0.9</td>
<td>No</td>
<td>02A+C</td>
<td>Esophagectomy</td>
<td>35-mm area with depressed base and raised edges at 34-37 cm extending 1/3 of the esophagus centered at the 9 o’clock position</td>
<td>Esophagectomy</td>
</tr>
<tr>
<td>IMC</td>
<td>5 (Fig. 4 A,B)</td>
<td>HGD</td>
<td>2.2</td>
<td>No</td>
<td>02A</td>
<td>10-mm area of nodularity at 37-38 cm at the 3-4 o’clock position with loss of mucosal pattern</td>
<td>EMR + RFA</td>
</tr>
<tr>
<td>6 (Fig. 5)</td>
<td>LGD</td>
<td>10.1</td>
<td>No</td>
<td>01S</td>
<td>EMR + RFA</td>
<td>20-mm nodule at 35-36 cm at the 12-2 o’clock position with loss of the mucosal pattern</td>
<td>EMR + RFA</td>
</tr>
<tr>
<td>7</td>
<td>HGD</td>
<td>5.9</td>
<td>No</td>
<td>02B</td>
<td>EMR + RFA</td>
<td>10 mm 33-36 cm at 3-4 o’clock subtly abnormal mucosa with loss of mucosal pattern</td>
<td>EMR + RFA</td>
</tr>
<tr>
<td>8 (Fig. 6)</td>
<td>HGD</td>
<td>2.3</td>
<td>Yes*</td>
<td>02A+C</td>
<td>EMR + RFA</td>
<td>10-mm nodular area at 39 cm 12 o’clock with irregular mucosal pattern</td>
<td>EMR + RFA</td>
</tr>
<tr>
<td>9 (Fig. 7)</td>
<td>HGD</td>
<td>4.6</td>
<td>Yes†</td>
<td>01S</td>
<td>EMR + RFA</td>
<td>10-mm nodule at 36 cm at the 3 o’clock position</td>
<td>EMR + RFA</td>
</tr>
<tr>
<td>10 (Fig. 8)</td>
<td>HGD</td>
<td>6.7</td>
<td>Yes†</td>
<td>02A</td>
<td>Esophagectomy</td>
<td>25-mm area of nodularity at 31 cm at the 8-2 o’clock position up to 28 cm at the 10-4 o’clock position with irregular mucosal pattern</td>
<td>Esophagectomy</td>
</tr>
</tbody>
</table>

SMC, Submucosal cancer; HGD, High-grade dysplasia; LGD, low-grade dysplasia; IMC, intramucosal cancer; RFA, radiofrequency ablation.

*One slight nodule at the 12 o’clock position.
†Irregular mucosa at 32 and 34 cm.
§Slightly raised area at 30 cm.

pathology in visible lesions that may not be identified in the community setting. This is particularly relevant when considering the controversy that exists in the medical literature over the significance of LGD in BE and its risk for progression to EAC. In a study reported in 2016, it was found that the incidence rate of HGD or EAC was 15.4% per patient per year for patients in whom the diagnosis of LGD was confirmed, a significantly higher rate than previously published. Our data raise the question as to whether these results reflect true progression of disease from LGD to advanced dysplasia or rather whether concomitant HGD/EAC was missed at original endoscopy. Concomitant HGD/EAC may also be missed where the BE segment sampling is inadequate. It is conceivable that
in some cases in this cohort, the referring endoscopist always intended to refer their case to a BE unit and therefore did not perform rigorous biopsies. However, where a bland BE segment was described at BE surveillance, adherence to the Seattle Protocol was only 28%. This figure, consistent with the published medical literature, suggests that a significant number of community endoscopists do not take rigorous biopsy specimens in routine BE surveillance, even when they may not be expecting to refer to a BE unit.  

In this study, the mucosal abnormality detection rate at BE unit assessment was 94% compared with 42% at referral. Many abnormalities were small, subtle lesions, and not all harbored dysplasia; however, there were
instances when even subtle lesions harbored advanced dysplasia. Three factors likely contribute to this large difference in lesion detection. First, most experts agree that high-resolution endoscopy is the preferred method for the endoscopic evaluation of BE as there is evidence that it has higher sensitivity for the detection of early BE neoplasia compared with standard video endoscopy systems. Additionally, NBI use has been shown to detect more areas of dysplasia than white light alone. HD-WLE and NBI were used in all BE unit assessments but in only 57% and 14% of the referral cohort, respectively. These results are consistent with a recently published survey of gastroenterologists in the United States, the majority of whom were community gastroenterologists. The results showed that less than one-third used advanced imaging techniques in BE surveillance.

Second, the median time to perform assessment endoscopy in the BE unit (in those patients who did not have CEM performed) was 40 minutes (range: 19-150 minutes),
compared with 10 minutes (range 5-60 minutes) at referral endoscopy. CEM was used in 40 patients who were enrolled in a study assessing the accuracy of HD-WLE, NBI, and CEM in identifying HGD and IMC, the results of which have been published. CEM had high accuracy rates for detecting HGD/IMC, but the benefit over HD-WLE/NBI was minimal, and using this modality required significantly increased time and cost. CEM resulted in finding 1 further point of HGD in a patient in whom HGD had already been detected by NBI, but this did not affect the clinical outcome. CEM has been shown in other studies to have high accuracy for detecting neoplastic BE and a high negative predictive value for endoscopically invisible neoplastic BE; therefore, some experts with specific expertise in CEM advocate its use to interrogate focal lesions identified with HD-WLE/NBI. Based on our cohort, we concluded that assessment by using HD-WLE/NBI, without CEM, was the most efficacious approach in our unit.

Third, although there are currently no studies showing that centers with expertise or high case volumes provide better quality care for BE patients with HGD/EAC, in other areas of gastroenterology, these factors are associated with better outcomes. Current expert consensus is that patients being considered for BE treatment with at least HGD be referred to a tertiary center. This study demonstrates that assessment by an endoscopist experienced in BE assessment will detect subtle lesions missed by endoscopists less experienced in this area. It is possible that the superior detection rates of mucosal abnormalities/EACs at a BE unit are influenced by knowing the referral histology, thereby resulting in modifying the assessment technique, eg, more thorough in patients with known advanced dysplasia. However, regardless of severity of previous dysplasia, all referred patients underwent the same process of careful mapping in both WLE and NBI modalities, and in all cases the predominant area of mucosal abnormality was targeted with biopsies with or without EMR.

Identification of these often subtle lesions was critical in finding the most advanced histology. Of the 28 cancers identified in our cohort, mucosal abnormalities were detected and targeted in all cases in the BE unit. Cancer detection rates at referral were significantly higher when a mucosal abnormality was targeted (81% vs 42%, P = 0.039), highlighting the importance of careful endoscopic assessment. In the 10 additional cases of cancer found at the BE unit, the median time from referral to assessment endoscopy was 5.2 months (range 0.9-10.1 months). Progression rates to EAC from HGD vary considerably in the medical literature: estimated at 6.6% per year for HGD and generally much lower for LGD, with the exception of the previously mentioned study that reported a 13.4% per year progression rate. Although it cannot be concluded that cancer developed in the interval from referral to assessment, in the majority of these 10 cases, it is thought unlikely.

This study also demonstrates the importance of EMR in accurately staging patients with cancer in BE. The most important predictor of lymph node metastasis in EAC is penetration depth of the tumor, which is most reliably determined by histopathological evaluation of the EMR specimen. Sixty-eight percent of our cohort underwent EMR for suspected neoplasia. EMR resulted in upstaging of the overall histology from baseline in 24 of 47 patients (51%), from dysplasia to cancer in 8 of 28 (29%) and IMC to SMC in 9 of 16 (56%). This had significant implications for subsequent management. In 12 patients with SMC, 11 were referred for esophagectomy or chemoradiotherapy because endoscopic therapy would not have been curative. In 5 patients, EMR detected endoscopically treatable IMC. This upstaging of pathology is reflected in the medical literature with EMR of visible lesions harboring HGD on biopsy being upgraded to cancer in 40% of cases.

This cohort of patients was referred to our BE unit for consideration of RFA treatment because RFA is currently only available in limited tertiary centers. It is likely, however, that RFA equipment will eventually become more widely available for use in the community setting. Based on the referral endoscopies and histology alone, an argument could have been made for proceeding directly to RFA in many cases. Our data clearly demonstrate the importance of careful assessment of focal lesions and resection of these before proceeding to RFA. We believe
that these data support the use of RFA only after detailed assessment and preferably in a BE unit because treatment may be suboptimal if RFA is delivered outside of expert BE centers. This study suggests that education programs for endoscopists should include methods for careful assessment, use of NBI, identification of subtle lesions, and performance of esophageal EMR.

CONCLUSION

Assessment of patients with dysplastic BE at a BE unit resulted in improved detection of mucosal abnormalities and EAC. EMR of these early cancers was a critical step in determining a patient's appropriateness for endoscopic therapy. We currently recommend that referral to a BE unit be considered in all patients with dysplastic BE to optimize detection of the most advanced histopathology and to perform EMR for staging of early cancers where necessary before embarking on a definitive management course.

REFERENCES


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Abbreviations: BE, Barrett’s esophagus; CEM, confocal endomicroscopy; EAS, esophageal adenocarcinoma; HGD, high-grade dysplasia; HD-WLE, high-definition white-light endoscopy; IM, intestinal metaplasia; IMC, intramucosal cancer; LGD, low-grade dysplasia; NBI, narrow-band imaging; RFA, radiofrequency ablation; SMC, submucosal cancer.

DISCLOSURE: All authors disclosed no financial relationships relevant to this article.

Received December 2, 2013. Accepted March 27, 2014.

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APPENDIX 1: PRAGUE CLASSIFICATION

A circumferential segment of Barrett’s esophagus (BE) 2 cm in length from the top of the mucosal folds and tongues of BE metaplasia extending an additional 3 cm superior would be classified as C2M5.
- Circumferential (C) length of BE
- Maximal (M) extent of any BE metaplasia.

APPENDIX 3: SEATTLE PROTOCOL

Random 4-quadrant biopsy specimens taken at 1- to 2-cm intervals along the Barrett’s esophagus segment by using jumbo biopsy forceps.

APPENDIX 2: Paris Classification of mucosal lesions

<table>
<thead>
<tr>
<th>Endoscopic appearance</th>
<th>Paris classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protruding lesions</td>
<td>1S</td>
<td>Nodule, sessile polyp</td>
</tr>
<tr>
<td>Flat elevated lesions</td>
<td>02A</td>
<td>Flat elevation of mucosa</td>
</tr>
<tr>
<td></td>
<td>02A+C</td>
<td>Flat elevation of mucosa with central depression</td>
</tr>
<tr>
<td>Flat lesions</td>
<td>02B</td>
<td>Flat mucosal change</td>
</tr>
<tr>
<td></td>
<td>02C</td>
<td>Mucosal depression</td>
</tr>
<tr>
<td></td>
<td>02C+A</td>
<td>Mucosal depression with raised edge</td>
</tr>
</tbody>
</table>
Chapter 5: Recurrent intestinal metaplasia at the gastroesophageal junction following endoscopic eradication of dysplastic Barrett’s esophagus may not be benign

5.1 Introduction and Summary

While there are many studies highlighting the efficacy and safety of endoscopic eradication of Barrett’s with radiofrequency ablation (RFA) with or without endoscopic mucosal resection (EMR), the data on recurrence rates of IM and dysplasia following eradication of BE are more limited and vary considerably. This is in part due to inconsistency in the definition of recurrence and additionally due to varying surveillance biopsy protocols employed between studies. While RFA is now generally accepted as the preferred method of ablation for dysplastic BE, all data on durability of outcomes have been in their relative infancy with minimal long-term data. There are also few data on durability of outcomes as applied to real world cohorts.

This study aimed to assess the effectiveness, safety and initial durability outcomes of RFA with or without EMR in one of the largest reported cohorts of patients with (predominantly advanced) dysplastic Barrett’s. We found that for this real world cohort, the rates of remission of Barrett’s and dysplasia were similar to, but on the lower end of the spectrum, of reported rates in the current literature. Additionally, there was a significant risk of recurrence of both intestinal metaplasia and dysplasia following successful eradication of BE, which appeared to occur most commonly in patients with advanced pre-treatment histology, and frequently at the gastroesophageal junction (GOJ). These findings have important clinical implications for the ongoing careful surveillance of this region, particularly in patients with prior advanced dysplasia or cancer.
5.2 PDF of Published Journal Article

Recurrent intestinal metaplasia at the gastro-esophageal junction following endoscopic eradication of dysplastic Barrett's esophagus may not be benign

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Background and study aims: Radiofrequency ablation (RFA) combined with endoscopic mucosal resection (EMR) is effective for eradicating dysplastic Barrett's esophagus. The durability of response is reported to be variable. We aimed to determine the effectiveness and durability of RFA with or without EMR for patients with dysplastic Barrett's esophagus.

Patients and methods: Patients with dysplastic Barrett's esophagus referred to two academic hospitals were assessed with high definition white-light endoscopy, narrow-band imaging, and Seattle protocol biopsies. EMR was performed in visible lesions. RFA was performed at 3-month intervals until complete remission of dysplasia (CR-D) and intestinal metaplasia (CR-IM) was achieved.

Results: In total, 137 patients received RFA (78 with EMR); 75 with over 12 months follow-up since commencing RFA. Pretreatment histology was intramucosal cancer (IMC): 21%; high grade dysplasia (HGD): 54%, low grade dysplasia (LGD) 25%. CR-D rates were 88%, 92%, and 100% at 1, 2, and 3 years; CR-IM rates were 69%, 74%, and 81%. Kaplan-Meier analysis showed increasing probability of achieving CR-D/CR-IM over time. Of 26 patients maintaining CR-IM for >12 months, five relapsed with intestinal metaplasia (19%), and three with dysplasia (12%). Recurrences occurred in patients with prior HGD/IMC, predominantly at the gastroesophageal junction (GE). None relapsed with cancer. Adverse events occurred in 4% of RFA and 6.5% of EMR procedures.

Conclusions: RFA combined with EMR is effective in achieving CR-D/CR-IM in the majority of patients with dysplastic Barrett's esophagus, with an incremental response over time. While durable in the majority, recurrent intestinal metaplasia and dysplasia, frequently occurring at the GE, suggest long-term surveillance is warranted in high risk groups.

Introduction

The AIM dysplasia trial, a randomized sham controlled trial published in 2008, showed that radiofrequency ablation (RFA) was highly effective in eradicating dysplastic Barrett's esophagus, and safe [1]. Subsequent studies have shown that combination endoscopic therapy (CET) (endoscopic mucosal resection (EMR) and RFA) provides a credible alternative to surgery in patients with early esophageal adenocarcinoma (EAC) [2–5]. EAC with submucosal invasion is still typically referred for esophagectomy due to risk of lymph node spread, which is reported to be between 10% and 50% depending on depth of mucosal invasion in the index EMR specimen [6–8]. RFA is now widely considered to be the preferred method for eradicating intestinal metaplasia and dysplasia within Barrett's esophagus [9].

While many studies report the efficacy of RFA in dysplastic Barrett's esophagus, there are fewer published data on the durability of treatment outcomes. A systematic review and meta-analysis of the efficacy and durability of RFA in Barrett's esophagus [10] analyzed 18 efficacy and six durability studies. The pooled recurrence rate of intestinal metaplasia after eradication was estimated at 13% (95% CI 9–18%) [10]. Outcomes of more recent studies continue to vary. Phoa et al. reported outcomes of 54 patients receiving RFA and EMR for Barrett's esophagus with high grade dysplasia (HGD) [11] and found sustained remission of intestinal metaplasia and neoplasia in 93% of those reaching 5 years of follow-up (n = 46) [11]. Others quote intestinal metaplasia recurrence rates between 20% and 32% [12, 13]. Recurrent or residual intestinal metaplasia typically occurs in distinct patterns: as visible islands in the tubular esophagus; buried intestinal metaplasia under neo-squamous mucosa; and at the gastroesophageal junction (GE). Korst et al. found the GE was the most common site for recurrent
intestinal metaplasia post-ablation (71%) [12]. The clinical significance of intestinal metaplasia at the GE is not well established. Several clinical trials assessing RFA efficacy did not routinely biopsy the GE at follow-up [1,14], partly accounting for the variation in quoted recurrence rates. In two studies where biopsies were routinely taken just below the non-squamous columnar junction post-RFA, one found persistent intestinal metaplasia in only 4% of 54 patients and no dysplasia; the other found recurrent dysplasia in 9% of 47 patients, with all dysplasia located at the GE [11,13]. With intestinal metaplasia thought to precede dysplasia, this suggests that intestinal metaplasia at the GE may not be a benign entity.

Aims

We aimed to assess the effectiveness and durability of RFA (with or without EMR) in patients with dysplastic Barrett's esophagus, by determining rates of complete remission of dysplasia (CR-D) and intestinal metaplasia (CR-IM), time to achieve these endpoints, number of RFA treatments required, and recurrence rates and location of intestinal metaplasia/dysplasia post-eradication. A secondary aim was to evaluate the safety of EMR and RFA.

Methods

Patients referred to St Vincent's Hospital Melbourne and The Royal Melbourne Hospital between 2008 and September 2013 were entered prospectively into a central database. Patient demographics, referral endoscopy details, prior treatment, and pre-assessment histology were recorded. Referral histology was reviewed by an expert gastrointestinal pathologist (RW). Patients underwent systematic assessment of their Barrett's segment with high definition (Olympus H-180) endoscopy using high definition white-light endoscopy (HD-WLE) and narrowband imaging (NBI). Forty patients (early in cohort) were also assessed with confocal endomicroscopy (CEM) as part of a study assessing the accuracy of HD-WLE, NBI, and CEM in identifying HGD/EAC. This study showed that CEM was accurate in confirming suspected dysplasia/neoplasia seen with HD-WLE/NBI, but did not significantly add to HGD/EAC detection or clinical outcome [15]. CEM was not routinely used for subsequent patient assessments. Barrett's extent was documented according to the Prague classification [16] (Appendix 1). Mucosal abnormalities were characterized according to size, Paris Classification [17] (Appendix 2), and mucosal pattern (irregularity/loss) [18]. Biopsies were taken according to the Seattle Protocol (Appendix 3). Biopsies of mucosal irregularities were labeled according to location (centimeters from mouth, o'clock position in neutral scope position), to facilitate location at subsequent endoscopy. Initially, where a lesion was thought to harbor HGD or neoplasia, biopsies were taken with EMR performed a few weeks later. In later cases, EMR was often performed at initial assessment. Biopsies were assessed for presence of intestinal metaplasia and grade of dysplasia using the revised Vienna classification (intestinal metaplasia without dysplasia (non-dysplastic Barrett's esophagus), indefinite for dysplasia (IND), low grade dysplasia (LGD), HGD or cancer) [19,20]. EMR specimens were evaluated for infiltration depth, vertical resection margins, tumor differentiation or grade of dysplasia, and lymphatic/vascular invasion.

Patients' subsequent management depended on the most advanced histology after assessment and suitability for CET. Those with submucosal cancer (SMC) were referred for esophagectomy or chemoradiotherapy as endoscopic therapy was not considered definitively curative. In those with intramucosal cancer (IMC) or nodular HGD, EMR was performed until we were confident no cancer remained before commencing RFA. In the treatment group, RFA was performed at 3-month intervals, unless delay occurred due to patient illness/social reasons. RFA was performed using the BARRXTM HALO system as previously described in the literature [21]. The HALO360 or HALO50 ablation catheters were used at the discretion of the endoscopist (AT, FM, C), GC depending on case specifics. Typically, HALO360 was used initially, unless the segment was patchy or significant narrowing existed from prior EMR scarring. Where HALO360 was used, the GE was overlapped and treated with the balloon. Where HALO90 was employed, the GE was focally ablated. In most cases, the esophagus was flushed with N-acetyl-cysteine pretreatment. Patients received double-dose proton pump inhibitor and topical anesthetic post-procedure. Interval EMR was performed where suspicious lesions were detected at subsequent endoscopy. Follow-up evaluation commenced at the first post-treatment endoscopy confirming CR-IM (no endoscopic Barrett’s and no intestinal metaplasia histologically, including at the GE). Recurrence was defined as intestinal metaplasia or dysplasia identified after achieving CR-IM. Intestinal metaplasia at the GE but not within the cardia was considered recurrence. Surveillance endoscopies were performed at 3, 6 and 12 months, then annually for those with prior IMC/HGD, and at 6 and 12 months, then annually for those with prior LGD. Surveillance biopsies were taken in four quadrants every 2 cm commencing just distal to the neo-squamous columnar junction at the GE, extending proximally for the original maximal (M) length of the segment. Where possible, patients were followed in the tertiary center for at least 1 year after achieving CR-IM; however, in some cases, due to social circumstances or distance from the hospital, patients were discharged to their referring endoscopist. Advice was given with regard to surveillance intervals and biopsy protocol. Subsequent endoscopy/histology reports were obtained for database entry. Patients with recurrent intestinal metaplasia or dysplasia were re-referred to the tertiary center for further management. EMR and RFA adverse events were defined as events requiring surgery, unplanned hospital admission, bleeding requiring transfusion or unplanned endoscopic procedure. For patients living remotely, admission for observation was arranged when minor intra-procedural bleeding occurred. These patients were included in adverse event rates.

Statistical analysis

Absolute remission rates and Kaplan–Meier estimates with 95% confidence intervals for achieving CR-D and CR-IM at 1, 2, and 3 years were calculated. Intestinal metaplasia/dysplasia recurrence rates (absolute, and Kaplan–Meier estimates) were calculated for those achieving CR-IM. Testing for statistical significance was performed using Chi squared/Fisher's exact test at a single tail significance level of 5%. This study was registered and approved by the hospitals’ Human Research Ethics Committees.
Results

There were 204 patient referrals (170 males; median age 67.7 years [31.2–89.6]). Following assessment, 50 patients were excluded from receiving CET predominantly due to advanced disease; 24 with SMC were referred for esophagectomy, and seven for chemoradiotherapy. Of 154 patients appropriate for CET, 17 were treated with EMR only. Of 264 patients assessed, 137 have received RFA or EMR.

Treatment group characteristics

In total, 137 patients (118 males) had commenced RFA treatment at time of analysis. Pretreatment histopathology after full assessment was IMC in 29 (21%), HGD in 74 (54%) and LGD in 34 (25%). Full assessment was defined as assessment endoscopy ± EMR and referral histology review. Median age was 65.9 years (34.8–87.5); median Barrett’s esophagus circumferenceal (C) and length were 3 cm (0–17) and 5 cm (0–18), respectively. In total, 78/137 had EMR in addition to RFA (69 pre-RFA, three between treatments, six post-RFA); of 38/137 had received Barrett’s esophagus treatment before referral: 22 with EMR, six argon plasma coagulation (APC), three laser, two cryotherapy, two photodynamic therapy, one gold probe, and two chemoradiotherapy (one for squamous cell carcinoma [SCC] of the esophagus).

Effectiveness

At the time of census, 85/137 were in post-treatment surveillance (five having achieved CR-D but not CR-IM); and 75 had at least 12 months of follow-up from first RFA to last endoscopy (therapeutic or surveillance). Of those with 12 months follow-up, nine had received HALO360 only (35 with HALO360/80, 31 with HALO90). In total, 80 patients achieved CR-D (median time 7.2 months [2.3–41.6]). 65 achieved CR-IM (median 8.6 months [2.3–30.5]). Median number of RFA to achieve these endpoints was 2 (1–6). Median follow-up from first RFA to last endoscopic procedure was 13 months (0–53.7). Absolute rates of CR-D and CR-IM for those with 1-, 2-, and 3-year follow-up were 88%, 92%, and 100%, and 69%, 74%, and 81%, respectively. Kaplan–Meier estimates for the same end points at 1, 2, and 3 years were 58%, 88%, and 95%, and 41%, 72%, and 92%, respectively. These results, with associated 95% confidence intervals are summarized in Table 1 and Fig. 2a, 2b. Of the 75 patients with at least 12 months of follow-up, at time of census, nine had not yet achieved CR-D (median follow-up 18.4 months [12.4–35.3]), and 24 had not yet achieved CR-IM (median follow-up 19.5 months [12.0–58.9]), both groups with median RFA (1–7). Of those not yet achieving CR-D, two were discharged from follow-up for social reasons without histological confirmation of CR-D. Two were awaiting follow-up biopsies. One was still receiving RFA after several intervening EMR procedures for nodular Barrett’s esophagus. Two had a poor endoscopic response to RFA thought due to visible volume reflux and were referred for fundoplication before continuing treatment. Two were referred for esophagectomy after cancer was found during RFA treatment. In one case, the pre-assessment histology was HGD with non-dysplastic Barrett’s esophagus found at referral and assessment. Three months following initial HALO90, a nodule harboring SMC was detected and completely excised with EMR. No residual cancer was found in the esophagectomy specimen. The second patient had IMC documented previously but HGD at referral and assessment. No EMR was performed. IMC was subsequently detected 3 months after two HALO360. Esophagectomy was curative. Five-year follow-up showed no endoscopic recurrence in either case. Of the 24 patients not yet achieving CR-IM, four had no visible Barrett’s esophagus, seven had less than 1 cm Barrett’s esophagus, 13 had smaller Barrett’s esophagus remaining. Location of intestinal metaplasia was focal at the GE in five, focal above the GE in four, diffuse in six patients, not specified in six, and in three there were no biopsies. There was no statistically significant difference in response to therapy based on pretreatment histology.

Durability

In total, 65 patients achieved CR-IM (median follow-up 12.4 months [0–64.4]). Of these, 12/65 had “possible Barrett’s” described at endoscopy (eight with tiny islands, one with possible rim at GE, three with possible Barrett’s tongues of <1 cm) but targeted biopsies of these areas did not reveal intestinal metaplasia. In total, 26 patients had at least 12 months follow-up after achieving CR-IM. Of those achieving CR-IM, five died before one HALO360 (one with EMR also); three of those had at least 12 months of follow-up since achieving CR-IM; 18/65 (28%) patients documented recurrent intestinal metaplasia after initial eradication, 72% at the GE; 10/18 had salvage therapy (APC 5, EMR 3, RFA 2). Nine had re-achieved CR-IM at time of census. Of the nine re-achieving CR-IM, median months in remission was 12.8 (0–38.9) (Fig. 3a, Table 2).
Table 1: Proportion of patients achieving CR-D and CR-M at 1, 2, and 3 years follow-up, with corresponding Kaplan-Meier estimates for the same end points.

<table>
<thead>
<tr>
<th>Time elapsed since commencing RFA treatment, years</th>
<th>Number reaching the amount of follow-up</th>
<th>Number achieving CR-D</th>
<th>Time to achieve CR-D, months</th>
<th>Kaplan-Meier estimate for achieving CR-D (with 95% confidence interval)</th>
<th>Number achieving CR-M</th>
<th>Time to achieve CR-M, months</th>
<th>Kaplan-Meier estimate for achieving CR-M (with 95% confidence interval)</th>
<th>Number of RFA required to achieve CR-D and CR-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>66 (88%)</td>
<td>7.8 (2.3-41.6)</td>
<td>57.6% (48.2%, 67.1%)</td>
<td>52 (69%)</td>
<td>10.6 (2.3-30.3)</td>
<td>41.3% (31.3%, 51.1%)</td>
<td>2 (1-6)</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>36 (92%)</td>
<td>7.8 (2.3-41.6)</td>
<td>57.6% (48.2%, 67.1%)</td>
<td>26 (78%)</td>
<td>10.6 (2.3-30.3)</td>
<td>41.3% (31.3%, 51.1%)</td>
<td>2 (1-6)</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>16 (100%)</td>
<td>12.5 (3.0-34.5)</td>
<td>95.2% (88.1%, 100.0%)</td>
<td>15 (93%)</td>
<td>12.2 (3.0-29.2)</td>
<td>81.8% (70.4%, 93.2%)</td>
<td>3 (1-6)</td>
</tr>
</tbody>
</table>

CR-D, complete remission of dysplasia; CR-M, complete remission of intramucosal neoplasia; RFA, radiofrequency ablation.

**Discussion**

We present a case series of patients with Barrett's esophagus treated with radiofrequency ablation (RFA). While RFA has shown promising results in the treatment of Barrett's esophagus, there is conflicting evidence in the literature regarding the durability of RFA-induced regression of dysplasia. The reported rates of regression of dysplasia after RFA are variable, with studies showing rates ranging from 0% to 100%. The duration of follow-up after RFA also varies, with studies reporting follow-up periods ranging from 1 to 10 years. While some studies have reported high rates of regression of dysplasia after RFA, others have not. Despite the variability in the reported rates of regression of dysplasia after RFA, there is a growing body of evidence suggesting that RFA may be an effective treatment option for patients with Barrett's esophagus with low-grade dysplasia.

**Safety of RFA and BMI**

The safety profile of RFA in patients with Barrett's esophagus is generally considered to be favorable. However, RFA has been associated with potential complications, including esophageal strictures, esophageal ulcerations, and esophageal perforations. The long-term safety and durability of RFA-induced regression of dysplasia remain to be determined, and further studies are needed to establish the safety profile of RFA in the treatment of Barrett's esophagus.

In conclusion, RFA is a promising treatment option for patients with Barrett's esophagus and low-grade dysplasia. Further research is needed to better understand the safety and durability of RFA-induced regression of dysplasia and to determine the optimal treatment strategy for patients with Barrett's esophagus.
incrementally over time with further RFA treatments: 84% CR-D/66% CR-IM within 18 months, and 95% CR-D/82% CR-IM within 36 months (Fig. 2a, Fig. 2b). It is likely that patients with complex Barrett’s esophagus segments (i.e., nodular, stricture, scarred or pouch-like esophagus) require more RFA sessions to achieve complete segment ablation due to technical difficulty in achieving total ablation at each session. A second subgroup of “slow responders” may exist who, despite complete ablation in a session, require a greater number of treatments to achieve CR-IM. We hypothesize that this group may include those with poorly controlled volume reflux resulting in ongoing mucosal insult between treatments. Further evaluation of our cohort is required to determine what proportion of patients yet to achieve CR-D/CR-IM respond eventually. Further studies are needed to assess predictors of poor/slow response.

Our cohort was heterogeneous, reflecting the broad spectrum of patients with dysplastic Barrett’s esophagus, and consisted of patients with predominantly advanced histology (>75% with HGD/JG). Cases included often complex Barrett’s esophagus segments and this was thought to contribute to slightly lower CR-D/CR-IM rates at early time points. Delay between RFA treatments due to medical/social reasons, or interval performance of EMR for suspicious lesions were also factors. Finally, 3/24 patients not achieving CR-IM and 4/9 patients not achieving CR-D after 12 months of treatment lacked histological confirmation at the time of census. It is therefore conceivable that the proportion of patients achieving CR-D/CR-IM is an underestimate.

**Progression of disease**

EAC was detected in two patients while receiving RFA, resulting in referral for esophagectomy (details in Results section). In both cases, given the time course, cancer likely went undetected at assessment rather than representing true progression on treatment. These cases highlight the importance of rigorous baseline evaluation to enable appropriate patient selection for CET. Both cases occurred very early in the cohort suggesting that a learning curve exists, even for experienced endoscopists, in recognizing subtle mucosal abnormalities that may harbor dysplasia or can-
Fig. 3  a Experience of 65 patients initially achieving CR-IM, showing recurrence of intestinal metaplasia and subsequent remission: Of 65 patients achieving CR-IM, 18 had recurrent intestinal metaplasia with nine of those re-achieving CR-IM.  

b Experience of 65 patients initially achieving CR-IM, showing recurrence of dysplasia and subsequent remission: Of 65 patients achieving CR-IM, six had recurrent dysplasia with five of those re-achieving CR-O and CR-IM.

Fig. 4  Durability of CR-IM over time.

<table>
<thead>
<tr>
<th>Patients with relapse of CR-IM</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>360</th>
<th>480</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without relapse of CR-IM</td>
<td>65</td>
<td>43</td>
<td>38</td>
<td>30</td>
<td>25</td>
<td>21</td>
<td>20</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

| Patient ID number with recurrent intestinal metaplasia/dysplasia\(^1\) after initial CR-IM | Months from initial CR-IM to when relapse intestinal metaplasia/dysplasia documented | Pretreatment histology | Type of recurrence | Location | Local/diffuse | Buried (yes/no) | Further treatment | Subsequent CR-D | Where CR-D re-achieved, months now in CR-D | Subsequent CR-IM | Where CR-IM re-achieved, months now in CR-IM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 2.0 | HGD | Intestinal metaplasia | GE | 3/4 biopsies | No | APC | N/A | N/A | N/A | Yes | 15 |
| 164 | 3.1 | HGD | Intestinal metaplasia | GE | Local | Yes | N/A | N/A | N/A | N/A | No | 0 |
| 23 | 3.2 | HGD | Intestinal metaplasia | Above GE | 2 biopsies | Yes | APC | N/A | N/A | N/A | Yes | 12.8 |
| 53 | 5.5 | HGD | Intestinal metaplasia | GE | Local | No | APC | N/A | N/A | N/A | Yes | 12.8 |
| 44 | 5.5 | HGD | Intestinal metaplasia | GE | Local | No | Surveillance | N/A | N/A | N/A | Yes | 0 |
| 38\(^1\) | 5.6 | IMC | HGD | GE | Local | No | EMR x 2 | No | N/A | N/A | No | 0 |
| 13 | 5.7 | HGD | Intestinal metaplasia | Above GE | Local | No | APC | N/A | N/A | N/A | No | 0 |
| 95 | 6.1 | HGD | Intestinal metaplasia | GE | Local | No | N/A | N/A | N/A | N/A | No | 0 |
| 69 | 6.7 | IMC | Intestinal metaplasia | GE | Local | No | Surveillance | N/A | N/A | N/A | No | 0 |
| 3699 | 7.7 | HGD | Intestinal metaplasia | GE | Local | No | N/A | N/A | N/A | N/A | No | 0 |
| 15\(^1\) \(15.7\) | 9.1/38.7 | IMC | Intestinal metaplasia initially; HGD subsequently | GE | 3 biopsy/focal | No | EMR + RFA | Yes | 0 | Yes | 0 |
| 14\(^1\) | 10.6 | HGD | LGD | GE | Local | No | No | Yes | 25.4 | Yes | 0 |
| 9\(^1\) | 11.0 | HGD | HGD | GE | Local | No | No | Yes | 15 | Yes | 15 |
| 16\(^1\) | 12.0 | HGD | LGD | N/A | N/A | No | No | Yes | 17.4 | Yes | 17.4 |
| 24 | 12.0 | HGD | Intestinal metaplasia | GE | Local | No | APC | N/A | N/A | N/A | Yes | 0 |
| 12 | 17.1 | HGD | Intestinal metaplasia | GE | Local | No | Surveillance | N/A | N/A | N/A | No | 0 |
| 5 | 19.5 | HGD | Intestinal metaplasia | Above GE | 2 biopsies | No | RFA | N/A | N/A | N/A | No | 0 |
| 15\(^1\) | 22.1 | LGD | LGD | GE | Local | No | No | Yes | 38.9 | Yes | 38.9 |

APC, argon plasma coagulation; CR-IM, complete remission of intestinal metaplasia; CR-D, complete remission of dysplasia; EMR, endoscopic mucosal resection; GE, gastroesophageal junction; HGD, high grade dysplasia; IMC, intramucosal cancer; LGD, low grade dysplasia; RFA, radiofrequency ablation.

\(^1\) Patients with recurrent dysplasia in addition to recurrent intestinal metaplasia.

\(^2\) This patient had recurrent intestinal metaplasia in > 1 biopsy at 9.1 months following initial CR-IM and recurrent focal high grade dysplasia at 38.7 months.
Table 3: Adverse events for RFA and EMR. Section a outlines the adverse event rate for RFA procedures and Section b outlines the adverse event rate for EMR procedures. Section c outlines the adverse event rate for RFA treatment (i.e., had combined endoscopic therapy). Section c outlines the EMR adverse event rate for the entire referral cohort, whether they had EMR for staging, sole treatment or as part of combination endoscopic therapy and thus includes patients from Sections a and b.

<table>
<thead>
<tr>
<th>Number</th>
<th>Type of adverse events</th>
<th>Number</th>
<th>%</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>a: RFA complications of the treatment cohort (RFA + EMR)</td>
<td>Patients undergoing RFA 137</td>
<td>12 (4.5%)</td>
<td>2</td>
<td>1.3%</td>
</tr>
<tr>
<td></td>
<td>Total RFA Procedures 305</td>
<td>2</td>
<td>0.6%</td>
<td>Required transfusion and repeat endoscopic procedure one in setting of revascularization</td>
</tr>
<tr>
<td></td>
<td>Procedures per patient Median 2 (1-7)</td>
<td>3</td>
<td>0.9%</td>
<td>Admitted for observation</td>
</tr>
<tr>
<td></td>
<td>Total adverse events</td>
<td>4</td>
<td>1.3%</td>
<td>2 requiring ≥2 dilations</td>
</tr>
<tr>
<td></td>
<td>Bleeding - requiring transfusion</td>
<td>2</td>
<td>0.6%</td>
<td>2 requiring single dilation</td>
</tr>
<tr>
<td></td>
<td>Bleeding - admitted for observation</td>
<td>1</td>
<td>0.3%</td>
<td>Admitted for observation</td>
</tr>
<tr>
<td>b: EMR complications occurring within the treatment cohort only (EMR + RFA)</td>
<td>Patients 78</td>
<td>1</td>
<td>0.8%</td>
<td>EMR of IMC at GE</td>
</tr>
<tr>
<td></td>
<td>Procedures 122</td>
<td>2</td>
<td>1.6%</td>
<td>Secondary to Mallory Weis tear</td>
</tr>
<tr>
<td></td>
<td>Procedures per patient Median 1 (1-8)</td>
<td>3</td>
<td>2.5%</td>
<td>Admitted overnight</td>
</tr>
<tr>
<td></td>
<td>Adverse events</td>
<td>4</td>
<td>1.3%</td>
<td>Requiring ≥2 dilations</td>
</tr>
<tr>
<td></td>
<td>Bleeding - requiring transfusion</td>
<td>1</td>
<td>0.8%</td>
<td>Admitted for observation</td>
</tr>
<tr>
<td>c: EMR complications occurring in the entire referral cohort (EMR at any time point)</td>
<td>Patients 140</td>
<td>1</td>
<td>0.5%</td>
<td>EMR of IMC at GE</td>
</tr>
<tr>
<td></td>
<td>Procedures 215</td>
<td>4</td>
<td>1.8%</td>
<td>Clipped</td>
</tr>
<tr>
<td></td>
<td>Procedures per patient Median 1 (1-8)</td>
<td>2</td>
<td>0.5%</td>
<td>Perforation excluded</td>
</tr>
<tr>
<td></td>
<td>Adverse events</td>
<td>8</td>
<td>3.7%</td>
<td>Secondary to Mallory Weis tear</td>
</tr>
<tr>
<td></td>
<td>Bleeding - requiring transfusion</td>
<td>1</td>
<td>1.4%</td>
<td>Admitted overnight</td>
</tr>
<tr>
<td></td>
<td>Bleeding - admitted for observation</td>
<td>2</td>
<td>0.9%</td>
<td>Requiring ≥2 dilations</td>
</tr>
<tr>
<td></td>
<td>Bleeding - admitted for observation</td>
<td>1</td>
<td>0.3%</td>
<td>Single dilation</td>
</tr>
<tr>
<td></td>
<td>Bleeding - admitted for observation</td>
<td>2</td>
<td>0.9%</td>
<td>Admitted for observation</td>
</tr>
</tbody>
</table>

EMR, endoscopic mucosal resection; GE, gastroesophageal junction; IMC, intramucosal cancer; RFA, radiofrequency ablation.

cer. This is supported by findings published on a subset of our cohort that found that Barrett’s assessment in specialized Barrett’s units resulted in improved detection of mucosal abnormalities and EAC [22].

Durability
Our data show that, in patients achieving CR-IM, there was a significant risk of recurrent intestinal metaplasia (28%) and dysplasia (9%). These documenting recurrence before 12 (and even 6) months (Fig. 3a), raise the possibility of the initial CR-IM diagnosis resulting from biopsy sampling error. Intestinal metaplasia and dysplasia recurrence rates in patients with at least 12 months in remission (possibly better representatives of true remission) were still 19% and 12%, respectively. These figures may be underestimated as a small number of patients achieving CR-IM were referred back to their original endoscopist for surveillance (as outlined in the Methods section). Based on receipt of patients’ subsequent endoscopy/histology reports, we believe that protocol was well adhered to outside the tertiary center and that potential classification bias was minimized.

There is currently inconsistency in the literature with regard to intestinal metaplasia recurrence, partly due to lack of consensus in its definition. A US Multicenter Consortium, whose definition included isolated intestinal metaplasia of the cardia[GE], found that, among patients achieving CR-IM by 24 months, 26% developed recurrent intestinal metaplasia within a year, and 33% after 2 years. Intestinal metaplasia of the cardia[GE] accounted for almost half of the recurrences reported [23]. Korst et al described recurrent intestinal metaplasia in 26% of 53 patients treated successfully with RFA. None relapsed with dysplasia though this
cohort consisted of predominantly non-dysplastic Barrett’s esophagus [12]. Vaccaro et al. found 26% cumulative intestinal metaplasia recurrence in 47 patients treated successfully with RFA [13]. This group had predominantly HGD pre-treatment, though it included non-dysplastic Barrett’s esophagus and IMC. Four (9%) had recurrent dysplasia at the neo-squamocolumnar junction; none were detected endoscopically. In Pibus et al.’s cohort, biopsies were obtained from the GE (median 20) which was focally ablated at least once [11]. Recurrent focal intestinal metaplasia was found in 19/54 patients (35%), however, this finding was not reproduced at subsequent endoscopies in 17/19 patients (median of five follow-up endoscopies), with no increase in intestinal metaplasia incidence at the GE over time [11]. In contrast, where intestinal metaplasia at the GE was not considered recurrence, reported recurrence rates were much lower. Of 139 patients achieving CR-IM in a UK study, only 9% had recurrent intestinal metaplasia by the end of follow-up; 47% of those, however, had recurrent dysplasia [24]. Whether intestinal metaplasia at the GE represents true recurrence, persistent intestinal metaplasia in an area not initially ablated, or cardiac intestinal metaplasia is uncertain.

Our intestinal metaplasia recurrence rates are in line with those studies routinely obtaining biopsies at the GE, as we did [11, 13, 23]. All patients with recurrent dysplasia/intestinal metaplasia had HGD or IMC as their most advanced pre-treatment histology. None recurred with a more advanced histology than previously documented, and importantly, none with recurrence developed cancer. Recurrences were predominantly focal, and were located at the GE in 72% with recurrent intestinal metaplasia and 82% with recurrent dysplasia. There was no statistically significant difference in recurrence rates between those who received HALO50 at the GE, and those who received HALO300 alone, however, the sample size of the latter group was small. The authors recognize the lack of focal ablation of the z-line with HALO50 in a few patients may have impacted overall recurrence rates and advocate focal GE ablation in all patients where possible. All with recurrence were able to be managed endoscopically with the majority re-achieving CR-D/CR-IM.

The risk for intestinal metaplasia progression to dysplasia/neoplasia at the GE is also unclear. Our data show that where dysplasia recurred, it did so at the GE in 83%, consistent with the finding of Vaccaro et al. [13]. Given that intestinal metaplasia precedes dysplasia, it follows that intestinal metaplasia at the GE, after RFA eradication of Barrett’s esophagus, is not necessarily benign. Like Vaccaro et al., our data indicate that, following successful ablation of dysplastic Barrett’s esophagus, the GE should be considered an area at risk for the development of dysplasia and potentially adenocarcinoma in high risk groups. It would suggest that the finding of intestinal metaplasia at the GE should not be excluded from reports of recurrence rates and durability.

Some early RFA cost analyses models incorporated the assumption that patients in whom dysplastic Barrett’s esophagus was eradicated with RFA did not require long-term surveillance [25]. Later models are including evidence such as ours that the potential for intestinal metaplasia and dysplasia recurrence may necessitate ongoing surveillance, particularly in high risk groups [26]. Further dedicated studies are required assessing the significance of intestinal metaplasia at the GE post-ablation.

Limitations of study
This study was a retrospective analysis of the outcomes of combined endoscopic treatment in a real-world cohort of Barrett’s patients. As previously mentioned, the cohort had predominantly advanced disease, with often complex segments, and several had received prior endoscopic treatment before their referral to a tertiary center. Decisions with regard to individual patient treatment were made at the discretion of the endoscopist, and while following recommended guidelines, adjustments to treatment were made when deemed clinically appropriate. These factors would have impacted CR-IM and CR-D rates. Additionally, the lack of focal ablation of the z-line with HALO50 in a few patients may have impacted overall recurrence rates and the authors advocate focal GE ablation in all patients where possible.

Finally, this study reports the preliminary data set of a cohort that is being followed long term. At the time of completion, there were only 16 patients who had reached at least 3 years of follow-up since first RFA and only 26 with at least 12 months follow-up since achieving the end point of CR-IM. The latter was in part due to some patients returning to their referring endoscopists for surveillance following CR-IM, again, a familiar scenario in real-world practice. We aim to reassess our durability outcomes in future analyses with a greater number of patients with long-term follow-up and additionally aim to recapture data for those patients previously returned to their referring specialist for surveillance.

Conclusion
• RFA, combined with EMR in select patients, is effective, safe, and durable for the majority of patients with dysplastic Barrett’s esophagus including those with complex disease. Dysplasia and intestinal metaplasia recurrence appears to occur more commonly in patients with advanced pre-treatment histology, and frequently at the GE. This suggests that recurrent intestinal metaplasia at the GE may not be a benign finding and that ongoing careful surveillance of this region should be considered in patients with previous advanced dysplasia.

Appendix 1
• Prague Classification [16]

A circumferential segment of Barrett’s esophagus of 2 cm in length from the top of the mucosal folds and tongues of Barrett’s metaplasia extending an additional 3 cm superior would be classified as C2M5.

• Circumferential (C) length of Barrett’s esophagus

• Maximal (M) extent of any Barrett’s metaplasia.
Appendix 2

Paris classification of mucosal lesions [17].

<table>
<thead>
<tr>
<th>Endoscopic appearance</th>
<th>Paris classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protruded lesions</td>
<td>15</td>
<td>Nodule, sessile polyp</td>
</tr>
<tr>
<td>Flat elevated lesions</td>
<td>02A</td>
<td>Flat elevation of mucosa</td>
</tr>
<tr>
<td></td>
<td>02A+C</td>
<td>Flat elevation of mucosa with central depression</td>
</tr>
<tr>
<td>Flat lesions</td>
<td>02B</td>
<td>Flat mucosal change</td>
</tr>
<tr>
<td></td>
<td>02C</td>
<td>Mucosal depression</td>
</tr>
<tr>
<td></td>
<td>02C+A</td>
<td>Mucosal depression with raised edge</td>
</tr>
</tbody>
</table>

Appendix 3

Seattle Protocol [27]

Random 4-quadrant biopsies taken at 1- to 2-cm intervals along the Barrett's segment using jumbo biopsy forceps.

Competing Interests: None

References

Chapter 6: Summary and Future Directions

This thesis aimed to further investigate several key areas in Barrett’s Oesophagus (BE) research where the current data is not conclusive or in its infancy. This included further characterising the risk of malignant potential for patients with low grade dysplasia (LGD), the importance of appropriate assessment prior to embarking on a definitive treatment course, and durability of outcomes following combined endoscopic therapy as applied in a real world setting.

6.1 Low Grade Dysplasia Study

The risk of malignant progression from Barrett’s oesophagus to oesophageal cancer is primarily influenced by the degree of dysplasia present within the segment. While some early studies suggested the risk of progression from LGD was low, more recent studies had shown that when there is an expert consensus diagnosis of LGD the risk of malignant progression is significantly higher than if expert pathologists disagree on the diagnosis or downgrade the degree of dysplasia.

I aimed to confirm this finding and additionally, hypothesised that patients’ risk for progression may be better predicted if LGD was further categorised as mild or moderate dysplasia, given degrees of dysplasia occur on a histological continuum with arbitrary cut-offs between low grade and high grade dysplasia (HGD). After identifying a cohort of 116 patients diagnosed with LGD over ten years ago, two expert GI pathologists separately reviewed the initial LGD histology in addition to subsequent episodes of dysplasia. For our cohort I found that while it was true that a consensus diagnosis of LGD did confer a higher risk of malignant progression it was a diagnosis of moderate dysplasia that was primarily responsible for this increase. This held true at the individual pathologist level also, prior to a consensus diagnosis being reached.

The findings from this study suggest that in addition to BE patients with LGD undergoing expert histological review, consideration should be given to further risk stratifying this group as having mild or moderate dysplasia, as this may influence a clinician’s decision to heighten surveillance or commence endoscopic therapy. This may be particularly relevant in cases where a second pathologist opinion is not readily available. In our study, the overall numbers with a consensus diagnosis of LGD (and particularly moderate LGD) were low and in the
future, further studies with larger cohorts of patients with consensus LGD, categorised as mild or moderate, are required to support our findings. This work has already commenced at the St Vincent’s Hospital Barrett’s Unit in Melbourne. The new study will identify current patients with confirmed HGD or EAC in whom a prior diagnosis of LGD has been made over year prior and again will have two expert GI pathologists reviewing this histology according to a two tier and three tier system. Results will be compared to a control group in whom LGD has not progressed to HGD or EAC. If our findings are supported by this study and future studies, then further characterising LGD as mild or moderate may be a simple addition to a histology report that may alert the clinician to a patient’s higher risk for progression and need for closer surveillance or consideration of endoscopic therapy.

6.2 Barrett’s Assessment Study

Combined endoscopic therapy with endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA) has been shown to be an effective alternative to oesophagectomy, with less morbidity and mortality, in eradicating HGD and even intramucosal cancer (IMC) in this group of patients. This only holds true if more advanced neoplastic lesions have been definitively excluded. While best practice suggests adequate mucosal inspection, use of high definition endoscopic equipment, adequate tissue sampling and mucosal resection of lesions suspicious for harbouring neoplasia, the literature suggests that many community endoscopists do not, or are unable to, adhere to best practice guidelines.

I hypothesised that assessment of patients with dysplastic Barrett’s at a Specialised Barrett’s unit (SBU) would result in improved detection of mucosal abnormalities and cancers. We found this to be true with a significantly higher number of mucosal lesions detected at our SBU and a 56% increase in cancer detection. This had significant clinical implications with several of the patients with cancer referred for chemoradiotherapy or surgery as curative endoscopic therapy was considered unlikely.

These data support the use of RFA only after detailed assessment and preferably in a BE unit as patient management may be suboptimal if RFA is delivered outside of expert BE centres. This study suggests that education programs for endoscopists wishing to manage dysplastic Barrett’s should include methods for careful assessment, use of NBI, identification of subtle lesions, and performance of oesophageal EMR. Further studies evaluating the impact of such
educational programs would be required to assess whether this translated to increased
detection of dysplasia/neoplasia in the community setting, and improved clinical outcomes
for the patients affected. Additional research into whether further training of community
endoscopists or pooling resources into tertiary Barrett’s Units is the more cost effective
approach.

6.3 Safety, Effectiveness and Durability of Endoscopic Treatment for Dysplastic
Barrett’s Oesophagus as Applied in a Real World Setting

Much has been published in the literature about the use of radiofrequency ablation in the
eradication of dysplastic Barrett’s oesophagus and it is now generally accepted as the
preferred method of ablation for flat dysplasia.393 There are fewer published studies on its
application in real world cohorts and additionally, on the durability of outcomes in such
groups. Studies on durability continue to vary significantly in their reported recurrence rates
of intestinal metaplasia and dysplasia.455, 487, 489

In this study I assessed the safety, effectiveness and initial durability outcomes of combined
endoscopic therapy (radiofrequency ablation and endoscopic mucosal resection) in one of
Australia’s largest cohorts of patients with dysplastic Barrett’s oesophagus. This group had
predominantly advanced histopathology and often complex Barrett’s segments. I found that
in this real world cohort, the effectiveness and safety profile are similar to published data but
that there was a significant risk of recurrence of both intestinal metaplasia and dysplasia. We
demonstrated that recurrence appeared to occur more commonly in patients with advanced
pre-treatment histology, and frequently at the gastroesophageal junction. We showed that
where dysplasia recurred it was usually detected early, never at a more advanced stage as
the prior most advanced histology, and was endoscopically manageable. These findings have
important clinical implications for the ongoing careful surveillance of this region, particularly
in patients with prior advanced dysplasia or cancer. As our Barrett’s Unit population continues
to grow in size and we have further durability outcomes, we will continue to follow our
patients’ durability outcomes prospectively and aim to re-analyse our results at the end of
2016, looking to support our current findings. We look with interest as greater numbers of
patients undergo endoscopic therapy for dysplastic BE as to other cohorts’ durability
outcomes and whether there are predictive risk factors for recurrence that may guide our
treatment and surveillance protocols.
## Appendices

### Appendix 1: Abbreviations and Definitions

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>ADC</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>AFI</td>
<td>Autofluorescence Imaging</td>
</tr>
<tr>
<td>ALA</td>
<td>5-aminolevulinic acid</td>
</tr>
<tr>
<td>BE</td>
<td>Barrett’s Oesophagus (esophagus)</td>
</tr>
<tr>
<td>CEM</td>
<td>Confocal Endomicroscopy</td>
</tr>
<tr>
<td>CET</td>
<td>Combined Endoscopic Therapy</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CRD</td>
<td>Complete Remission of Dysplasia</td>
</tr>
<tr>
<td>CRIM</td>
<td>Complete Remission of Intestinal Metaplasia</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised Tomography</td>
</tr>
<tr>
<td>EMR</td>
<td>Endoscopic Mucosal Resection</td>
</tr>
<tr>
<td>ETMI</td>
<td>Endoscopic Trimodal Imaging</td>
</tr>
<tr>
<td>EUS</td>
<td>Endoscopic Ultrasound</td>
</tr>
<tr>
<td>EAC</td>
<td>Oesophageal (esophageal) Adenocarcinoma</td>
</tr>
<tr>
<td>GOJ</td>
<td>Gastroesophageal Junction</td>
</tr>
<tr>
<td>GORD</td>
<td>Gastroesophageal Reflux Disease</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin-stained</td>
</tr>
<tr>
<td>HGD</td>
<td>High Grade Dysplasia</td>
</tr>
<tr>
<td>HD-WLE</td>
<td>High Definition White Light Endoscopy</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>IM</td>
<td>Intestinal Metaplasia</td>
</tr>
<tr>
<td>IMC</td>
<td>Intramucosal Cancer</td>
</tr>
<tr>
<td>IND</td>
<td>Indefinite for Dysplasia</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
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<td>LGD</td>
<td>Low Grade Dysplasia</td>
</tr>
<tr>
<td>MBM</td>
<td>Multiband Mucosectomy</td>
</tr>
<tr>
<td>MIO</td>
<td>Minimally Invasive (Laparoscopic) Oesophagectomy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MPEC</td>
<td>Multipolar Electrocoagulation</td>
</tr>
<tr>
<td>NBI</td>
<td>Narrow Band Imaging</td>
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<td>Non-dysplastic Barrett’s Oesophagus (esophagus)</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
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<td>Photodynamic Therapy</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>QALY</td>
<td>Quality Adjusted Life Year</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Control Trial</td>
</tr>
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<td>Radiofrequency Ablation</td>
</tr>
<tr>
<td>SD-WLE</td>
<td>Standard Definition White Light Endoscopy</td>
</tr>
<tr>
<td>SMC</td>
<td>Submucosal Cancer</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
</tr>
<tr>
<td>VCR</td>
<td>Victorian Cancer Registry</td>
</tr>
</tbody>
</table>
1.2 Barrett’s Oesophagus

British Society of Gastroenterology definition of Barrett’s Oesophagus: an endoscopically apparent area above the oesophagogastric junction that is suggestive of Barrett oesophagus (salmon-coloured mucosa) which is supported by the finding of columnar lined oesophagus on histology.

1.3 Paris Classification of mucosal lesions

<table>
<thead>
<tr>
<th>Endoscopic appearance</th>
<th>Paris classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protruded lesions</td>
<td>1S</td>
<td>Nodule, sessile polyp</td>
</tr>
<tr>
<td>Flat elevated lesions</td>
<td>02A</td>
<td>Flat elevation of mucosa</td>
</tr>
<tr>
<td></td>
<td>02A + C</td>
<td>Flat elevation of mucosa with central depression</td>
</tr>
<tr>
<td>Flat lesions</td>
<td>02B</td>
<td>Flat mucosal change</td>
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<tr>
<td></td>
<td>02C</td>
<td>Mucosal depression</td>
</tr>
<tr>
<td></td>
<td>02C + A</td>
<td>Mucosal depression with raised edge</td>
</tr>
</tbody>
</table>

1.4 Prague Classification – A circumferential segment of Barrett’s esophagus of 2cm in length from the top of the mucosal folds and tongues of Barrett’s metaplasia extending an additional 3cm superior would be classified as C2M5.

- Circumferential (C) length of Barrett’s oesophagus
- Maximal (M) extent of any Barrett’s metaplasia.

1.5 Seattle Protocol – Random 4-quadrant biopsies taken at 1 to 2-cm intervals along the Barrett’s segment using jumbo biopsy forceps
1.6 Vienna Classification for classifying dysplasia

The standardised criteria for each category is as follows:

1. Non-dysplastic Barrett’s oesophagus (NDBE): metaplastic columnar epithelium containing goblet cells with uniform glandular architecture, basally located nuclei with smooth membranes, minimal anisonucleosis, and preserved polarity, normal nuclear/cytoplasmic ratio; greater nuclear alterations and partial mucin depletion were acceptable when associated with evidence of inflammation, erosion, or ulceration.

2. Indefinite for dysplasia (IND): cytologic changes similar to those seen in LGD but with surface maturation or presence of inflammation.

3. Low grade dysplasia (LGD): glandular proliferation and crowding without complex branching, cribriform or villous architecture, hyperchromatic, enlarged nuclei with mild irregularity of nuclear membrane, and nuclear stratification extending to the surface epithelium.

4. High grade dysplasia (HGD): complex cribriform or villous architecture, marked nuclear pleomorphism and irregularity of contour, increased nuclear/cytoplasmic ratio, large and irregular nucleoli, full-thickness nuclear stratification, loss of polarity, and prominent mucin depletion.

5. Oesophageal adenocarcinoma (EAC): malignant cells, singly or in groups, infiltrating beyond the basement membrane, with or without associated stromal desmoplasia. A distinction between intramusosal and submucosal invasive cancer was not performed.

1.7 Three tier system of classification

1. Non-dysplastic BE: As above

2. IND: As above

3. Mild – Features consistent with LGD

4. Moderate – Features of LGD (listed above) with features suspicious for but falling short of HGD.

5. Severe – As for HGD

6. ADC: As above
## Appendix 2: Sample from master list of patient outcomes for Chapter 3

| Labno  | Name   | Patient | Sex | Age first in DB | Tcode | Mcode | Summary                        | Worst Histol in | Focal/Multi focal | Number of | First LGD | Incident or | Worst Follow | Date of worst | Last histol | Last Follow | Total F/U (years) | Total Gas Episodes |
|--------|--------|---------|-----|-----------------|-------|-------|--------------------------------|-----------------|-------------------|------------|-----------|-------------|--------------|--------------|-------------|-------------|--------------|-------------------|-------------------|
| 2001000001 | Patient A | 10111 | M  | 75.05 | 62  | 42100 | 1. Biopsy of Oesophagus       | LGD             | 4                  |            | 2001 P    | LGD         | 2003         | LGD          | 2003         | 2.18         | 3            |
| 2002100005 | Patient A | 10112 | M  | 76.12 | 62  | 74000 | Oesophagus a x all in (several) | LGD             | 4                  |            | 2001 P    | LGD         | 2003         | LGD          | 2003         | 2.18         | 3            |
| 2003000005 | Patient A | 10113 | M  | 77.23 | 62  | 73330 | Oesophagus a x all in (several) | LGD             | 4                  |            | 2001 P    | LGD         | 2003         | LGD          | 2003         | 2.18         | 3            |
| 1997600009 | Patient B | 10211 | M  | 78.2  | 62  | 43000 | 40006 | Oesophagus LGD                | 1997 P          | HGD         | 1999 LGD   | LGD          | 1999         | LGD          | 2.74         | 5            |
| 1997600009 | Patient B | 10212 | M  | 78.84 | 62  | 73330 | 69720 | Oesophagus LGD                | 1997 P          | HGD         | 1999 LGD   | LGD          | 1999         | LGD          | 2.74         | 5            |
| 1999600002 | Patient B | 10213 | M  | 79.87 | 62  | 73330 | Oesophagus ND8E                | 1997 P          | HGD         | 1999 LGD   | LGD          | 1999         | LGD          | 2.74         | 5            |
| 1999600009 | Patient B | 10214 | M  | 80.87 | 62  | 73330 | 76008 | Oesophagus HGD                | 1997 P          | HGD         | 1999 LGD   | LGD          | 1999         | LGD          | 2.74         | 5            |
| 1999600007 | Patient B | 10215 | M  | 80.94 | 62  | 73330 | Oesophagus ND8E                | 1997 P          | HGD         | 1999 LGD   | LGD          | 1999         | LGD          | 2.74         | 5            |
| 2008000010 | Patient C | 10311 | M  | 58.96 | 62  | 73330 | Oesophagus LGD                | 2000 P          | LGD         | 2000 IND   | 2005         | 5.05         | 2005         | 5.05         | 7            |
| 2008000037 | Patient C | 10312 | M  | 57.8  | 62  | 73330 | Oesophagus ND8E                | 2000 P          | LGD         | 2000 IND   | 2005         | 5.05         | 2005         | 5.05         | 7            |
| 2009000021 | Patient C | 10313 | M  | 59.85 | 62  | 73330 | Oesophagus ND8E                | 2000 P          | LGD         | 2000 IND   | 2005         | 5.05         | 2005         | 5.05         | 7            |
| 2009000027 | Patient C | 10314 | M  | 61.92 | 62  | 73330 | Oesophagus ND8E                | 2000 P          | LGD         | 2000 IND   | 2005         | 5.05         | 2005         | 5.05         | 7            |
| 2002000019 | Patient D | 10411 | F  | 61.05 | 62  | 73330 | Oesophagus LGD                | 2002 P          | IND         | 2004 ND8E | 2007         | 4.7          | 2007         | 4.7          | 6            |
| 2005000020 | Patient D | 10414 | F  | 63.75 | 62  | 73330 | Oesophagus ND8E                | 2002 P          | IND         | 2004 ND8E | 2007         | 4.7          | 2007         | 4.7          | 6            |
| 2006000019 | Patient D | 10415 | F  | 64.71 | 62  | 73330 | Oesophagus ND8E                | 2002 P          | IND         | 2004 ND8E | 2007         | 4.7          | 2007         | 4.7          | 6            |
| 2007000028 | Patient D | 10416 | F  | 65.75 | 62  | 73330 | Oesophagus ND8E                | 2002 P          | IND         | 2004 ND8E | 2007         | 4.7          | 2007         | 4.7          | 6            |
## Appendix 3: Sample from master list of slide locations

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Labno</th>
<th>Name (known by PI, deidentified for appendix)</th>
<th>Slide Identifier</th>
<th>Worst Histology in episode</th>
<th>Number of slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>20028016947</td>
<td>A</td>
<td>20311</td>
<td>LGD</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>20118020471</td>
<td>B</td>
<td>14119</td>
<td>ADC</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>19968001344</td>
<td>C</td>
<td>17411</td>
<td>NDBE</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>19968002132</td>
<td>D</td>
<td>21611</td>
<td>NDBE</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>19968003248</td>
<td>E</td>
<td>18911</td>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>19968004193</td>
<td>F</td>
<td>13811</td>
<td>NDBE</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>19968005385</td>
<td>G</td>
<td>19111</td>
<td>NDBE</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>19968005572</td>
<td>H</td>
<td>10911</td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>19968005864</td>
<td>I</td>
<td>13812</td>
<td>Mod</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>19968006645</td>
<td>J</td>
<td>16511</td>
<td>NDBE</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>19968007837</td>
<td>K</td>
<td>19011</td>
<td>HGD</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>19978000180</td>
<td>L</td>
<td>18712</td>
<td>LGD</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>19978000395</td>
<td>M</td>
<td>17412</td>
<td>Mild dysplasia</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>19978000976</td>
<td>N</td>
<td>10211</td>
<td>LGD</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>1997802047</td>
<td>O</td>
<td>11211</td>
<td>Mod</td>
<td>2</td>
</tr>
</tbody>
</table>
Appendix 4: Sample from excel spreadsheet for pathologists to enter findings

<table>
<thead>
<tr>
<th>Pathologist</th>
<th>Study number</th>
<th>Labno</th>
<th>No of cases</th>
<th>Side order</th>
<th>Total number of dysplasia</th>
<th>Number of cases with dysplasia</th>
<th>Side number from episode with histology</th>
<th>Side number from episode without histology</th>
<th>Worst Hist in episode (Two-tier system)</th>
<th>Worst Hist in episode (Three-tier system)</th>
<th>Focal or Multifocal</th>
<th>Presence of inflammation (in epithelium)</th>
<th>Presence of inflammation (in lamina propria)</th>
<th>Comment</th>
<th>Two-tier drop down</th>
<th>Three-tier drop down</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Drop down boxes included:**

- Cardia
- Cardia
- Focal
- Yes
- Yes
- Squamous
- Squamous
- Multifocal
- No
- No
- ND BE
- ND BE
- IN D
- IN D
- LG D
- Mild
- LG D
- Moderate
- IM C/AD C
- Severe
- Other
- IM C/AD C
- Other
Appendix 5: Barrett’s Database Information

Appendix 5.1: Homepage and referral endoscopy details

<table>
<thead>
<tr>
<th>Date of referral</th>
<th>Barrett’s Length C (cm)</th>
<th>Barrett’s Length M (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/11/2013</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Scope Findings:
- Comments: The gastro-oesophageal mucosal junction (z-line) was irregular (ZAP grade 2) with a distinct, obvious tongue of columnar appearing epithelium 7 cm in breast. In addition, moderate to severe

- Number of Bx: 10
- Most Advanced Pathology Findings: Barrett’s with early cancer
- Pathology Findings Comments: Esophagus 5.3 cm severe dysplasia/IMC (1/9 Bx)
### Appendix 5.2: Assessment endoscopy details

<table>
<thead>
<tr>
<th>Referral Scope</th>
<th>Assessment Scope at Entry</th>
<th>Pre-Procedure Tests (not EMR)</th>
<th>EMR Procedures</th>
<th>Halo Procedures</th>
<th>Follow Up Gastroscopy Findings</th>
<th>MDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Gastroscopy</td>
<td>15/11/2013</td>
<td>Barrett's Length C (cm)</td>
<td>1</td>
<td></td>
<td>Is there upstaging</td>
<td></td>
</tr>
<tr>
<td>Stricture</td>
<td>No</td>
<td>Barrett's Length M (cm)</td>
<td>2</td>
<td></td>
<td>Molecular Bx taken</td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td>Yes</td>
<td>Radius (cm)</td>
<td></td>
<td></td>
<td>Picture Hyperlink</td>
<td></td>
</tr>
<tr>
<td>Nodule</td>
<td>No</td>
<td>Hiatus Hernia</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VMA</td>
<td>Yes</td>
<td>Hiatus hernia size (cm)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gastroscopy Findings Comments**: 2cm segment of Barrett's (C1M2) from 35-37cm with 3cm hernia below this. The major lesion is a...

**Most Advanced Pathology Findings**: Barrett's with high grade dysplasia

**Pathology Findings Comments**: 34/36 6-7 EMR - There is inflamed columnar lined mucosa with widespread intestinal metaplasia, in continuity with oesophageal...
### Appendix 5.3: EMR procedures

<table>
<thead>
<tr>
<th>EMR Date</th>
<th>Procedure by</th>
<th>Performed Where</th>
<th>Structure</th>
<th>Ulceration</th>
<th>XN</th>
<th>VNA</th>
<th>Number of lesions</th>
<th>Method</th>
<th>Pieces removed</th>
<th>Sedation Type</th>
<th>Gastroscopy findings</th>
<th>Pathology result</th>
<th>Pathology Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/05/13</td>
<td>Dr Georgia Cameron</td>
<td>St Vincent’s Hospital</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>5</td>
<td>Duet - Band and Share</td>
<td>Sedation</td>
<td>Diaphragm 42, top of folds 30cm. Barrett’s C1M4.</td>
<td>Mucosal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14/06/13</td>
<td>Dr Georgia Cameron</td>
<td>St Vincent’s Hospital</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>3</td>
<td>Duet - Band and Share</td>
<td>Sedation</td>
<td>Diaphragm 42, top of folds 30cm. EMR site today 11-0</td>
<td>Barrett’s with low grade dysplasia</td>
<td>Barret’s with low grade dysplasia</td>
<td></td>
</tr>
<tr>
<td>22/12/14</td>
<td>Dr P Mahinda</td>
<td>St Vincent’s Hospital</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>Duet - Band and Share</td>
<td>Sedation</td>
<td>Top of gastric folds is at 38cm, with 2cm hiatus</td>
<td>Barrett’s with low grade dysplasia</td>
<td>EMR 37/39 - 5/9. Sections of the EMR specimen examined at</td>
<td></td>
</tr>
</tbody>
</table>

### Appendix 5.4: RFA procedures

<table>
<thead>
<tr>
<th>Ref No.</th>
<th>Date</th>
<th>Procedure by</th>
<th>Performed Where</th>
<th>Type of Catheter (used)</th>
<th>Catheter Balloon Diameter (mm)</th>
<th>Number of overlapping stapes</th>
<th>Sedation Type</th>
<th>Gastroscopy findings</th>
<th>Pathology result</th>
<th>Pathology Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8/01/13</td>
<td>Dr Georgia Cameron</td>
<td>St Vincent’s Hospital</td>
<td>366</td>
<td>4/6 with intubation</td>
<td>10</td>
<td>Sedation</td>
<td>Diaphragm is at 42cm. Top of folds at 38cm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14/12/13</td>
<td>Dr Georgia Cameron</td>
<td>St Vincent’s Hospital</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Appendix 5.5: Pre-procedure Tests

<table>
<thead>
<tr>
<th>Date of Procedure</th>
<th>Name of Procedure</th>
<th>Result</th>
<th>EMR Procedures</th>
<th>Halo Procedures</th>
<th>Follow Up Gastroscopy Findings</th>
<th>MDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/05/13</td>
<td>CT</td>
<td>Abnormal</td>
<td></td>
<td></td>
<td>Few subcentimeter LNs on left of mediastinum</td>
<td></td>
</tr>
<tr>
<td>12/06/13</td>
<td>PET Scan</td>
<td>Normal</td>
<td></td>
<td></td>
<td>No PET/CT evidence of disease.</td>
<td></td>
</tr>
<tr>
<td>07/08/13</td>
<td>Endoscopic Ultrasound</td>
<td>Normal</td>
<td></td>
<td></td>
<td>No nodes</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 6: Letter to referring Doctors (Chapter 4)

Dear Doctor,

Thank you for involving us in the care of your patient ...............................................

We are currently writing up our experience of assessing treating over 150 patients with dysplastic Barrett’s oesophagus. Part of this assessment involves looking at any impact of the mapping protocol and use of NBI and confocal endomicroscopy compared to standard evaluation prior to referral. We have your endoscopy report but would be very grateful if you could provide us with the following additional information regarding the endoscopy that was performed on ...............................................

1. Code of the endoscope used (e.g. GIF-H180 2909502, Olympus H180 etc.) to ascertain whether high definition or standard definition gastroscope was used.

2. Anaesthetic received (General, sedation with propofol, sedation without propofol)

If you could please fill in the bottom of this page and return via fax to the Department of Gastroenterology we would be most grateful.

Many thanks for your assistance.

Yours sincerely,

Dr Georgina Cameron
Endoscopy Fellow
St Vincent’s Hospital
9288 2211
Fax: 9288 3590
Mobile 0407091987
Royal Melbourne Hospital

Dr Andrew Taylor
Gastroenterologist
St Vincent’s Hospital

Dr Chatura Jayasekera
Gastroenterologist
Royal Melbourne Hospital

Gastroscope Type

1. Endoscope Code (if known):
2. or if not known, circle either:
   a. High Definition endoscope/ Standard Definition endoscope

Anaesthetic Type

1. Anaesthetist: Yes/No
2. General/Sedation
3. If sedation: with propofol/without propofol
Appendix 7: Sample of description of lesions found at assessment endoscopy (Chapter 4)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date of assessment</th>
<th>Duration of procedure</th>
<th>Lesion at assessment</th>
<th>Number Size of lesions [mm]</th>
<th>Location of lesion [cm]</th>
<th>In relation to Barrett's (G01, lower, mid, or top)</th>
<th>Left wall</th>
<th>Right wall</th>
<th>Overlap</th>
<th>DESCRIPTION</th>
<th>Parts classification (flat, irregular, normal)</th>
<th>Mucosal pattern</th>
<th>Presence of inflammation</th>
<th>Pathology after full assessment scope (NB: +/- EMR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19/07/2010</td>
<td>Y</td>
<td>G01</td>
<td>5</td>
<td>5</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Y</td>
<td>VMA</td>
<td>02B mild loss</td>
<td>Y</td>
<td>Slight irregular v G01</td>
<td>IMC</td>
</tr>
<tr>
<td>B</td>
<td>19/07/2010</td>
<td>Y</td>
<td>Lower</td>
<td>25; 21-34 cm</td>
<td>Lower</td>
<td>Yes</td>
<td>Y</td>
<td>No</td>
<td>Y</td>
<td>FLAT NODULAR (FARIS 02c)</td>
<td>02C irregular Y</td>
<td>Y</td>
<td>Abnormal looking mucosa IMC</td>
<td>SMC</td>
</tr>
<tr>
<td>C</td>
<td>27/05/2009</td>
<td>nY</td>
<td>Top</td>
<td>22; 25-27 cm</td>
<td>Top</td>
<td>Yes</td>
<td>Y</td>
<td>No</td>
<td>Y</td>
<td>NODULE</td>
<td>02A Crohn's disease Y</td>
<td>N</td>
<td>Barrett's from 22-25 cm</td>
<td>SMC</td>
</tr>
<tr>
<td>D</td>
<td>15/08/2011</td>
<td>B5 Y</td>
<td>G01</td>
<td>5</td>
<td>5-8 cm</td>
<td>Yes</td>
<td>Y</td>
<td>No</td>
<td>Y</td>
<td>VMA</td>
<td>02B loss irregular N</td>
<td>N</td>
<td>Slightly irregular G01</td>
<td>IMC</td>
</tr>
<tr>
<td>E</td>
<td>24/05/2010</td>
<td>Y9 Y</td>
<td>Lower</td>
<td>7</td>
<td>5-40 cm</td>
<td>Yes</td>
<td>N</td>
<td>No</td>
<td>Y</td>
<td>RAISED NODULAR</td>
<td>01S loss N</td>
<td>N</td>
<td>5 m raised nodule VMC</td>
<td>IMC</td>
</tr>
<tr>
<td>F</td>
<td>6/12/2009</td>
<td>Y7 Y</td>
<td>Lower</td>
<td>10</td>
<td>5-37 cm</td>
<td>Yes</td>
<td>N</td>
<td>No</td>
<td>Y</td>
<td>NODULE</td>
<td>02A irregular Y</td>
<td>N</td>
<td>10 m area of nodule IM</td>
<td>IM</td>
</tr>
<tr>
<td>G</td>
<td>7/12/2007</td>
<td>55 Y</td>
<td>Lower</td>
<td>10</td>
<td>20-50 cm</td>
<td>Yes</td>
<td>Y</td>
<td>No</td>
<td>Y</td>
<td>NODULE</td>
<td>02A irregular loss N</td>
<td>N</td>
<td>Shallow ulcer with CRGD</td>
<td>IMC</td>
</tr>
<tr>
<td>H</td>
<td>18/07/2011</td>
<td>60 Y</td>
<td>Top</td>
<td>10</td>
<td>20-27 cm</td>
<td>Yes</td>
<td>Y</td>
<td>No</td>
<td>Y</td>
<td>NODULE</td>
<td>01S irregular Y</td>
<td>Y</td>
<td>Raised nodule with CRGD</td>
<td>SMC</td>
</tr>
<tr>
<td>I</td>
<td>13/12/2010</td>
<td>150 Y</td>
<td>Lower</td>
<td>5</td>
<td>5-34 cm</td>
<td>Yes</td>
<td>N</td>
<td>No</td>
<td>Y</td>
<td>CRGD</td>
<td>02A irregular loss N</td>
<td>N</td>
<td>Bumpy mucosa IM</td>
<td>HGD</td>
</tr>
<tr>
<td>J</td>
<td>22/07/2009</td>
<td>sup n/a (late laser EMR)</td>
<td>G01</td>
<td>3</td>
<td>37 cm</td>
<td>Yes</td>
<td>N</td>
<td>No</td>
<td>Y</td>
<td>NODULE</td>
<td>02A irregular Y</td>
<td>N</td>
<td>CRGD</td>
<td>HGD</td>
</tr>
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</table>
Bibliography

Please note that references for the previously published Chapters 4 and 5 are listed at the end of their respective PDF files.


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