THE ROLE OF CAPSULE SPONGE TESTING IN EARLY DETECTION OF BARRETT'S OESOPHAGUS AND OESOPHAGEAL ADENOCARCINOMA

By

Siobhan Chien

BMSc MBChB MRCS (Ed)

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School of Cancer Sciences

College of Medicine, Veterinary & Life Sciences

University of Glasgow

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Abstract

Barrett's oesophagus is the precursor lesion to oesophageal adenocarcinoma (OAC). Barrett's oesophagus can transform through a stepwise series of dysplastic change prior to evolving into invasive OAC. High-quality surveillance programmes permit early diagnosis of dysplasia, at which point treatment can potentially be endoscopic and improve outcomes in a disease with a dismal 5-year prognosis of <20%. Furthermore, the development of population-based screening programmes for this condition could present an additional opportunity to transform clinical outcomes. At present, Barrett's surveillance is performed using upper gastrointestinal (UGI) endoscopy. However, endoscopy services are under significant strain across the country, with demand outstripping capacity on a national level. Furthermore, UGI endoscopy is not a perfect test: the rate of abnormalities missed at endoscopy is not insignificant, highlighting the difficulty in maintaining consistent quality with this investigation. Innovative technologies are undoubtedly required to aid endoscopy services.

Capsule sponge testing is one such novel technique that offers promising results in the detection of Barrett's oesophagus (and potentially OAC) within the clinical trial setting. However, real-world data supporting its use has been lacking to date, and its role in the cohort of patients with an established diagnosis of Barrett's oesophagus has yet to be critiqued in clinical practice. Scotland was the first country to introduce capsule sponge testing into clinical practice in a national pilot programme triggered by the Coronavirus pandemic for patients undergoing Barrett's surveillance, as well as those referred to secondary care with symptomatic reflux. This thesis aims to present the results of this national programme and evaluate the role of capsule sponge testing beyond the trial setting, addressing the hypothesis that capsule sponge testing could be utilised as a safe and effective triage tool to UGI endoscopy in the above populations long-term beyond the pandemic.

Chapter 2 presents the results of the Scottish pilot programme in a national pragmatic implementation study in the context of Barrett's surveillance. Of the 4204 capsule sponge tests performed for Barrett's surveillance, 608 patients

proceeded to UGI endoscopy within 12 months, with 50/608 patients (8.2%) identified as having high grade dysplasia (HGD) or cancer on endoscopic biopsies. 46/50 patients (92.0%) were identified as high-risk on capsule sponge testing, triggering urgent endoscopy. This rose to 100% when insufficient tests were removed from analysis, demonstrating that capsule sponge testing is effectively identifying high-risk patients for dysplasia or cancer that require further investigation with urgent endoscopy.

Chapter 3 then evaluates the impact of delayed Barrett's surveillance on endoscopic pathology yield, as well as determining if the Scottish pilot programme has reduced delays to surveillance. As surveillance delay increased beyond 24 months, patients were significantly more likely to develop dysplasia or malignancy ($p<0.001$). In Year 1 versus Year 2 of the programme, there was a longer median delay to surveillance (9 vs. 5 months; $p<0.001$) and an increased proportion of patients with delayed surveillance (72.6% vs. 57.0%; $p<0.001$), implying that the Scottish capsule sponge programme has reduced delays to usual Barrett's surveillance imposed by the pandemic.

Chapter 4 then assesses the impact of capsule sponge testing on Barrett's dysplasia yield in a single Scottish health board, by comparing the 2-year period pre- and post-implementation of the service, as well as directly comparing endoscopic biopsy results in patients undergoing capsule sponge testing (+/- subsequent endoscopy) versus those undergoing traditional endoscopic surveillance in a single health board. These results demonstrated no significant differences in the rates of HGD, intramucosal cancer (IMC) or invasive cancer diagnosed between the groups, although yield of indefinite for dysplasia (IND) and low grade dysplasia (LGD) cases was higher in the endoscopic surveillance cohort.

Chapter 5 analyses the impact of the introduction of capsule sponge testing on Barrett's surveillance endoscopy services in a single Scottish health board. These results demonstrated that dysplasia or cancer was significantly more likely to be present in endoscopic biopsies after capsule sponge testing was implemented ($p<0.001$). This implies that the introduction of capsule sponge testing has refined the local endoscopy service to focus on those most likely to have pathology, with a concurrent reduction in the proportion of endoscopies

performed for non-dysplastic Barrett's oesophagus only. In addition, 28.0% fewer endoscopies were performed for Barrett's surveillance after capsule sponge testing was introduced.

Chapter 6 then evaluates longer-term follow-up outcomes in the lower risk patients initially undergoing capsule sponge testing for Barrett's surveillance by comparing those who had UGI endoscopy versus repeat capsule sponge testing as their next follow-up. Again, there were no significant differences in the rates of HGD, IMC and OAC between the groups. Traditional endoscopy detected more cases of IND (4.2% vs. 0.9%; p=0.012) and LGD (3.6% vs. 0.9%; p=0.032). Chapter 6 also compares use of the Cytosponge™ versus EndoSign® capsule sponge devices for the first time. The EndoSign® device detected a higher proportion of trefoil factor 3 (TFF3) positive cases compared to Cytosponge™ (72.3% vs. 48.2%; p<0.001), with both devices demonstrating sound reproducibility.

Moving beyond the Barrett's surveillance population, Chapter 7 presents the first real-world results of a national prospective cohort study evaluating the clinical application of capsule sponge testing in symptomatic reflux disease based on endoscopic biopsy results. With insufficient tests excluded, 16.6% of patients undergoing UGI endoscopy were found to have IM on endoscopic biopsies, which strongly correlated with positive biomarkers (88.5% vs. 11.5%; p<0.001), including one case of dysplasia. These results suggest that capsule sponge testing is also an effective triage tool to endoscopy in symptomatic reflux patients when combined with clinical assessment.

Finally, Chapter 8 aims to establish whether combining TFF3 result on capsule sponge testing with markers of systemic inflammation, specifically raised neutrophil-lymphocyte ratio (NLR), can effectively rule out Barrett's oesophagus within the symptomatic reflux population. This study concluded that the combination of TFF3 negativity and normal NLR excluded IM in 99.6% of cases, providing excellent reassurance to clinicians aiming to discharge patients from secondary care and prioritise access to endoscopy resources.

In summary, this thesis has demonstrated positive results for capsule sponge testing in clinical practice on a national level, provisionally supporting its use as a triage tool to endoscopy beyond the pandemic. However, further work focusing

on long-term follow-up of these patients is required before capsule sponge testing can be formally integrated into clinical pathways in the future.

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Author's Declaration

I confirm that all work presented in this thesis is my own, unless stated otherwise. This work has not been submitted for consideration of any other degree, either at the University of Glasgow or any other institution.

The work presented in this thesis was undertaken during a period of research between 2022 and 2024 in the Centre for Sustainable Delivery at the Golden Jubilee National Hospital. The work was completed whilst working as a General Surgery Specialty Registrar in the West of Scotland deanery.

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List of Publications

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British Journal of Surgery (BJS) Prize (3rd place) at ASGBI Congress 2024
"Endoscopic histopathology results in patients undergoing oesophageal cell collection device and biomarker testing for Barrett's oesophagus surveillance"

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"National adoption of an oesophageal cell collection device for Barrett's oesophagus surveillance: impact on delay to investigation and pathological findings"

Best Service Poster (1st place) at BSG Live 2022
"The introduction of Cytosponge to NHS Scotland: initial reports"

List of Abbreviations

ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ANIA	Accelerated National Innovation Adoption
ASGE	American Society for Gastrointestinal Endoscopy
AspECT	Aspirin and Esomeprazole Chemoprevention in Barrett's metaplasia Trial
AUC	Area under the curve
AUS	Atypia of uncertain significance
BCSP	Bowel cancer screening programmes
BEST	Barrett's oEsophagus Screening Trial
BOSS	Barrett's Oesophagus Surveillance Versus Endoscopy at Need Study
BSG	British Society of Gastroenterology
BMI	Body mass index
BMP-4	Bone morphogenetic factor 4
C	Circumferential
CfSD	Centre for Sustainable Delivery
CI	Confidence interval
CM	Centimetre
COVID-19	Coronavirus disease 2019
COX	Cyclooxygenase
CytoSCOT	Cyto Sponge Cytology Oesophageal Test
DELTA	IntegrateD diagnostic solution for EarLy deTection of oesophageal cAncer
DNA	Deoxyribonucleic acid
EET	Endoscopic eradication therapy
EMR	Endoscopic mucosal resection
ESD	Endoscopic submucosal dissection
ESGE	European Society of Gastrointestinal Endoscopy
FBC	Full blood count
FFPE	Formalin-fixed paraffin-embedded
FLOT	5-Fluorouracil, Leucovorin, Oxaliplatin and Docetaxel
GOJ	Gastro-oesophageal junction
GORD	Gastro-oesophageal reflux disease
H&E	Haematoxylin and eosin
HGD	High grade dysplasia
HR	Hazard ratio
IM	Intestinal metaplasia
IMC	Intramucosal adenocarcinoma
IND	Indefinite for dysplasia
IQR	Interquartile range
LGD	Low grade dysplasia
M	Maximal
M-Beret	Michigan Barrett's oEsophagus pREdiction Tool
MCV	Mean corpuscular volume
MDM	Methylated DNA marker
MDT	Multidisciplinary team
NAC	Neoadjuvant chemotherapy
NBI	Narrow band imaging
ND	Negative for neoplasia
NHS	National Health Service

NHSE	National Health Service England
NHS GGC	National Health Service Greater Glasgow & Clyde
NLR	Neutrophil-lymphocyte ratio
NNS	Number needed to scope
NOGCA	National Oesophago-Gastric Cancer Audit
NPV	Negative predictive value
NSAID	Non-steroidal anti-inflammatory drug
OAC	Oesophageal adenocarcinoma
OCCD	Oesophageal cell collection device
OG	Oesophagogastric
OR	Odds ratio
OSCC	Oesophageal squamous cell carcinoma
PBPP	Public Benefit and Privacy Panel
PEUGIC	Post endoscopy upper gastrointestinal cancer
PHS	Public Health Scotland
PLR	Platelet-lymphocyte ratio
PPI	Proton pump inhibitor
PPIE	Patient and public involvement and engagement
PPV	Positive predictive value
qFIT	Quantitative faecal immunochemical test
QoL	Quality of life
RCT	Randomised controlled trial
RFA	Radiofrequency ablation
ROC	Receiver-operator characteristics
SC	Squamocolumnar
SCI	Scottish Care Information
Shh	Sonic hedgehog
SHTG	Scottish Health Technologies Group
SIMD	Scottish Index of Multiple Deprivation
TFF3	Trefoil factor 3
TNE	Transnasal endoscopy
UGI	Upper gastrointestinal
UK	United Kingdom
USA	United States of America
VOC	Volatile organic compound
WATS-3D	Wide-area transepithelial sampling with 3-dimensional computer analysis
WHO	World Health Organization
WLE	White light endoscopy

Chapter 1 Introduction

Barrett's oesophagus is an acquired pre-malignant condition in which columnar epithelium replaces the usual stratified squamous epithelial lining of the distal oesophagus (Barrett, 1950; Jankowski et al., 2010). Barrett's oesophagus is of clinical significance as it is the precursor lesion to oesophageal adenocarcinoma, a deadly disease of rising incidence and prevalence yet abysmal 5-year survival rates of <20% (Arnold et al., 2019). The stepwise progression of Barrett's oesophagus through metaplastic and dysplastic cellular changes has powered clinical interest in this field and provides the impetus for the endoscopic surveillance programme, as early diagnosis of dysplasia or cancer may facilitate endoscopic treatment and thereby improve survival rates (Pech et al., 2008; Pouw et al., 2010). New technologies, such as non-endoscopic oesophageal cell collection devices, have been the focus of research interest in recent years, with capsule sponge testing demonstrating most significant promise in the diagnosis of Barrett's oesophagus within the clinical trial setting (Fitzgerald et al., 2020). This first chapter undertakes a review of the literature surrounding Barrett's oesophagus and the use of novel diagnostic technologies (including capsule sponge testing), highlighting recent advances, current clinical challenges and areas for future study.

1.1 Overview of oesophageal cancer

1.1.1 Global epidemiology

Oesophageal cancer is the eighth most common malignancy worldwide: at present, it is the sixth leading cause of global cancer mortality, resulting in over 500,000 cancer deaths on an annual basis (Bray et al., 2018; Fitzmaurice et al., 2017; Uhlenhopp et al., 2020). The incidence and prevalence of oesophageal cancer have followed a rapid upward trajectory over the past four decades (Hur et al., 2013; Siegel, Miller, & Jemal, 2019). There is global variation in the prevalence and disease burden of oesophageal cancer, however approximately 80% of cases are diagnosed in more deprived countries (Wong et al., 2018). Oesophageal cancer is four times more common amongst the male population (Zhang, 2013) and is most frequently associated with advanced age, with diagnoses uncommon below the age of 40 years (Liu et al., 2023).

1.1.2 Epidemiology in the United Kingdom and Scotland

In the United Kingdom (UK), oesophageal cancer is the fourteenth most common malignancy. Approximately 9,400 new cases of oesophageal cancer were diagnosed in the UK per year between 2017-2019. It is the seventh leading cause of cancer mortality, accounting for 5% of all cancer deaths nationally (Cancer Research UK, 2023). The incidence of oesophageal cancer peaks in the elderly population: the disease is most frequently diagnosed in those over 85-89 years of age, with 41% of all new diagnoses in patients aged 75 years and over (Cancer Research UK, 2023).

These epidemiological patterns are mirrored in Scotland, where oesophageal cancer accounts for 3.0% of all cancer cases and 5.4% of cancer deaths. Although mortality rates have improved over time, the 5-year relative survival rate for patients diagnosed with oesophageal cancer between 2007 and 2011 was just 12.3% in the Scottish population (Public Health Scotland, 2020). Historically, oesophageal cancer rates have exhibited a positive correlation with higher levels of social deprivation in Scotland (McKinney et al., 1995). Oesophageal cancer is irrefutably an important public health issue on both a national and global level due to its high disease-related mortality.

1.1.3 Presentation and prognosis

Despite modern advances in oncological treatments and surgical technique, the prognosis of oesophageal cancer remains dismal, with an overall 5-year survival rate of less than 20%, owing to its propensity for late presentation with advanced disease (Arnold et al., 2019; Domper Arnal, Ferrández Arenas, & Lanas Arbeloa, 2015; Pohl, Sirovich, & Welch, 2010). Patients commonly present with progressive dysphagia, odynophagia, unintentional weight loss or upper gastrointestinal (UGI) bleeding. Additionally, fatigue is common amongst patients due to disease burden or anaemia in the context of chronic occult bleeding. Locally advanced or metastatic disease is frequently present at time of diagnosis, as symptoms of malignant obstruction or stricture emerge late in the disease process due to the expansive muscular nature of the oesophagus (Smyth et al., 2017). Resultantly, the most common stage at presentation is stage IV, at which point treatment options are limited (Then et al., 2020).

Prognosis is intrinsically linked to tumour stage at diagnosis, relative to both depth of tumour invasion and presence of lymph node metastases. Long-term prognosis for oesophageal cancer is substantially more optimistic when the disease is detected early. There is an inverse relationship between survival and tumour stage: 5-year survival rates rise to over 90% when the disease is staged as T1 at diagnosis (Liu et al., 2005), compared to 5-year survival rates of <25% in patients with T4 disease or lymph node metastases (Kim et al., 2011). Early detection is therefore imperative to improve patient outcomes and mortality rates.

1.1.4 Impact of the COVID-19 pandemic on oesophageal cancer survival

The rapid spread of Coronavirus disease 2019 (COVID-19) resulted in the declaration of an international pandemic by the World Health Organization (WHO) in March 2020, eliciting an unprecedented major global health crisis. The COVID-19 pandemic challenged the infrastructure of the National Health Service (NHS) in the UK to an extraordinary degree and saw the temporary cessation of many standard clinical pathways across the country. This included usual endoscopy services, resulting in significant delays to diagnosis (Edwards,

Penman, & Coleman, 2020). This disruption to both elective and emergency healthcare services had a profoundly detrimental effect on cancer diagnosis, treatment and survival (Baxter et al., 2021; The Lancet Oncology, 2020; Watt, Sullivan, & Aggarwal, 2022).

Recent literature has demonstrated this impact on patients with oesophagogastric (OG) cancer in Scotland. A pilot study undertook initial analysis using data from the West of Scotland regional OG cancer multidisciplinary team (MDT): this study demonstrated a decline in new OG cancer referrals with longer time to diagnostic UGI endoscopy as the COVID-19 pandemic evolved. Furthermore, the following trends were also observed after the first national lockdown was implemented: increased metastatic disease at presentation; increased treatment with non-curative rather than curative intent; shorter median survival time (Khan et al., 2022). These initial results triggered the expansion of the study on a national level, which corroborated these findings and clearly demonstrated the adverse impact of the COVID-19 pandemic on patient outcomes in OG cancer across Scotland (Baxter et al., 2023).

1.1.5 Pathology of oesophageal cancer

Oesophageal cancer has two main histological subtypes: oesophageal squamous cell carcinoma (OSCC) and oesophageal adenocarcinoma (OAC) (Pennathur et al., 2013). OSCC and OAC have important distinctions in tumorigenesis dependent on the cancer's cell of origin (Zhang, 2013). Other less frequent types of oesophageal carcinoma include leiomyosarcoma, melanoma, neuroendocrine carcinoma and small cell carcinoma (Enzinger & Mayer, 2003).

1.1.6 Oesophageal squamous cell carcinoma

OSCC accounts for the majority of oesophageal cancer cases globally (Wong et al., 2018). OSCC arises directly from the usual stratified squamous epithelial lining, typically affecting the upper or middle one-third of the oesophagus (Jemal et al., 2010). Associated risk factors for OSCC include cigarette smoking, alcohol consumption, caustic injury, male gender, positive family history, poor oral hygiene and nutritional deficiencies (Eslick, 2009; Pennathur et al., 2013). Definitive chemoradiotherapy (with or without salvage oesophagectomy) remains

the mainstay of treatment for locally advanced OSCC, with novel immunotherapeutic agents utilised as an additional strategy within current OSCC treatment algorithms (Puhr, Prager, & Ilhan-Mutlu, 2023).

1.1.7 Oesophageal adenocarcinoma

In the developed world, there has been a dramatic epidemiological shift, with the prevalence of OSCC greatly surpassed by OAC. OAC accounts for approximately two-thirds of all newly diagnosed oesophageal cancers in the UK (Huang et al., 2021). Indeed, the UK has the highest incidence of OAC reported worldwide (Arnold et al., 2015).

Historically, the incidence of OAC has followed a sharp upward trajectory. Its incidence has risen more rapidly than any other cancer in the United States of America (USA) (Pohl & Welch, 2005). Similar concerning trends had previously been observed in England and Wales (Lepage et al., 2008). However, more recent research from the UK has suggested that the OAC age-standardised incidence rate has slowed over the past three decades and is predicted to remain stable until 2032, as demonstrated in Figure 1.1 (Offman, Pesola, & Sasieni, 2018).

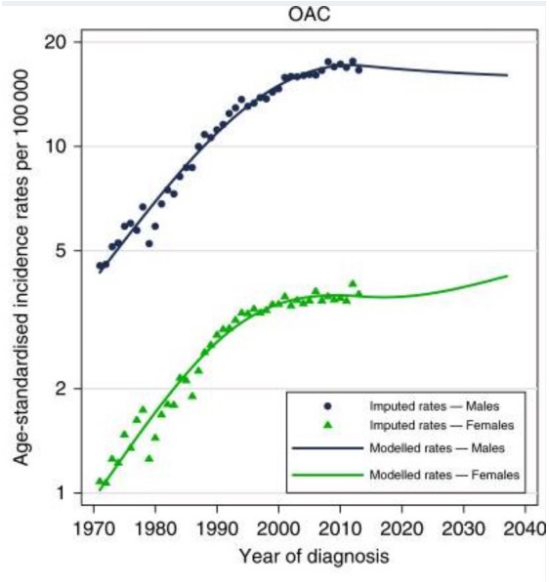

Figure 1.1 Annual and modelled OAC incidence rates for men and women projected until 2037
Figure 1.1 taken from Offman, Pesola & Sasieni, 2018 (Offman, Pesola, & Sasieni, 2018).

OAC is most prevalent amongst the white population, with higher mortality rates in this patient cohort compared to non-white ethnic groups (Baquet et al., 2005; Cook, Chow, & Devesa, 2009). While OAC predominantly affects older patients, its incidence in the younger population is climbing. Recent literature has demonstrated younger patients below the age of 50 years are significantly more likely to present with advanced disease, resulting in inferior 5-year disease-free survival rates compared to older patients (Codipilly et al., 2021).

OAC most frequently occurs in the lower one-third of the oesophagus or within the gastro-oesophageal junction (GOJ) (Jemal et al., 2010). The main risk factors for OAC include gastro-oesophageal reflux disease (GORD), cigarette smoking, obesity, male sex and increasing age (Thrift, 2021). Symptomatic GORD is considered one of the strongest risk factors for OAC, although symptoms may be infrequent or absent in up to 40% of patients (Pennathur et al., 2013). However, of paramount clinical importance, is the precursor lesion to OAC: Barrett's oesophagus (Barrett, 1950; Haggitt et al., 1978).

1.2 Overview of Barrett's oesophagus

1.2.1 Definition of Barrett's oesophagus

Barrett's oesophagus is an acquired pre-malignant condition and is a sequela of reflux oesophagitis (Moersch, Ellis, & McDonald, 1959), affecting 3-15% of all patients with GORD (Dulai et al., 2002; Saha et al., 2024; Westhoff et al., 2005). In addition to chronic exposure to gastric refluxate, male sex, obesity, advancing age, white ethnicity and tobacco use are further risk factors for the development of this disease (Edelstein et al., 2007; Shaheen & Richter, 2009). However, given the majority of patients with reflux oesophagitis do not evolve to Barrett's oesophagus, it is hypothesised that genetic predisposition may also play a vital role (Ek et al., 2013; Gharahkhani et al., 2016). The prevalence of Barrett's oesophagus within the general population is estimated at 1-2% (Eusebi et al., 2021; Marques de Sá et al., 2021; Marques de Sá et al., 2020; Ronkainen et al., 2005), although its exact incidence remains uncertain. Diagnosis can be hindered by the fact that reflux symptoms may be absent in up to 46% of all Barrett's oesophagus patients (Reid et al., 2010).

In Barrett's oesophagus, the normal squamous epithelial lining of the distal oesophagus is damaged by chronic exposure to gastric acid and is replaced by glandular ("columnar") cells (Barrett, 1950; Jankowski et al., 2010). Macroscopically, this is visualised as the replacement of the normal oesophageal squamous cells by salmon-coloured mucosa of a minimum length of 1 centimetre (cm) in a cephalad direction from the GOJ (Fitzgerald et al., 2014; Shaheen et al., 2022).

At present, a universally accepted definition of Barrett's oesophagus remains elusive: there exists heterogeneity in current societal guidelines as to the criteria for diagnosis. In addition to the described visual mucosal changes, the American Society for Gastrointestinal Endoscopy (ASGE), American College of Gastroenterology (ACG) and American Gastroenterological Association (AGA) state that a histopathological diagnosis of intestinal metaplasia (IM) by biopsy of the tubular oesophagus is a requirement for the diagnosis of Barrett's oesophagus (Qumseya et al., 2019; Shaheen et al., 2022; Sharma et al., 2020). This recommendation is also reiterated within the European Society of Gastrointestinal Endoscopy (ESGE) guidelines (Weusten et al., 2023). The evidence base for these guidelines is derived from a large population-based study of 8,522 patients with Barrett's oesophagus from the Northern Ireland Cancer Registry, demonstrating a significantly higher risk of OAC in those with IM at index endoscopy compared to those without IM (0.38% vs. 0.07%/year; hazard ratio [HR] 3.54; 95% confidence interval [CI] 2.09-6.00) (Bhat et al., 2011). Furthermore, analysis of clinical outcomes in a recent UK-based novel cohort study of 244 patients demonstrated that disease progression was significantly higher in patients with IM compared to those without IM ($p=0.03$), with the former cohort also displaying a significantly higher mutational burden on genomic analysis compared to the latter ($p<0.01$) (Black et al., 2024).

However, evidence supporting the association between presence of IM and development of OAC is inconsistent. Similar cancer risks for patients with or without IM were demonstrated in a single-centre UK study of 688 patients (0.37% vs. 0.30%/year) (Kelty et al., 2007), as well as a multi-centre UK study of 1,751 patients (HR 1.36; 95% CI 0.53-2.96) (Gatenby et al., 2008). Furthermore, there was no significant difference in the frequency of deoxyribonucleic acid (DNA) content abnormalities in cells with and without IM (Liu et al., 2009), inferring

that these groups have similar potential to develop neoplasia. Additionally, the distribution of IM within the Barrett's segment may be patchy: sampling error at endoscopic biopsy may lead to a false-negative result. Biopsies may also have been acquired from the proximal stomach or may reflect poor sampling of the Barrett's segment (Harrison et al., 2007; Riddell & Odze, 2009). For this reason, the British Society of Gastroenterology (BSG) guidelines state that the diagnosis of Barrett's oesophagus is not reliant on the presence of IM: metaplastic differentiation to columnar mucosa alone is sufficient for diagnostic purposes (Fitzgerald et al., 2014).

1.2.2 Endoscopic evaluation of Barrett's oesophagus

Diagnosis of Barrett's oesophagus is dual component, requiring both direct endoscopic visualisation of the oesophageal mucosa and histopathological review of obtained oesophageal biopsy samples. In the normal oesophagus, the squamocolumnar (SC) junction (i.e. the confluence of the oesophageal squamous epithelium and gastric columnar epithelium) and GOJ are found at the same level. As previously mentioned, Barrett's oesophagus results in the proximal migration of the SC junction, with the normal white or light pink coloured squamous mucosa replaced by salmon pink coloured mucosa of the columnar epithelium in a caudocranial direction (Figure 1.2) (Fitzgerald et al., 2014; Shaheen et al., 2022). The GOJ is a crucial landmark to confirm the presence of Barrett's oesophagus and is ascertained by the convergence of the distal palisade vessels and proximal gastric folds. However, identification of these landmarks can be challenging at endoscopy: peristalsis, oesophagitis, excessive insufflation and presence of an irregular Z-line can trigger diagnostic uncertainty and introduce potential for inter- and intra-observer variation (Dekel et al., 2003; Kim et al., 1994; Sharma, Morales, & Sampliner, 1998).

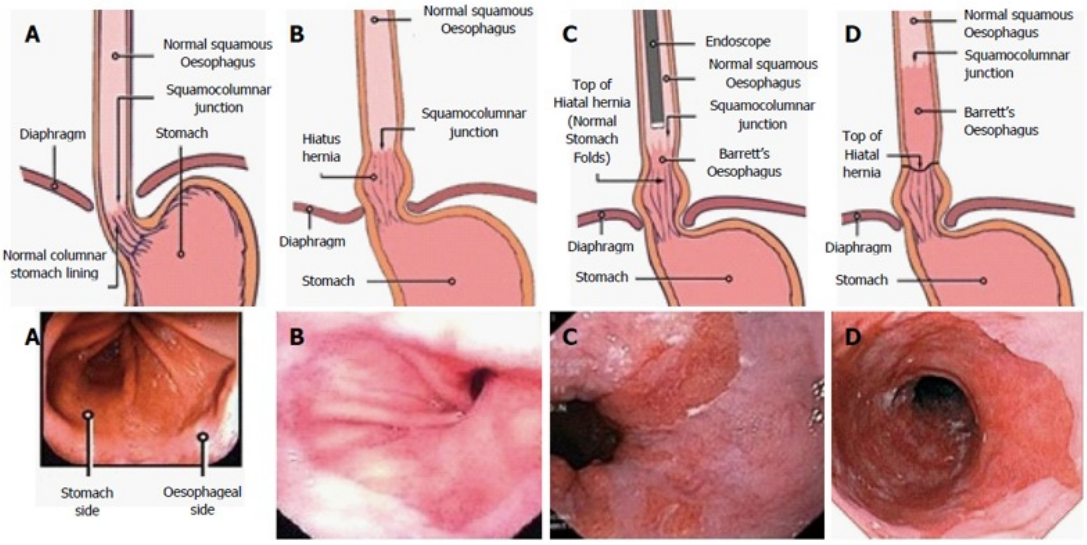

Figure 1.2 Barrett's oesophagus at endoscopy
A) Normal oesophagus and stomach B) Hiatus hernia C) Short segment Barrett's oesophagus D) Long segment Barrett's. Figure 1.2 taken from D Samuel, 2021 (Samuel, 2021).

At present, UGI endoscopy is universally recommended as the gold standard diagnostic test for Barrett's oesophagus. Transnasal endoscopy (TNE) is an alternative for diagnosis: TNE without sedation is less invasive, well-tolerated and demonstrates comparable performance characteristics to standard transoral endoscopy, with a sensitivity of 98% and specificity of 100% (Saeian et al., 2002; Shariff et al., 2012). The current ESGE guidelines advise that TNE is deemed an acceptable alternative technique for Barrett's oesophagus case-finding (Weusten et al., 2023).

The validated Prague classification has been widely adopted to facilitate standardised reporting of disease recognition and extent. The Prague classification is generally recognised as the best method for description of the Barrett's segment: this incorporates measurement of both the circumferential (C) and maximal (M) extent of the columnar epithelium within the oesophagus in cm, utilising the proximal margin of the gastric folds and the diaphragmatic hiatus as anatomical landmarks (Figure 1.3). The Prague classification not only provides standardised terminology, but also exhibits excellent inter-user reliability coefficients for both the C (0.95) and M (0.94) extent of the Barrett's segment (Sharma et al., 2006).

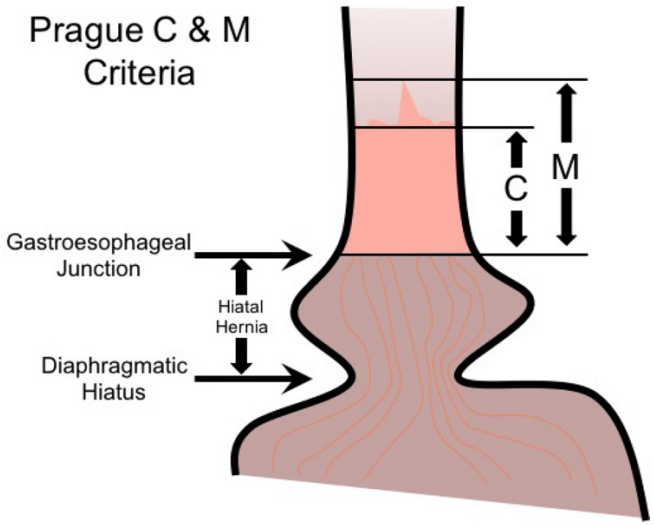

Figure 1.3 Gastro-oesophageal landmarks for Prague classification
Figure 1.3 taken from Triggs & Falk, 2021 (Triggs & Falk, 2021).

Current societal guidelines advise thorough mucosal inspection of the oesophagus using high-resolution white light endoscopy (WLE), with meticulous attention paid to key anatomical landmarks including the SC junction, GOJ and a retroflexed view of the cardia of the stomach (Fitzgerald et al., 2014; Shaheen et al., 2022). Adequate inspection time is critical: a positive correlation exists between longer mucosal inspection time and the ability to detect Barrett's dysplasia or OAC (Gupta et al., 2012). Similarly, the use of high-resolution WLE has established links to improved detection of Barrett's dysplasia and early neoplastic lesions versus standard definition WLE (Kara et al., 2005; Sami et al., 2015; Spechler et al., 2011).

However, despite adherence to best-practice technique with careful white light inspection, subtle lesions can be overlooked. To minimise the risk of "missed" dysplasia, advanced imaging techniques have been developed as adjuncts to high-resolution WLE to guide targeted biopsy of concerning lesions. Chromoendoscopy is one such technique which employs the use of dyes to enrich pathology yield at endoscopy. For example, uptake of methylene blue is observed in columnar epithelial cells, thus facilitating the macroscopic detection of IM and aiding targeted biopsies of the affected area (Breyer et al., 2003; Canto, 1999; Canto et al., 2001; Kiesslich et al., 2001; Sharma et al., 2001). However, there exists conflicting evidence on its efficacy, with a recent meta-analysis failing to demonstrate an incremental yield of IM or dysplasia compared to usual endoscopic techniques (Ngamruengphong, Sharma, & Das, 2009).

Furthermore, it is hypothesised that methylene blue used at such concentrations as required for chromoendoscopy may damage DNA (Davies et al., 2007). Indigo carmine is another such agent which facilitates identification of characteristic mucosal patterns for IM, dysplasia or cancer. However, the use of indigo carmine is limited by the requirement for high magnification to the detriment of narrowing the endoscopic field of view (Sharma et al., 2003). Acetic acid has also demonstrated 96.7% sensitivity and 66.5% specificity for detection of high-grade dysplasia (HGD) or cancer (Pohl et al., 2010): dysplasia yield is also significantly increased compared to use of standard endoscopy with random biopsies (Longcroft-Wheaton et al., 2010). However, data supporting the use of acetic acid have been derived from single-centre studies. The BSG guidelines therefore do not recommend that such techniques are utilised routinely for Barrett's endoscopy at this juncture (Fitzgerald et al., 2014).

To circumvent the use of harmful dyes, recent technological advances have encouraged the pursuit of virtual chromoendoscopy techniques. Narrow band imaging (NBI) is the most well-studied of these techniques, utilising light filters to differentiate subtle differences in oesophageal mucosal pattern and superficial vasculature. Mannath et al. published a meta-analysis including eight studies, concluding that NBI demonstrates 96% sensitivity and 94% specificity for the diagnosis of HGD, in addition to 95% sensitivity and 65% specificity for the diagnosis of IM (Mannath et al., 2010). This technique has also shown an incremental diagnostic yield for dysplasia in three additional studies, although this improvement was only demonstrated in the per-biopsy analysis in two studies (as opposed to per-patient) (Kara et al., 2005; Sharma et al., 2013; Wolfsen et al., 2008). The current literature is fortified by two recent studies demonstrating that NBI can improve detection of early Barrett's neoplasia in both expert and non-expert endoscopist cohorts when compared to high-resolution WLE alone (de Groof et al., 2020; Everson et al., 2019). Whilst clearly a valuable adjunct to usual high-resolution WLE and biopsies, NBI is not a substitute for standard practice: as such, routine use of NBI is yet to be formally incorporated into national guidelines (Fitzgerald et al., 2014; Qumseya et al., 2019; Shaheen et al., 2022). Rapid technological advances have facilitated research into additional virtual chromoendoscopy techniques (such as autofluorescence imaging, endoscopic trimodal imaging, confocal laser

endomicroscopy, spectroscopy and optical coherence tomography), which may shape the future approach to Barrett's endoscopy in coming years (Fitzgerald et al., 2014).

After judicious endoscopic inspection, adherence to a systematic protocol for endoscopic biopsies has been shown to increase pathology yield compared to a random biopsy approach (Abela et al., 2008; Fitzgerald et al., 2001). It is thus recommended that biopsies of the Barrett's segment are taken following the Seattle biopsy protocol: this protocol demands the acquisition of four-quadrant random biopsies every 2cm, in addition to targeted biopsies of macroscopically visible lesions (Fitzgerald et al., 2014; Levine et al., 2000). The ACG guidelines recommend a minimum of 8 biopsies at the time of index endoscopy, with Seattle biopsy protocol followed for those with Barrett's segments >4cm (Shaheen et al., 2022). Although recognised to be time-consuming, costly and associated with variable adherence rates of 10-79% (notably poorer in longer Barrett's segments), the Seattle biopsy protocol is associated with improved detection of early dysplasia (Abrams et al., 2009; Curvers et al., 2008; Das et al., 2008; Fitzgerald et al., 2001; Ramus et al., 2008).

The BSG guidelines mandate that "visible lesions should be considered malignant until proven otherwise", therefore targeted biopsies should be obtained (Fitzgerald et al., 2014). The Paris classification should be used to describe the macroscopic appearance and lesion morphology of mucosal irregularities within the Barrett's segment (Figure 1.4) (Endoscopic Classification Review Group, 2005).

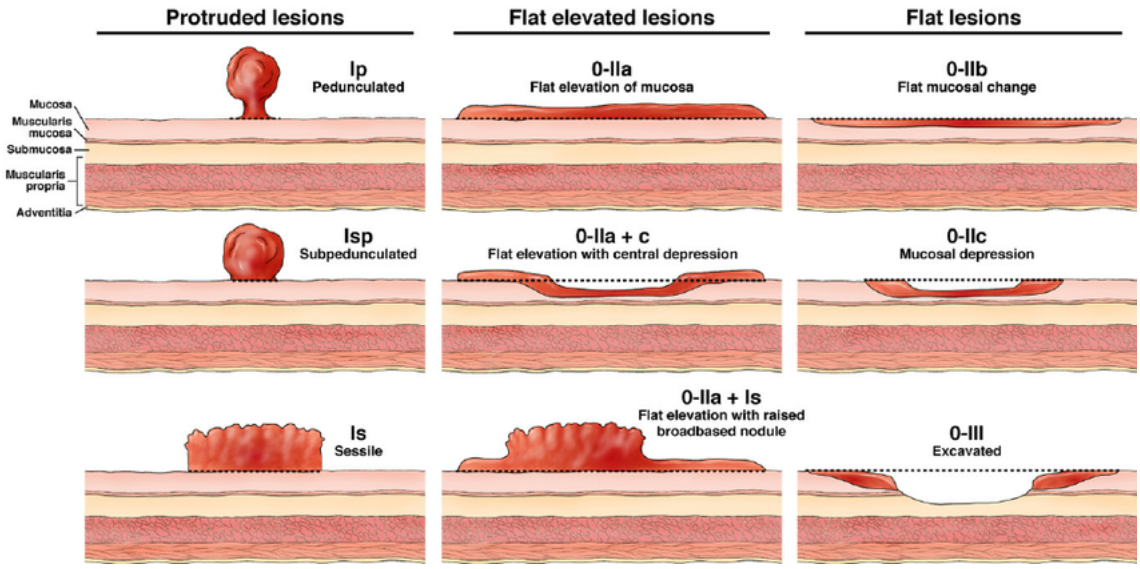

Figure 1.4 Paris classification of mucosal neoplasia
Figure 1.4 taken from the Belgian Society of Gastrointestinal Endoscopy, 2024 (Belgian Society of Gastrointestinal Endoscopy, 2024).

1.2.3 Histological evaluation of Barrett's oesophagus

Precise histological assessment of obtained biopsy material is imperative to facilitate accurate clinical diagnosis and determine ongoing follow-up. Ideally, this should be performed by a pathologist with a specialist interest in the condition. In cases where dysplasia is suspected, this should be confirmed or refuted by a second pathologist, as interobserver variability of Barrett's dysplasia is high (Fitzgerald et al., 2014; Odze, 2009). Haematoxylin and eosin (H&E) staining to identify columnar epithelium is the conventional method used to diagnose Barrett's oesophagus histologically: Alcian blue stain has also been recommended by some authors as an additional method to detect acid mucin within goblet cells (Falk & Goldblum, 2007).

The histology of Barrett's mucosa can be complex and heterogeneous, making standardised reporting of the condition challenging. In recent years, there has been deviation from historical descriptions of the condition as columnar epithelium located within the tubular oesophagus: it has since become apparent that there exists a mosaic of cell types observed within Barrett's epithelium. This includes those typically located within the stomach (i.e. gastric surface mucous cells) and intestine (i.e. goblet cells, although also occasionally enterocytes, endocrine cells and Paneth cells) (Naini, Souza, & Odze, 2016; Paull et al., 1976).To improve and standardise histological reporting for the condition,

the BSG guidelines recommend the following elements are recorded by the pathologist as a minimum: number of biopsy samples analysed at each level; type of mucosa present (squamous or columnar); presence of any native oesophageal structures; presence of gastric (cardiac/fundic) or intestinal type metaplasia; presence and grade of dysplasia (Fitzgerald et al., 2014).

1.2.4 Metaplasia-dysplasia-carcinoma sequence

Barrett's oesophagus is of clinical relevance in cancer prevention as it presents a unique opportunity to transform poor outcomes from OAC by detection of pre-malignant cells at an early stage. It is recognised that the columnar cells demonstrate the propensity to evolve through a stepwise progression of cellular changes through metaplasia to low-grade dysplasia (LGD), high-grade dysplasia (HGD), intramucosal adenocarcinoma (IMC) and ultimately OAC. This is known as the metaplasia-dysplasia-carcinoma sequence (Jankowski et al., 1999).

It is widely acknowledged that most patients with Barrett's oesophagus will not progress to dysplasia or cancer. The overall risk of malignant transformation to OAC in Barrett's oesophagus is estimated to be low at 0.3% (Desai et al., 2012). However, this risk rises exponentially to 16-30% when dysplasia is present within the Barrett's segment (Januszewicz & Fitzgerald, 2019; Shaheen et al., 2011) and is maximal when HGD is present (Pennathur et al., 2013). The weighted incidence of OAC in patients with HGD within the Barrett's segment was 6.58 per 100 person-years in a published meta-analysis (Rastogi et al., 2008). Presence of dysplasia is the single best predictor of OAC risk progression in Barrett's oesophagus (Whitson & Falk, 2015). In addition, increasing segment length has been shown to be an independent risk factor for development of dysplasia (Anaparthy et al., 2013). Recognition of early dysplasia is essential in these patients to reduce the risk of cancer development and progression: its presence also has ramifications for follow-up and ongoing management options.

Dysplasia signifies the presence of genetic and epigenetic cellular changes indicative of epithelial damage, resulting in clonal proliferation of damaged cells which favour neoplasia and exhibit malignant predisposition. Histological findings required to diagnose Barrett's dysplasia include the following: nuclear

enlargement; hyperchromatism; surface maturation; atypical mitosis; loss of cytoplasmic maturation (Goldblum, 2003).

Barrett's dysplasia is broadly subcategorised into LGD and HGD: most pathologists utilise the revised Vienna classification to grade dysplasia, based on the histological changes described above (Table 1.1) (Schlemper, Kato, & Stolte, 2001). The diagnosis "indefinite for dysplasia" (IND) is reserved for those cases in which the cellular changes described above are present, but not sufficient to confirm presence of dysplasia: the clinical significance of this is yet to be fully established (Thota et al., 2016). This classification system has demonstrated sound reproducibility, specificity and positive predictive value (PPV) amongst gastrointestinal pathologists (Kaye et al., 2009).

Table 1.1 Revised Vienna classification

Category	Diagnosis
1	Negative for neoplasia (ND)
2	Indefinite for neoplasia/dysplasia (IND)
3	Mucosal low-grade neoplasia (LGD) Low-grade adenoma Low-grade dysplasia
4 4.1 4.2 4.3 4.4	Mucosal high-grade neoplasia (HGD) High-grade adenoma/dysplasia Non-invasive carcinoma (carcinoma *in situ*) Suspicious for invasive carcinoma Intramucosal carcinoma
5	Submucosal invasion by carcinoma

Challenges to the histological diagnosis of Barrett's dysplasia are multifocal and may often present a clinical management conundrum. For example, reflux oesophagitis can induce reactive changes within the oesophageal mucosa, mimicking LGD. Identification of LGD is subject to considerable interobserver variability: historically, rates of interobserver agreement between pathologists regarding LGD have been reported as less than 50% (Falk, 2017; Kerkhof et al., 2007; Spechler, 2005). Furthermore, diagnostic inaccuracy is florid in the hands of non-expert pathologists: previous literature from the Netherlands has demonstrated that as many as 85% of LGD cases diagnosed by general pathologists were downgraded to "no dysplasia" following review by an expert panel (Curvers et al., 2010).

Additionally, dysplastic lesions can be flat, discrete and relatively inconspicuous in Barrett's oesophagus with patchy distribution (Montgomery et al., 2001). Despite diligent adherence to the Seattle biopsy protocol and use of advanced imaging techniques such as NBI, sampling bias and user error dictate that regions of dysplasia or small foci of invasive cancer can be missed at endoscopy. Furthermore, it is estimated that the rate of OAC missed within 1 year of index Barrett's endoscopy may be as high as 23-30% (van Putten et al., 2018; Visrodia, Singh, et al., 2016b; Wani et al., 2023). Similarly, invasive OAC has been identified within the resected specimen in up to 30-40% of patients undergoing oesophagectomy for HGD (Collard, 2002).

Finally, there may be disparity between presence of dysplasia and clinical outcome. While presence of HGD poses a significant risk of progression to cancer (6-20%) (Lekakos et al., 2011), the magnitude of this risk in LGD is debatable (Krishnamoorthi et al., 2018). There is a recognised correlation between LGD and progression to HGD or invasive cancer: a meta-analysis including 24 studies demonstrated the annual incidence to be 1.73% per patient-year (Singh et al., 2014). However, dysplasia can be absent within subsequent endoscopic biopsies in patients previously documented as having Barrett's dysplasia. In a large multi-centre randomised controlled trial (RCT) of radiofrequency ablation (RFA) versus surveillance in patients with LGD, no further LGD was identified in 28% of patients in the surveillance only arm during follow-up (although notably this study has been criticised for the possible inclusion of HGD cases at recruitment, therefore its results must be interpreted with caution) (Phoa et al., 2014). However, it remains unclear whether these patients truly regress within the metaplasia-dysplasia-carcinoma sequence or whether this is due to initial overdiagnosis and sampling error (Jagadesham & Kelty, 2014). Whilst many patients do not progress through this pathway to invasive cancer, the converse phenomenon is also observed: OAC may be found in patients without dysplasia at previous endoscopy (Reid et al., 2010). Dubiety exists as to whether this is due to rapid disease progression during the time interval between surveillance endoscopies, sampling error or if the dysplastic stage is bypassed altogether in some cases (Montgomery et al., 2001). There is a clear requirement for new technology to improve consistency in the diagnosis of Barrett's dysplasia.

1.2.5 Pathogenesis of Barrett's metaplasia

The pathogenesis of Barrett's metaplasia is not fully understood, although chronic exposure to acid refluxate is accepted to play a key role. It has been proposed that cell damage to the squamous epithelium in response to reflux of gastric and bile acids results in a metaplastic response and the acquisition of columnar epithelium (Chandrasoma et al., 2000). Apoptosis, cellular senescence or carcinogenesis are possible sequelae of the DNA damage and free radical formation that may be triggered by the oxidative stress response within oesophageal cells following exposure to acid and bile reflux (Dvorak et al., 2007). Free radical generation may also induce increased expression of various cytokines and upregulation of genes, resulting in further proliferation of this metaplastic process (Reid et al., 2010).

The origin of metaplastic columnar cells in Barrett's oesophagus is poorly understood: the inability to observe the process of metaplastic conversion *in vivo* and lack of reliable physiological animal models have contributed to this uncertainty (Naini, Souza, & Odze, 2016). Several theories have previously been proposed to explain this process, as demonstrated in Figure 1.5 (Naini, Souza, & Odze, 2016).

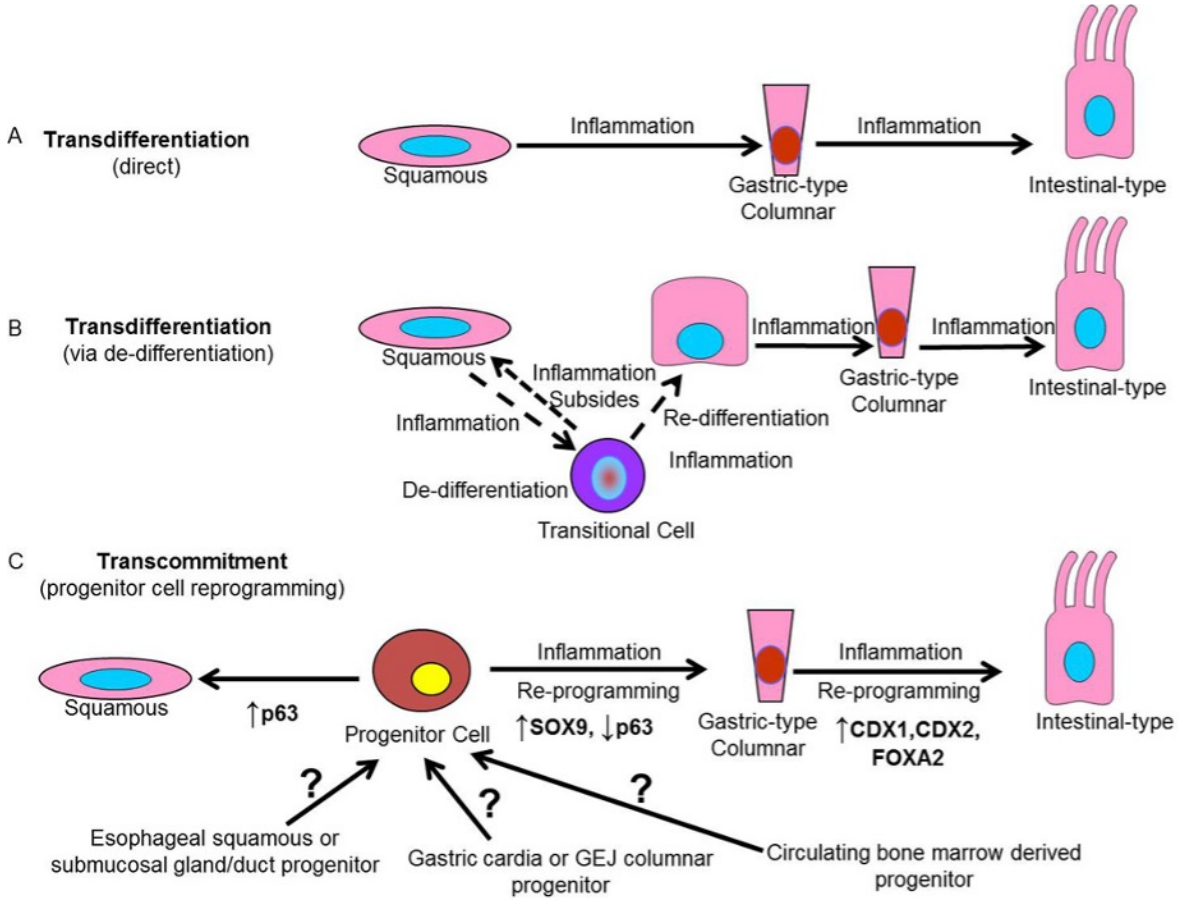

Figure 1.5 Pathways of cellular reprogramming in Barrett's metaplasia
Figure 1.5 taken from Naini, Souza & Odze, 2016 (Naini, Souza, & Odze, 2016).

Observational studies in embryonic mouse models and histological assessment of human oesophageal biopsies have suggested a potential role for transdifferentiation in Barrett's oesophagus in response to gastric refluxate (Naini, Souza, & Odze, 2016). Transdifferentiation is a process which occurs at an individual cellular level, in which one fully differentiated cell type transforms directly into another, without entering an intermediate pluripotent state (i.e. squamous cells transform directly into the columnar variety) (Slack, 2007). However, this theory seems less likely as full phenotypic conversion of cultured mature squamous cells has yet to be observed (Geboes & Hoorens, 2021).

Another possible theory is that metaplasia arises from undifferentiated progenitor cells displaying the ability to produce and maintain multiple cell lines (i.e. pluripotency) (Slack, 2007). In this hypothesis, immature progenitor cells undergo cellular reprogramming to express columnar developmental transcription factors in a process termed transcommitment, facilitating subsequent differentiation into another cell type (Wang & Souza, 2016). This

concept is supported by research undertaken in embryonic models of the mouse oesophagus (Beresford, 1990; Slack, 2007). Columnar epithelium expressing Sonic hedgehog (Shh) typically lines the embryonic mouse oesophagus. Expression of Shh in turn stimulates expression of bone morphogenetic factor 4 (BMP-4) within the oesophageal stromal cells: BMP-4 signalling is responsible for the maintenance of a columnar phenotype within the oesophageal epithelial cells (Litingtung et al., 1998; Wang et al., 2010). After birth, Hedgehog signalling ceases, with a consequent decline in stromal BMP-4 levels in the mouse oesophagus: as a result, the columnar epithelium reverts to stratified squamous epithelium shortly after birth (Litingtung et al., 1998; Que et al., 2006; Que et al., 2007; Yu, Slack, & Tosh, 2005). When mice are genetically engineered to maintain oesophageal stromal BMP-4 signalling, presence of columnar epithelium persists in the oesophagus, implying that reactivation of Hedgehog signalling (with a concomitant rise in BMP-4 levels) is responsible for the reprogramming of oesophageal squamous cells to a columnar phenotype via the process of transcommitment (Rodriguez et al., 2010). To expand on this theory, oesophageal squamous cell lines and tissues demonstrate increased expression of the transcription factors SOX9 and FOXA2 when exposed to increased acid and bile salts during both *in vitro* and *in vivo* studies. These transcription factors are targets of both the Hedgehog pathway (thus implicated in the development of columnar cells) and CDX2 (which is also involved in the expression of an intestinal phenotype) (Huo et al., 2010; Mari et al., 2014; Milano et al., 2007; Tatsuta et al., 2005; Wang et al., 2010; Wang et al., 2014).

The origin of such progenitor cells remains ambiguous. Potential oesophageal resident progenitor cells have been speculated to arise from either the interpapillary zone located within the basal layer of squamous epithelium (Pan et al., 2013; Seery, 2002) or the ducts of the submucosal glands (Glickman et al., 2001; Leedham et al., 2008). Mouse models have also provided convincing evidence that immature progenitor cells may be located within the proximal stomach: Lgr5+ progenitor cells located within the gastric cardia have been implicated in the development of metaplastic columnar epithelium in lineage tracing experiments (Quante et al., 2012; Wang et al., 2011). Finally, several studies support the role of multipotent progenitor cells from bone marrow as

contributors to the development of Barrett's oesophagus (Hutchinson et al., 2011; Sarosi et al., 2008; Spechler et al., 2010).

There is also some evidence to suggest that the first step in this metaplastic transformation requires the development of gastric type mucinous columnar epithelium, before additional cellular reprogramming ultimately triggers intestinal differentiation, and finally the presence of goblet cells (Chaves et al., 2005; Dias Pereira & Chaves, 2012; Hahn et al., 2009; Mari et al., 2014). Inflammation and tissue injury induced by exposure of the squamous epithelium to gastric and bile acids results in activation of the Hedgehog, BMP-4 and NF-κB signalling pathways, whilst downregulating Notch signalling. Again, these signals upregulate expression of SOX9 (responsible for induction of columnar differentiation), as well as FOXA2, CDX1 and CDX2 (which induce intestinal differentiation) (Souza, Krishnan, & Spechler, 2008; Wang & Souza, 2011; Wang et al., 2014). Intestinalisation has been shown to preclude the morphological appearance of goblet cells in several studies: CDX2 and MUC2 play early and late roles in this process, respectively (Chaves et al., 2005; Glickman et al., 2001; Wang & Souza, 2011; Wang et al., 2014).

A more recent study undertaking comprehensive phenotyping and multiomic profiling of different epithelial cell types from the normal oesophagus, gastric epithelium (including the cardia), Barrett's oesophagus and OAC from human participants has yielded promising results. This study concluded that Barrett's oesophagus originates from the gastric cardia. The initiation of c-MYC and HNF4A-driven transcriptional pathways modifies the gastric cardia cells to express an intestinal phenotype. In addition, this study also demonstrated that the origin of OAC can be traced back to undifferentiated Barrett's oesophagus cells, even in the absence of visible metaplastic precursor lesions (Nowicki-Osuch et al., 2021). This recent novel breakthrough in the understanding of the pathogenesis of Barrett's oesophagus is hoped to aid diagnosis, risk stratification and treatment of the disease in the future.

1.2.6 Biomarkers in Barrett's oesophagus

Risk stratification is a key concept in the management of Barrett's oesophagus, with the aim of identifying those most likely to develop malignancy. As

previously discussed, the presence of dysplasia is currently the most reliable clinical predictor of risk (Souza & Spechler, 2021). The role of biomarkers (including chromosomal abnormalities, genetic mutations, protein expression and epigenetic phenomena) for risk stratification in Barrett's oesophagus is a rapidly expanding area of interest. Historically, this has relied on the acquisition of endoscopic tissue biopsies. However, novel computer software has been developed over recent years as an adjunct to traditional histological assessment (Konda & Ellison, 2021).

The trefoil factor family are a group of small stable peptides, which play a role in intestinal mucosal defence and repair, as well as tumorigenesis (Dignass et al., 1994). Trefoil factor 3 (TFF3) is a member of this group and is a stable peptide biomarker highly expressed in intestinal metaplasia within the UGI tract (Leung et al., 2002). TFF3 was identified as a suitable biomarker for IM in a microarray experiment comparing mRNA expression of a broad range of genes in the normal squamous oesophagus, Barrett's oesophagus and normal gastric cardia. At the luminal surface of Barrett's oesophagus, this biomarker was expressed to high levels but was absent in adjacent tissues ($p < 0.001$) (Lao-Sirieix et al., 2009). TFF3 was therefore deemed the optimal biomarker candidate for identification of true IM, as immunohistochemistry for TFF3 demonstrated strong staining at the mucosal surface in Barrett's oesophagus biopsies (Paterson et al., 2020). The application of TFF3 immunohistochemistry to detect IM has been extensively analysed and validated in the Barrett's oEsophagus Screening Trials (BEST), which will be discussed in further detail later in this thesis.

The positive correlation between p53 and Barrett's dysplasia is well established. Mutations in the p53 gene occur early in the carcinoma sequence when whole exome sequencing of paired samples of Barrett's oesophagus and OAC are compared: this is then followed by whole genome doubling and oncogenic amplifications (Stachler et al., 2015). Absent or aberrant p53 expression within oesophageal tissue increases the risk of progression to HGD or OAC in patients with Barrett's oesophagus. Consequently, p53 is the most widely studied biomarker in Barrett's oesophagus. Immunohistochemistry staining for p53 can be widely performed by histopathologists (Konda & Ellison, 2021). In a recent meta-analysis including 36 studies and 2260 patients, a significant association

was demonstrated between aberrant p53 expression and increased risk of neoplastic progression in both non-dysplastic and LGD Barrett's patients (odds ratio [OR] 7.04; 95% CI 3.68-13.46). These results imply that p53 immunohistochemistry staining could be used to risk-stratify patients with Barrett's oesophagus (Janmaat et al., 2017). A further meta-analysis has demonstrated that a positive association exists between p53 aberrancy and neoplastic progression in Barrett's oesophagus, with an OR of 3.84 (95% CI 2.79-5.27; $p<0.001$) in 8 retrospective case-control studies and a relative risk of 17.31 (95% CI 9.35-32.08; $p<0.001$) in 7 cohort studies (Snyder et al., 2019). A further recent study has corroborated that abnormal p53 immunohistochemistry demonstrates a strong correlation with neoplastic progression (HR 5.03; 95% CI 3.88-6.50), including in those without histological evidence of dysplasia ($p<0.001$) (Redston et al., 2022). Therefore, the BSG guidelines acknowledge the clinical utility of p53 immunohistochemistry in addition to usual histological assessment of dysplasia (Fitzgerald et al., 2014). However, its routine clinical application has been hindered by the need for clarification on cases and inter-observer variability in its interpretation (Srivastava et al., 2017).

Chromosomal derangements, including aneuploidy and tetraploidy, have also been implicated as markers of progression to OAC. Using flow cytometry, previous literature has shown the relative risk of progression to oesophageal cancer to be 7.5 (95% CI , 4.0-14.0) and 5.0 (95% CI, 2.7-9.4) for those with tetraploidy and aneuploidy respectively, when compared to those without such abnormalities (Reid et al., 2000). A multicentre study demonstrated that presence of aneuploidy on targeted biopsies obtained at index endoscopy reliably predicted progression from non-dysplastic Barrett's oesophagus to any grade of neoplasia ($p=0.013$) and to HGD/OAC ($p=0.002$) (Hadjinicolaou et al., 2020). However, at present, flow cytometry is limited by the need for fresh tissue samples. Novel techniques utilising DNA flow cytometry performed on formalin-fixed paraffin-embedded (FFPE) slides, in addition to image cytometry, may be promising modalities for future study to assess for DNA content abnormalities that are more easily integrated into clinical practice (Choi et al., 2018; Dunn et al., 2010; Wang et al., 2015).

Chromosomal derangements have also been evaluated using shallow whole genome sequencing assays. In a retrospective case-control study using FFPE

tissue (matched for sex, segment length, age and smoking status), an increase in copy number alterations was observed in those who developed dysplasia, compared to the non-progressor cohort (Killcoyne et al., 2020).

The TissueCypher® Barrett's Esophagus Assay (Cernostics, Pittsburgh, Philadelphia, USA) is a new technique which has been validated in five multi-centre studies. It has been shown to predict progression from samples demonstrating non-dysplastic Barrett's, IND and LGD to HGD and OAC, and may also detect HGD or OAC in Barrett's segments initially missed on surveillance endoscopy (Critchley-Thorne et al., 2017; Critchley-Thorne et al., 2016; Davison et al., 2020; Frei et al., 2021; Frei et al., 2020). TissueCypher® labels 9 relevant biomarkers (including p53, p16, AMACR, HER2, cytokeratin-20, CD68, COX-2, HIF-1 alpha, and CD45RO) using a multiplexed fluorescence imaging platform (Konda & Ellison, 2021). This then facilitates extraction of objective quantitative data on multiple epithelial, stromal and morphometric features within intact Barrett's tissue specimens (DeWard & Critchley-Thorne, 2018; Prichard et al., 2015). The integration of quantitative image analysis data generates a risk score ranging from 0-10 which is then utilised to provide a three-tier risk stratification approach to classify patients as low-, intermediate- or high-risk for progression to HGD or OAC within 5 years. The HR for the 5-year probability of progression to HGD or cancer was 4.19 (95% CI 1.52-11.57) for intermediate- versus low-risk, and 14.73 (95% CI 6.55-33.16) for high- versus low-risk in the nested case control study (Critchley-Thorne et al., 2016). A single-blinded case-control study demonstrated that patients with a high-risk TissueCypher® score had a 4.7-fold increased risk of progression to HGD or OAC within 5 years compared to the low-risk group (Davison et al., 2020). Furthermore, TissueCypher® has been shown to outperform traditional histological review by pathologists for LGD. When a blinded cohort study was conducted on biopsies of patients with LGD within the screening cohort of an RCT, TissueCypher® demonstrated a higher sensitivity for identifying those who progressed to HGD or OAC compared to independent review across 30 pathologists (71% vs. mean 63%, range 33-88%; p=0.012) (Khoshiwal et al., 2023). This precision medicine technology has the potential to identify those patients who have high-risk TissueCypher® scores but no histological evidence of dysplasia at endoscopy who may warrant consideration for more rigorous surveillance (e.g. in a similar manner to LGD) (Konda & Ellison,

2021). It is proposed that TissueCypher® may also provide adjunctive evidence to support a surveillance-only approach or extension of surveillance intervals in patients with low-risk scores (Diehl et al., 2021). However, its performance characteristics are yet to be externally validated in a prospective study, and the technology is not yet available within the UK (Trindade et al., 2019).

Another source of potential predictive markers for Barrett's progression is found within the hypermethylation of tumour suppressor genes. Multivariate analysis has demonstrated the methylation of promoter regions P16 (OR 1.74; 95% CI 1.33-2.20), RUNX3 (OR 1.80; 95% CI 1.08-2.81) and HPP1 (OR 1.77, 95% CI 1.06-2.81) to be independent risk factors for progression to HGD and OAC (Schulmann et al., 2005). In addition, analysis of 8 methylation biomarkers (p16, RUNX3, HPP1, NELL1, TAC1, SST, AKAP12, and CDH13) demonstrated an AUC (area under the curve) of 0.84 ($p<0.001$) in a retrospective, multicentre, double-blinded variation study of 50 progressors versus 145 non-progressors (Jin et al., 2009).

Wide-area transepithelial sampling with 3-dimensional computer analysis (WATS-3D; CDx Diagnostics, Suffern, New York, USA) is an additional method to detect Barrett's metaplasia and dysplasia. The abrasive brush collects tissue fragments from a wide area of the oesophageal mucosa during endoscopy, with sampling of the full thickness epithelium performed by the pathology-based marker platform. A neural network computer system then undertakes scanning and reconstruction of the specimen into 3-dimensional images, which can undergo analysis to detect dysplasia, subsequently alerting the pathologist to perform detailed histological analysis of the area of concern (Konda & Ellison, 2021). This technique has been shown to increase yield of IM and dysplasia when used as an adjunct to endoscopic biopsies (Gross, Smith, & Kaul, 2018; Smith et al., 2019). In a recent large prospective registry study of 23,933 patients with GORD, 19.3% of IM cases were detected by WATS-3D alone (i.e. traditional endoscopic biopsies were negative for IM). Similarly, 107/240 patients with dysplasia (44.6%) were identified solely by WATS-3D (Shaheen et al., 2024). However, WATS-3D technology is yet to be formally incorporated into standard clinical practice.

Clearly biomarkers are an evolving area of interest and present a unique opportunity for new technologies to enhance the diagnosis of Barrett's dysplasia and OAC.

1.2.7 Treatment of Barrett's dysplasia and OAC

As previously discussed, OAC frequently presents at a late or advanced stage with distant metastases. This observation is substantiated by the 2024 National Oesophago-Gastric Cancer Audit (NOGCA), in which only 39% of oesophageal cancer patients were suitable to undergo treatment with curative intent (National Oesophago-Gastric Cancer Audit, 2024). Presently, the best curative option for early stage or locally advanced OAC is neoadjuvant chemotherapy (NAC) followed by surgical resection (D'Journo & Thomas, 2014; Shapiro et al., 2015).

Administration of preoperative NAC has been shown to infer a 5-15% improvement in 5-year survival rates (Allum et al., 2009; Cunningham et al., 2006; Medical Research Council Oesophageal Cancer Working Group, 2002; Ychou et al., 2011). A recent randomised controlled, open-label, phase 2/3 trial (FLOT4-AIO) has demonstrated that the pre-operative FLOT (5-Fluorouracil, Leucovorin, Oxaliplatin and Docetaxel) regimen increased median overall survival by 15 months ($p=0.012$) and median disease-free survival by 12 months ($p=0.004$): FLOT has subsequently become a standard NAC regimen in OAC (Al-Batran et al., 2019).

Oesophagectomy involves dissection and removal of the oesophagus, followed by restoration of digestive tract continuity, most commonly with a gastric tube interposition (Haverkamp et al., 2017). This is a complex surgical procedure subjecting the patient to significant physiological insult, with the potential for major perioperative morbidity. A recent study reported that 39.8% of patients undergoing oesophagectomy developed postoperative complications classified as Clavien-Dindo Grade III or IV in severity (Bundred et al., 2020). Administration of NAC potentiates the risk of early postoperative complications, such as anastomotic leak, formation of chylothorax, pulmonary complications, wound infection and increased 30-day mortality (Ruol et al., 2007).

Over recent years, steady advances in endoscopic techniques have resulted in fewer patients requiring systemic therapy and surgical intervention for early-stage OAC. When neoplasia is confined to the mucosal layer of the oesophagus, the risk of lymphatic spread is low: this forms the basic principle for endoscopic

eradication therapy (EET). Larger blood and lymph vessels are located within the lower submucosa of the oesophagus; therefore, the risk of lymph node metastasis rises in line with the degree of submucosal invasion. Provided the muscular layer is not breached, the oesophageal mucosa and submucosa can both be safely resected endoscopically (Noordzij, Curvers, & Schoon, 2019). In patients with T1a lesions, the risk of lymph node metastasis is <2%: EET using a targeted local approach is preferential to oesophagectomy in these cases (Sharma et al., 2020). Notably, the risk of lymph node metastasis rises to 20% in patients with T1b lesions (i.e. denoting submucosal invasion) (Graham et al., 2018). Oesophagectomy is reserved for these patients, those with multifocal or advanced carcinoma, or lesions not amenable to EET (Sharma et al., 2020).

Complete eradication of IM and dysplasia is the definitive goal of EET (i.e., the absence of macroscopically visible Barrett's oesophagus at endoscopy and histological eradication of IM from the SC junction along the entirety of the length of the pre-treatment Barrett's segment) (Kolb & Wani, 2020). Unfortunately, recurrence of IM or dysplasia on endoscopic biopsies does occur at a rate of 8-10% per year (Kahn, Shaheen, & Iyer, 2020), although this risk of recurrence is reduced when EET is performed in high-volume centres (Soroush et al., 2019; Tan et al., 2019; Wani et al., 2020). It is therefore advocated that EET is performed in tertiary care referral centres by expert Barrett's endoscopists: the BSG guidelines also recommend that all patients undergoing treatment for dysplasia or early cancer should be discussed at the local specialist OG MDT (Fitzgerald et al., 2014).

RFA is the modality of choice for flat dysplastic Barrett's oesophagus. The most compelling evidence for its use is derived from the multi-centre, sham-controlled trial by Shaheen et al., in which 127 patients with Barrett's dysplasia were randomly assigned in a 2:1 ratio to RFA or a sham procedure. When RFA was compared to the sham procedure, complete eradication of dysplasia was more frequent in both the LGD (90.5% vs. 22.7%; $p<0.001$) and HGD (81.0% vs. 19.0%; $p<0.001$) groups respectively. The RFA group saw complete eradication of IM in 77.4% compared to 2.3% in the control group ($p<0.001$) and was also associated with reduced disease progression (3.6% vs. 16.3%; $p=0.03$) and reduced number of cancers (1.2% vs. 9.3%; $p=0.045$) (Shaheen et al., 2011; Shaheen et al., 2009). Whilst its safety and efficacy are evident, RFA is

expensive, requires an expert operator, necessitates multiple treatment sessions to eradicate dysplasia and causes tissue destruction, therefore samples cannot be sent for histological analysis. Other ablative techniques such as balloon cryotherapy, photodynamic therapy, argon plasma coagulation or hot avulsion also exist, but are yet to yield such convincing results and be incorporated into routine clinical practice (Wolfsen, 2018).

For visible neoplastic lesions, endoscopic resection obtains a larger and deeper tissue specimen, enabling more accurate tumour staging by calculating depth of invasion. The two main techniques utilised are endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). EMR utilises snare-wiring and is usually performed in a piecemeal fashion (Terheggen et al., 2017). However, piecemeal removal of the lesion may compromise lateral margins, impair precise histological diagnosis due to the theoretical risk of missing advanced neoplastic areas, and is associated with a higher incidence of recurrence (Cao et al., 2009; Pech et al., 2008). Conversely, ESD uses an improved needle-knife technique to allow en-bloc resection of larger lesions for accurate pathological analysis of lateral and deep margins (Guo et al., 2014). ESD has been shown to be superior to EMR in achieving higher rates of histologically complete resection. However, it is more technically demanding and associated with higher complication rates than EMR (Terheggen et al., 2017). Thus, whilst ESD is gaining traction in western countries, EMR remains the mainstay of endoscopic resection for Barrett's dysplasia or early cancer.

EET has demonstrated low complication rates, favourable histological outcomes and excellent long-term survival rates in early OAC (Pech et al., 2008; Pouw et al., 2010; Wang & Sampliner, 2008), thus reducing surgical morbidity and mortality associated with oesophagectomy whilst also enabling organ preservation. In addition, EET is associated with lower costs, improved patient tolerance and better postoperative quality of life (QoL) (Guo et al., 2014). These clear advantages of EET emphasise the importance of early detection of dysplastic Barrett's lesions.

Recent work has focused on chemoprevention of Barrett's dysplasia or OAC with acid-suppressant drugs. It has been suggested that downregulation of cyclooxygenase (COX) by proton pump inhibitors (PPIs) may be protective against

neoplasia (de Bortoli et al., 2011): this hypothesis also implies that the inhibitive effect of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) on COX may achieve the same outcome. The current BSG guidelines published in 2014 advocate the use of gastric acid suppressant medication for symptom control, noting that, of the available oral agents, PPIs have the best clinical profile. However, the current evidence base does not support the use of PPIs, aspirin or NSAIDs as chemopreventive agents (Fitzgerald et al., 2014). In 2018, Jankowski et al. published the long-awaited results of the Aspirin and Esomeprazole Chemoprevention in Barrett's metaplasia Trial (AspECT) trial. This large RCT demonstrated that high-dose PPI therapy (80mg esomeprazole daily) was superior to low-dose PPI (20mg esomeprazole daily) by prolonging the time to reach the composite endpoint of all-cause mortality, OAC or HGD with minimal side effects (Jankowski et al., 2018). However, these results were hindered by several significant study limitations: lack of double-blinding; small effect size; low event rate (Muthusamy et al., 2022). Therefore, the evidence base for PPI administration as chemoprevention for Barrett's dysplasia remains insufficient to draw definitive conclusions.

1.2.8 Endoscopic Barrett's surveillance programmes

Despite discrepancies in their definition of Barrett's oesophagus, all major gastroenterology societies currently advocate for endoscopic surveillance of the disease (Fitzgerald et al., 2014; Qumseya et al., 2019; Shaheen et al., 2022). Its pre-malignant disposition and stepwise progression through the metaplasia-dysplasia-carcinoma sequence renders Barrett's oesophagus an ideal condition in which to undertake surveillance to reduce risk of cancer progression (Katona & Falk, 2011). However, the merit of Barrett's surveillance programmes has previously been questioned, given the organisation required and costs incurred for a reasonably low pathology yield. Despite this, observational studies have shown improved prognosis in those with surveillance-detected cancers compared to those presenting with clinical symptoms: these cancers are detected at an earlier stage, at which point EET may be offered as a curative treatment (Corley et al., 2002; Streitz, Andrews, & Ellis, 1993; van Sandick et al., 1998; Wright et al., 1996). This principle of early cancer detection at a treatable stage forms the foundation for the Barrett's surveillance programme.

Following detection of Barrett's oesophagus, patients should be fully informed of their diagnosis and its implications and receive a formal invitation to join the surveillance programme. Patients should receive an initial clinical consultation detailing the risks, benefits and limitations of endoscopic surveillance. Suitability for entry into the surveillance programme should be assessed: factors to consider include patient age, additional comorbidities, life expectancy and ability to undergo repeated endoscopies or other treatment options (Fitzgerald et al., 2014).

Surveillance endoscopy should be performed employing the same techniques as described for diagnostic Barrett's transoral endoscopy. Of note, TNE is not recommended for routine Barrett's surveillance at present (Weusten et al., 2023). Thorough mucosal inspection identifying relevant anatomical landmarks is again essential, using advanced imaging techniques where possible. The Prague classification system should be used to describe the Barrett's segment and biopsies taken in accordance with the Seattle protocol. The obtained biopsies undergo histological assessment in the same manner as previously discussed, using the minimum dataset advised by the BSG and the revised Vienna classification (Table 1.1) (Schlemper, Kato, & Stolte, 2001). This process of pathological assessment is of particular importance, as the presence or absence of dysplasia dictates timing of subsequent surveillance intervals, as well as the requirement for additional treatment.

Time interval between surveillance endoscopy is dictated by length of the Barrett's segment, in addition to the presence or absence of dysplasia at histological assessment. Guidelines from the major gastrointestinal societies regarding surveillance intervals are again incongruous: interestingly, their recommendations are not based on results from RCTs. The general principles advise that patients with non-dysplastic Barrett's oesophagus should undergo surveillance endoscopy every 2-5 years, although this interval is shortened when dysplasia is present within the Barrett's segment (Fitzgerald et al., 2014; Qumseya et al., 2019; Shaheen et al., 2022; Sharma et al., 2020; Weusten et al., 2023).

In Scotland, recommendations from the BSG guidelines are followed to determine Barrett's surveillance intervals. Patients with an irregular Z-line (i.e.

macroscopic changes <1cm) do not require regular surveillance as the risk of progression to HGD/OAC is considered low (Jung et al., 2011; Thota et al., 2017). Where patients demonstrate columnar or intestinal metaplasia only, those with short segments (i.e., M <3cm using the Prague classification) should undergo surveillance endoscopy every 3-5 years, whilst those with long segments (i.e., M ≥3cm) should undergo surveillance endoscopy every 2-3 years. In patients whose biopsies are IND, acid suppression therapy should be optimised and repeat endoscopy should be performed at 6 months (at this point, if dysplasia is present, patients follow the according protocol; if dysplasia is absent, patients follow the recommended surveillance interval for metaplasia only). As previously discussed, a second pathologist should review all biopsies positive for dysplasia. Endoscopy should be repeated at 6-monthly surveillance intervals where LGD is present: the BSG guidelines state there is insufficient evidence at present to recommend EET for patients with persistent LGD. Where two consecutive sets of biopsies show no evidence of dysplasia, the patient should return to the non-dysplastic pathway. Where HGD is present, the patient should be discussed at the specialist OG MDT and formally referred to a tertiary centre for ongoing treatment (Fitzgerald et al., 2014). Figure 1.6 demonstrates the recommended surveillance pathway for non-dysplastic Barrett's oesophagus, whilst Figure 1.7 demonstrates the flowchart for dysplastic Barrett's oesophagus, as determined by the BSG guidelines (Fitzgerald et al., 2014).

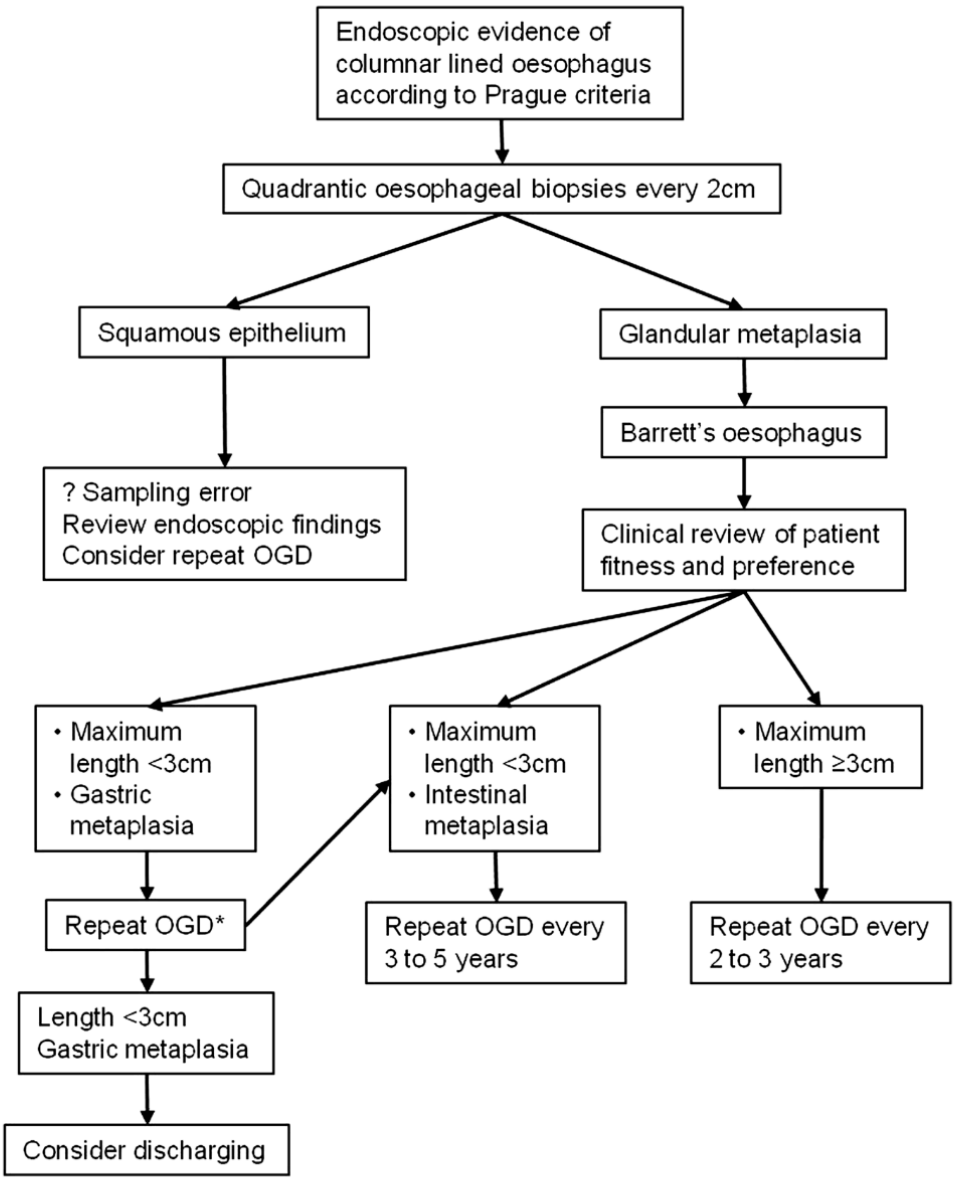

Figure 1.6 Surveillance flowchart for non-dysplastic Barrett's oesophagus
Figure 1.6 taken from BSG guidelines, 2014 (Fitzgerald et al., 2014).

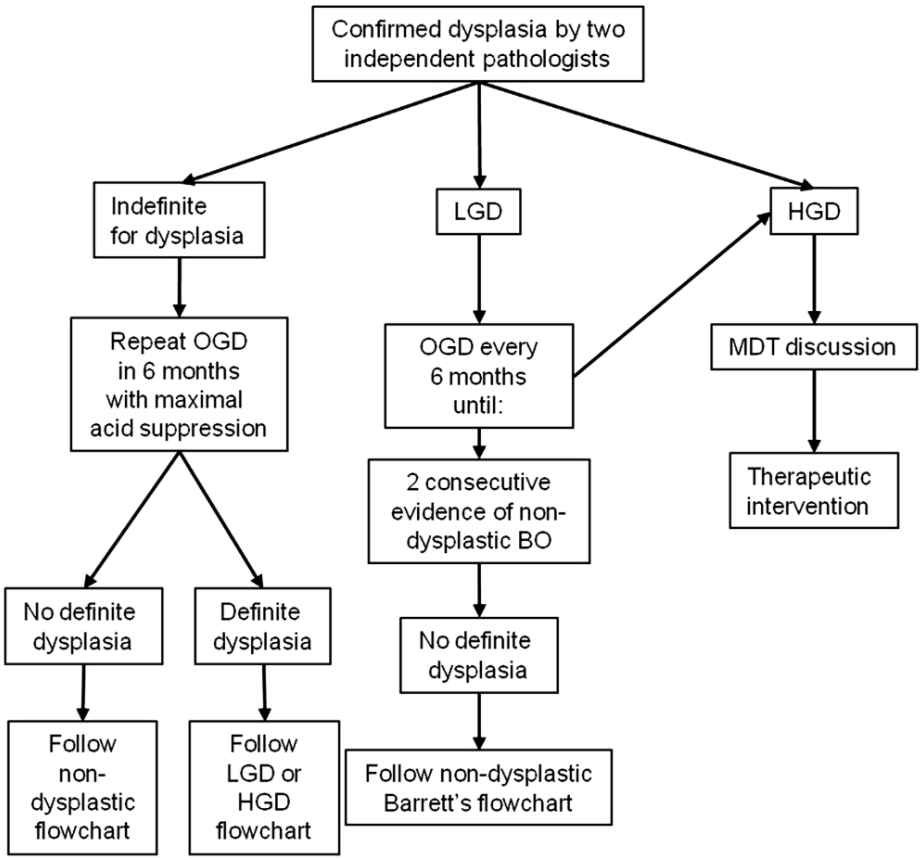

Figure 1.7 Surveillance flowchart for dysplastic Barrett's oesophagus
Figure 1.7 taken from BSG guidelines, 2014 (Fitzgerald et al., 2014).

The current endoscopic Barrett's surveillance programme is not without limitation. Whilst generally considered a safe procedure, UGI endoscopy is not devoid of complications: these include bleeding, perforation and cardiopulmonary complications associated with the use of sedative agents (Agostoni et al., 2011; Ben-Menachem et al., 2012; Quine et al., 1995; Sieg, Hachmoeller-Eisenbach, & Eisenbach, 2001). Undertaking Seattle protocol biopsies in long Barrett's segments can be time-consuming and uncomfortable for patients: endoscopy lists should be prioritised to accommodate a longer time slot for such patients (Graham et al., 2016). Furthermore, UGI endoscopy is not a perfect test: some subtle lesions are not macroscopically visible, dysplasia may be focal and patchy, and endoscopic punch biopsies can be fraught with user error and subject to sampling bias. In a recently published population-based cohort study, Wani et al. noted the rate of "missed" OAC within 1 year of index endoscopy for Barrett's surveillance to be as high as 24% (Wani et al., 2023). Post-endoscopy incident cancers are estimated to constitute 14% of the total oesophageal cancer burden (Vajravelu et al., 2022). As previously mentioned,

adherence to the Seattle protocol can be substandard (Abrams et al., 2009; Das et al., 2008) and interpretation of dysplasia is subject to significant interobserver variability (Odze, 2009), which may detrimentally impact pathology yield. Finally, it is estimated that less than 20% of patients with Barrett's oesophagus receive a formal diagnosis, as the majority of OAC cases arise *de novo*, limiting the benefits of surveillance and potential for early diagnosis of dysplasia or cancer (Dulai et al., 2002; El-Serag et al., 2016; Visrodia, Singh, et al., 2016b). Patients with a prior diagnosis of Barrett's oesophagus were more likely to be diagnosed with early-stage OAC (OR 3.68; 95% CI 1.30-10.40) and demonstrated improved survival (HR 0.45; 95% CI 0.25-0.80) compared to those diagnosed *de novo* (Cooper, Kou, & Chak, 2009).

Controversy surrounding the current Barrett's surveillance programme was further compounded by the recent publication of the Barrett's Oesophagus Surveillance Versus Endoscopy at Need Study (BOSS). This large RCT included 3453 patients recruited from 109 centres across the UK, with a minimum follow-up period of 10 years. This landmark study demonstrated no significant difference in overall survival between the surveillance arm (333 deaths in 1733 patients) versus the at-need arm (356 deaths in 1719 patients; HR 0.95; 95% CI 0.82-1.10). In addition, traditional surveillance did not improve cancer-specific survival, time to diagnosis of OAC, or cancer stage at diagnosis. However, within the surveillance arm, detection of HGD was double that of the at-need arm: the implications of this finding beyond the 10–15-year follow-up period of this trial remain uncertain (Old et al., 2025). Furthermore, a concurrent economic evaluation alongside the BOSS study concluded that regular endoscopic surveillance every 2-3 years was unlikely to be cost-effective (Deidda et al., 2025). Evidently, the findings from these studies have questioned the merit of continuing the resource-intensive endoscopic Barrett's surveillance programme in its current format.

Clearly, it is evident that the Barrett's surveillance programme has scope for improvement. Developments in the field of advanced imaging techniques may enhance dysplasia detection at endoscopy as discussed previously. Risk stratification combining clinical details and biomarkers is another promising area of development. Novel non-endoscopic technologies, such as sponge cytology

devices, are being adopted into surveillance programmes: this will be explored in greater detail later in this thesis.

1.2.9 Screening for Barrett's oesophagus

Cancer screening aims to identify pre-malignant or early neoplastic lesions across a healthy asymptomatic population at a point in the disease trajectory where treatment is likely to result in cure (Dobrow et al., 2018), with established population-based screening programmes demonstrating positive results for breast, cervical and colorectal cancers (Siegel et al., 2021). It has been speculated that OAC is an ideal target for screening for the following reasons: it has a well-defined precursor lesion in the form of Barrett's oesophagus; dysplasia and early cancers are amenable to EET; EET is associated with reduced rates of cancer progression (Phoa et al., 2014; Shaheen et al., 2009).

At present, major gastroenterological societies advise consideration of screening for Barrett's oesophagus in the presence of risk factors such as chronic GORD symptoms, age 50 years or older, positive family history, white race, male sex and obesity (Fitzgerald et al., 2014; Qumseya et al., 2019; Shaheen et al., 2022). However, it is noted that, given the large volume of patients with reflux symptoms yet low associated pathology yield, screening with UGI endoscopy is neither a feasible nor justified option at present (Fitzgerald et al., 2014). Chronic GORD symptoms alone may not be sufficient to identify the relevant patient cohort. A recent publication from the USA has demonstrated suboptimal sensitivity (39-43%) and specificity (67-77%) for current societal guidelines recommending screening for Barrett's oesophagus or OAC inclusive of GORD, with over half of their cohort diagnosed with Barrett's oesophagus in the absence of reflux symptoms (Nguyen et al., 2021).

More recent work has focused on identifying an optimal population to screen for Barrett's oesophagus and early OAC. A post-hoc analysis from the BEST 3 RCT focused on enriching the population for screening. This study identified that >70% of Barrett's oesophagus and early OACs would be detected by undertaking targeted screening of individuals with a 5% probability of having the described pathology. From this study, the relevant populations identified to maximise

diagnostic yield and minimise harm from overdiagnosis were women aged ≥ 65 years and men aged ≥ 55 years with chronic reflux symptoms, which forms the basis of the BEST 4 screening trial (Tan et al., 2024).

Additionally, several clinical prediction algorithms have been developed in recent years, amalgamating risk factors for Barrett's oesophagus and OAC. These include the Michigan Barrett's oEsophagus pREdiction Tool (M-Beret) (Rubenstein et al., 2013), Kunzmann tool (Kunzmann et al., 2018), Thrift tool (Thrift et al., 2012), Locke tool (Locke, Zinsmeister, & Talley, 2003), Gerson tool (Gerson et al., 2001) and the Nord-Trøndelag Health Study (HUNT) (Xie et al., 2018). However, clinical application of these tools has been limited by their lack of external validation. A recent study from the Michigan group demonstrated that the ability to predict Barrett's oesophagus of these six tools was only modest (AUC 0.660-0.695), with no tool superior to the others. However, it is noteworthy that all risk algorithms were more reliable at predicting presence of Barrett's oesophagus compared to presence of reflux symptoms alone (Rubenstein et al., 2020). Data assessing the clinical application of these tools in a prospective study is lacking but may pave the way to enrich the desired population for Barrett's screening in the future.

1.3 Potential screening techniques for Barrett's oesophagus

New non-endoscopic cell sampling devices have emerged over recent years, which may shape the future landscape of screening for Barrett's oesophagus and OAC. However, it is yet to be established whether non-endoscopic screening for OAC will reduce cancer mortality: this is to be investigated by the ongoing BEST 4 trial. As mentioned above, this large RCT aims to evaluate whether capsule sponge biomarker testing in the symptomatic reflux population (i.e. males aged 55-79 years and women aged 65-79 years requiring at least 6 months of acid-suppressant medication use in the previous year) is a useful screening tool to reduce OAC-related mortality (National Institute for Health and Care Research, 2022).

A variety of non-endoscopic screening devices have been developed over recent years: these devices have exhibited good performance in the detection of

Barrett's oesophagus, demonstrate improved patient acceptability over endoscopy, and have shown good cost-effectiveness on economic evaluation when compared to endoscopic screening (Benaglia et al., 2013; Fitzgerald et al., 2020; Iyer et al., 2020; Iyer et al., 2021; Moinova et al., 2018; Swart et al., 2021). Given the considerable developments in the evidence base for non-endoscopic cell-sampling devices, the ACG guidelines have been updated to include a conditional recommendation that "a swallowable, non-endoscopic capsule sponge device combined with biomarker testing is a suitable alternative to endoscopy for screening for Barrett's oesophagus" (Shaheen et al., 2022). This is mirrored in the ESGE guidelines, which state "a swallowable, non-endoscopic cell collection device such as the Cytosponge™, combined with a cytopathological assessment and biomarker TFF3, can be used as an alternative to endoscopy for case finding of Barrett's oesophagus". The ESGE guidelines do note that there is insufficient evidence to recommend other non-endoscopic technologies at present (Weusten et al., 2023).

In addition, other techniques such as TNE and breath sampling have been garnering increasing research interest in recent years (Tan et al., 2021). The advantages and limitations of such screening techniques are summarised in Table 1.2 (Tan, 2023).

Table 1.2 Advantages and limitations of screening techniques for Barrett's oesophagus and OAC
Adapted from original manuscript by Tan, 2023 (Tan, 2023).

Technology	Advantages	Limitations
Standard transoral endoscopy	Current gold standard	Invasive; expensive; operator-dependent; requires histological assessment of biopsies
Prediction scores	Low cost; ease of use	Requires accurate data collection; awaits validation in prospective studies
Transnasal endoscopy	Similar diagnostic performance to WLE; can be performed in outpatient setting	Requires expert operator; cost of hardware; small biopsies/limited sampling
Capsule sponge testing using TFF3 immunohistochemistry	Minimally invasive; can be performed in office or community setting; minimal training required; more cost-effective than endoscopy; objective TFF3 immunohistochemistry amenable to artificial intelligence automation; presence of columnar cells provides quality control for adequate sampling of GOJ; offers pan-oesophageal sampling and can diagnose other pathologies not limited to Barrett's oesophagus	Low signal-to-noise ratio due to pan-oesophageal sampling (focal IM can be missed); triage test that requires follow-on endoscopy for confirmation; slide-based assay may be limited for massive scale-up
EsophaCap™ device using methylated DNA markers	Minimally invasive; can be performed in outpatient setting; minimal training; potential to be more cost-effective than endoscopy; quantitative results that could be automated; assay suitable for mass testing	Cut-off for diagnosis of Barrett's oesophagus not completely standardised; low signal-to-noise ratio due to pan-oesophageal sampling (focal IM can be missed); triage test that requires follow-on endoscopy for confirmation; further evidence required in intended-to-screen population; formal health economic studies required
EsoCheck™ device using EsoGuard assay	Less invasive than endoscopy; can be	Difficult to know when to deflate balloon (early

	performed in outpatient setting; potential to be more cost-effective than endoscopy; selective sampling of Barrett's segment so could avoid contamination with other cells; assay suitable for mass testing	deflation may lead to inadequate sampling of Barrett's segment); triage test that requires follow-on endoscopy for confirmation; further evidence required in intended-to-screen population; formal health economic studies required
Volatile organic compounds	Ease of administration (no need for expert operator); potential to be more cost-effective than endoscopy	Clinical studies and accuracy data only in early stages; may be affected by patient diet or environmental factors; triage test that requires follow-on endoscopy for confirmation; diagnostic performance requires validation in reflux population not enriched for Barrett's oesophagus; formal health economic studies required

1.3.1 Capsule sponge testing

Capsule sponge testing using an oesophageal cell collection device (OCCD) such as Cytosponge™ (Medtronic, Minneapolis, USA) or Endosign® (Cyted Ltd, Cambridge, UK) currently possesses the most substantial body of evidence for use in Barrett's screening. This minimally invasive, cell-sampling device was developed by the Fitzgerald laboratory in Cambridge and has been rigorously tested in several clinical trials over the past 15 years. Initially designed for use in the primary care setting, the capsule sponge device consists of a 3cm-diameter medical grade mesh sponge on a string compressed within a vegetarian coating (Kadri et al., 2010). The device is swallowed by the patient whilst sitting upright, under the supervision of a trained practitioner. The capsule coating dissolves after a 7-minute interval, permitting expansion of the sponge within the patient's UGI tract. The sponge is then removed by pulling the string: on its retrieval, the sponge collects a pan-oesophageal sampling of more than 1 million cells which can then undergo cytological analysis (Fitzgerald, 2015; Paterson et al., 2020; Ross-Innes et al., 2017). Contraindications to capsule sponge testing include: presence of dysphagia; swallowing disorders; anatomical abnormalities

of the oesophagus or stomach; RFA, EMR or OG surgery within the previous 2 months; portal hypertension or oesophageal varices; pregnancy; anticoagulation (Scottish Health Technologies Group, 2023).

Following its retrieval, the sponge is immediately immersed in preservative fluid (BD SurePath™) before samples are sent to a single UK-based laboratory (Cyted Ltd) for processing. The collected cells are removed from the sponge and centrifuged to a homogenous clot, before being retrieved and processed to FFPE blocks. Superficial and deep sections are cut and stained for H&E, as well as the identification of the immunohistochemical biomarker TFF3 and p53 (Figure 1.8) (Lao-Sirieix et al., 2009).

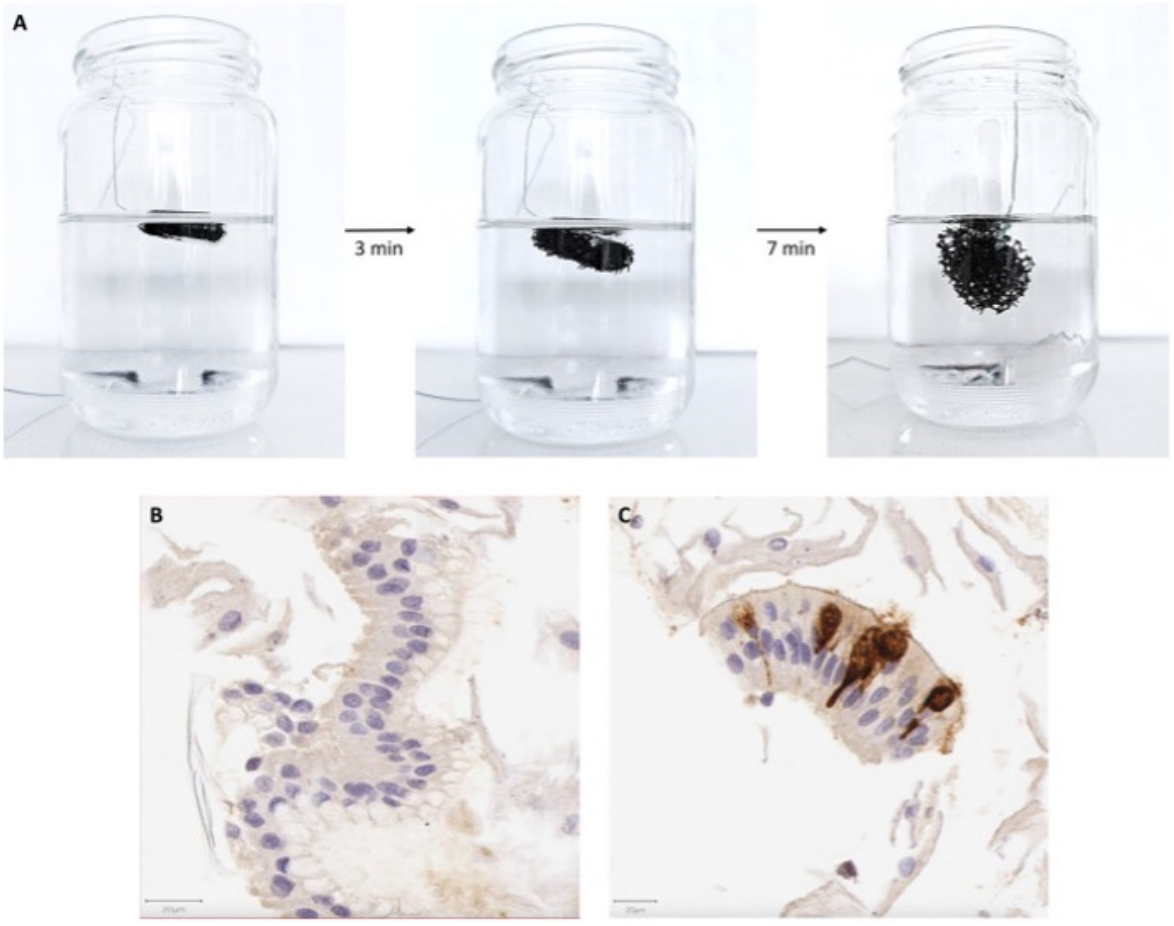

Figure 1.8 Capsule sponge device demonstrating detection of TFF3
A) Submerged capsule sponge device (Cytosponge™) dissolving with expansion of sponge. B) 40x magnification of an FFPE Cytosponge™ slide demonstrating TFF3-negativity of gastric cardia epithelium in a patient without Barrett's oesophagus. C) 40x magnification of an FFPE Cytosponge™ slide demonstrating TFF3 positivity of goblet cells in a patient with Barrett's oesophagus. Figure 1.8 taken from Tan, 2023 (Tan, 2023).

A trained pathologist will first assess the sample is adequate for analysis by ensuring that an abundance of squamous cells is present within the sample:

samples may be rendered inadequate due to contamination with food substances or other artefacts. The sample is then assessed to ensure the sponge has reached the stomach. This is confirmed by the presence of columnar cells (i.e. the sample is deemed insufficient in the absence of columnar groups). In the event of an insufficient or inadequate sample, repeat capsule sponge testing or routine endoscopy is advised (Angel et al., 2026).

As discussed previously, TFF3 is a biomarker for IM: TFF3 positivity indicates the presence of goblet cells (synonymous with IM) and hence a diagnosis of Barrett's oesophagus (Ross-Innes et al., 2015). The BEST 1, 2 and 3 trials have seen capsule sponge testing (using the Cytosponge™ device) rigorously scrutinised for the diagnosis of Barrett's oesophagus. BEST 1 was a proof-of-concept trial assessing the feasibility of capsule sponge testing among patients prescribed acid-suppressant medication for >6 months. This initial trial demonstrated that the test could be safely and feasibly administered in the primary care setting with encouraging sensitivity and specificity (although notably the study was not powered for this) (Kadri et al., 2010). This was followed by BEST 2, a case-control study of 1110 patients powered for sensitivity and specificity for Barrett's oesophagus. In intention-to-treat analyses, capsule sponge testing demonstrated an overall sensitivity of 80.0% for Barrett's, rising to 87.2% in those with a circumferential Barrett's segment ≥3cm. Interestingly, this per-protocol analysis also included tests which did not reach the stomach (i.e. no gastric cardia cells were sampled): this is now a quality control measure whereby lack of gastric cardiac mucosa indicates an unsuccessful swallow. When such insufficient cases were excluded, the sensitivity and specificity of capsule sponge testing for Barrett's oesophagus improved to 94.0% and 92.4% respectively (Ross-Innes et al., 2015).

Following on from this, the BEST 3 trial was a landmark paper in expanding the evidence base for capsule sponge testing as a screening tool for Barrett's oesophagus. This multi-centre, pragmatic RCT recruited >13,000 patients, who were then randomised to either capsule sponge testing using Cytosponge™ versus usual care (acid-suppressant medication and endoscopy only if deemed appropriate by the primary care physician). In the group randomised to receive Cytosponge™, capsule sponge testing increased detection of Barrett's

oesophagus over ten times the usual standard of care (rate ratio = 10.6; 95% CI 6.0-18.8; p<0.0001). This group were also found to have a higher proportion of early-stage Barrett's dysplasia, although the number of cases was admittedly small (Fitzgerald et al., 2020).

Additional work has proven capsule sponge testing to be safe, minimally invasive and cost-effective, with high levels of tolerance and acceptability by patients (Freeman et al., 2017; Januszewicz et al., 2019; Kadri et al., 2010; Lao-Sirieix et al., 2007; Maroni et al., 2022; Ross-Innes et al., 2015; Swart et al., 2021). A recent systematic review including 2672 capsule sponge tests reported only two significant adverse events (one case of sponge detachment and one case of pharyngeal bleeding), both of which were resolved without any ill or long-term effects on the patient. In addition, over 96% of patients were able to successfully swallow the device (Januszewicz et al., 2019). Capsule sponge testing also outperforms endoscopy in terms of ease of administration and patient convenience: it can be undertaken within 10 minutes in the outpatient setting and does not require recovery time, with no restrictions on the remainder of the patient's day (unlike endoscopy with sedation) (Fitzgerald et al., 2020). Whilst the current slide-based assay still requires individual review by a trained histopathologist, recent work has demonstrated that weakly supervised deep learning models can detect goblet cells (and hence Barrett's oesophagus) from H&E slides. The proposed semi-automated clinical workflow is estimated to reduce expert pathologists' workload by 48% without sacrificing diagnostic performance, which may make large-scale application of the capsule sponge device for screening purposes more feasible from a logistical perspective (Bouzid et al., 2024).

Furthermore, a biomarker panel including atypia (including clear-cut dysplasia and atypia of uncertain significance) and p53 has been added to capsule sponge testing. The detection of atypia on H&E staining implies possible inflammation or pre-malignant dysplasia, whilst p53 is the most prevalent biomarker for malignant transformation in Barrett's oesophagus, as previously discussed. The addition of this biomarker profile to capsule sponge testing has been shown to be effective in the detection of dysplasia and neoplasia within the trial setting and enables risk-stratification of this patient cohort to appropriately prioritise endoscopy resources (Pilonis et al., 2022; Ross-Innes et al., 2017). A cross-

sectional study of 891 participants found a sensitivity of 89% and specificity of 84% for HGD, IMC or OAC when biomarkers for atypia and p53 were overexpressed on capsule sponge testing (Pilonis et al., 2022).

The combination of capsule sponge test result with clinical data to classify patients into risk groups (i.e., low, moderate or high-risk) has also demonstrated positive results within a recent large multicentre, prospective, pragmatic implementation study undertaken in the UK. When applied to 910 participants from the integrated diagnostic solution for early detection of oesophageal cancer (DELTA) and NHS England (NHSE) implementation pilot studies, this risk algorithm has been shown to substantially enrich for dysplasia. Results demonstrated a PPV for any dysplasia or cancer in the high-risk group (i.e., atypia and/or p53 positivity on capsule sponge testing) of 37.7% (95% CI 29.7-46.4). Within the low-risk group, the prevalence of HGD or cancer was 0.4% (95% CI 0.1-1.6), and the negative predictive value (NPV) for any grade dysplasia or cancer was 97.8% (95% CI 95.9-98.8). Furthermore, patients who demonstrated both atypia and p53 positivity on capsule sponge testing were found to be at highest risk of HGD or cancer (relative risk 135.8 [95% CI 32.7-564.0] relative to the low-risk group) (Tan et al., 2025). Based on these results, the authors proposed that surveillance strategies could be tailored to a capsule sponge test only method within the low-risk group, although societal guidelines are yet to provide formal recommendations on this topic. Of note, this study was published after the bulk of the work presented in this thesis was undertaken.

Endosign® is a new capsule sponge device using the same technologies as Cytosponge™ although manufactured by Cyted Ltd (Cambridge, UK). It is administered and retrieved in the same manner as described above, also utilising the biomarker panel of TFF3, atypia and p53 to risk stratify patients and undertake similar diagnostic analyses to the Cytosponge™ device (Cyted Ltd, 2024). It has been assumed that, as similarities exist in the design and function, the laboratory analysis has remained unchanged and the sponge manufacturer (Europlaz™) is the same, the existing evidence can be generalised to both devices (Europlaz, 2025; Scottish Health Technologies Group, 2023).

It is evident that capsule sponge testing has the potential to become a powerful diagnostic triage tool for Barrett's oesophagus and related dysplasia, however

evidence for its role beyond the trial setting remains limited. Furthermore, the current literature supporting capsule sponge testing originates from a research group based at the University of Cambridge and is yet to be externally validated. Moreover, its role as a surveillance strategy in patients with a previous diagnosis of Barrett's oesophagus has yet to be tested in a real-world setting.

1.3.2 Other non-endoscopic cell collection devices

The EsophaCap™ (CapNostics, Concord, North Carolina, USA) is another similar cell collection device: it uses MUC2 immunohistochemistry and methylated DNA markers (MDMs) to screen for IM, Barrett's dysplasia and OAC. The EsophaCap™ device has previously demonstrated 68% sensitivity and 91% specificity with insufficient samples (i.e. without columnar cells) excluded from the analysis. This device is very similar to Cytosponge™, however has a smaller 25mm diameter (Zhou et al., 2019).

EsoCheck™ (Lucid Diagnostics, New York, USA) is an inflatable surface-textured balloon device of 16 x 9mm attached to a silicon catheter. When the balloon is swallowed and presumed to be within the stomach, it is inflated with air and withdrawn to reach the lower oesophageal sphincter. It is then pulled for a further 6cm through the distal oesophagus, aiming to collect a more targeted oesophageal epithelial sample. On deflation of the balloon, negative pressure retracts epithelial samples into the device. The balloon tip is severed from the catheter on retrieval and placed in preservative solution before being sent for analysis. EsoCheck™ uses a 2-marker MDM panel of mVIM and mCCNA1 (EsoGuard assay, Lucid Diagnostics) to detect Barrett's oesophagus. The EsoGuard assay obtained 90.3% sensitivity and 91.7% specificity in a pilot study of 86 patients (Moinova et al., 2018).

Unfortunately, the EsophaCap™ and EsoCheck™ devices are not currently available for clinical use in the UK due to a lack of evidence base (Scottish Health Technologies Group, 2023). Furthermore, these devices are not approved for use within the ESGE guidelines (Weusten et al., 2023). EsophaCap™ was in fact recalled from clinical practice in the USA in 2024 following a series of device failures due to string detachment (Lucid Diagnostics, 2024).

1.3.3 Transnasal endoscopy for Barrett's screening

As previously discussed, TNE is permitted as an alternative investigation for diagnosis of Barrett's oesophagus (Weusten et al., 2023). TNE facilitates inspection of the oesophagus through nasal intubation: with the use of a narrower endoscope of <6mm diameter, TNE negates the need for sedation, improves patient tolerability and enables investigation within the outpatient setting (Sami et al., 2015). When compared to standard transoral endoscopy in a randomised cross-over study of 82 patients (49 Barrett's oesophagus compared to 33 controls), TNE demonstrated a sensitivity of 98% and specificity of 100% for the endoscopic diagnosis of Barrett's oesophagus, with 60% of patients reporting a preference for TNE (Shariff et al., 2012). More recently, a single-centre randomised cross-over study demonstrated 100% sensitivity and specificity for the endoscopic diagnosis of Barrett's oesophagus using TNE over standard endoscopy, but was limited by poorer image quality and reduced yield of IM on biopsies (Shariff et al., 2016). However, TNE as a population-based screening technique is limited by the ongoing requirement for an expert operator, therefore efforts to expedite its rollout into clinical practice have stalled (Tan, 2023).

1.3.4 Volatile organic compounds

Volatile organic compounds (VOCs) are gaseous by-products of cellular metabolism detected in a person's breath. Two devices that detect VOCs are available: the Hanna device and the electric nose or "e-nose" device. The Hanna device uses selected ion flow tube mass spectrometry to quantify VOCs (Belluomo et al., 2021). When tested in a diagnostic validation study including 163 OG cancers and 172 controls, the Hanna device demonstrated 80% sensitivity and 81% specificity for OG cancers, with an AUC of 0.85 (Markar et al., 2018). The e-nose device generates digital signals when VOCs interact with metal-oxide sensor arrays. Analysis through artificial neural networks extracts breath print differences, allowing for discrimination of Barrett's oesophagus, GORD and controls. The e-nose device demonstrated 82% sensitivity, 80% specificity and an AUC of 0.79 in a pilot study of 122 patients under endoscopic surveillance with a previous diagnosis of Barrett's dysplasia (Chan et al., 2017). A more recent study of 402 patients (129 Barrett's oesophagus, 141 GORD and 132 controls)

demonstrated 91% sensitivity and 74% specificity for Barrett's oesophagus, with an AUC of 0.91. Sensitivity improved to 96% in patients with long segment Barrett's ≥3cm (Peters et al., 2020). Although use of VOCs for Barrett's screening seems promising, these devices are yet to undergo large-scale testing and validation on a screening population not enriched for Barrett's oesophagus, limiting their clinical application at present.

1.3.5 Methylated DNA markers

Previous research has demonstrated methylation of CpG islands in various genes have been associated with many cancers: as such, MDMs have been proposed as a method of detecting Barrett's oesophagus or OAC. Benefits of such techniques include: the method is quantitative (therefore eliminates subjective bias); the process can be automated and scalable; it does not require histopathological expertise to interpret specimens (Iyer et al., 2021). Detection of MDMs in Barrett's oesophagus has been tested using the Cytosponge™ and EsophaCap™ devices. 4 MDMs (TFPI2, TRIST1, ZNF345 and ZNF569) have been shown to identify Barrett's oesophagus amongst Barrett's patients and reflux controls within a retrospective validation study using Cytosponge™ specimens from the BEST 2 case-control trial. TFPI2 demonstrated the most promise with 82.2% sensitivity and 95.7% specificity (Chettouh et al., 2018). A multicentre case-control marker elimination study using the EsophaCap™ device identified 5 MDMs (ZNF682, VAV3, NDRG4, FER1L4 and ZNF568) able to discriminate patients with Barrett's oesophagus compared to controls using random forest modelling with cross-validation. This panel demonstrated 92% sensitivity and 94% specificity for Barrett's oesophagus, with 95% of patients preferring EsophaCap™ to endoscopy (Iyer et al., 2020). More recently, this panel has been recalibrated on 86 patients and condensed to 3 best-performing MDMs (NDRG4, ZNF682, VAV3) (Iyer et al., 2021). This 3-MDM algorithm has recently been shown to demonstrate 100% sensitivity for HGD and 82% sensitivity for non-dysplastic Barrett's oesophagus in a study of 352 EsophaCap™ samples (overall sensitivity 88% and 84% specificity) (Iyer et al., 2024). An additional separate investigator study identified an alternative panel of MDMs (p16, NELL1, TAC1 and AKAP12) using the EsophaCap™ device, demonstrating 78.6% sensitivity, 92.8% specificity and an AUC of 0.93 in diagnosing Barrett's oesophagus over controls (Wang et al., 2019).

Although the combination of non-endoscopic cell collection devices and quantitative nature of MDMs make this technique appealing for targeted screening of Barrett's oesophagus, the current evidence base within an enriched cohort for Barrett's oesophagus or dysplasia has the potential to introduce systematic bias, which could overestimate the performance of biomarkers. Before this method can be adopted into clinical practice, detection of MDMs requires testing within a larger scale RCT conducted on the intended screening population (i.e. not enriched for Barrett's oesophagus or dysplasia) (Tan et al., 2021).

1.4 Introduction of capsule sponge testing in Scotland

1.4.1 Endoscopy service pressures

Clearly, significant inroads have been made in the development of less invasive techniques to detect Barrett's oesophagus, although current UK societal guidelines maintain UGI endoscopy is the gold standard investigation for diagnosis and surveillance of Barrett's oesophagus (Fitzgerald et al., 2014). Unfortunately, endoscopy services across Scotland have been under considerable strain in recent years. Current demand for endoscopy surpasses capacity: pressures on endoscopy services were significantly aggravated by the COVID-19 pandemic. As discussed previously, the COVID-19 pandemic saw the temporary cessation of all routine endoscopy procedures across the country, due to concerns surrounding infection risks from the aerosol-generating nature of the procedure and reallocation of resources during this challenging period (Edwards, Penman, & Coleman, 2020). This reduced accessibility to endoscopy resources resulted in large numbers of patients experiencing delays to planned Barrett's surveillance.

Although routine UGI endoscopy has subsequently resumed in Scotland, waiting lists remain substantial across the country: in September 2022, more than 2000 additional patients were awaiting UGI endoscopy and 30% fewer patients were meeting the recommended 6-week waiting time targets for urgent UGI endoscopy referrals compared to September 2019 (Figure 1.9) (Public Health Scotland, 2023b). This limited access to endoscopic services highlighted the increasing requirement to streamline the surveillance strategy for Barrett's

oesophagus and ensure appropriate prioritisation of resources for those high-risk individuals that would benefit most from endoscopic surveillance (Parasa et al., 2018). These system pressures also appeared to confirm that, as predicted, a future endoscopy-based screening strategy to detect Barrett's oesophagus in patients with chronic reflux symptoms would not be a feasible option financially or logistically (Fitzgerald et al., 2014). Therefore, there was a clear need for innovative, non-endoscopic technology to ease this burden on both patients and the healthcare system (Britton et al., 2018) and alternatives to UGI endoscopy for Barrett's diagnosis and surveillance were sought in Scotland.

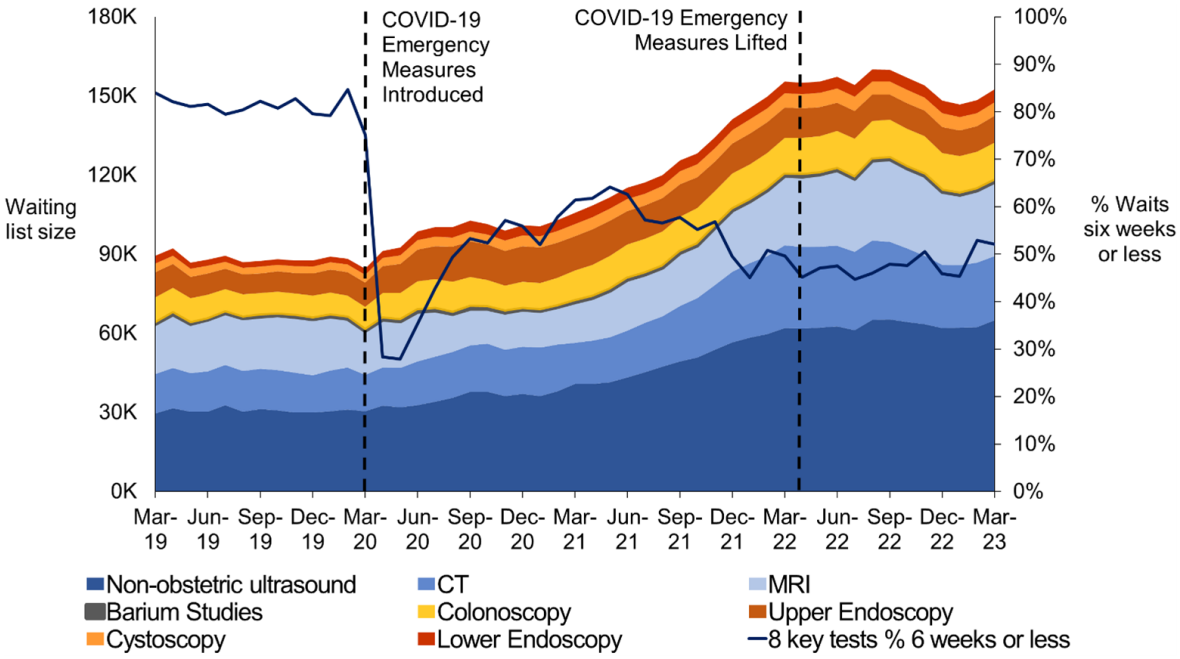

Figure 1.9 Scottish waiting list size by test
Figure 1.9 taken from Public Health Scotland demonstrating trends in the waiting list size and percentage of waits ongoing for 6 weeks or less at month-end, categorised by test, from 31 March 2019 to 31 March 2023 (Public Health Scotland, 2023b).

1.4.2 CytoSCOT programme

Trial data have clearly demonstrated that capsule sponge testing has the potential to become a vital diagnostic triage tool to detect those Barrett's patients at high-risk of dysplasia and OAC requiring UGI endoscopy. In 2020, the National Endoscopy Programme board was established in Scotland to address some of the issues described above. The Endoscopy and Urology Diagnostic Recovery and Renewal Plan, in conjunction with the Centre for Sustainable Delivery (CfSD), was formulated on the basis of the following themes: balancing demand and capacity; optimising clinical pathways; improving quality and

efficiency; workforce training and development; infrastructure, innovation and redesign (Scottish Government, 2021). This proposal incorporated the use of capsule sponge testing.

The national rollout of capsule sponge testing was expedited at pace across NHS Scotland in 2020 to reduce pressure on endoscopy services and facilitate recovery from the COVID-19 pandemic as part of a pilot scheme in the form of the CytoSCOT (SpongeCytologyOesophagealTest) programme (Scottish Government, 2021). With the financial support of full funding from the Scottish government, the CytoSCOT programme saw capsule sponge testing temporarily replace UGI endoscopy as the first-line investigation in the following patient cohorts: (1) patients undergoing surveillance for non-dysplastic Barrett's oesophagus; (2) low-risk symptomatic reflux patients referred to secondary care in the absence of red-flag symptoms. The device is currently used as a triage tool to identify patients requiring further investigation with UGI endoscopy based on biomarker panel results, with trained UGI endoscopy nurses facilitating service delivery within secondary care in the outpatient setting. Uptake of capsule sponge testing was embraced across eleven mainland Scottish health boards (NHS Ayrshire & Arran; NHS Borders; NHS Dumfries & Galloway; NHS Fife; NHS Forth Valley; NHS Grampian; NHS Greater Glasgow & Clyde; NHS Highland; NHS Lanarkshire; NHS Lothian; NHS Tayside). The CytoSCOT clinical subgroup was formed to implement delivery of the programme, including a named consultant responsible for each health board, nurse endoscopists administering the device and representatives from the CfSD (Scottish Health Technologies Group, 2023). Similar pilot programmes using capsule sponge testing were subsequently introduced in NHS England after the Scottish programme was initiated (Morris et al., 2024; NHS England, 2024; Tan et al., 2025).

Due to the unique emergent situation of the COVID-19 pandemic, capsule sponge testing was implemented nationally across Scotland without a convincing body of evidence to support its use in clinical practice, particularly in the context of Barrett's surveillance. A recent study by Landy et al., published after the CytoSCOT programme was implemented, presented the initial results from 10,577 capsule sponge tests performed across the UK (including 4639 tests in Scotland), categorising patients into risk groups and summarising the predicted impact on endoscopy services based on recommendations for referral as per the

capsule sponge test result and clinical details (Landy et al., 2023). Although this study hypothesised that capsule sponge testing was protecting scarce endoscopy resources by reducing the number of referrals for further investigation, it lacked follow-up endoscopic biopsy results to validate these claims and was unable to draw conclusions as to the true clinical effectiveness of capsule sponge testing as a triage tool.

Given the innovative nature of this technology, analysis of clinical outcomes in those patients undergoing capsule sponge testing is essential to improve understanding of the clinical application of the device and its ability to detect pre-cancerous change and early OAC within the real-world setting. Moreover, the continuation of the CytoSCOT programme and Scottish capsule sponge testing service beyond the pandemic was conditional, based on appropriate close audit and follow-up of the involved patient cohort to ensure significant pathology was not missed by capsule sponge testing in this group in the long-term (Scottish Government, 2021).

1.5 Aims of thesis

Capsule sponge testing has the potential to significantly benefit patient care by offering a simpler, faster alternative to UGI endoscopy, as well as reducing pressure on endoscopy services and enabling appropriate allocation of resources for high-risk patients. This thesis will present the results of the CytoSCOT programme and address the current void in the literature related to the use of capsule sponge testing in the real-world setting.

The overall aims of this thesis are as follows:

1) To assess whether capsule sponge testing is a safe and effective alternative to UGI endoscopy in Barrett's surveillance and symptomatic reflux patients by evaluating the correlation between capsule sponge biomarker test result and endoscopic histopathology biopsies

2) To present the first body of work analysing the use of capsule sponge testing in the real-world setting with a large patient cohort over a wide geographical location

3) To evaluate the impact of capsule sponge testing on the Scottish endoscopy service

The body of this thesis is comprised of seven chapters, the combination of which intend to address the three overall aims described above. The individual aim of each chapter is documented below:

- Chapter 2 aims to evaluate whether capsule sponge testing is effectively detecting high-risk Barrett's surveillance patients requiring urgent endoscopy within the real-world setting in the context of the CytoSCOT programme

- Chapter 3 aims to analyse whether the introduction of the CytoSCOT programme reduced delays to Barrett's surveillance, as well as evaluate whether delayed surveillance negatively impacts endoscopic histopathology results

- Chapter 4 aims to establish the impact of the introduction of capsule sponge testing on dysplasia detection rates and compare rates of dysplasia detection between those undergoing capsule sponge testing versus endoscopic surveillance in clinical practice in a single Scottish health board

- Chapter 5 aims to examine the effect the capsule sponge testing service had on Barrett's surveillance endoscopy services in a single Scottish health board

- Chapter 6 aims to analyse results of patients' next follow-up using either UGI endoscopy or repeat capsule sponge testing in the lower risk cohort of CytoSCOT patients previously undergoing capsule sponge testing for Barrett's surveillance, as well as evaluate whether results were comparable using the Cytosponge™ versus the EndoSign® capsule sponge devices

- Chapter 7 aims to evaluate whether capsule sponge testing is a safe and effective diagnostic triage tool for the investigation of reflux symptoms in the real-world setting in the context of the CytoSCOT programme

- Chapter 8 aims to establish whether presence of IM at endoscopy demonstrates a positive association with systemic inflammation and therefore whether the combination of capsule sponge result and serum markers of systemic inflammation can effectively rule out Barrett's oesophagus within the symptomatic reflux population

This thesis will aim to expand current understanding of the clinical application of capsule sponge biomarker testing with a view to establishing an evidence base that may permit formalisation of a clinical pathway for capsule sponge testing. If successful, this may potentially enable capsule sponge testing to become the standard of care for Barrett's surveillance and investigation of low-risk symptomatic reflux patients in Scotland in the future.

Chapter 2 Capsule sponge and biomarker testing to identify high-risk Barrett's patients requiring endoscopic investigation

The BEST 3 trial delivered a compelling argument for the use of capsule sponge testing in the detection of Barrett's oesophagus (Fitzgerald et al., 2020), with Chapter 1 exploring the evidence base for capsule sponge and biomarker testing in greater detail. However, the role of capsule sponge testing as a triage tool for surveillance in the cohort of patients with an established Barrett's diagnosis has yet to be fully evaluated: there is a paucity of evidence within the current available literature exploring the clinical application of this investigation in the real-world setting. Chapter 2 aims to address this by presenting the findings of a national pragmatic study analysing the results of the CytoSCOT programme. This study aims to ascertain whether capsule sponge testing is successfully identifying high-risk Barrett's patients requiring urgent endoscopy based on subsequent endoscopic histopathology biopsies.

This chapter is based on a publication in the British Journal of Surgery in 2024 (Chien, Glen, Penman, Cruickshank, et al., 2024). Permission to include this work was granted by the editorial team.

2.1 Introduction

As previously discussed, high-quality endoscopic Barrett's surveillance programmes not only facilitate early detection of neoplastic change but also enable therapeutic intervention in cases of dysplasia or early cancer. Although UGI endoscopy is presently the gold standard for Barrett's surveillance, this invasive procedure is not without limitation, as extensively discussed in Chapter 1. Furthermore, Barrett's surveillance programmes demand substantial clinical and administrative resources, and the endoscopy service in Scotland was under significant strain following the COVID-19 pandemic. The BOSS study has added further uncertainty to the debate surrounding the merits of current endoscopic surveillance (Old et al., 2025). However, there was scope for improvement and a requirement for new innovative technologies to enhance current Barrett's surveillance strategies even prior to the publication of the BOSS study, in addition to tackling the resultant endoscopy backlog from the pandemic. The CytoSCOT programme therefore provided an ideal opportunity to embrace change in the Scottish approach to Barrett's surveillance, and saw capsule sponge testing introduced across Scotland as an emergency response to the COVID-19 pandemic (Scottish Government, 2021).

Although the BEST 3 trial data have shown capsule sponge testing increases detection of Barrett's oesophagus ten times over the current standard of care within the reflux screening population (Fitzgerald et al., 2020), its role as a triage tool specifically within the Barrett's surveillance population remains unclear and the CytoSCOT programme was commenced in Scotland without a robust body of evidence to support its use in the real-world setting. This study is the first to analyse the correlation between capsule sponge test result and subsequent endoscopic histopathology biopsies in clinical practice. The aim of this study was to evaluate whether capsule sponge testing is effectively detecting high-risk Barrett's oesophagus patients requiring urgent endoscopy in the real-world setting to validate the ongoing use of capsule sponge testing for Barrett's oesophagus surveillance in clinical practice within the context of the CytoSCOT programme.

2.2 Methods

During the two-year dedicated research period, a master CytoSCOT database was devised using Microsoft Excel. This included all capsule sponge tests performed using the Cytosponge™ device across NHS Scotland between 14 September 2020 and 30 April 2023 identified from prospectively maintained local databases from 11 mainland Scottish health boards. 5590 tests were included in the master database, with 66 data variables collected per test performed. Online clinical systems including Clinical Portal, Scottish Care Information (SCI) Store (Version 8.5) and SCI Gateway (R 20.0) were utilised for data collection, with national access granted remotely through the Centre for Sustainable Delivery. This master database was manually re-checked every six months during the research period to ensure endoscopic and follow-up data were updated and accurate for each test performed for CytoSCOT audit purposes and prior to data analysis for each chapter in this thesis. Indication for capsule sponge test was defined as either: 1) Barrett's surveillance; 2) investigation of reflux symptoms.

The master CytoSCOT database was utilised for data analysis in Chapters 2, 3, 6, 7 and 8 of this thesis. There is therefore a degree of overlap in the samples included in the data analysis of the above chapters. However, each respective chapter clearly states which cohort of patients were eligible for inclusion within its Methods section, with variables including indication for test, date of test and result of initial capsule sponge test clearly documented as the relevant inclusion criteria for each chapter, obtained from the master database.

For the purpose of this chapter, all patients who underwent capsule sponge testing using the Cytosponge™ OCCD for surveillance of Barrett's oesophagus between 14 September 2020 and 30 April 2023 were identified from the CytoSCOT database. Patients were recruited for capsule sponge testing if previously entered in local Barrett's surveillance programmes, where prior endoscopy demonstrated macroscopic changes consistent with Barrett's oesophagus (i.e. salmon-coloured mucosa progressing cephalad from the GOJ). The presence of IM on endoscopic biopsies was not considered a prerequisite for entry into surveillance, as per the BSG guidelines (Fitzgerald et al., 2014). All patients who subsequently underwent UGI endoscopy within 12 months of capsule sponge test with available histopathology results were identified and

included in this analysis. Forward referral for endoscopy was subject to clinicians' discretion at local health board level.

All samples included in this analysis were processed centrally in a single UK-based diagnostic laboratory (Cyted Ltd). As previously described in the trial context and within Chapter 1, all capsule sponge devices were placed in BD SurePath Preservative Fluid, with cells retrieved and processed to paraffin blocks (Fitzgerald et al., 2020). Superficial and deep sections were used to prepare both H&E slides to assess cellular atypia (including clear-cut dysplasia and atypia of uncertain significance) and FFPE slides to perform TFF3 and p53 immunohistochemistry simultaneously. p53 staining with an intensity of three was considered significant as previously published (Fitzgerald et al., 2020). In accordance with BSG guidance for reporting of dysplasia, all samples with suspected atypia or p53 positivity were reviewed by a second pathologist. Processed Cyted Ltd pathology reports were then returned to each health board, with ongoing clinical management decided locally.

Individual patient records were interrogated, with baseline demographics, previous Barrett's endoscopic morphology and pathology, and capsule sponge test result recorded retrospectively to form the national CytoSCOT registry. Indication for UGI endoscopy, endoscopic biopsy results and subsequent clinical management were also documented. Barrett's segment length was determined using the Prague classification: short segment was defined as M <3cm; long segment was defined as M ≥3cm to <10cm; ultra-long segment was defined as M ≥10 cm. Patients were further subcategorised into ultra-low, low, medium and high-risk groups based on capsule sponge test results and clinical details as previously published by Landy et al. (Landy et al., 2023). This is summarised in Figure 2.1.

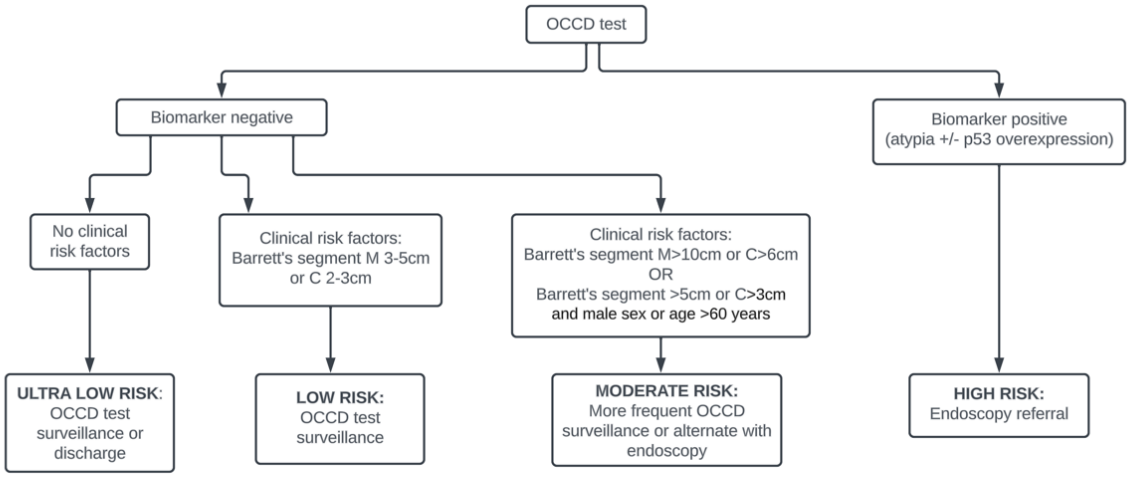

Figure 2.1 Decision tree to categorise risk group based on biomarker status

The primary outcome was presence of HGD or cancer within endoscopic biopsies. The secondary outcome was diagnosis of any grade of dysplasia within the endoscopic biopsies, based on Barrett's risk group. Exclusion criteria included: capsule sponge test for reflux symptoms; patients who did not undergo UGI endoscopy within 12 months of capsule sponge test; outstanding histopathology results at the time of analysis; previous endoscopic treatment for dysplasia.

Patients were not actively involved in study design or analysis. Whilst Patient and Public Involvement and Engagement (PPIE) is acknowledged to be of crucial importance in real-world implementation studies, the rapid roll-out of this service in response to the COVID-19 pandemic unfortunately precluded PPIE in the studies undertaken within the CytoSCOT programme. Ethical approval was obtained for this study via information governance teams in each health board, in addition to national Public Benefit and Privacy Panel (PBPP) approval from the Caldicott guardian.

Continuous parameters were presented as median and interquartile range (IQR) and categorical data as counts and percentages. The Chi-squared test was performed for comparison of categorical variables, where appropriate. A p value of ≤ 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 28.0 (SPSS Inc., Chicago, Illinois, USA).

2.3 Results

5590 capsule sponge tests were performed across 11 Scottish health boards within the study period; 4204 tests (75.2%) were performed on 3745 patients for Barrett's surveillance. The additional 459 capsule sponge tests were repeat procedures for insufficient samples (i.e. insufficient glandular groups for analysis in the surveillance setting; n=229) or clarification of initial results, where the first test showed TFF3 negativity (n=230).

Figure 2.2 demonstrates patient workflow and initial clinical management, categorised by risk group as demonstrated in Figure 2.1. Of note, Figure 2.2 includes all tests performed for Barrett's surveillance (n=4204). 322/327 high-risk patients (98.5%) were referred for urgent endoscopy. 697/3745 patients (18.6%) were discharged from Barrett's surveillance based on capsule sponge test result and clinical details: this included those within the ultra-low risk group with 2 negative tests for IM or those >80 years of age. 75/853 patients (8.8%) were discharged within the moderate risk group: in all cases, this was due to age and comorbidity.

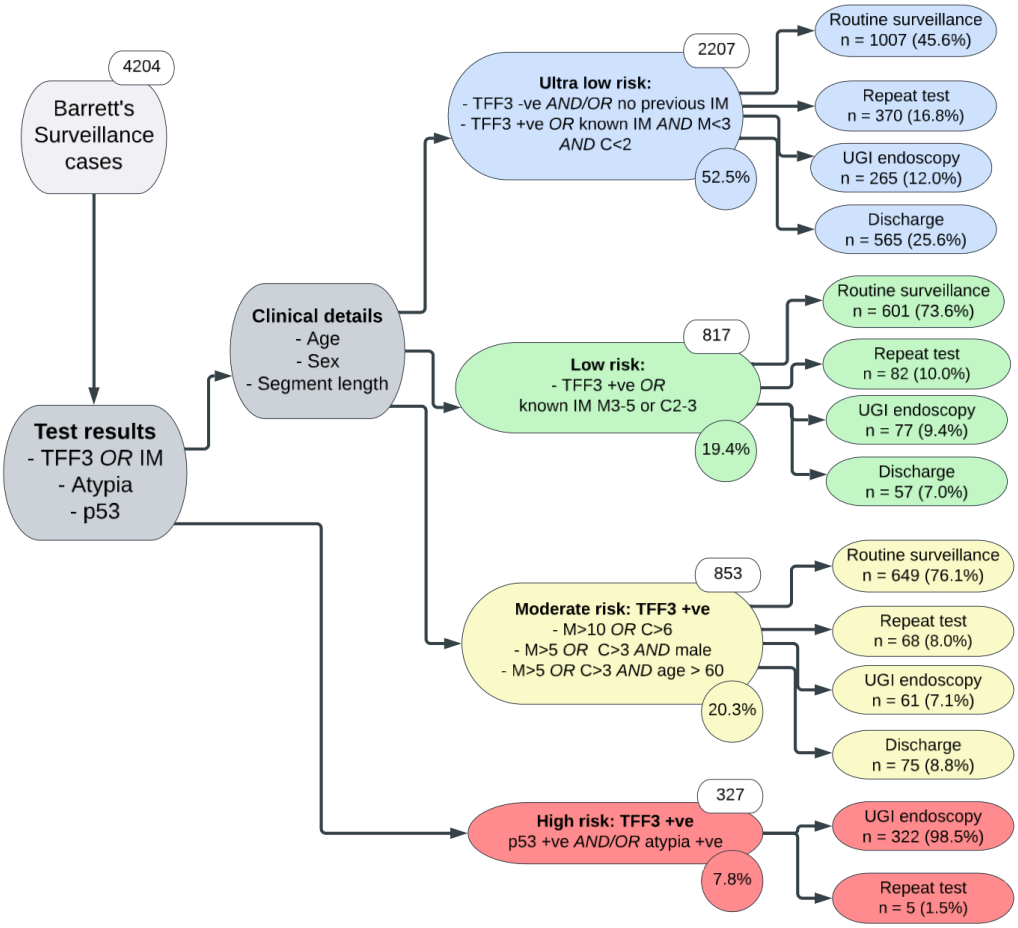

Figure 2.2 Patient workflow and clinical management within the Barrett's surveillance cohort, categorised by risk group (n=4204)

608/3745 patients (16.2%) underwent UGI endoscopy within 12 months of capsule sponge test and were then included in this analysis (Figure 2.3). The median age was 67 years (IQR 60-73) and 427/608 patients (70.2%) were male. The median follow-up time was 14 months (IQR 8-22). The median time from last surveillance endoscopy to capsule sponge test was 38 months (IQR 29-48), with 509/608 patients (83.7%) demonstrating IM on previous endoscopic biopsies.

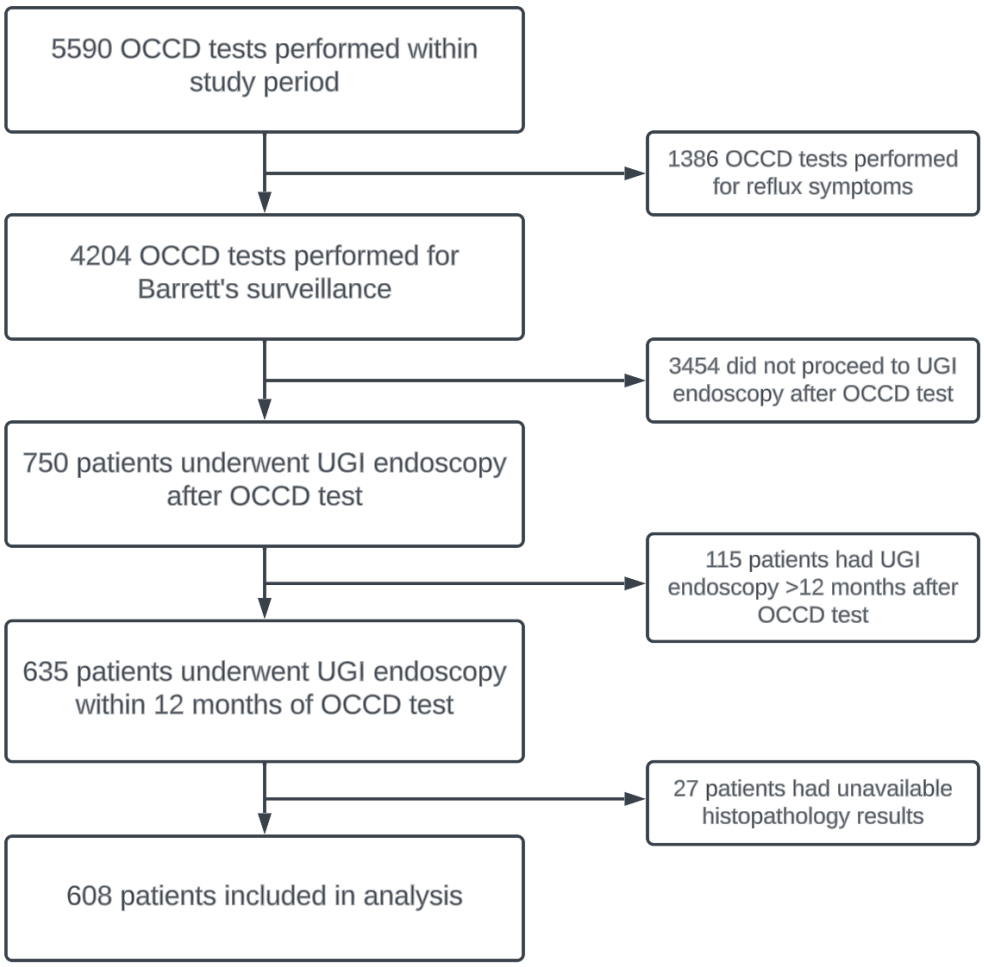

Figure 2.3 Inclusion criteria for data analysis

Table 2.1 summarises capsule sponge test results in the whole Barrett's surveillance cohort (n=4204). Table 2.2 demonstrates capsule sponge biomarker result according to Barrett's segment length within the cohort of patients included within this analysis. Within this cohort, 124/608 tests (20.4%) were returned as insufficient: in the whole Barrett's cohort, 456/4204 tests (10.8%) were deemed insufficient for analysis (Table 2.1). 229/456 initial insufficient tests (50.2%) underwent repeat capsule sponge testing, 131 (28.7%) proceeded direct to UGI endoscopy and 96 (21.1%) awaited repeat testing at the time of analysis. Of the 229 repeated insufficient tests, 184 (80.3%) yielded a satisfactory sample on second test: all patients with a second insufficient test were referred for UGI endoscopy. Of the 608 capsule sponge tests included, 325 (53.5%) demonstrated TFF3 positivity (including those who were also atypia and/or p53 positive). Patients were significantly more likely to demonstrate

TFF3 positivity with increasing segment length. TFF3 was positive in 80/282 short segment patients (28.4%); 198/278 long segment patients (71.2%); and 47/48 ultra-long segment patients (97.9%) (p <0.001). Patients with long or ultra-long Barrett's segments were significantly more likely to be high risk (atypia and/or p53 positive) compared to those with short segments (214/326 vs. 86/282 patients: 65.6% vs. 30.5%; p <0.001). 43/48 patients (89.6%) with ultra-long Barrett's segments were within the high-risk group.

Table 2.1 Capsule sponge test results in the whole Barrett's surveillance cohort (n=4204)

Capsule sponge test result	N (%)
TFF3 negative	1451 (34.5%)
TFF3 positive only	1970 (46.8%)
Atypia only	191 (4.5%)
p53 only	30 (0.7%)
Atypia *and* p53 positive	106 (2.5%)
Insufficient	456 (10.8%)

Table 2.2 Capsule sponge test results according to length of Barrett's segment (n=608)

Capsule sponge test result	All (n=608)	Short segment (n=282)	Long segment (n=278)	Ultra-long segment (n=48)	P value
TFF3 negative	136 (22.4%)	110 (39.0%)	26 (9.4%)	0 (0%)	<0.001
TFF3 positive only	48 (7.9%)	13 (4.6%)	30 (10.8%)	5 (10.4%)	0.020
Atypia only	179 (29.4%)	58 (20.6%)	94 (33.8%)	27 (56.3%)	<0.001
p53 only	24 (3.9%)	7 (2.5%)	15 (5.4%)	2 (4.2%)	0.208
Atypia *and* p53	97 (16.0%)	21 (7.4%)	62 (22.3%)	14 (29.2%)	<0.001
Insufficient	124 (20.4%)	73 (25.9%)	51 (18.3%)	0 (0%)	<0.001

The median time to endoscopy from capsule sponge test was 2 months (IQR 1-5). Table 2.3 summarises indication for endoscopy. 108/136 patients (79.4%) with a TFF3 negative capsule sponge test result underwent UGI endoscopy to assess suitability for discharge from the surveillance programme. 300/608 endoscopies (49.3%) were performed in response to atypia and/or p53 positivity on capsule sponge testing (i.e. high-risk group). Seattle protocol biopsies were followed in 150/300 (50.0%) high-risk cases (22/300 had missing data). 1 patient (0.2%) presented with a delayed UGI bleed 6 months after capsule sponge testing. 27 high-risk patients were not included in this analysis: 12 declined invitation to UGI endoscopy, 8 underwent UGI endoscopy >12 months after capsule sponge

test, 3 had repeat capsule sponge testing in the first instance, 3 died from unrelated causes, and 1 did not have available biopsy results at the time of analysis.

Table 2.3 Indication for UGI endoscopy (n=608)

Indication	N (%)
Abnormal capsule sponge result (atypia +/- p53)	300 (49.3%)
Insufficient capsule sponge result	123 (20.2%)
TFF3 negative capsule sponge result	108 (17.8%)
Investigation of red flag UGI symptoms	46 (7.6%)
Assessment of ulcer healing (detected by capsule sponge)	28 (4.6%)
Capsule sponge detachment	2 (0.3%)
Delayed UGI bleed	1 (0.2%)

Table 2.4 demonstrates endoscopic biopsy results by Barrett's segment length. 50/608 patients (8.2%) had endoscopic biopsies showing HGD, IMC or invasive cancer: this equated to 50/300 patients (16.7%) within the high-risk group. When extrapolated to the whole Barrett's cohort (n=3745), the prevalence of HGD, IMC or invasive cancer was 1.3%. 88/3745 patients (2.3%) had a normal capsule sponge test result (i.e. TFF3, atypia and p53 negative) but had endoscopic biopsies demonstrating IM, IND or LGD: 22/88 of these patients (25.0%) had long-segment Barrett's. No patients had a normal capsule sponge test result but subsequently had endoscopic biopsies demonstrating HGD, IMC or invasive cancer. Analysis of endoscopic biopsy results by capsule sponge test result is demonstrated in Table 2.5.

Table 2.4 Endoscopic biopsy results according to Barrett's segment length (n=608)

Endoscopic biopsy result	All (n=608)	Short segment (n=282)	Long segment (n=278)	Ultra-long segment (n=48)	P value
No IM	95 (15.6%)	80 (28.4%)	14 (5.0%)	1 (2.1%)	<0.001
Non-dysplastic Barrett's oesophagus	375 (61.7%)	157 (55.7%)	189 (68.0%)	29 (60.4%)	0.011
IND	35 (5.7%)	18 (6.4%)	15 (5.4%)	2 (4.2%)	0.781
LGD	53 (8.7%)	13 (4.6%)	32 (11.5%)	8 (16.6%)	0.002
HGD	26 (4.3%)	9 (3.2%)	13 (4.7%)	4 (8.3%)	0.241
Intramucosal carcinoma	12 (2.0%)	2 (0.7%)	8 (2.9%)	2 (4.2%)	0.095
Adenocarcinoma	11 (1.8%)	3 (1.0%)	6 (2.1%)	2 (4.2%)	0.276
Squamous cell carcinoma	1 (0.2%)	0 (0%)	1 (0.4%)	0 (0%)	0.552

Table 2.5 Endoscopic biopsy results compared to capsule sponge test result (n=608)

Endoscopic biopsy result	Capsule sponge test result					
	TFF3 negative (n=136)	TFF positive only (n=48)	Atypia only (n=179)	p53 only (n=24)	Atypia and p53 (n=97)	Insufficient (n=124)
No IM	48 (35.3%)	8 (16.7%)	11 (6.1%)	0 (0%)	0 (0%)	28 (22.6%)
Non-dysplastic Barrett's oesophagus	80 (58.8%)	37 (77.1%)	121 (67.6%)	17 (70.8%)	35 (36.1%)	85 (68.5%)
IND	5 (3.7%)	1 (2.1%)	15 (8.4%)	1 (4.2%)	11 (11.3%)	2 (1.6%)
LGD	3 (2.2%)	2 (4.2%)	20 (11.2%)	5 (20.8%)	18 (18.6%)	5 (4.0%)
HGD	0 (0%)	0 (0%)	6 (3.4%)	0 (0%)	17 (17.5%)	3 (2.4%)
IMC	0 (0%)	0 (0%)	2 (1.1%)	1 (4.2%)	9 (9.3%)	0 (0%)
OAC	0 (0%)	0 (0%)	3 (1.7%)	0 (0%)	7 (7.2%)	1 (0.8%)
OSCC	0 (0%)	0 (0%)	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)

Table 2.6 demonstrates endoscopic biopsy results by Barrett's surveillance risk group (n=484): patients with an insufficient capsule sponge test result were excluded, as an insufficient result triggered a different clinical decision-making pathway (i.e. repeat capsule sponge test or routine endoscopy). Of the 50 patients with HGD, IMC or invasive cancer, 46 (92.0%) were high-risk (i.e. atypia and/or p53 overexpression on capsule sponge testing), triggering urgent

endoscopy. The remaining 4 patients underwent endoscopy due to insufficient capsule sponge test results (Table 2.5). 43/48 (89.6%) patients with LGD were identified as high-risk on capsule sponge testing. 37/50 (74.0%) patients underwent EMR and/or RFA, 7/50 (14.0%) underwent oesophagectomy, 3/50 (6.0%) were treated with radical chemoradiotherapy, and 3/50 (6.0%) were palliated due to additional co-morbidities. 2/3745 Barrett's surveillance patients (0.1%) died following a diagnosis of OAC.

Table 2.6 Endoscopic biopsy results compared with Barrett's surveillance risk group (n=484)

Endoscopic biopsy result	All patients (n=484)	Ultra-low risk (n=126)	Low risk (n=37)	Moderate risk (n=21)	High risk (n=300)
No IM	67 (13.8%)	48 (38.1%)	5 (13.5%)	3 (14.3%)	11 (3.7%)
Non-dysplastic Barrett's oesophagus	290 (59.9%)	70 (55.5%)	31 (83.8%)	16 (76.2%)	173 (57.7%)
Indefinite for dysplasia	33 (6.8%)	5 (4.0%)	0 (0%)	1 (4.8%)	27 (9.0%)
LGD	48 (9.9%)	3 (2.4%)	1 (2.7%)	1 (4.8%)	43 (14.3%)
HGD	23 (4.8%)	0 (0%)	0 (0%)	0 (0%)	23 (7.7%)
Intramucosal carcinoma	12 (2.5%)	0 (0%)	0 (0%)	0 (0%)	12 (4.0%)
Adenocarcinoma	10 (2.1%)	0 (0%)	0 (0%)	0 (0%)	10 (3.3%)
Squamous cell carcinoma	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)

2.4 Discussion

The present study is the first in clinical practice across a wide geographical location to demonstrate the diagnostic accuracy of capsule sponge testing and feasibility of its ongoing use as a diagnostic triage tool to UGI endoscopy within the Barrett's surveillance cohort.

Previous literature demonstrates incidence rates of 8%, 4% and 1-2% of LGD, HGD and OAC in patients undergoing endoscopic Barrett's surveillance respectively (de Jonge et al., 2010; Peters et al., 2019). These initial results present similar overall incidence rates at endoscopic biopsy, implying that capsule sponge

testing is successfully identifying those patients requiring urgent UGI endoscopy, although longer-term endoscopic data are required to validate this claim.

Prior to the COVID-19 pandemic, all Barrett's patients underwent endoscopic surveillance, whereas only 16.2% of the capsule sponge cohort required endoscopy within 12 months of capsule sponge test. Estimates suggest the CytoSCOT programme has saved NHS Scotland in excess of £200,000 for Barrett's surveillance since its inception in September 2020 (National Institute for Health and Care Excellence, 2020), while safely identifying those at increased cancer risk. These financial savings are expected to multiply with the continued expansion of capsule sponge testing in Scotland.

Within the HGD, IMC or invasive cancer cohort, 92.0% had an abnormal capsule sponge test result (atypia and/or p53 overexpression), triggering urgent referral for UGI endoscopy: this increased to 100% once insufficient tests were removed. Previous trial data have supported the use of the capsule sponge biomarker panel in conjunction with clinical details to generate a Barrett's risk group, thereby enabling identification of patients at increased risk of dysplasia or malignancy (Landy et al., 2023; Pilonis et al., 2022). These real-world results support this and demonstrate Barrett's risk group is a useful predictor of presence of dysplasia or cancer, which could aid prioritisation of limited endoscopy resources. The present data suggest capsule sponge testing has a PPV of 16.7% for HGD, IMC or invasive cancer, compared to 30.8% in previously published literature (Pilonis et al., 2022): this may be due to the lower adherence to Seattle protocol biopsies within this cohort, and reflects the real-world nature of this retrospective analysis. Furthermore, as previously discussed, glandular atypia can be classified as clear-cut dysplasia or atypia of uncertain significance (AUS). AUS can be due to inflammation or be considered IND: when these cases are excluded, the PPV of these biomarkers for HGD, IMC or invasive cancer increases (Tan et al., 2025). Due to small numbers of cases of glandular atypia with clear-cut dysplasia within this cohort, the atypia positive patients were not sub-categorised into these groups. The inclusion of AUS cases may also explain the lower PPV seen in this cohort. This highlights the importance of stringent follow-up of the remaining 83.3% of the high-risk group: this cohort is likely to benefit from more frequent surveillance than the current recommended intervals by the BSG (Fitzgerald et al., 2014).

Increasing segment length is an independent risk factor for the development of dysplasia (Anaparthy et al., 2013). LGD was significantly more common in those with longer segments, but not HGD or cancer. Patients with longer segments were significantly more likely to demonstrate atypia and/or p53 overexpression on capsule sponge testing, again suggesting that these patients may benefit from more stringent and frequent surveillance to detect dysplastic change.

Notably, the 608 patients included within the analysis are a higher-risk group compared to the overall Barrett's surveillance population: 300/608 patients (49.3%) demonstrated positive capsule sponge biomarkers for atypia and/or p53. Within the described cohort undergoing UGI endoscopy, only 48/608 patients (7.9%) had a capsule sponge test demonstrating TFF3 positivity alone. Most patients with a TFF3 positive only result within the whole Barrett's cohort were pushed to their next surveillance interval as per BSG guidelines (Fitzgerald et al., 2014): they did not undergo UGI endoscopy and were not included in this analysis. This also explains the higher proportion of insufficient (20.4%) and TFF3 negative (22.4%) results within this cohort compared to the wider Barrett's group (Table 2.1), as repeat insufficient tests or a TFF3 negative result may trigger referral for endoscopy. Where the capsule sponge test was insufficient for analysis, repeat capsule sponge testing was recommended in the first instance, although the decision to proceed directly to UGI endoscopy was left at clinicians' discretion. In TFF3 negative cases, there was clinical variability on decision to continue surveillance or offer endoscopy to clarify safe discharge from the programme. Notably, patients were only discharged from surveillance if IM was not identified on endoscopic biopsies.

Of the 608 patients, 22.4% had a TFF3 negative result, including 9.4% of patients with long-segment Barrett's. This may be due to sampling error, as well as the inclusion of cases without true Barrett's oesophagus (e.g. those with an irregular Z-line) and short segments <1cm in this dataset. This is supported by the fact that the proportion of TFF3 positive tests increased with segment length. Repeat capsule sponge testing was recommended in the first instance for longer Barrett's segments to clarify if the result was spurious. The dataset also highlights a small proportion of patients (88/3745, 2.3%) within the TFF3 negative group who had endoscopic biopsies confirming either IM or LGD (i.e. "missed" pathology on capsule sponge testing). This is complicated by the fact

that patients with a TFF3 negative result did not all routinely undergo UGI endoscopy. The aim of capsule sponge testing is to risk stratify to reduce burden on patients and endoscopy services while not missing cancer diagnoses: previous literature has suggested HGD may be detected in <2% in the low risk group and approximately 8% in the moderate risk group (Pilonis et al., 2022). Although UGI endoscopy remains the gold standard for Barrett's surveillance, as discussed in Chapter 1, endoscopic biopsies are subject to significant sampling bias and may be fraught with user error. Post-endoscopy incident oesophageal cancer is estimated to account for 14% of the oesophageal cancer burden (Vajravelu et al., 2022), with published missed OAC rates in the Barrett's surveillance population within 1 year of index endoscopy of 23-30% (van Putten et al., 2018; Visrodia, Singh, et al., 2016a; Wani et al., 2023). Thus, neither UGI endoscopy nor the capsule sponge is the perfect test, with both investigations posing the small risk of missed pathology. Reassuringly, there were no cases of missed cancers within 1 year of capsule sponge testing within this cohort; however, more follow-up data are required in the low and moderate risk groups, as well as those in the high-risk group with no evidence of dysplasia within endoscopic biopsies, before definitive conclusions can be drawn.

This study has several limitations. Forward referral for endoscopy was not standardised nationally, with this decision left at clinicians' discretion locally. This resulted in significant heterogeneity in decision-making pathways between local health boards, reflecting the real-world nature of this work and the requirement for pragmatism when interpreting its results. Development of national guidelines for appropriate surveillance methods will be important as follow-up data emerge. In the present study, the median follow-up time is currently only 14 months: continued follow-up of this cohort will be required to ensure no cancer diagnoses are missed over a longer time period. It is also not possible to draw definitive conclusions as to whether future follow-up should be with capsule sponge testing versus endoscopy at this juncture. Although it may be hypothesised that capsule sponge testing could replace endoscopy as the first-line investigation for Barrett's surveillance, analysis of patients undergoing repeat capsule sponge testing at their next surveillance interval will be critical to formalise this pathway long term and will be discussed in further detail later in this thesis. Furthermore, endoscopic biopsy results and length of surveillance

period prior to the CytoSCOT programme were not accessible on a national level to enable accurate comparison between the two Barrett's surveillance modalities. Finally, there was not access to national data for the patients who proceeded direct to endoscopic surveillance, either due to contraindications to capsule sponge testing or inability to swallow the capsule sponge device: this number is hypothesised to be small compared to the proportion who underwent capsule sponge testing, as the latter became the standard of care for Barrett's surveillance during the study period across Scotland.

To conclude, within the Scottish real-world setting, capsule sponge testing demonstrates sound diagnostic accuracy as a triage tool in highlighting high-risk Barrett's surveillance patients requiring urgent UGI endoscopy. Moving forward, this evidence suggests that capsule sponge testing should have a standardised role in clinical practice to support overwhelmed endoscopy and histopathology services within the Barrett's population. While urgent investigation is recommended for high-risk patients, further data are required to establish definitive future surveillance guidelines.

Chapter 3 Effect of delayed Barrett's surveillance on histopathological findings

As previously mentioned, traditional endoscopic Barrett's surveillance programmes were significantly impacted by the COVID-19 pandemic, following the national recommendation that all routine endoscopy services should be temporarily paused across the country while the health service focused on tackling the pandemic (Edwards, Penman, & Coleman, 2020). With delays to Barrett's surveillance becoming increasingly commonplace across Scotland, this provided the impetus for the national rollout of capsule sponge testing in the context of the CytoSCOT programme. Furthermore, whilst delayed Barrett's surveillance was presumed to be detrimental and result in increased cases of dysplasia and cancer anecdotally, the true impact of delayed surveillance on histopathology yield has yet to be formally established in the literature. While Chapter 2 provided compelling evidence for the use of capsule sponge testing in clinical practice, this chapter aims to analyse whether the introduction of the CytoSCOT programme successfully confronted this clinical challenge and reduced delays in Barrett's surveillance, whilst also evaluating the impact of delayed surveillance on endoscopic histopathology results.

This chapter is based on a publication in Diseases of the Esophagus in 2024 (Chien, Glen, Penman, Bryce, et al., 2024). Permission to include this work was granted by the editorial team.

3.1 Introduction

The stepwise progression of neoplastic change demonstrated in dysplastic Barrett's oesophagus renders the disease an ideal target for dedicated surveillance programmes. However, this service demands significant resources for a reasonably low pathology yield and surveillance programmes faced further difficulties when routine endoscopy was curtailed during the COVID-19 pandemic (Edwards, Penman, & Coleman, 2020).

Therefore, as discussed in previous chapters, the CytoSCOT programme was launched in September 2020, as alternatives to endoscopy were sought to safely enable the continuation of the Scottish Barrett's surveillance programme and minimise the risk of cancer progression. As a result, Scotland was one of the first countries to facilitate the clinical application of capsule sponge testing for Barrett's surveillance (Scottish Government, 2021), and was the first to evaluate its diagnostic accuracy in the real-world setting (Chien, Glen, Penman, Cruickshank, et al., 2024), as described in Chapter 2. To reiterate, capsule sponge testing using the OCCD is currently used as a triage tool to identify high-risk Barrett's patients requiring urgent endoscopy (Chien, Glen, Penman, Cruickshank, et al., 2024; Vieth & Neurath, 2022), therefore allowing continuation of Barrett's surveillance by reducing endoscopy demand on an already over-stretched service and improve endoscopy waiting lists.

As referenced in Chapter 1, Barrett's surveillance is performed at regular time intervals in accordance with societal guidelines but a solid evidence base to support the timings of such surveillance intervals does not exist. Whilst anticipated to be unfavourable anecdotally, the true impact of delayed Barrett's surveillance on endoscopy pathology pattern has not been reported to date within the current literature. The COVID-19 pandemic, coupled with the introduction of the CytoSCOT programme, provided a unique opportunity to evaluate the impact of delayed surveillance on patient outcomes.

Chapter 3 aims to evaluate whether the CytoSCOT programme has improved the delays to Barrett's surveillance inflicted by the COVID-19 pandemic, as well as whether delayed Barrett's surveillance has negatively impacted endoscopic pathology patterns within this patient cohort.

3.2 Methods

Prospectively maintained local databases were used to identify all patients who underwent Barrett's surveillance using the OCCD (Cytosponge™) across 11 mainland Scottish health boards, as described in Chapter 2. All patients signed an NHS consent form before undergoing capsule sponge testing. All capsule sponge tests were processed centrally in a UK-based diagnostic laboratory (Cyted Ltd), as previously described in Chapter 2. Processed results were returned to the requesting hospital, with ongoing management decided locally.

Patients who underwent capsule sponge testing for Barrett's surveillance within a 2-year period between 14 September 2020 and 13 September 2022 were included in this retrospective analysis, with all capsule sponge tests for investigation of reflux symptoms excluded. Patients were dichotomised into two groups, with those undergoing capsule sponge testing in Year 1 (14/9/2020 to 13/9/2021) compared to those in Year 2 (14/9/2021 to 13/9/2022).

Individual patient electronic records were interrogated to record baseline patient demographics, previous Barrett's endoscopic morphology and pathology (including Prague classification), and capsule sponge test result to form the national CytoSCOT registry. Long segment Barrett's oesophagus was defined as M >3cm using the Prague classification. Patients were defined as high-risk if capsule sponge test biomarkers were positive for atypia and/or p53.

The time interval from last endoscopy to capsule sponge test in months was recorded. In addition, the recommended Barrett's surveillance interval in months from last endoscopy was also recorded from patients' notes as per local guidelines: this was subtracted from the time interval from last endoscopy to capsule sponge test to calculate the delay to Barrett's surveillance. This was defined as delayed if the difference between these dates was greater than 3 months, making allowances for reasonable patient-directed or administrative delays given the real-world context of this study. All repeat capsule sponge tests were excluded from analysis. Patients who proceeded to undergo endoscopy within 12 months of capsule sponge test were identified, with indication for endoscopy, endoscopic biopsy results, time to pathology result, and ongoing clinical management recorded.

Continuous parameters were presented as median and IQR, and categorical data as counts and percentages. The Chi-squared test was performed for comparison of categorical variables, and the Mann-Whitney U test was performed for comparison of continuous variables, where appropriate. A p value of ≤0.05 was considered statistically significant.

Ethical approval was obtained on a national level from the Caldicott guardian, as well as from local information governance teams within each health board, to undertake this analysis.

3.3 Results

A total of 3223 capsule sponge tests were included in this analysis: 1478 tests were performed in Year 1; 1745 tests were performed in Year 2. The median follow-up time was 16 months (IQR 10-21). Table 3.1 demonstrates patient demographics, which were largely comparable between the two cohorts. Of note, there was a significantly longer median delay in Year 1 compared to Year 2 (9 months vs. 5 months; p <0.001), and the proportion of patients with delayed surveillance was significantly higher in Year 1 compared to Year 2 (72.6% vs. 57.0%; p <0.001).

Table 3.1 Patient demographics by year of study

Demographic	Year 1	Year 2	Significance (p value)
Number of capsule sponge tests performed for Barrett's surveillance	1478	1745	-
Proportion male, n (%)	1010 (68.3%)	1187 (68.0%)	0.849
Median age (years), median (IQR)	67 (59 – 73)	66 (58 – 73)	0.409
Median body mass index ($kg/m^{2)}$, median (IQR)	28.0 (25.2 – 31.3)	27.9 (25.0 – 31.5)	0.853
Proportion positive smoking history, n (%)	638 (48.3%)	732 (46.8%)	0.433
Proportion PPI use, n (%)	1418 (95.9%)	1673 (95.9%)	0.924
Long segment Barrett's (Prague M >3cm)	636 (43.0%)	586 (33.6%)	<0.001
Median delay to Barrett's surveillance, months (IQR)	9 (3 – 13)	5 (1 – 16)	<0.001
Proportion delayed Barrett's surveillance, n (%)	1073 (72.6%)	995 (57.0%)	<0.001

Table 3.2 demonstrates capsule sponge test results by worst pathology result. Patients were significantly more likely to have an abnormal capsule sponge test result (i.e. atypia and/or p53 positive), and therefore be deemed high-risk, in Year 1 compared to Year 2 (12.0% vs. 5.3%; p <0.001).

Table 3.2 Capsule sponge test results by year of study

Capsule sponge test result	All (n=3223)	Year 1 (n=1478)	Year 2 (n=1745)	Significance (p value)
TFF3 negative	1031 (32.0%)	398 (26.9%)	633 (36.3%)	<0.001
TFF3 positive	1598 (49.6%)	775 (52.5%)	823 (47.2%)	0.003
Atypia	151 (4.7%)	98 (6.6%)	53 (3.0%)	<0.001
p53	120 (3.7%)	80 (5.4%)	40 (2.3%)	<0.001
Insufficient	323 (10.0%)	127 (8.6%)	196 (11.2%)	0.013

425/3223 patients (13.2%) were further investigated with UGI endoscopy and had available histopathology results at the time of analysis; 225/425 endoscopies (52.9%) were in the Year 1 cohort; 200/425 endoscopies (47.1%) were in the Year 2 cohort. Of note, this includes endoscopies performed for all indications: this is summarised in Table 3.3. 246/425 endoscopies (57.9%) were performed in response to an abnormal capsule sponge result (i.e. atypia and/or p53 positive). 1 patient (0.2%) experienced detachment of the capsule sponge device: this was retrieved endoscopically within 4 hours, and the patient came to no further harm. 1 patient (0.2%) underwent endoscopy after presenting with an UGI bleed >6 months after the capsule sponge test, rather than a direct complication of the investigation. The median time from capsule sponge test to endoscopy was 2 months (IQR 1-5). The median time from endoscopy to histopathology result was 16 days (IQR 8-35).

Table 3.3 Indication for UGI endoscopy (n=425)

Indication	N (%)
Abnormal capsule sponge result (atypia +/- p53 positive)	246 (57.9%)
Insufficient capsule sponge result	67 (15.8%)
TFF3 negative capsule sponge result	47 (11.1%)
Investigation of red flag UGI symptoms	40 (9.4%)
Assessment of ulcer healing	23 (5.4%)
Capsule sponge detachment	1 (0.2%)
Delayed UGI bleed	1 (0.2%)

Figure 3.1 demonstrates endoscopic pathology results categorised by delay time in Barrett's surveillance, where p relates to the proportion of patients with confirmed dysplasia or malignancy. As delay to Barrett's surveillance increases >24 months, the proportion of patients with dysplastic or malignant Barrett's oesophagus changes significantly increases (p <0.001). Malignant transformation of Barrett's oesophagus to either IMC or OAC was observed in 21/3223 patients (0.7%).

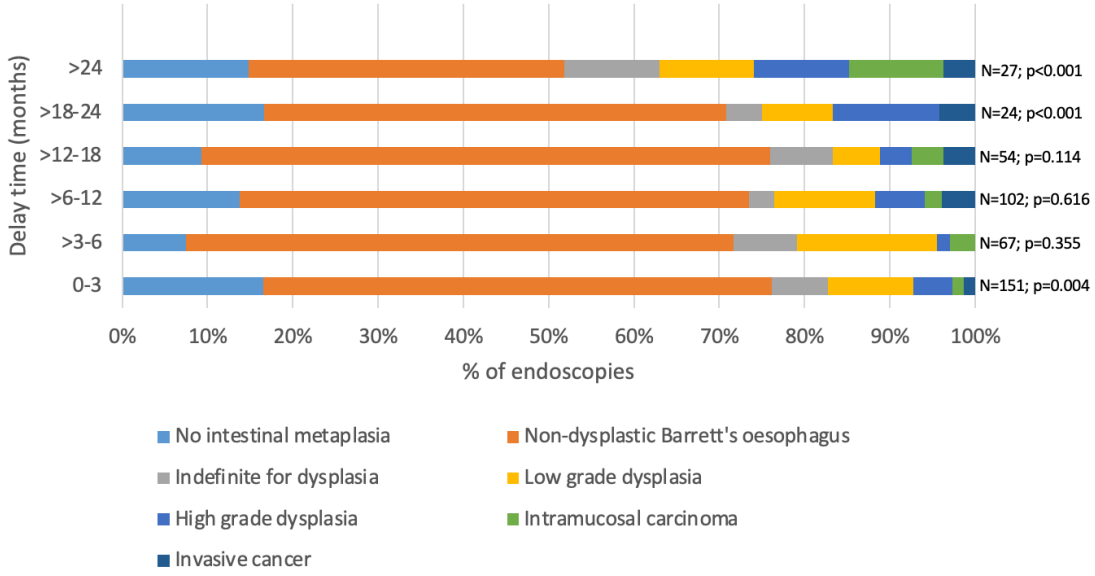

Figure 3.1 Endoscopic biopsy results categorised by delay to Barrett's surveillance (n=425)

271/3223 patients (8.4%) were within the high-risk group (i.e. atypia and/or p53 positive on capsule sponge testing). 246/271 patients (90.8%) underwent urgent UGI endoscopy within 12 months of capsule sponge test. The median time to endoscopy within this high-risk cohort was 2 months (IQR 1-3). 168/246 high-risk patients (68.3%) had delayed Barrett's surveillance. Figure 3.2 demonstrates endoscopic biopsy results within this high-risk group categorised by delay in Barrett's surveillance, where p relates to the proportion of patients with confirmed dysplasia or malignancy. 10/18 patients (55.5%) in this group demonstrated dysplasia when Barrett's surveillance was delayed >24 months (p = 0.009).

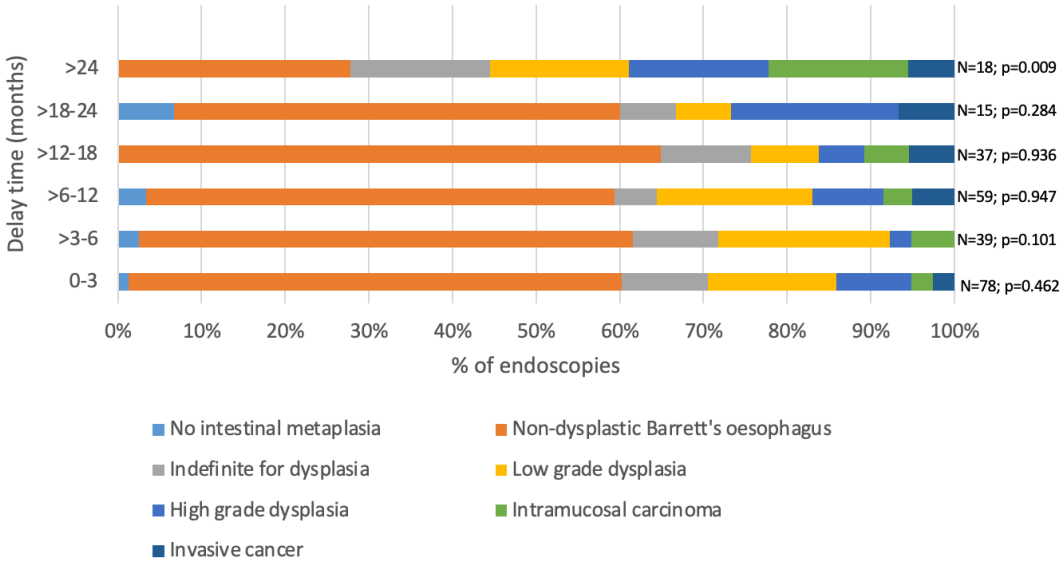

Figure 3.2 Endoscopic biopsy results categorised by delay to surveillance in patients with atypia and/or p53 positivity on capsule sponge testing (n=246)

A total of 25 high-risk patients were not included in the analysis of endoscopy results. 10/25 patients declined invitation to endoscopy. 8/25 patients underwent UGI endoscopy >12 months after capsule sponge test: 3/8 had IM, 2/8 had no IM, 2/8 had IND and 1/8 had LGD on endoscopic biopsies. 3/25 patients underwent repeat capsule sponge testing in the first instance: 2/3 were TFF3 positive and returned to routine surveillance, and 1/3 was atypia positive (subsequent endoscopic biopsies showed IM). 3/25 patients died from unrelated causes before endoscopy. 1/25 patients underwent UGI endoscopy but did not have biopsies taken due to bleeding risk (follow-up remains outstanding). One high-risk patient declined invitation to UGI endoscopy, subsequently re-presented with dysphagia, and was diagnosed with OAC 18 months after initial capsule sponge test.

More patients with long segment Barrett's underwent capsule sponge testing in Year 1 vs. Year 2 (43.0% vs. 33.6%; p <0.001) (Table 3.1). 1089/1577 patients with long segment Barrett's had delayed surveillance, compared to 975/1646 patients with short segment Barrett's (69.1% vs. 59.2%; p <0.001). Table 3.4 demonstrates impact of delayed surveillance on endoscopy pathology in short versus long segment Barrett's oesophagus. HGD, IMC and invasive cancer were more likely to be present in patients with long segment disease with delayed

surveillance. 402/1646 patients (24.4%) with short segment Barrett's were discharged from routine surveillance following capsule sponge test.

Table 3.4 Impact of delayed Barrett's surveillance on endoscopy pathology in short versus long segment Barrett's oesophagus

Pathology result at endoscopic biopsy	Short segment (n=166)		Long segment (n=259)	
	No delay (n=61)	Delay >3 months (n=105)	No delay (n=90)	Delay >3 months (n=169)
No intestinal metaplasia	20 (32.8%)	25 (23.8%)	5 (5.6%)	7 (4.1%)
Non-dysplastic Barrett's oesophagus	33 (54.1%)	56 (53.3%)	57 (63.3%)	107 (63.3%)
Indefinite for dysplasia	4 (6.5%)	8 (7.6%)	6 (6.7%)	8 (4.7%)
Low grade dysplasia	2 (3.3%)	8 (7.6%)	13 (14.4%)	23 (13.6%)
High grade dysplasia	2 (3.3%)	4 (3.8%)	5 (5.6%)	11 (6.5%)
Intramucosal carcinoma	0 (0%)	2 (1.9%)	2 (2.2%)	7 (4.1%)
Invasive cancer	0 (0%)	2 (1.9%)	2 (2.2%)	6 (3.6%)

In total, 43 patients were diagnosed with HGD, IMC or invasive cancer at endoscopic biopsy. 32/43 patients (74.4%) had delayed Barrett's surveillance. 24/32 patients with delayed surveillance were suitable for endoscopic treatment with either RFA or EMR, compared to 9/11 patients who underwent surveillance within the recommended time interval (75.0% vs. 81.8%). Of the remaining 8 patients within the delayed group, 4 patients underwent surgical resection, 2 underwent chemoradiotherapy and 2 were palliated. In the group without delayed surveillance, only 1 patient had surgery and 1 was palliated.

3.4 Discussion

The effect of delayed Barrett's surveillance on the distribution of pathology findings has not previously been described in the literature. This analysis presents the first real-world assessment of the effect of delayed Barrett's surveillance on endoscopy pathology pattern, as a direct consequence of the COVID-19 pandemic on endoscopy services.

As discussed in Chapter 1, the pandemic negatively impacted stage migration of newly diagnosed oesophageal cancers (Khan et al., 2022). This effect is observed in Public Health Scotland (PHS) cancer stage data from 2019 to 2021, demonstrating more oesophageal cancer patients presented with advanced or metastatic disease (Public Health Scotland, 2022, 2023a). This study demonstrates that delays to Barrett's surveillance resulted in significantly higher rates of dysplasia and malignancy when surveillance is delayed 24 months beyond the recommended time interval, thereby suggesting timely Barrett's surveillance does remain of value to improve patient outcomes and reduce cancer-related morbidity. At a juncture where the BOSS study has questioned the utility of endoscopic surveillance programmes for cancer detection, it is important to note that the number of dysplasia cases was significantly higher within the surveillance arm of the trial (Old et al., 2025). This raises the question that surveillance in a different format (i.e. with capsule sponge testing) could still play a vital role: this is substantiated by the above findings demonstrating increased rates of dysplasia when surveillance is delayed. Furthermore, within the HGD, IMC and invasive cancer patient cohort, these results have demonstrated that disease is more likely to be advanced and less amenable to endoscopic treatment where Barrett's surveillance is delayed.

Although capsule sponge testing has been demonstrated to be effective within the trial setting (Fitzgerald et al., 2020), evidence supporting its use in real-world clinical practice is continuing to evolve. This preliminary data was amongst the first to suggest that capsule sponge testing could safely be used as a Barrett's surveillance method to identify patients at risk of dysplasia and malignancy in the real-world setting, having been published prior to the work presented in Chapter 2.

This data successfully demonstrated that both the proportion of Barrett's oesophagus patients with delayed surveillance (72.6% in Year 1 vs. 57.0% in Year 2; $p < 0.001$) and median delay time to Barrett's surveillance have significantly improved (9 months in Year 1 vs. 5 months in Year 2; $p < 0.001$). Undoubtedly, the CytoSCOT programme played a critical role in this phenomenon: the programme was better established, with increased capacity and wider geographical availability in Year 2, with Scottish capsule sponge testing for Barrett's surveillance increasing by 18.0% between the time intervals. This

expansion was reinforced by the publication of the national Endoscopy and Urology Diagnostic Recovery and Renewal Plan in November 2021, which encouraged implementation of new technologies (specifically including capsule sponge testing) to support endoscopy services across Scotland (Scottish Government, 2021). Although the BSG published guidance to support safe resumption of routine UGI endoscopy to enable post-pandemic service recovery (Rees et al., 2020), this was slow to be implemented across NHS Scotland. Although the total number of patients on endoscopy waiting lists across Scotland improved in Year 1 compared to Year 2 (12,277 in September 2021 vs. 9635 in September 2022), this remained significantly higher than pre-pandemic numbers (7365 in September 2019). This was mirrored in the proportion of patients meeting 6-week waiting time targets in September 2019 (69.6%), September 2021 (31.7%) and September 2022 (41.4%) (Public Health Scotland, 2023b), highlighting that the improvement in delayed surveillance in Year 2 was unlikely due to increased capacity of endoscopy services alone, further highlighting the crucial role of the CytoSCOT programme in facilitating Barrett's surveillance and supporting service recovery.

In addition to delays related to endoscopy waiting lists, the CytoSCOT programme has alleviated pressures on local histopathology services. The median time to urgent histopathology result from endoscopy in this cohort remained longer than the previously reported median turnaround time to capsule sponge test result (16 vs. 8 days) (Pilonis et al., 2022). Although many Barrett's oesophagus patients are still experiencing delays in surveillance, it is hypothesised that this will improve with time as the CytoSCOT programme advances.

Increasing segment length is acknowledged as a risk factor for progression to Barrett's dysplasia (Anaparthy et al., 2013). In this cohort, there was an increased number of patients with longer segments in Year 1 compared to Year 2 (43.0% vs. 33.6%; $p < 0.001$). Furthermore, these results demonstrate that this pattern of worsening pathology is amplified within the long segment Barrett's group. Not only are these patients more likely to develop dysplasia compared to the short segment cohort, but the impact of delayed surveillance toward worsening pathology patterns becomes more pronounced in patients with long segment Barrett's. These results also demonstrate reduced numbers of abnormal

capsule sponge test results (i.e. atypia and/or p53 positive) with time (12.0% in Year 1 vs. 5.3% in Year 2; p <0.001). Clinicians may have processed higher-risk, long segment patients within Year 1 of the CytoSCOT programme, thereby explaining the improvement in capsule sponge test results pattern over time. 1031/3223 capsule sponge test (32.0%) returned demonstrating TFF3 negative results in the Barrett's cohort. This may be due to the inclusion of non-Barrett's cases within the dataset, in addition to short segments <1cm: the increased proportion of TFF3 negative results combined with the increased proportion of short segment Barrett's in Year 2 supports this theory. Furthermore, the proportion of capsule sponge tests yielding insufficient results increased in Year 2 (8.6% vs. 11.2%; p=0.013). As the CytoSCOT programme expanded in Scotland between Year 1 and Year 2, more endoscopy nurses were trained to perform capsule sponge testing: it is possible that the increased proportion of insufficient tests may be due to the learning curve associated with undertaking this new technique.

This study is not without limitations, many of which were also discussed regarding the cohort included in Chapter 2. Firstly, although BSG guidelines provide national recommendations, Barrett's surveillance intervals were variable between health boards based on local guidelines, introducing heterogeneity amongst the study group. This cohort only included those undergoing Barrett's surveillance with capsule sponge testing in the first instance: the group who underwent Barrett's surveillance with UGI endoscopy alone was not included due to limited access to national endoscopy data. Therefore, it is difficult to definitively prove that these improvements in delay time were due to the CytoSCOT programme alone. Additionally, as the sensitivity of capsule sponge testing is 92% (Fitzgerald et al., 2020; Ross-Innes et al., 2017) and patients with a TFF3 negative capsule sponge test result did not routinely undergo UGI endoscopy, it cannot be proven that these are true negative results: this raises the possibility of "missed" pathology within the cohort. As previously discussed, although UGI endoscopy is the gold standard for Barrett's surveillance, endoscopic punch biopsies are subject to significant sampling bias. This highlights that there is no perfect test for Barrett's surveillance. Finally, the median follow-up time was only 16 months. Further work is required to

determine if these improvements in delayed surveillance are sustained with the continued development of the CytoSCOT programme.

In conclusion, delayed Barrett's oesophagus surveillance beyond 24 months is associated with increased rates of sinister pathology. The CytoSCOT programme is a valuable resource to enable the continuation of Barrett's surveillance at lower cost and reduce the burden on endoscopy services, focussing endoscopy on patients at higher risk and enabling earlier detection of precursor lesions.

Chapter 4 Impact on Barrett's dysplasia yield following the introduction of capsule sponge testing versus traditional endoscopic surveillance

While the preceding two chapters have provided compelling evidence that capsule sponge testing is a useful triage tool in Barrett's surveillance and is successfully identifying patients at high-risk of dysplasia, these analyses failed to consider the cohort of patients that proceeded direct to endoscopic Barrett's surveillance, due to lack of access to national endoscopy data. Therefore, capsule sponge testing has yet to be directly compared to traditional endoscopy in clinical practice beyond the trial setting. Chapter 4 explores this by aiming to establish the impact of the introduction of capsule sponge testing on dysplasia detection rates at endoscopic biopsy in a single health board by directly comparing patients who underwent capsule sponge testing for Barrett's surveillance (+/- subsequent UGI endoscopy) to those undergoing surveillance with traditional endoscopy alone, as well as comparing the overall dysplasia yield in the 2-year periods pre and post implementation of capsule sponge testing.

This chapter is based on a publication in Diseases of the Esophagus in 2025 (Chien & Glen, 2025). Permission to include this work was granted by the editorial team.

4.1 Introduction

As discussed in previous chapters, the current recommended Barrett's surveillance strategy in the UK is with UGI endoscopy performed at specific time-points dependent on segment length and previous histopathology results (Fitzgerald et al., 2014). However, this investigation is not without drawbacks: not only is UGI endoscopy an invasive procedure, it is subject to user error and sampling bias, as well as being exceptionally resource-intensive to identify the small proportion of patients who progress to dysplasia and require further treatment (Lin et al., 2019). These limitations, as well as system pressures on over-stretched endoscopy services, provided the impetus to explore capsule sponge testing as a method to support the Barrett's surveillance service.

The preceding two chapters have presented the findings of the national CytoSCOT programme and have effectively demonstrated that capsule sponge testing is successfully facilitating the identification of individuals high-risk for dysplasia requiring further investigation with urgent endoscopy within the Barrett's surveillance cohort (Chien, Glen, Penman, Cruickshank, et al., 2024). However, due to ethics committee restrictions, access to national data were not available for those patients who went direct to endoscopy rather than capsule sponge testing.

Therefore, the impact of the introduction of the capsule sponge testing service on dysplasia detection rates in clinical practice has yet to be established. This chapter aims to address this by comparing dysplasia diagnoses in a pre-intervention cohort with an implementation cohort after capsule sponge testing was introduced in a single Scottish health board. Furthermore, it is yet to be demonstrated whether capsule sponge testing is comparable to traditional endoscopic surveillance beyond the trial setting. This cohort study also aimed to compare rates of dysplasia detection between those undergoing capsule sponge testing for Barrett's surveillance (with or without subsequent endoscopy) versus those undergoing endoscopic surveillance alone.

4.2 Methods

All patients undergoing endoscopic surveillance for Barrett's oesophagus in a single Scottish health board (NHS Greater Glasgow and Clyde; NHS GGC) were identified from a prospectively maintained database of all pathology reports coded as "Barrett's oesophagus" from 1/1/2018 to 31/12/2022. Individual electronic records were interrogated to collect baseline demographics at the time of surveillance, previous Barrett's morphology, histopathology results and ongoing clinical management using the health board's online clinical systems, including Clinical Portal, SCI Store (Version 8.5) and SCI Gateway (R 20.0). This was cross-checked with the prospectively maintained local capsule sponge database, where the same demographics were collected for each patient, to formulate a global Barrett's surveillance database for NHS GGC on Microsoft Excel. Patients were invited to undertake capsule sponge testing in lieu of surveillance endoscopy in the absence of red flag symptoms. They were recruited for capsule sponge testing using the Cytosponge™ device (Fitzgerald et al., 2020), via an opt-in system through letter invitation. All capsule sponge tests were administered by a trained practitioner within the outpatient setting as previously described within the Scottish capsule sponge cohort (Chien, Glen, Penman, Bryce, et al., 2024; Chien, Glen, Penman, Cruickshank, et al., 2024).

Processing and reporting of results was performed centrally at one UK-based laboratory (Cyted Ltd™). All capsule sponge tests were processed and analysed as described in Chapter 2, with processed results returned to the requesting hospital and ongoing management decided locally by the requesting clinician.

Patients included in the analysis required a minimum of one previous endoscopy with biopsies: oesophageal biopsies either confirmed the presence of IM and/or prior endoscopy demonstrated macroscopic changes consistent with Barrett's oesophagus (i.e. salmon-coloured mucosa progressing cephalad from the GOJ). The presence of IM on endoscopic biopsies was not considered a precondition for entry into surveillance, as per the BSG guidelines (Fitzgerald et al., 2014). The Prague classification was used to determine Barrett's segment length: short segment was defined as M ≤3cm; long segment was defined as M >3cm to ≤10cm; ultra-long segment was defined as M >10cm. Patients were coded by worst pathology on oesophageal biopsies using the following descriptors: no intestinal

metaplasia; intestinal metaplasia; indefinite for dysplasia; low grade dysplasia; high grade dysplasia; intramucosal adenocarcinoma; invasive cancer (including oesophageal adenocarcinoma and squamous cell carcinoma).

To compare whether capsule sponge testing had affected dysplasia numbers overall, the 2-year periods before and after implementation were compared. The “pre-intervention” group included all patients undergoing endoscopic surveillance from 1/1/2018 to 31/12/2019. Capsule sponge testing was formally introduced to the health board on 14/9/2020: due to its aerosol-generating nature, routine endoscopy services had been temporarily halted during 2020 in line with guidance related to the COVID-19 pandemic. For this reason, surveillance undertaken in 2020 was discarded from analysis. The 2-year period from 1/1/2021 to 31/12/2022 was used as the “implementation” cohort, including those who underwent surveillance with both endoscopy and capsule sponge testing.

All patients undergoing surveillance over the 2-year period 1/1/2021 to 31/12/2022 were identified and dichotomised into two groups: capsule sponge test (+/- subsequent endoscopy) versus traditional endoscopic surveillance only. Endoscopic biopsy results were compared for both groups, with the pathology results included for the capsule sponge +/- endoscopy cohort taken from the biopsies obtained if patients proceeded to UGI endoscopy. Onward referral for endoscopy in the capsule sponge group was decided at the clinicians’ discretion. The endoscopic surveillance cohort was utilised as a control group for previous best practice.

Exclusion criteria included: paediatric patients under the age of 18 years; previous EET with RFA or EMR; previous oesophagectomy; presence of dysplasia in last endoscopic biopsies; IMC or invasive cancer in previous oesophageal biopsies; EMR or oesophagectomy specimens; slides referred from other health boards; squamous dysplasia; repeat endoscopy for mapping biopsies in cases of confirmed HGD or IMC.

Patients were not actively involved in study design or analysis. Ethical approval was obtained for this study from the Caldicott guardian for NHS GGC. National PBPP approval was also granted for the collection of capsule sponge data.

Continuous parameters were presented as median and IQR, and categorical data as counts and percentages. The Fisher exact and Chi-squared tests were performed for comparison of categorical variables, where appropriate. The Mann-Whitney U test was performed for comparison of continuous variables. A p value of ≤ 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 28.0 (SPSS Inc., Chicago, Illinois, USA).

4.3 Results

4.3.1 Impact of change in practice on overall dysplasia rates

1568 patients underwent endoscopic Barrett's surveillance in the 2-year period between 1/1/2018 and 31/12/2019: these patients were the pre-intervention group. 1791 patients underwent Barrett's surveillance with either traditional endoscopy or capsule sponge testing in the 2-year period between 1/1/2021 and 31/12/2022, comprising the implementation group. Table 4.1 demonstrates evenly matched baseline demographics between the two cohorts, implying that the two groups were comparable. Patients in the implementation group demonstrated a longer time from last surveillance (35 vs. 25 months; $p<0.001$) and were more likely to have no IM on their last biopsies (23.2% vs. 17.9%; $p<0.001$).

Table 4.1 Baseline patient demographics in the pre-intervention versus implementation groups
*** Missing data in 11 cases. ** Missing data in 15 cases. † 920 patients had capsule sponge testing +/- endoscopy; 871 patients had traditional endoscopic surveillance.**

Demographic		Pre-intervention group (n=1568)	Implementation group (n=1791†)	P value
Age (years), median (IQR)		65 (57 - 72)	66 (57 – 73)	0.248
Sex	Male	1008 (64.3%)	1145 (63.9%)	0.831
	Female	560 (35.7%)	646 (36.1%)	
Scottish Index of Multiple Deprivation (SIMD) *	Most deprived (SIMD 1-5)	902 (57.7%)	1020 (57.1%)	0.741
	Least deprived (SIMD 6-10)	661 (42.3%)	765 (42.9%)	
Smoking status **	Never smoker	741 (47.5%)	853 (47.8%)	0.898
	Ex smoker	584 (37.4%)	654 (36.7%)	
	Current	236 (15.1%)	276 (15.5%)	
PPI use	Yes	1499 (95.6%)	1697 (94.8%)	0.254
	No	69 (4.4%)	94 (5.2%)	
Barrett's segment length	Short	916 (58.4%)	1052 (58.7%)	0.064
	Long	587 (37.4%)	635 (35.4%)	
	Ultra-long	65 (4.1%)	104 (5.8%)	
Last endoscopic pathology result	No IM	281 (17.9%)	415 (23.2%)	<0.001
	IM	1287 (82.1%)	1376 (76.8%)	
Time from last endoscopic surveillance (months), median (IQR)		25 (23 – 34)	35 (27 – 45)	<0.001

Table 4.2 compares the rates of dysplasia detection between the two cohorts. There was no significant difference in the rates of HGD, IMC or invasive cancer with the introduction of capsule sponge testing in the implementation group. There were more cases of LGD diagnosed in the pre-intervention group (3.4% vs. 2.2%; p=0.033). However, when the actual numbers are compared, there were 107 patients with IND or LGD on biopsies and 22 patients with HGD, IMC or invasive cancer in the pre-intervention group, compared to 105 patients with IND or LGD on biopsies and 23 patients with HGD, IMC or invasive cancer in the implementation group.

Table 4.2 Comparison of dysplasia rates in the pre-intervention versus implementation groups
† 920 patients had capsule sponge testing +/- endoscopy; 871 patients had traditional endoscopic surveillance.

Endoscopic biopsy result	Pre-intervention group (n=1568)	Implementation group (n=1791 †)	P value
Indefinite for dysplasia	54 (3.4%)	66 (3.7%)	0.707
Low grade dysplasia	53 (3.4%)	39 (2.2%)	0.033
High grade dysplasia	15 (1.0%)	9 (0.5%)	0.151
Intramucosal adenocarcinoma	3 (0.2%)	5 (0.3%)	0.731
Invasive cancer	4 (0.3%)	9 (0.5%)	0.280

4.3.2 Capsule sponge surveillance versus traditional endoscopic surveillance

In total, 1791 patients underwent Barrett's surveillance over the 2-year period between 1/1/2021 and 31/12/2022: 871 patients had traditional endoscopic surveillance only; 920 patients had capsule sponge surveillance, with 157 patients (17.1%) proceeding to endoscopy after capsule sponge based on clinical assessment and interpretation of capsule sponge result. The median length of follow-up was 22 months (IQR 16-29). Patients who opted for capsule sponge testing were more likely to be younger (65 vs. 67 years; p=0.013), male (67.5% vs. 60.2%; p<0.001), ever smokers (52.8% vs. 51.5%; p=0.025) and from less deprived areas (45.7% vs. 39.9%; p=0.013). The capsule sponge cohort also demonstrated a longer median time from last endoscopic surveillance compared to the endoscopy only cohort (38 vs. 31 months; p<0.001). Patient demographics are summarised in Table 4.3.

Table 4.3 Baseline patient demographics in the endoscopy only versus capsule sponge +/- endoscopy groups in years 2021 and 2022
*** Missing data in 6 cases. ** Missing data in 8 cases.**

Demographic		Endoscopy only (n=871)	Capsule sponge +/- endoscopy (n=920)	P value
Age (years), median (IQR)		67 (57 – 74)	65 (57 – 72)	0.013
Sex	Male	524 (60.2%)	621 (67.5%)	<0.001
	Female	347 (39.8%)	299 (32.5%)	
SIMD *	Most deprived (SIMD 1-5)	523 (60.1%)	497 (54.3%)	0.013
	Least deprived (SIMD 6-10)	347 (39.9%)	418 (45.7%)	
Smoking status **	Never smoker	420 (48.5%)	433 (47.2%)	0.025
	Ex smoker	324 (37.5%)	330 (35.9%)	
	Current smoker	121 (14.0%)	155 (16.9%)	
PPI use	Yes	822 (94.4%)	875 (95.1%)	0.486
	No	49 (5.6%)	45 (4.9%)	
Barrett's segment length	Short	501 (57.5%)	551 (59.9%)	0.541
	Long	320 (36.7%)	315 (34.2%)	
	Ultra-long	50 (5.7%)	54 (5.9%)	
Last endoscopic pathology result	No IM	186 (21.4%)	229 (24.9%)	0.076
	IM	685 (78.6%)	691 (75.1%)	
Time from last endoscopic surveillance (months), median (IQR)		31 (24 – 41)	38 (30 – 47)	<0.001

Table 4.4 compares the rates of dysplasia diagnosed between the two cohorts. There were no statistically significant differences between the proportion of cases of LGD, HGD, IMC and invasive cancer between the two cohorts, implying that capsule sponge surveillance is non-inferior to endoscopic surveillance in the identification of Barrett's dysplasia and early cancer. However, there were more IND cases identified within the endoscopy only group (5.3% vs. 2.2%; p<0.001).

Table 4.4 Comparison of dysplasia rates in the endoscopy only versus capsule sponge +/- endoscopy groups in years 2021 and 2022

Endoscopic biopsy result	Endoscopy only (n=871)	Capsule sponge +/- endoscopy (n=920)	P value
Indefinite for dysplasia	46 (5.3%)	20 (2.2%)	<0.001
Low grade dysplasia	25 (2.9%)	14 (1.5%)	0.051
High grade dysplasia	6 (0.7%)	3 (0.3%)	0.331
Intramucosal adenocarcinoma	1 (0.1%)	4 (0.4%)	0.375
Invasive cancer	7 (0.8%)	2 (0.2%)	0.100

Table 4.5 compares the rates of dysplasia diagnosed between all patients undergoing endoscopic surveillance over all 4 years (i.e. 2018, 2019, 2021 and 2022), with the 920 patients who had capsule sponge surveillance (+/- endoscopy) over the 2-year period from 2021-2022. Again, there were no significant differences in the yield of HGD, IMC and invasive cancer between the two cohorts. However, more patients were diagnosed with IND (4.1% vs. 2.2%; p=0.007) and LGD (3.2% vs. 1.5%; p=0.008) in the cohort undergoing traditional endoscopic surveillance compared to capsule sponge testing.

Table 4.5 Comparison of dysplasia rates in the endoscopy only versus capsule sponge +/- endoscopy groups in years 2018, 2019, 2021 and 2022

Endoscopic biopsy result	Endoscopy only (n=2439)	Capsule sponge +/- endoscopy (n=920)	P value
Indefinite for dysplasia	100 (4.1%)	20 (2.2%)	0.007
Low grade dysplasia	78 (3.2%)	14 (1.5%)	0.008
High grade dysplasia	21 (0.9%)	3 (0.3%)	0.101
Intramucosal adenocarcinoma	4 (0.2%)	4 (0.4%)	0.151
Invasive cancer	11 (0.5%)	2 (0.2%)	0.331

4.4 Discussion

Although biomarker risk stratification with capsule sponge testing has been demonstrated to predict presence of dysplasia within the trial setting (Fitzgerald

et al., 2020; Pilonis et al., 2022; Ross-Innes et al., 2017), real-world data supporting this hypothesis is limited. Furthermore, trial data has advocated its use as a potential screening tool in the diagnosis of Barrett's oesophagus, yet only two previous studies have evaluated the use of capsule sponge testing as a Barrett's surveillance method (Chien, Glen, Penman, Cruickshank, et al., 2024; Tan et al., 2025). This study is the first work to directly compare differences in pathology yield with endoscopic versus capsule sponge surveillance for Barrett's oesophagus in clinical practice.

These results have demonstrated that there was no significant difference in overall rates of HGD, IMC and invasive cancer when the pre-intervention and implementation groups were compared, implying that capsule sponge testing has not negatively impacted dysplasia yield overall. Although there were more cases of LGD diagnosed in the pre-intervention group (3.4% vs. 2.2%; p=0.033), the overall numbers diagnosed were comparable each year. If it is assumed that the prevalence of Barrett's oesophagus remains constant within the population with a constant progression rate and therefore a constant number of patients will develop dysplasia each year, these findings are more reassuring. Endoscopy is also not a perfect test: previous studies have reported that, in patients with non-dysplastic Barrett's, 8-25% of LGD cases were missed at index endoscopy (Nguyen et al., 2022; Visrodia, Iyer, et al., 2016). Over the 2-year period, 763 fewer UGI endoscopies were performed without the overall dysplasia numbers reducing, providing clinicians' reassurance that dysplasia or cancer cases were not missed with capsule sponge testing.

Furthermore, over a 2-year period, there were no statistically significant differences in the rates of detection of LGD, HGD, IMC and invasive cancer between patients undergoing capsule sponge testing versus traditional endoscopy for Barrett's surveillance. However, the endoscopy only cohort had a higher prevalence of IND cases (5.3% vs. 2.2%; p<0.001), as well as nearly twice that for LGD cases (2.9% vs. 1.5%; p=0.051), although the latter did not reach statistical significance. This does raise the question of "missed pathology" with capsule sponge testing. However, current literature and understanding around the clinical significance of IND or LGD cases remains debatable: there is considerable interobserver variability and over-diagnosis at endoscopy (Falk, 2017; Thota et al., 2016), implying that UGI endoscopy is also not without its

limitations in this specific cohort. When comparing the capsule sponge surveillance group to the whole cohort undergoing endoscopic surveillance only over the 4-year study period, the increased yield of LGD in the endoscopy only cohort became statistically significant (3.2% vs. 1.5%; p=0.008), and the higher prevalence of IND cases was again seen in this group (4.1% vs. 2.2%; p=0.007). However, these results should be interpreted with caution given that the two groups were observed over a different study period and the implementation group (i.e. 2021-2022) were subject to delayed surveillance due to the COVID-19 pandemic, which has been shown to negatively impact pathology yield (Chien, Glen, Penman, Bryce, et al., 2024). There is scope for repeating this analysis once longer-term follow-up is available for both groups to determine whether these trends are observed in a larger population undergoing capsule sponge testing.

There were also significantly fewer cases of LGD diagnosed in the implementation versus pre-intervention groups (3.4% vs. 2.2%; p=0.033). As discussed above, diagnosis of LGD remains variable at endoscopy and there is clinical uncertainty regarding the natural history and its ongoing management as, although LGD is associated with a higher risk of malignant transformation, it is unclear whether LGD warrants therapeutic intervention. Current BSG guidelines therefore advocate for more intense surveillance of these patients at 6-monthly intervals only (Fitzgerald et al., 2014). With limited evidence for its use in Barrett's surveillance at present, there is dubiety about the frequency of surveillance required in the cohort undergoing capsule sponge testing. Longer term follow-up and comparison of these cohorts is required to definitively state whether capsule sponge testing is less likely to detect cases of LGD than traditional endoscopy, although increasing intensity of surveillance when using the capsule sponge device may be a hypothetical solution to reduce the risk of missing early dysplasia diagnoses.

Overall, these results imply that capsule sponge testing is non-inferior to the current gold standard investigation of UGI endoscopy for Barrett's surveillance in the detection of HGD, IMC and cancer, and supports the existing evidence that capsule sponge testing could be used as an alternative to traditional endoscopic surveillance when used as a triage tool to risk-stratify patients (Chien, Glen, Penman, Cruickshank, et al., 2024). Importantly, these promising results were

obtained with only 17.1% of the capsule sponge cohort requiring endoscopy, enabling prioritisation of scarce endoscopy resources. This reduction in endoscopy workload is similar to previously published results from the Scotland-wide cohort (Chien, Glen, Penman, Cruickshank, et al., 2024). This 2-year period was selected as a control group as both the endoscopic surveillance cohort and the capsule sponge cohort were exposed to the same impact of delayed Barrett's surveillance related to the COVID-19 pandemic (Chien, Glen, Penman, Bryce, et al., 2024). This effect is highlighted by the fact that the implementation group demonstrated a longer time from last surveillance compared to the pre-intervention group prior to the pandemic (35 vs. 25 months; $p<0.001$).

The health board employed an opt-in system for capsule sponge testing. Previous literature has demonstrated that participation in clinical research and engagement with new healthcare technologies is more commonly seen in men (Hawke et al., 2024) and those with higher socioeconomic status (Kim et al., 2024). This may explain why this dataset includes a higher proportion of males and those from more affluent postcodes included within this dataset who agreed to "opt in" for capsule sponge testing over traditional endoscopic surveillance. Despite capsule sponge testing being adopted across the country as an endoscopy alternative during the COVID-19 pandemic, there remained a reasonable proportion of patients who declined invitation to capsule sponge testing and opted for endoscopic surveillance instead. Capsule sponge testing has subsequently expanded further with continued funding from the Scottish government (Scottish Government, 2021; Scottish Health Technologies Group, 2023). The capsule sponge cohort also demonstrated a longer median time from last endoscopic surveillance compared to the endoscopy only cohort (38 vs. 31 months; $p<0.001$). Patients may have been more likely to opt for this technique if their surveillance interval was delayed, as endoscopy services in Scotland had not recovered to pre-pandemic capacity, as previously published (Chien, Glen, Penman, Bryce, et al., 2024).

This dataset did not include indication for endoscopy: unlike the capsule sponge group, some patients within the endoscopy only group may have been referred for endoscopy prior to their recommended surveillance interval due to red flag UGI symptoms. This may explain the higher absolute number of invasive cancers diagnosed within the endoscopy only group, although this did not reach

statistical significance. If the dataset removed this subset of patients and included only those due Barrett's surveillance, it is hypothesised that the number of patients with HGD, IMC or invasive cancer would be lower than currently demonstrated in the endoscopy only group and there would be minimal difference in pathology yield between the two cohorts. As patients with red flag symptoms were specifically excluded from capsule sponge testing, this does highlight that these results must be interpreted pragmatically due to the inclusion of this cohort within the endoscopy only group, reflecting the real-world nature of this study.

There are further limitations to this dataset. 763 patients (82.9%) of the capsule sponge surveillance group did not undergo endoscopy, as capsule sponge testing was adopted in Scotland as a real-world implementation pilot, aiming to reduce the number of endoscopies performed. There is therefore the possibility of missed pathology within this cohort. Furthermore, although surveillance was performed in multiple centres, this analysis only includes data from one health board: including patients from additional health boards would strengthen these findings. In addition, as discussed above, it is not possible to comment on the recommended surveillance intervals for risk stratification for capsule sponge testing at this juncture, as there is currently insufficient guidance for this at present. Within NHS GGC, patients with TFF3 positivity only on capsule sponge testing were recommended repeat surveillance at 3 years for long segment and 5 years for short segment Barrett's. However, it was felt that longer segments (i.e. M >8cm) should not undergo repeat surveillance with capsule sponge testing and should undergo endoscopic surveillance by an endoscopist with an interest in Barrett's oesophagus at the next recommended interval. This advice was purely anecdotal without a strong evidence base; therefore, ongoing follow-up of this cohort is being undertaken to continue to assess this recommendation. Finally, the follow-up time remains less than the current recommended BSG surveillance intervals. Longer term follow-up will be vital to validate these findings and ensure pathology is not missed once patients re-attend for their next surveillance.

Chapter 5 Impact of the introduction of capsule sponge testing on the Barrett's surveillance endoscopy service

It is evident that over-stretched endoscopy services have been struggling to support the workload generated by the Barrett's oesophagus surveillance programme for many years. The change in national process reactive to the COVID-19 pandemic from traditional endoscopy to capsule sponge testing for Barrett's surveillance instituted in 2020 across Scotland has been well-documented in the preceding chapters. However, the impact of the introduction of this new surveillance strategy on the pre-existing Barrett's surveillance endoscopy services has yet to be determined, and this will be explored further in Chapter 5 by examining the effect this new clinical pathway had on Barrett's surveillance endoscopy services in a single Scottish health board.

5.1 Introduction

The preceding chapters have provided an in-depth analysis of the results from the CytoSCOT programme, which saw a national clinical subgroup approve capsule sponge testing for Barrett's surveillance as an alternative to endoscopy following publication of promising trial results (Fitzgerald et al., 2020; Pilonis et al., 2022), with support and funding from the Scottish government (Scottish Government, 2021). While Chapters 2 and 4 have demonstrated that capsule sponge testing is identifying patients at high-risk for dysplasia appropriately (Chien, Glen, Penman, Cruickshank, et al., 2024) and its introduction has not negatively impacted dysplasia yield overall (Chien & Glen, 2025), Chapter 3 also highlighted that the evolution of the CytoSCOT programme helped to reduce the proportion of patients with delayed Barrett's surveillance (Chien, Glen, Penman, Bryce, et al., 2024).

Surveillance endoscopy demands judicious inspection of the oesophageal mucosa as well as multiple biopsies. Therefore, Barrett's surveillance endoscopies are usually allocated longer time slots when planning endoscopy lists (Beg et al., 2017). For an in-demand service such as UGI endoscopy in which resources must be prioritised and productivity maximised, it is important to direct the service towards those most likely to have significant pathology. This is particularly relevant in the Barrett's surveillance patient cohort, in which the vast majority of patients do not demonstrate progression to dysplasia or cancer (Hussein et al., 2021; Whitson & Falk, 2015). Capsule sponge testing is of value as a triage tool in this context, as it can identify those patients who are most likely to require endoscopic investigation and enrich the likelihood of identifying concerning pathology, as well as saving patients from unnecessary invasive procedures.

However, the effect of capsule sponge testing on local endoscopy services has yet to be formally evaluated. This chapter aims to determine the impact of the introduction of capsule sponge testing on the Barrett's surveillance endoscopy service by analysing whether capsule sponge testing has reduced the number of endoscopies performed for Barrett's surveillance in a single Scottish health board.

5.2 Methods

A prospectively maintained database of all endoscopic pathology reports coded as “Barrett’s oesophagus” was used to identify all patients undergoing Barrett’s surveillance in NHS GGC over a 5-year study period from 1 January 2018 to 31 December 2022. Each record was manually re-checked using the NHS GGC electronic patient record systems to ensure patients met the eligibility criteria and record baseline demographics using Microsoft Excel.

In a similar manner to Chapter 4, patients included in this analysis required a minimum of one previous endoscopy with biopsies: oesophageal biopsies either confirmed the presence of IM and/or prior endoscopy demonstrated macroscopic changes consistent with Barrett’s oesophagus as previously described. The Prague classification was used to determine Barrett’s segment length: short segment was defined as M ≤3cm; long segment was defined as M >3cm to ≤10cm; ultra-long segment was defined as M >10cm.

Patients were coded by worst pathology result on oesophageal biopsies using the following descriptors: no intestinal metaplasia; intestinal metaplasia; indefinite for dysplasia; low grade dysplasia; high grade dysplasia; intramucosal adenocarcinoma; adenocarcinoma; squamous cell carcinoma. This database was then cross-checked with the national capsule sponge testing database for Scotland to identify all NHS GGC patients undergoing capsule sponge testing within the study period. Patients were recruited for capsule sponge testing using the Cytosponge™ device in NHS GGC as previously described in Chapter 4.

The traditional endoscopic surveillance service ran from January 2018 to March 2020, when the COVID-19 pandemic was declared. As described above, 2020 saw routine endoscopy temporarily halted due to the pandemic, followed by the slow reintroduction of reduced endoscopy services after the first lockdown. Capsule sponge testing was formally introduced into NHS GGC on 14/9/2020. For the purposes of this analysis, 1/1/2018 to 31/12/2019 were considered pre-intervention years, whilst 1/1/2021 to 31/12/2022 were considered implementation years to allow for this disruption to normal services in 2020.

Exclusion criteria included: paediatric patients under the age of 18 years; previous EET with RFA or EMR; previous oesophagectomy; presence of IMC or invasive cancer in previous oesophageal biopsies; EMR or oesophagectomy specimens; slides referred from other health boards; squamous dysplasia; repeat endoscopy for mapping biopsies in cases of HGD or IMC.

Patients were not actively involved in study design or analysis. Ethical approval was obtained for this study from the Caldicott guardian for NHS GGC. National PBPP approval was also granted for the collection of capsule sponge testing data.

Continuous parameters were presented as median and IQR, and categorical data as counts and percentages. The Chi-squared and Kruskall-Wallis tests were performed for comparison of categorical and continuous variables respectively. A p value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 28.0 (SPSS Inc., Chicago, Illinois, USA).

5.3 Results

5.3.1 Baseline demographics

During the 5-year study period, 3804 endoscopies were performed for Barrett's surveillance in NHS GGC in 2483 patients. The median follow-up time was 44 months (IQR 25-59). Baseline demographics were evenly matched across the 5 years included in the study period (Table 5.1). Patients were significantly more likely to have a history of dysplasia on last endoscopy in the post-pandemic years ($p=0.027$). The proportion of patients demonstrating non-dysplastic Barrett's oesophagus only on their last endoscopy also decreased in 2021 and 2022, with a concurrent increase in cases of IND and LGD ($p<0.001$).

Table 5.1 Baseline demographics by year (n=3804)
*** Missing data in 7 cases. ** Missing data in 20 cases.**

Demographic		Barrett's surveillance endoscopies by year					P value
		2018 (n=976)	2019 (n=989)	2020 (n=425)	2021 (n=612)	2022 (n=802)	
Age (years), median (IQR)		66 (59 - 73)	66 (58 - 73)	67 (58 - 74)	68 (59 - 74)	67 (58 - 75)	0.095
Sex, n (%)	Male	641 (65.7%)	640 (64.7%)	290 (68.2%)	378 (61.8%)	519 (64.7%)	0.287
	Female	335 (34.3%)	349 (35.3%)	135 (31.8%)	234 (38.2%)	283 (35.3%)	
SIMD, n (%) *	Most deprived (SIMD 1-5)	555 (57.0%)	577 (58.5%)	252 (59.4%)	351 (57.4%)	472 (58.9%)	0.894
	Least deprived (SIMD 6-10)	418 (43.0%)	410 (41.5%)	172 (40.6%)	260 (42.6%)	330 (41.1%)	
Smoking status, n (%) **	Never smoker	443 (45.6%)	472 (48.0%)	193 (45.8%)	282 (46.4%)	367 (45.9%)	0.573
	Ex smoker	372 (38.3%)	381 (38.7%)	156 (37.1%)	245 (40.3%)	309 (38.7%)	
	Current smoker	157 (16.1%)	131 (13.3%)	72 (17.1%)	81 (13.3%)	123 (15.4%)	
Antacid use, n (%)	PPI only	843 (86.4%)	868 (87.8%)	361 (84.9%)	543 (88.7%)	715 (89.2%)	0.247
	H2 antagonist only	17 (1.7%)	19 (1.9%)	14 (3.3%)	11 (1.8%)	13 (1.6%)	
	PPI + H2 antagonist	92 (9.4%)	80 (8.1%)	41 (9.6%)	42 (6.9%)	50 (6.2%)	
	None	24 (2.5%)	22 (2.2%)	9 (2.1%)	16 (2.6%)	24 (3.0%)	
Last biopsy result, n (%)	No intestinal metaplasia	156 (16.0%)	164 (16.6%)	70 (16.5%)	105 (17.2%)	130 (16.2%)	< 0.001
	Intestinal metaplasia	744 (76.2%)	704 (71.2%)	307 (72.2%)	412 (67.3%)	502 (62.6%)	
	Indefinite for dysplasia	23 (2.4%)	63 (6.4%)	28 (6.6%)	51 (8.3%)	98 (12.2%)	
	Low grade dysplasia	46 (4.7%)	53 (5.4%)	17 (4.0%)	43 (7.0%)	67 (8.4%)	
	High grade dysplasia	7 (0.7%)	5 (0.5%)	3 (0.7%)	1 (0.2%)	5 (0.6%)	
Number of previous surveillance endoscopies, median (IQR)		2 (1-3)	2 (1-3)	2 (1-4)	2 (2-4)	2 (1-4)	< 0.001
Previous history of dysplasia, n (%)		90 (9.2%)	108 (10.9%)	40 (9.4%)	68 (11.1%)	112 (13.7%)	0.027

5.3.2 Impact of capsule sponge testing on pathology pattern

Table 5.2 presents the Barrett's surveillance endoscopy findings by year. As the study period progressed, dysplasia (of any grade) or cancer was significantly more likely to be present within endoscopic biopsies (p<0.001), with significantly fewer scopes performed for simple IM only. In the pre-intervention years, 164/1965 endoscopies performed resulted in a diagnosis of dysplasia or cancer, compared to 184/1414 in the implementation years (8.3% vs. 13.0%; p<0.001). There was no significant difference in the proportion of LGD versus HGD and cancer (115/164 in the pre-intervention years vs. 123/184 in the implementation years; 70.1% vs. 66.8%; p=0.512).

Table 5.2 Endoscopy findings by year (n=3804)

Endoscopy findings		Barrett's surveillance endoscopies by year					P value
		2018 (n=976)	2019 (n=989)	2020 (n=425)	2021 (n=612)	2022 (n=802)	
Barrett's segment length, n (%)	Short	528 (54.1%)	557 (56.3%)	261 (61.4%)	378 (61.8%)	483 (60.2%)	0.022
	Long	395 (40.5%)	380 (38.4%)	146 (34.4%)	197 (32.2%)	288 (35.9%)	
	Ultra-long	43 (4.4%)	52 (5.3%)	18 (4.2%)	37 (6.0%)	31 (3.9%)	
Biopsy result, n (%)	No IM	163 (16.7%)	172 (17.4%)	70 (16.5%)	117 (19.1%)	151 (18.8%)	<0.001
	IM	708 (72.5%)	663 (67.0%)	301 (70.8%)	369 (60.3%)	462 (57.6%)	
	IND	34 (3.5%)	61 (6.2%)	22 (5.2%)	54 (8.8%)	77 (9.6%)	
	LGD	40 (4.1%)	75 (7.6%)	20 (4.7%)	50 (8.2%)	73 (9.1%)	
	HGD	23 (2.4%)	13 (1.3%)	11 (2.6%)	12 (2.0%)	26 (3.2%)	
	IMC	2 (0.2%)	2 (0.2%)	0 (0%)	5 (0.8%)	4 (0.5%)	
	OAC	6 (0.6%)	2 (0.2%)	1 (0.2%)	4 (0.7%)	8 (1.0%)	
	OSCC	0 (0%)	1 (0.1%)	0 (0%)	1 (0.2%)	1 (0.1%)	

Of the 184 cases of dysplasia or cancer diagnosed in the implementation years, 32 patients (17.4%) had undergone capsule sponge testing prior to endoscopy. 27/32 patients (84.4%) had either atypia and/or p53 positivity on capsule sponge testing and were referred for urgent endoscopy (15/32 were atypia and p53 positive; 9/32 were atypia positive only; 3/32 were p53 positive only; 2/32 were TFF3 positive only; 2/32 were TFF3 negative; 1/32 had an insufficient result).

5.3.3 Impact of capsule sponge testing on number of endoscopies performed

Table 5.3 demonstrates the impact of capsule sponge testing on the Barrett's surveillance endoscopy service in NHS GGC. The number of capsule sponge tests performed per year increased as the service developed across NHS GGC. The introduction of capsule sponge testing saw a decrease of 28.0% in the number of endoscopies performed (1965 in the pre-intervention years vs. 1414 in the implementation years). However, a further 669 patients who would have been due surveillance endoscopy underwent capsule sponge testing alone without additional investigation: when these patients are included, the number of surveillance endoscopies performed is reduced by 62.0%.

Table 5.3 Impact of capsule sponge testing on Barrett's endoscopy service
*** Excludes patients subsequently referred for follow-up endoscopy or repeat capsule sponge test.**

	Year				
	2018	**2019**	**2020**	**2021**	**2022**
Total number of Barrett's surveillance endoscopies performed	976	989	425	612	802
Total number of Barrett's surveillance capsule sponge tests performed	0	0	55	501	597
Barrett's surveillance endoscopies performed after capsule sponge test	N/A	N/A	5	44	118
Barrett's surveillance with capsule sponge test only *	N/A	N/A	41	337	332

5.3.4 Clinical outcomes

235/2483 patients (9.5%) were discharged from the Barrett's surveillance programme over the study period, either due to age, comorbidity or initial diagnostic uncertainty of Barrett's oesophagus. In the total cohort, 133/2483 patients (5.4%) underwent treatment for persistent dysplasia or cancer: 123/133 (92.5%) had RFA +/- EMR; 7/133 (5.3%) underwent oesophagectomy; 3/133 (2.2%) had radical chemoradiotherapy. 34/2483 Barrett's surveillance patients (1.4%) demonstrated malignant transformation into intramucosal or invasive adenocarcinoma. The median time to malignant transformation from first diagnosis of Barrett's oesophagus was 76 months (IQR 24-102). 11/34 patients

(32.4%) were treated with EET; 5/34 patients (14.7%) underwent oesophagectomy; 3/34 (8.8%) were treated with radical chemoradiotherapy; 15/34 patients (44.1%) were palliated due to additional comorbidities. The 3 patients who developed oesophageal squamous cell carcinoma were all treated with oncological therapy alone with curative intent. 22/2483 patients (0.9%) died due to oesophagogastric cancer.

5.4 Discussion

This study is the first real-world assessment of the shift in Barrett's surveillance method from traditional endoscopy to capsule sponge testing on endoscopy services. Although initial trial results for capsule sponge testing were promising (Fitzgerald et al., 2020; Pilonis et al., 2022), the introduction of this service in Scotland occurred without a body of evidence to suggest this could be done in the real-world setting: unique circumstances around the endoscopy service caused by the COVID-19 pandemic have allowed this change and work to be done. However, for these clinical pathways to be formally adopted, there needs to be a clearly documented positive impact on service delivery.

These results have demonstrated that the proportion of endoscopies performed demonstrating dysplasia (all grades) and cancer increased after the pandemic and following the introduction of capsule sponge testing. The number of endoscopies performed with biopsies demonstrating simple IM only fell as a result. This implies that capsule sponge testing is enabling the surveillance endoscopy service to be tailored towards those patients at higher risk of dysplasia, increasing diagnostic yield of concerning pathology and reducing burden on patients with simple IM or no IM who can be reassured with capsule sponge testing and avoid surveillance endoscopy altogether. This has recently been suggested as a possible surveillance strategy for low-risk patients by Tan et al. (Tan et al., 2025).

It is hypothesised that the institution of capsule sponge testing has changed the approach to Barrett's surveillance patients that proceed to endoscopy. A positive finding of atypia and/or p53 positivity on capsule sponge testing allows these patients to be directed to certain endoscopists with a specialist interest in Barrett's oesophagus. Furthermore, endoscopists are prepared for the increased

likelihood of pathology in those with a positive biomarker panel on capsule sponge testing (Pilonis et al., 2022), much in the same way that screening colonoscopies with positive quantitative faecal immunochemical test (qFIT) have a higher yield of pathology (McSorley et al., 2021). The endoscopist may therefore approach the procedure with the preconception that pathology is likely to be present, rather than most Barrett's surveillance endoscopies in the past where the likelihood was the presence of simple non-dysplastic Barrett's oesophagus only. However, it is possible that this shift may be multifactorial: recent literature has shown that delayed Barrett's surveillance due to the COVID-19 pandemic negatively impacted pathology patterns overall, as discussed in Chapter 3 (Chien, Glen, Penman, Bryce, et al., 2024).

These results have also demonstrated that capsule sponge testing has resulted in a reduction in the number of surveillance endoscopies performed in NHS GGC. Although services have been reinstated following the pandemic, demand for endoscopy continues to outstrip capacity. Therefore, accessibility to an alternative investigation for Barrett's surveillance has enabled the service to be targeted towards those at higher risk of dysplasia or cancer who may benefit from more frequent surveillance and direct visual inspection of the oesophageal mucosa by endoscopists with a special interest in Barrett's oesophagus.

This study has several limitations. This study is unable to address whether patients with non-dysplastic Barrett's oesophagus on capsule sponge testing are being appropriately reassured and moved to their next surveillance interval without follow-up endoscopy for confirmation. The close audit and measure of outcomes in the capsule sponge group is integral to the continued rollout of this service. Furthermore, it was not possible to access Scotland-wide endoscopy data and therefore limited analysis to within a single health board: although inclusive of multiple hospitals within the health board, this may reduce but not eliminate the inter-departmental reporting variation of pathology. It would be useful to evaluate whether these findings on the impact on endoscopy services are mirrored in other health boards. Finally, this dataset is limited to only 28 months following the introduction of capsule sponge testing: the longer-term impact of capsule sponge testing on endoscopy services has yet to be established.

In conclusion, these preliminary results suggest that capsule sponge testing has resulted in a reduction in the number of surveillance endoscopies performed, with increased yield of concerning pre-cancerous pathology. However, dysplasia was not diagnosed at an earlier stage. This chapter provides further evidence that capsule sponge testing is enabling prioritisation of scarce endoscopy resources for those individuals at increased risk of dysplasia or cancer, although further data is required to substantiate these findings in the longer term.

Chapter 6 Long-term follow-up outcomes in low and moderate risk patients previously undergoing capsule sponge testing for Barrett's surveillance

From the evidence presented in the preceding chapters, it is undeniable that capsule sponge testing for Barrett's surveillance has garnered increasing popularity of late. However, its ongoing use has been hindered by the paucity of long-term follow-up data in this patient cohort and lack of formal clinical guidelines. This chapter aims to analyse results of patients' next follow-up using either UGI endoscopy or repeat capsule sponge testing in the lower risk cohort of patients undergoing capsule sponge testing for Barrett's surveillance in the context of the CytoSCOT programme. This chapter also aims to evaluate whether results were comparable using the Cytosponge™ versus the EndoSign® capsule sponge device, as the current literature only evaluates the use of the former and data directly comparing the two devices is lacking at present.

This chapter is based on a publication in Frontline Gastroenterology in 2026 (Chien et al., 2026). Permission to include this work was granted by the editorial team.

6.1 Introduction

Momentum for capsule sponge testing as an alternative triage investigation for Barrett's surveillance has been escalating over the past few years. As discussed, the CytoSCOT programme was a revolutionary clinical pathway initiated in Scotland in response to the COVID-19 pandemic and has demonstrated positive results as presented in previous chapters. The pilot study from the CytoSCOT programme (Chapter 2) presented the first real-world data utilising the Cytosponge™ device in clinical practice and was the first to examine its utility in a population with a previous diagnosis of Barrett's oesophagus enrolled in surveillance programmes (as opposed to the screening population targeted in existing literature) (Chien, Glen, Penman, Cruickshank, et al., 2024).

However, to modify existing clinical pathways, implementation of new technology requires buy-in and support from clinicians and policy makers (Flessa & Huebner, 2021). While the COVID-19 pandemic provided the impetus to instigate capsule sponge testing for Barrett's surveillance in Scotland, the initial results have been limited by the lack of long-term follow-up data to support its safety and ensure cases of dysplasia and cancer were appropriately detected using the device. This has impeded the formal incorporation of recommendations on the use of capsule sponge testing into recognised clinical guidelines for Barrett's surveillance.

This chapter therefore aims to evaluate long-term outcomes in the lower risk cohort of patients who were pushed to their next surveillance interval after initial capsule sponge test, by analysing results of their next follow-up using either traditional UGI endoscopy or repeat capsule sponge testing. This chapter also aims to evaluate whether results were comparable using the Cytosponge™ versus the EndoSign® capsule sponge device, which replaced Cytosponge™ as the solitary means of capsule sponge testing across Scotland in 2023.

6.2 Methods

The national CytoSCOT database was devised to incorporate patient demographics, previous Barrett's endoscopy and pathology results, initial capsule sponge test result and ongoing clinical management for all patients with

a prior diagnosis of Barrett's oesophagus who were previously enrolled in local endoscopic surveillance programmes and subsequently underwent capsule sponge testing for surveillance across 11 mainland Scottish health boards (Chien, Glen, Penman, Bryce, et al., 2024; Chien, Glen, Penman, Cruickshank, et al., 2024). Inclusion criteria defining a diagnosis of Barrett's oesophagus and the definition of segment length using the Prague classification were as described in Chapters 2, 4 and 5.

Delivery of capsule sponge testing was performed locally by trained individuals in the outpatient setting in participating health boards, with all capsule sponge tests processed and analysed in a single UK-based laboratory (Cyted Ltd.) as previously described (Chien, Glen, Penman, Bryce, et al., 2024; Fitzgerald et al., 2020). All patients included in this study underwent initial capsule sponge testing using the Cytosponge™ device. The results were returned to the requesting hospital and ongoing clinical management following the initial capsule sponge test (including length of next surveillance interval) was determined at local health board level. Following the initial capsule sponge test, patients were subcategorised into Barrett's risk groups (ultra-low, low, moderate, and high risk) based on the capsule sponge test result and clinical details as previously published and discussed within Chapter 2 (Figure 2.1) (Chien, Glen, Penman, Cruickshank, et al., 2024; Landy et al., 2023). Of note, although this clinical risk score was developed from capsule sponge cohort data, it is not routinely used in clinical practice.

The national CytoSCOT database was used to identify all patients who underwent capsule sponge testing for Barrett's surveillance between 14/9/2020 and 31/12/2021 to ensure that patients had a minimum of 3 years' follow-up at the time of data collection. Patients were included in this analysis if they had an initial capsule sponge test result demonstrating TFF3 negativity only or TFF3 positivity only (i.e. patients within the ultra-low, low and moderate risk groups only). Patients with initial capsule sponge tests demonstrating atypia and/or p53 positivity (i.e. high risk) were excluded as this triggered a different clinical decision-making pathway. Additional exclusion criteria included patients undergoing traditional endoscopic surveillance without available histopathology results at the time of analysis, patients undergoing capsule sponge testing for

investigation of reflux symptoms and all insufficient capsule sponge tests with missing biomarker data.

Clinical data were retrieved from electronic records using Clinical Portal and SCI Store for patients' next Barrett's follow-up results. Patients were trichotomized into groups based on the method of follow-up: capsule sponge testing (+/- endoscopy, based on capsule sponge results and clinical decision-making); traditional endoscopic surveillance following the BSG guidelines (Fitzgerald et al., 2014); awaiting follow-up. The former two cohorts were then compared to assess whether clinical outcomes were similar, with the group awaiting follow-up excluded from this analysis.

Further analysis was performed on the cohort of patients undergoing repeat capsule sponge testing to determine if outcomes were comparable between those undergoing repeat testing with the Cytosponge™ versus the EndoSign® device. As described in Chapter 1, the EndoSign® device is a new capsule sponge test using the same technologies as Cytosponge™ but manufactured by Cyted Ltd (Cambridge, UK). The device is administered and retrieved in the same manner as described for Cytosponge™, also utilising the biomarker panel of TFF3, atypia and p53 to risk-stratify patients and undertake similar diagnostic analyses to the Cytosponge™ device (Cyted Ltd, 2024). The results were also processed in a single UK-based laboratory (Cyted Ltd) and returned to the requesting hospital to arrange ongoing management.

Patients were not actively involved in study design, recruitment or analysis. Ethical approval for this study was obtained via local information governance teams in each health board, in addition to PBPP approval on a national level from the Caldicott guardian. All patients signed an NHS consent form prior to the procedure.

Continuous parameters were presented as median and IQR, and categorical data as counts and percentages. The Chi-squared test was performed for comparison of categorical variables, where appropriate. The Mann-Whitney U test and Kruskall-Wallis tests were performed for comparison of continuous parameters, where appropriate. A p value of ≤ 0.05 was considered statistically significant.

Statistical analysis was performed using SPSS software version 29.0 (SPSS Inc., Chicago, Illinois, USA).

6.3 Results

1812 patients were identified from the national CytoSCOT database as having an initial capsule sponge test for Barrett's surveillance demonstrating TFF3 negativity or TFF3 positivity only between 14/9/2020 and 31/12/2021. At the time of analysis, 1132/1812 patients (62.5%) had not had follow-up; 330/1812 patients (18.2%) underwent traditional endoscopic follow-up; 350/1812 patients (19.3%) had follow-up with repeat capsule sponge testing.

The 680 patients who had undergone follow-up with either UGI endoscopy or capsule sponge testing were included in this analysis. Baseline demographics are summarised in Table 6.1. Patients who underwent repeat capsule sponge testing follow-up were more likely to be male (74.9% vs. 67.0%; p=0.023), have a higher body mass index (BMI) (28.4 vs. 27.5; p=0.040), have long or ultra-long segment Barrett's (75.5% vs. 56.3%; p<0.001), have confirmed IM on biopsies at last endoscopy (89.1% vs. 79.1%; p<0.001) and be within the moderate risk group based on previous capsule sponge result (38.9% vs. 25.2%; p<0.001). There was also a longer time from initial capsule sponge test in the repeat capsule sponge testing group compared to the endoscopy only cohort (35 vs. 27 months; p<0.001).

Table 6.1 Patient demographics for those undergoing follow-up for Barrett's oesophagus after initial capsule sponge testing (n=680)
*** Data missing for 136 patients. ** Data missing for 2 patients. *** Data missing for 55 patients.**

Demographic		Endoscopy only (n=330)	Capsule sponge testing +/- endoscopy (n=350)	P value
Age (years), median (IQR)		66 (57 – 72)	66 (59 – 72)	0.887
Sex, n (%)	Male	221 (67.0%)	262 (74.9%)	0.023
	Female	109 (33.0%)	88 (25.1%)	
BMI (kg/m^2), median (IQR) *		27.5 (25.2 – 30.6)	28.4 (25.4 – 32.0)	0.040
SIMD, n (%) **	Most deprived (SIMD 1-5)	152 (46.1%)	162 (46.6%)	0.898
	Least deprived (SIMD 6-10)	178 (53.9%)	186 (53.4%)	
Smoking status, n (%) ***	Never smoker	164 (55.4%)	175 (53.2%)	0.579
	Ever smoker	132 (44.6%)	154 (46.8%)	
Barrett's segment length, n (%)	Short	144 (43.6%)	86 (24.6%)	<0.001
	Long	173 (52.4%)	240 (68.6%)	
	Ultra-long	13 (3.9%)	24 (6.9%)	
IM present at last endoscopy, n (%)		261 (79.1%)	312 (89.1%)	<0.001
Barrett's risk group (based on initial capsule sponge result), n (%)	Ultra-low	145 (43.9%)	103 (29.4%)	<0.001
	Low	102 (30.9%)	111 (31.7%)	
	Moderate	83 (25.2%)	136 (38.9%)	
Time from initial capsule sponge test to next follow-up (months), median (IQR)		27 (16 – 36)	35 (21 – 36)	<0.001
Median length of follow-up (months), median (IQR)		38 (35 – 43)	39 (35 – 42)	0.774

Table 6.2 summarises endoscopic biopsy results for the 330 patients whose next follow-up was performed with endoscopy only, summarised by worst pathology result. Within this cohort, 3.6% of patients progressed to LGD, 1.8% progressed to HGD, 0.3% progressed to IMC and 0.3% progressed to invasive OAC. There were 11/330 cases of HGD, IMC and invasive UGI malignancy within this cohort (3.3%). 4/11 patients underwent either EMR and/or RFA; 2/11 patients were awaiting EMR and/or RFA at the time of analysis; 2/11 patients underwent radical chemoradiotherapy with curative intent; 1/11 patient with OAC had undergone

Ivor-Lewis oesophagectomy. The 2 patients with gastric adenocarcinoma were palliated due to additional comorbidities and died as a result. There were no cases of HGD, IMC or invasive OAC diagnosed within 12 months of initial capsule sponge result.

Table 6.2 Endoscopic biopsy results for patients undergoing Barrett's follow-up with traditional endoscopy (n=330)

Endoscopic biopsy result	N (%)	Time from initial capsule sponge (months), median (IQR)
No intestinal metaplasia	84 (25.5%)	25 (16 – 34)
Intestinal metaplasia	209 (63.3%)	27 (14 – 36)
Indefinite for dysplasia	14 (4.2%)	35 (26 – 37)
Low grade dysplasia	12 (3.6%)	30 (19 – 37)
High grade dysplasia	6 (1.8%)	36 (33 – 37)
Intramucosal adenocarcinoma	1 (0.3%)	18 (18 – 18)
Oesophageal adenocarcinoma	1 (0.3%)	22 (22 – 22)
Oesophageal squamous cell carcinoma	1 (0.3%)	19 (19 – 19)
Gastric adenocarcinoma	2 (0.6%)	19 (15 – 22)

For the 350 patients who underwent repeat capsule sponge testing as their next form of follow-up, the results of the second capsule sponge test are summarised in Table 6.3. Patients with insufficient tests and hence missing biomarker data were excluded. Most patients had a reassuring repeat capsule sponge test demonstrating TFF3 positivity only (62.9%) or TFF3 negativity (28.3%). 31/350 patients (8.8%) had an abnormal repeat capsule sponge test (i.e. atypia and/or p53 positive).

Table 6.3 Capsule sponge test results for patients undergoing Barrett's follow-up with repeat capsule sponge testing (n=350)

Repeat capsule sponge test result	N (%)
TFF3 negative	99 (28.3%)
TFF3 positive only	220 (62.9%)
Atypia only	13 (3.7%)
p53 only	12 (3.4%)
Atypia + p53	6 (1.7%)

67/350 patients (19.1%) proceeded to UGI endoscopy following repeat capsule sponge testing (either based on an abnormal capsule sponge result or clinical judgement) and had available histopathology results at the time of analysis. This

is summarised within Table 6.4. Within the total cohort undergoing repeat capsule sponge testing as the next form of follow-up, 3/350 (0.9%) progressed to LGD, 3/350 (0.9%) progressed to HGD and 1/350 (0.3%) progressed to IMC. There were no cases of invasive UGI malignancy diagnosed within this group. Of the 4 cases of HGD or IMC within this cohort, all 4 cases were diagnosed at a minimum of 34 months after first capsule sponge test and detected on capsule sponge testing with an abnormal result demonstrating atypia and/or p53 positivity. 3 patients with HGD underwent RFA, whilst the 1 patient with IMC was deemed unfit for intervention due to additional comorbidities. Only 1 patient diagnosed with LGD was not identified with an abnormal capsule sponge test result: this test was performed using the Cytosponge™ device. Of note, 6 patients with an abnormal capsule sponge test result were awaiting urgent UGI endoscopy at the time of analysis and were not included in Table 6.4. Capsule sponge testing using atypia and/or p53 biomarkers had a PPV of 24.0% for dysplasia (any grade) and cancer in this cohort. When cases of LGD were excluded, the PPV of capsule sponge testing using atypia and/or p53 biomarkers was 16.0% for HGD and cancer. Figure 6.1 demonstrates the workflow of patients based on final endoscopic pathology results for both cohorts.

Table 6.4 Endoscopic biopsy results for patients within the repeat capsule sponge test cohort categorised by capsule sponge result (n=67)

Endoscopic biopsy result	N (%)	Repeat capsule sponge test result					P value
		TFF3 negative	TFF3 positive only	Atypia only	p53 only	Atypia + p53	
No intestinal metaplasia	17 (25.4%)	13 (76.5%)	3 (17.6%)	1 (5.9%)	0 (0%)	0 (0%)	0.041
Intestinal metaplasia	40 (59.7%)	20 (50.0%)	6 (15.0%)	8 (20.0%)	3 (7.5%)	5 (12.5%)	0.417
Indefinite for dysplasia	3 (4.5%)	1 (33.3%)	0 (0%)	1 (33.3%)	1 (33.3%)	0 (0%)	0.631
Low grade dysplasia	3 (4.5%)	1 (33.3%)	0 (0%)	0 (0%)	1 (33.3%)	1 (33.3%)	0.361
High grade dysplasia	3 (4.5%)	0 (0%)	0 (0%)	1 (33.3%)	2 (66.7%)	0 (0%)	0.030
IMC	1 (1.5%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0.112
OAC	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-

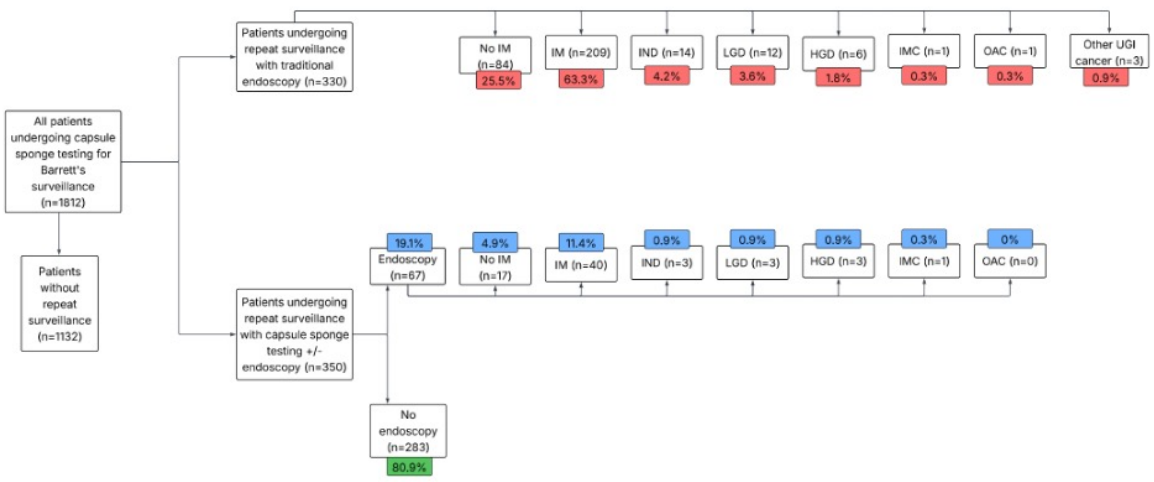

Figure 6.1 Workflow of patients based on final endoscopic pathology results for both cohorts

Inclusive of both follow-up cohorts, 397/680 patients (58.4%) proceeded to UGI endoscopy. 47/680 patients (6.9%) had biopsies demonstrating IND, LGD, HGD, IMC, OAC or OSCC. Table 6.5 compares these patients by method of Barrett's follow-up. As most patients with a TFF3 positive or TFF3 negative only repeat capsule sponge result did not undergo UGI endoscopy routinely, cases of no IM and IM only were not included in Table 6.5. There was no significant difference in the detection of HGD, IMC or invasive cancer between the two cohorts. Traditional endoscopic surveillance diagnosed more cases of IND (4.2% vs. 0.9%; p=0.012) and LGD (3.6% vs. 0.9%; p=0.032) compared to repeat capsule sponge testing.

Table 6.5 Incidence of dysplasia and cancer categorised by Barrett's follow-up method

Endoscopic biopsy result	Method of Barrett's follow-up		P value
	UGI endoscopy only (n=330)	Repeat capsule sponge testing (+/- endoscopy) (n=350)	
Indefinite for dysplasia	14 (4.2%)	3 (0.9%)	0.012
Low grade dysplasia	12 (3.6%)	3 (0.9%)	0.032
High grade dysplasia	6 (1.8%)	3 (0.9%)	0.273
Intramucosal adenocarcinoma	1 (0.3%)	1 (0.3%)	0.967
Oesophageal adenocarcinoma	1 (0.3%)	0 (0%)	0.303
Oesophageal squamous cell carcinoma	1 (0.3%)	0 (0%)	0.303

Of the 350 patients who underwent repeat capsule sponge testing, the Cytosponge™ device was utilised in 137/350 cases (39.1%) and the EndoSign® device was utilised in 213/350 cases (60.9%). Table 6.6 compares results between the two devices. As demonstrated in Table 6.1, 312/350 patients (89.1%) of the repeat capsule sponge cohort had confirmed IM on last endoscopic biopsies: this was higher in the EndoSign® cohort versus the Cytosponge™ cohort but did not reach statistical significance (195/213 patients (91.5%) vs. 117/137 patients (85.4%); p=0.071). EndoSign® detected a higher proportion of TFF3 positive cases compared to the Cytosponge™ device (72.3% vs. 48.2%; p<0.001). There were significantly more patients with abnormal capsule sponge results (i.e. atypia and/or p53 positive) in the EndoSign® group. Both devices demonstrated sound and comparable reproducibility when the test was repeated: 265/350 patients (75.7%) had the same result on repeat capsule sponge testing compared to their initial result. However, there was a significantly longer time from initial capsule sponge test in the EndoSign® group compared to the Cytosponge™ group (36 vs. 14 months; p<0.001). In the group undergoing repeat capsule sponge testing with Cytosponge™, there was a temporal association with TFF3 result: the median time from last capsule sponge test was longer in the TFF3 positive group versus the TFF3 negative group (23 months (IQR 14-25) vs. 7 months (IQR 11-15); p<0.001). This association was not observed within the EndoSign® group (p=0.536). Patients within the EndoSign®

repeat sponge cohort were significantly more likely to have longer Barrett's segments (p<0.001). The association between Barrett's segment length and TFF3 positivity is further analysed in Table 6.7, confirming that repeat tests were significantly more likely to be TFF3 positive in longer Barrett's segments for both devices (p<0.001).

Table 6.6 Repeat capsule sponge test results comparing both devices

		Oesophageal cell collection device used		P value
		Cytosponge™ (n=137)	EndoSign® (n=213)	
Capsule sponge test result, n (%)	TFF3 negative	71 (51.8%)	28 (13.1%)	<0.001
	TFF3 positive only	66 (48.2%)	154 (72.3%)	<0.001
	Atypia only	0 (0%)	13 (6.1%)	0.003
	p53 only	0 (0%)	12 (5.6%)	0.005
	Atypia + p53	0 (0%)	6 (2.8%)	0.048
Patients with same result on second test, n (%)		106 (77.4%)	159 (74.6%)	0.562
Time from initial capsule sponge test (months), median (IQR)		14 (8 – 24)	36 (35 – 37)	<0.001
Barrett's segment length, n (%)	Short	71 (51.8%)	15 (7.1%)	<0.001
	Long	61 (44.5%)	179 (84.0%)	
	Ultra-long	5 (3.7%)	19 (8.9%)	

Table 6.7 Correlation between TFF3 result and Barrett's segment length for both devices

Proportion of capsule sponge tests demonstrating TFF3 positivity by oesophageal cell collection device	Barrett's segment length			P value
	Short	Long	Ultra-long	
Cytosponge™, n (%)	12 (16.9%)	49 (80.3%)	5 (100%)	<0.001
EndoSign®, n (%)	6 (40.0%)	160 (89.4%)	18 (94.7%)	<0.001

6.4 Discussion

There has been a paucity in the long-term data available in the current literature to support the ongoing use of capsule sponge testing for Barrett's

surveillance beyond the pandemic. This study is the first to analyse patients' subsequent Barrett's follow-up after initially undergoing capsule sponge testing for Barrett's surveillance as part of the CytoSCOT pilot programme launched during the COVID-19 pandemic.

This dataset includes analysis of 680 patients whose initial capsule sponge test result returned as either TFF3 negative or TFF3 positive only. At the time of next follow-up, this study has demonstrated rates of progression to LGD, HGD, IMC and OAC of 3.6%, 1.8%, 0.3% and 0.3% respectively within the endoscopy only cohort and 0.9%, 0.9%, 0.3% and 0% respectively within the repeat capsule sponge cohort. The overall incidence rates for the 680 patients included in this analysis were 2.2% for LGD, 1.3% for HGD, 0.3% for IMC and 0.1% for invasive OAC. These incidence rates are lower than previously reported in the literature for LGD (8%), HGD (4%) and OAC (1-2%) for patients undergoing traditional endoscopic Barrett's surveillance (de Jonge et al., 2010; Peters et al., 2019). The overall incidence rates of these cases may be lower than expected as the high-risk group of patients who had previously tested positive for atypia and/or p53 on initial capsule sponge testing were not included in this analysis. It is postulated that this high-risk group may be more likely to develop dysplastic Barrett's change compared to the ultra-low, low and moderate risk groups presented in this study, although further long-term data is lacking at present to support this hypothesis. Previous work from the CytoSCOT programme has demonstrated that 91.7% of this high-risk group in Scotland proceeded direct to urgent endoscopy after initial capsule sponge result and have been subject to increased frequency of surveillance compared to recommended BSG guidelines (Chien, Glen, Penman, Cruickshank, et al., 2024): future research focused on outcomes in this cohort will be imperative to improve understanding of the potential of capsule sponge testing in the Barrett's surveillance population.

When follow-up methods were compared, traditional surveillance with UGI endoscopy detected more cases of IND (4.2% vs. 0.9%; p=0.012) and LGD (3.6% vs. 0.9%; p=0.032) compared to repeat capsule sponge testing. This does raise potential concerns that capsule sponge testing may not be as accurate at detecting cases of early dysplasia compared to UGI endoscopy, as discussed in Chapter 4 (Chien & Glen, 2025). Notably, these results must be interpreted with caution, as there were 283 patients within the repeat capsule sponge group who

did not undergo endoscopy, and it therefore cannot confidently be stated that these patients did not have significant pathology on endoscopic biopsies. However, the clinical significance of these pathologies remains uncertain in the current literature: although increased frequency of surveillance of LGD is recommended, the current BSG guidelines imply that there is dubiety about the natural progression of LGD to HGD or cancer at present and EET is not routinely recommended in the British societal guidelines (Fitzgerald et al., 2014). As discussed in Chapter 4, there is also considerable interobserver variability and over-diagnosis of IND and LGD at endoscopy (Falk, 2017; Thota et al., 2016), implying that there are also limitations to diagnosis at UGI endoscopy within this cohort and UGI endoscopy is also not a perfect test.

However, it is reassuring that there were no significant differences in the detection of HGD, IMC or invasive cancer between the endoscopy only and repeat capsule sponge testing cohorts. These results are encouraging for the repeat capsule sponge cohort, as this group had more patients within the moderate risk group (38.9% vs. 25.2%; $p<0.001$) with longer Barrett's segments (75.5% vs. 56.3%; $p<0.001$) so were hypothetically at increased risk of developing dysplasia, as longer Barrett's segments are more likely to progress to dysplasia (Anaparthy et al., 2013). It is also reassuring that the incidence rates of HGD, IMC and OAC are not higher than expected at follow-up, as this implies that capsule sponge testing was unlikely to have misdiagnosed these cases at the time of initial testing: this is reinforced by the longer median time to diagnosis of these pathologies as demonstrated in Table 6.2. This finding also reduces the likelihood of missing cases of true early dysplasia at first capsule sponge test, as these patients could potentially have progressed to more advanced disease over the minimum 3-year follow-up period for this study, although further research is again required to substantiate this hypothesis.

The current literature supporting the use of capsule sponge testing in cases of Barrett's oesophagus is currently limited to use of the Cytosponge™ device (Chien, Glen, Penman, Cruickshank, et al., 2024; Fitzgerald et al., 2020; Pilonis et al., 2022; Ross-Innes et al., 2017). It has been assumed that, since both devices retain the same design and function and laboratory analysis remains consistent, the existing evidence base could be generalised to both devices (Scottish Health Technologies Group, 2023). This is the first study to directly

analyse results of the EndoSign® device compared to Cytosponge™, which is no longer utilised in the UK. A previous study from the CytoSCOT programme presented a PPV of 16.7% for HGD, IMC and cancer for capsule sponge testing using the Cytosponge™ device (Chien, Glen, Penman, Cruickshank, et al., 2024). In this cohort, capsule sponge testing demonstrated a PPV for 24.0% for all cases of confirmed dysplasia or cancer: these cases were all detected by the EndoSign® device, as there was no atypia and/or p53 positive tests in the Cytosponge™ group. However, the PPV dropped to 16.0% when cases of LGD were excluded, implying that the devices perform comparably in the detection of HGD, IMC and cancer. Reassuringly, there were once again no cases of HGD, IMC or invasive UGI cancer missed in the repeat capsule sponge cohort, which validates the findings of the initial CytoSCOT study (Chien, Glen, Penman, Cruickshank, et al., 2024). The solitary case of LGD that was misdiagnosed on repeat capsule sponge testing with a TFF3 negative test was using the Cytosponge™ device, providing further reassurance that EndoSign® is safe for clinical use. Repeat capsule sponge testing with both devices also show good reproducibility, with 75.7% of tests demonstrating the same result on repeat testing.

Overall, the cohort undergoing repeat capsule sponge testing with the EndoSign® device appeared to have significantly more atypia and/or p53 positive results. This would initially imply that EndoSign® may have improved dysplasia detection rates. However, the time from initial capsule sponge test was much longer in the EndoSign® group compared to the Cytosponge™ group (36 vs. 14 months; $p<0.001$). Dysplasia may have developed over this longer time interval within the former cohort, making these results difficult to interpret and invalidating this initial assumption.

However, EndoSign® detected significantly more TFF3 positive tests than the Cytosponge™ device (72.3% vs. 48.2%; $p<0.001$). Given that 89.1% of patients within the repeat capsule sponge testing group had confirmed IM at previous endoscopy and there was no significant difference between the two cohorts as to proportion of patients with IM at last endoscopy, this provisionally implies that the EndoSign® device may be superior at detecting Barrett's oesophagus than Cytosponge™. This could be explained by three possible hypotheses. Firstly, previous research in Chapter 2 has demonstrated that TFF3 positivity increases

with increasing Barrett's segment length (Chien, Glen, Penman, Cruickshank, et al., 2024). Patients undergoing repeat capsule sponge testing with the EndoSign® device were significantly more likely to have longer Barrett's segments compared to the Cytosponge™ device ($p<0.001$), which may explain the increased proportion of TFF3 positive cases. This was confirmed in Table 6.7, which again demonstrated the positive correlation between increasing segment length and TFF3 positivity with both devices. Secondly, there was a temporal correlation between TFF3 positivity and longer median time from last capsule sponge test in the Cytosponge™ group, which may indicate disease progression, and the EndoSign® cohort had a significantly longer time from initial capsule sponge test than the Cytosponge™ group. Finally, there is a slight difference in the expanded diameter of the capsule sponge devices: while both are reported to be 30mm in diameter, the EndoSign® device has the capacity to expand by a further 2mm in some instances, which may lead to increased cell pick-up (Cyted Ltd, 2024; Kadri et al., 2010). It is also worth noting that the Cytosponge™ device was slightly modified in May 2023: only one repeat test using the Cytosponge™ device was included after this time, which demonstrated a TFF3 positive result. It is worth noting that this test was performed on 1 June 2023 and could potentially have been from an older batch of Cytosponge™ devices using the previous design. Further research using the EndoSign® device on a larger scale is required to determine whether these initial findings can be validated or if they are related to true differences in these real-world cohorts as described above.

There are further limitations to this dataset, many of which are also discussed in previous chapters. After initial capsule sponge testing, ongoing clinical management (including timing and method of next surveillance) was decided at local health board level. Consequently, there was significant heterogeneity between health boards, which further highlights the need for national recommendations and reflects the real-world nature of this study. It was not possible to obtain access to national data for the patients who underwent Barrett's surveillance with traditional endoscopy over the same period as initial capsule sponge testing (14/9/2020 to 31/12/2021) due to data restrictions. Comparison of rates of progression to dysplasia and cancer across the country in these two cohorts could have consolidated these findings and provided further

reassurance for capsule sponge testing across similar surveillance populations. Additionally, indication for endoscopy was not included in this dataset: a subset of patients within both the endoscopy and repeat capsule sponge testing group were referred for endoscopy due to the development of red-flag UGI symptoms. This may have falsely elevated the cancer detection rates, particularly within the endoscopy only group, compared to a purely surveillance only population and may also explain the detection of 2 cases of gastric adenocarcinoma within the endoscopy only cohort (red-flag UGI symptoms are a contraindication to capsule sponge testing). Finally, whilst this data does reinforce that capsule sponge testing appears to be safe and effective at detecting HGD and cancer cases, the missing endoscopy data for the 283 patients in the repeat capsule sponge testing group means that robust conclusions to suggest that capsule sponge testing could formally replace UGI endoscopy as the recommended form of Barrett's surveillance moving forward cannot be drawn at this juncture. Whilst this study adds to the current literature, ongoing follow-up of this cohort is still required before formal clinical pathways and guidelines can be modified.

Chapter 7 Clinical application of capsule sponge testing in symptomatic reflux patients

While the previous chapters have focused on the impact of capsule sponge testing on the Scottish Barrett's surveillance population, capsule sponge testing (using the OCCD with biomarkers) was also implemented nationally across Scotland in 2020 for symptomatic reflux patients referred to secondary care for non-urgent endoscopy. The aim of this pathway within the CytoSCOT programme was to use capsule sponge testing as a case-finding triage tool to reduce pressures on the endoscopy service during COVID-19, focus endoscopy resources on those most likely to have pathology and streamline the patient pathway for reflux symptoms. Chapter 7 presents the first real-world results and evaluates the clinical application of capsule sponge testing in symptomatic reflux disease based on endoscopic biopsy results.

This chapter is based on a publication in BMC Gastroenterology in 2024 (Chien, Glen, Bryce, et al., 2024). Permission to include this work was granted by the editorial team.

7.1 Introduction

Gastro-oesophageal reflux disease (GORD) is a common chronic gastrointestinal condition, affecting up to 20% of adults in the western population (El-Serag et al., 2014). GORD is predominantly characterised by heartburn symptoms, triggered by regurgitation of gastric contents into the lower oesophagus. As discussed in Chapter 1, GORD has been linked to the development of Barrett's oesophagus, currently the only identifiable precursor to OAC (Lagergren et al., 1999) and identified in 7% of patients with reflux symptoms (Saha et al., 2024). Improved detection of Barrett's oesophagus offers the potential for earlier diagnosis of both dysplasia and early cancer, leading to improved patient outcomes (El-Serag et al., 2016). Currently, all major gastroenterology societies recommend screening for Barrett's oesophagus in patients with risk factors (e.g. chronic reflux symptoms; male sex; age >50 years; Caucasian race; smoking history; obesity; family history of Barrett's oesophagus or OAC) (Fitzgerald et al., 2014; Shaheen et al., 2022).

Despite being the current gold standard for investigation of reflux symptoms, UGI is not without limitations, as discussed extensively in the preceding chapters. In addition to the drawbacks previously described, the majority of UGI endoscopies performed for reflux symptoms do not yield pathology, placing unnecessary burden on both patients and healthcare systems (Lin et al., 2019).

Evidently, there is scope for innovative diagnostic tools which are easily accessible, minimally invasive and cost-effective to ease the burden on endoscopy services. While the previous chapters have concentrated on the role of capsule sponge testing in Barrett's surveillance patients, most of the research published thus far has in fact focused on the detection of Barrett's oesophagus in the reflux population. Trial data from a large, multicentre RCT have suggested that capsule sponge testing is a useful triage tool to UGI endoscopy in individuals on medication for reflux symptoms who are proactively invited for a screening test (Fitzgerald et al., 2020; Ross-Innes et al., 2017). However, current literature fails to extrapolate these promising results to real-world clinical practice and correlate capsule sponge result with endoscopic biopsies (Landy et al., 2023).

The CytoSCOT programme offered patients capsule sponge testing using an OCCD as an alternative investigation to UGI endoscopy, aiming to reduce pressures on endoscopy services and aid recovery from the COVID-19 pandemic, with the service delivered by trained UGI endoscopy nurses within secondary care in the outpatient setting (Scottish Government, 2021). Whilst predominantly used to risk-stratify those undergoing Barrett's surveillance in Scotland (Chien, Glen, Penman, Cruickshank, et al., 2024), capsule sponge testing was also used as a case-finding triage tool to UGI endoscopy for patients referred to secondary care with persistent GORD symptoms.

This national prospective cohort study aims to evaluate whether capsule sponge testing is a safe and effective diagnostic triage tool in the investigation of reflux symptoms and present the first real-world results of capsule sponge testing for GORD beyond the trial setting in the context of the CytoSCOT programme.

7.2 Methods

Prospectively maintained local databases were used to identify all patients who underwent capsule sponge testing using the Cytosponge™ OCCD (Medtronic, Minnesota, USA) following referral to secondary care for investigation of reflux symptoms in the absence of red flag symptoms (i.e. dysphagia, weight loss, anaemia) between 14 September 2020 and 30 April 2023. All patients referred from primary care on the routine reflux pathway were considered eligible: reflux symptoms generally included burning sensation, acid taste, waterbrash and/or regurgitation. There were no specific inclusion criteria. 11 Scottish health boards were included in this analysis.

Capsule sponge testing was carried out as described in Chapter 1 by a trained practitioner in the outpatient clinic setting. All capsule sponge tests were processed centrally in a UK-based diagnostic laboratory (Cyted Ltd), as previously described in Chapter 2. Cyted Ltd returned all processed pathology reports to the requesting hospital, with ongoing clinical management decided by the UGI specialist team within the parent health board.

Individual patient electronic records were interrogated to record baseline demographics, capsule sponge test result, indication for endoscopy, endoscopic

biopsy results and ongoing clinical management. In addition to capsule sponge testing, all patients underwent clinical assessment either by UGI specialist nurses or consultants in secondary care to aid decision-making and ongoing management: this was performed either virtually via telephone consultation or face-to-face in the outpatient clinic setting. Triage assessment was performed prior to capsule sponge test to identify presence of red flag symptoms as discussed above and suitability for capsule sponge testing, as well as after capsule sponge testing to ensure resolution of symptoms and safety for discharge from secondary care. Further analysis was performed on all patients who subsequently underwent UGI endoscopy and had available histopathology results.

Exclusion criteria included: inability to tolerate capsule sponge testing; capsule sponge testing for Barrett's surveillance; those with outstanding histopathology results at time of analysis; paediatric population (age <18 years). Contraindications to capsule sponge testing were specified by the manufacturer as previously published (Fitzgerald et al., 2020) and included: pregnancy; liver disease including cirrhosis; oesophageal varices; significant dysphagia; previous oesophageal tumour; oesophageal surgery (including endoscopic therapy). Patients were not actively involved in study design, recruitment or analysis.

Ethical approval was obtained via local information governance teams in each health board, in addition to PBPP approval on a national level from the Caldicott guardian. All patients signed an NHS consent form prior to the procedure.

Continuous parameters were presented as median and IQR, and categorical data as counts and percentages. The Chi-squared test was performed for comparison of categorical variables, where appropriate. A p value of ≤ 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 28.0 (SPSS Inc., Chicago, Illinois, USA).

7.3 Results

During the study period, 1385 capsule sponge tests were performed for symptomatic reflux across NHS Scotland, on 1305 patients (80 tests were repeat tests performed due to insufficient first samples or assessment of inflammation healing). The median follow-up time was 20 months (IQR 12-27). Sponge

detachment was reported in 2/1385 cases (0.1%), with both devices retrieved endoscopically within 4 hours with no additional complications reported. Patient demographics are recorded in Table 7.1. 42.4% of the patients were male, with a median age of 56 years (IQR 46-65). However, the age range of patients included in this analysis was 18-93 years. The median BMI was 28.1, although data were missing for 411 patients. 519/1385 patients (37.5%) had a positive smoking history, although data were missing for 26 patients. 88.2% of patients were on a PPI prior to test.

Table 7.1 Patient demographics (n=1385)

Patient demographic	
Male sex, n (%)	587 (42.4%)
Age (years), median (IQR)	56 (46 – 65)
BMI (kg/m^2), median (IQR)	28.1 (25.0 – 32.4)
Positive smoking history, n (%)	519 (37.5%)
PPI use, n (%)	1221 (88.2%)

Table 7.2 summarises capsule sponge test results within the symptomatic reflux cohort by worst pathology result. Most capsule sponge tests (1103/1385; 79.6%) were biomarker negative. Figure 7.1 demonstrates patient workflow and clinical management as per capsule sponge test result. 913/1305 patients (70.0%) were discharged from secondary care with no additional investigations planned following capsule sponge testing and clinical assessment.

Table 7.2 Capsule sponge test results (n=1385)

Capsule sponge test result	**N (%)**
TFF3 negative	1103 (79.6%)
TFF3 positive only	116 (8.4%)
Atypia positive only	20 (1.4%)
p53 positive only	1 (0.1%)
Atypia and p53 positive	3 (0.2%)
Insufficient	142 (10.3%)

Figure 7.1 Patient workflow and clinical management based on capsule sponge test result within the symptomatic reflux group (n=1385)

355/1305 patients (27.2%) proceeded to undergo UGI endoscopy following capsule sponge testing. The median time to UGI endoscopy after capsule sponge test was 5 months (IQR 2-10). 279/355 (78.6%) of UGI endoscopies were performed within 12 months of capsule sponge testing. Indications for UGI endoscopy within this group are summarised in Table 7.3. 120/355 UGI endoscopies (33.8%) were performed in response to a capsule sponge result demonstrating TFF3, atypia and/or p53 positivity, whereas the other endoscopies were performed at clinical discretion due to ongoing symptoms or lack of reassurance by the test. Table 7.4 demonstrates endoscopic biopsy results by worst pathology result. Where the UGI tract appeared macroscopically normal at UGI endoscopy, no biopsies were taken. Overall, 55/1305 (4.2%) in the reflux group (55/355 of those who underwent endoscopy; 15.5%) were diagnosed with Barrett's oesophagus, after endoscopic biopsies demonstrated IM (including 1 patient who also exhibited LGD and 1 patient with neuroendocrine carcinoma of the oesophagus).

Table 7.3 Indication for UGI endoscopy (n=355)

Indication	N (%)
Ongoing reflux symptoms	124 (34.9%)
TFF3 positive capsule sponge result	100 (28.2%)
Insufficient capsule sponge result	39 (11.0%)
Atypia +/- p53 positive capsule sponge result	20 (5.6%)
Investigation of red flag UGI symptoms	43 (12.1%)
Assessment of ulcer healing	27 (7.6%)
Capsule sponge detachment	2 (0.6%)

Table 7.4 Endoscopic biopsy results (n=355)

Endoscopic biopsy result	N (%)
No biopsies	111 (31.3%)
No intestinal metaplasia	184 (51.8%)
Intestinal metaplasia	53 (14.9%)
Low grade dysplasia	1 (0.3%)
Oesophageal adenocarcinoma	1 (0.3%)
Gastric adenocarcinoma	3 (0.8%)
Gastric lymphoma	1 (0.3%)
Neuroendocrine carcinoma of the oesophagus	1 (0.3%)

Table 7.5 demonstrates endoscopic biopsy results compared with biomarker status (i.e. TFF3, atypia, and/or p53) on capsule sponge testing. All insufficient capsule sponge tests (and thus missing biomarker data) were excluded. Endoscopists were significantly more likely to obtain biopsies in cases where capsule sponge testing showed positive biomarkers (83.9% vs. 16.1%; $p < 0.001$). Inclusive of the 2 patients with LGD and neuroendocrine carcinoma, 52/314 patients (16.6%) demonstrated IM on endoscopic biopsies: biomarkers for TFF3, atypia and/or p53 were significantly more likely to be positive in this group (46/52; 88.5% vs. 11.5%, $p < 0.001$). In total, 45/52 patients with confirmed IM (86.5%) demonstrated TFF3 positivity on capsule sponge testing.

Table 7.5 Endoscopic biopsy results compared with biomarker status (n=314)

Endoscopic biopsy result	All, n (%) (n=314)	TFF3 negative, n (%) (n=190)	TFF3 +/- atypia +/- p53 positive, n (%) (n=124)	P value
No biopsies	93 (29.6%)	78 (83.9%)	15 (16.1%)	<0.001
No intestinal metaplasia	165 (52.5%)	102 (61.8%)	63 (38.2%)	0.618
Intestinal metaplasia	50 (15.9%)	6 (12.0%)	44 (88.0%)	<0.001
Low grade dysplasia	1 (0.3%)	0 (0%)	1 (100%)	0.215
Oesophageal adenocarcinoma	1 (0.3%)	1 (100%)	0 (0%)	0.418
Gastric adenocarcinoma	2 (0.6%)	2 (100%)	0 (0%)	0.252
Gastric lymphoma	1 (0.3%)	1 (100%)	0 (0%)	0.418
Neuroendocrine carcinoma of the oesophagus	1 (0.3%)	0 (0%)	1 (100%)	0.215

Whilst 116/1385 capsule sponge tests demonstrated TFF3 positivity only, this increased to 123/1385 (8.9%) TFF3 positive tests when inclusive of those also demonstrating atypia and/or p53 positivity. When the 142 insufficient tests were removed, this equated to 9.9% of the total symptomatic reflux cohort with a TFF3 positive capsule sponge result (123/1243), suggesting a possible underlying diagnosis of Barrett's oesophagus. Within this TFF3 positive group, 111/123 patients (90.2%) had undergone UGI endoscopy with available biopsy results as follows: 52/111 (46.8%) demonstrated no IM; 43/111 (38.7%) demonstrated IM; 1/111 (0.9%) demonstrated LGD; 1/111 (0.9%) demonstrated neuroendocrine carcinoma of the oesophagus; and 14/111 (12.6%) had a macroscopically normal UGI tract and therefore no biopsies were taken.

24/1385 capsule sponge tests (1.7%) demonstrated atypia and/or p53 positivity on biomarker testing: 20/24 of these patients (83.3%) subsequently underwent UGI endoscopy. Of the 4 patients missing endoscopy data in this high-risk group, 2/4 declined invitation to endoscopy, 1/4 was awaiting endoscopy at the time of analysis, and 1/4 died from unrelated causes before endoscopy was performed. Endoscopic biopsy results within this high-risk group were as follows: 14/20 (70.0%) demonstrated no IM; 4/20 (20.0%) demonstrated IM; 1/20 (5.0%)

demonstrated neuroendocrine carcinoma; and 1/20 (5.0%) had a macroscopically normal UGI tract with no biopsies obtained.

1103/1385 capsule sponge tests (79.6%) were negative for TFF3, atypia and p53: these tests therefore did not prompt automatic referral for endoscopy and required sound clinical decision-making to assess appropriateness for discharge. 10/1103 TFF3 negative patients (0.9%) proceeded to have an UGI endoscopy and were found to have significant pathology: 6/10 demonstrated IM; 1/10 demonstrated oesophageal adenocarcinoma; 2/10 demonstrated gastric adenocarcinoma and 1/10 demonstrated gastric lymphoma. Endoscopy was triggered by the subsequent development of red flag symptoms on clinical assessment in all 10 cases.

The patients with a cancer diagnosis are discussed in more detail here. The patient diagnosed with OAC initially had a TFF3 negative result but was referred to the surgical outpatient clinic for further review due to the development of red flag UGI symptoms following capsule sponge testing. This was identified on telephone consultation with the local UGI specialist nurse. The patient was lost to follow-up without an outpatient clinic appointment being made and was re-referred from primary care with progressive dysphagia, undergoing UGI endoscopy and receiving the diagnosis of OAC 27 months after capsule sponge testing. There were no cases of oesophageal squamous cell carcinoma within our cohort.

4/1305 patients (0.3%) were found to have a gastric malignancy at UGI endoscopy. Gastric pathology cannot be detected by a capsule sponge test unless it is sampled from the gastric cardia. Therefore, checking for any gastric symptoms is an important part of patient assessment before deciding that capsule sponge is an appropriate test. One case triggered endoscopy due to an insufficient capsule sponge result, which is the appropriate action as part of the clinical guidance and serendipitously had a gastric lymphoma diagnosed. However, the other three cases with a negative capsule sponge result re-presented at a later date with progressive red flag symptoms (although all 3 had persistent reflux symptoms and epigastric discomfort, 2 patients also described significant weight loss, whilst 1 patient presented with new iron deficiency anaemia). All cancer patients were over the age of 55 years. The patient with

gastric lymphoma underwent oncological treatment with curative intent. 1 patient underwent neoadjuvant chemotherapy followed by surgical resection but was found to have distant metastases intra-operatively. 2 patients were palliated due to additional co-morbidities. Overall, 3/4 patients died due to gastric malignancy: this equates to 0.2% when extrapolated to the whole population within this study. Figure 7.2 summarises the overall workflow and decision-making pathway of all valid capsule sponge results (n=1243).

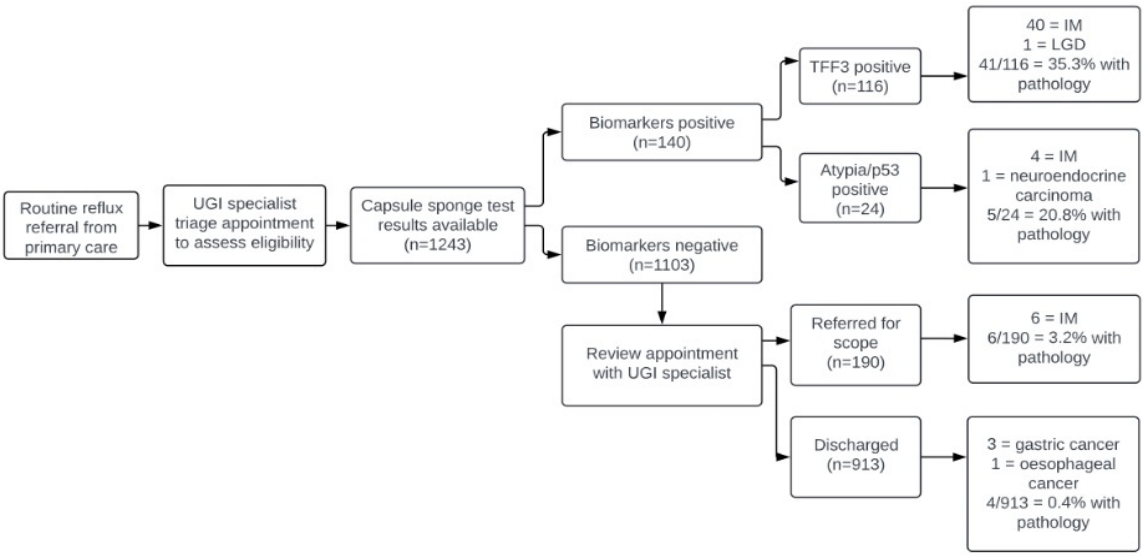

Figure 7.2 Summary of workflow of all valid capsule sponge results (n=1243)

7.4 Discussion

While evidence for the role of capsule sponge testing is sound within the trial setting (Fitzgerald et al., 2020; Pilonis et al., 2022) and emerging in the context of Barrett's surveillance (Chien, Glen, Penman, Cruickshank, et al., 2024), its application within clinical practice for symptomatic reflux disease has yet to be critiqued. This is the first study to analyse the use of capsule sponge testing in symptomatic reflux patients across a wide geographical location in the real-world setting and evaluate the association between capsule sponge test and endoscopic biopsy results.

Within the symptomatic reflux population referred to secondary care without alarm symptoms, 1.7% were recommended urgent endoscopy and 11.9% were advised routine endoscopy based on biomarker panel results on capsule sponge

testing: this is in line with previously published national figures (Landy et al., 2023). 4.2% of this cohort had IM present within endoscopic biopsies and were diagnosed with Barrett's oesophagus: again, this correlates with previously published literature (Fitzgerald et al., 2020). This was initially detected with TFF3 positivity on capsule sponge testing in 45/52 cases (86.5%). When insufficient tests with missing biomarker data were excluded, the correlation between positive biomarkers on capsule sponge testing and confirmed IM at endoscopy showed statistical significance ($p < 0.001$), suggesting that capsule sponge testing is successfully identifying those patients requiring additional investigation with UGI endoscopy. There were 6/190 patients (3.2%) who had a TFF3 negative sponge but were found to have IM at endoscopy. Previous results have demonstrated that TFF3 positivity is associated with increasing segment length and a perfect screening test does not exist (Chien, Glen, Penman, Cruickshank, et al., 2024). This may account for these false negative results.

Overall, 27.2% of the reflux population subsequently underwent UGI endoscopy after capsule sponge testing: this is a substantial reduction in diagnostic UGI endoscopies in a cohort in which this would historically be the first-line investigation of choice. Given the yield of significant pathology at endoscopy was low within the reflux group overall, this implies that capsule sponge testing could be a useful adjunct triage tool within the GORD population referred to secondary care to protect and prioritise scarce endoscopy resources. In addition to financial savings, this also reduces burden on patients who are spared exposure to unnecessary invasive investigation. These results have also demonstrated that this has been achieved whilst maintaining high clinical safety, with a missed pathology rate (for IM, cancer and lymphoma) of 0.9%. There were 4/1305 (0.3%) cancer diagnoses (including 3 gastric cancers), highlighting the importance of assessing for symptoms related to gastric (rather than oesophageal) pathology before referring for capsule sponge testing. This highlights that capsule sponge testing is primarily to assess oesophageal disease and should not be used as a stand-alone test: concurrent clinical assessment remains of utmost importance. These results imply that all patients undergoing capsule sponge testing for reflux symptoms should undergo consultation with an UGI specialist nurse or medical practitioner within secondary care to assess the need for additional investigation before discharge is instigated. Particular

attention must be paid to the presence of key red flag symptoms such as dysphagia, weight loss and anaemia, as mentioned previously. However, in the context of gastric pathology, other signs and symptoms to consider include palpable abdominal mass, ongoing upper abdominal pain, nausea and/or vomiting, raised platelet count and treatment-resistant dyspepsia, particularly in those aged 55 years or over (National Institute for Health and Care Excellence, 2015). In addition to capsule sponge testing and optimisation of medical therapy, clinicians may wish to consider additional investigation with UGI endoscopy and/or cross-sectional imaging in these circumstances.

It should also be noted that the current gold standard (UGI endoscopy) is not a perfect test: the overall rates of post endoscopy upper gastrointestinal cancer (PEUGIC) vary in the literature from 8-13% (Kamran et al., 2025; Menon & Trudgill, 2014; Pimenta-Melo et al., 2016). UGI endoscopy is not the solitary investigation for reflux symptoms: such patients may be better investigated with alternative tests, such as mucosal impedance or manometry studies, dependent on symptom profile (Kahrilas & Sifrim, 2008). Capsule sponge testing combined with expert clinical assessment may be sufficient to suggest these alternative investigations (in lieu of UGI endoscopy), although data is lacking at present to draw such conclusions.

The Barrett's diagnosis rate in this study is lower than in the BEST 3 capsule sponge screening trials (Fitzgerald et al., 2020). This is likely because the screening trials recruited individuals at higher risk, based on age >50 years and at least 6 months' history of PPI medication, unlike the unselected cohort obtained from endoscopy waiting lists in this study. This analysis contains a higher proportion of female patients (57.6%), despite both Barrett's oesophagus and OAC being more prevalent in males (Krishnamoorthi et al., 2018). This likely reflects increased health-seeking behaviours in women compared to men, with younger women <50 years more likely to attend primary care (Thompson et al., 2016). To mitigate this female preponderance within the CytoSCOT cohort, the inclusion criteria could have been tailored to meet the risk factors recommended by societal guidelines for screening in the future. This nuance reflects the real-world nature of this work and it must be emphasised that proactive screening for Barrett's oesophagus was not the purpose of this pilot implementation study: the CytoSCOT programme was implemented to aid

struggling endoscopy waiting lists as described in the Endoscopy and Urology Diagnostic Recovery and Renewal Plan (Scottish Government, 2021). The BEST 4 trial aims to address these real-world discrepancies by proactively recruiting individuals at increased risk of Barrett's into screening programmes: the trial will recruit men aged 55-79 years and women aged 65-79 years with GORD and minimum 6 months' acid-suppressant medication use in the past year to specifically account for this increased risk in men (National Institute for Health and Care Research, 2022).

This study demonstrates that capsule sponge testing can be used as a simple, low-cost diagnostic test to identify Barrett's oesophagus in a secondary care population allowing concentration of endoscopy services to high-risk cases. More work is required to determine how best to fit capsule sponge testing into diagnostic pathways for GORD with direct referrals from primary care to reduce triage time and reinforce appropriate case selection. In the long term, increasing diagnosis of Barrett's oesophagus at population level may be possible via more systematic primary care screening: this is the purpose of the BEST 4 screening trial to determine whether this could reduce morbidity and mortality from OAC (National Institute for Health and Care Research, 2022). Clearly, a priority is to establish new clinical pathways for patients with symptomatic GORD using capsule sponge testing to rationalise endoscopy use. Although these initial results are promising, the outcome of future studies will be imperative to the adoption and expansion of capsule sponge testing within diagnostic pathways in the future.

This study is not without limitations. The need for further investigation with UGI endoscopy after capsule sponge testing was decided at local health board level. Therefore, there was significant clinical heterogeneity in the ongoing management of patients across the country, with variation in the threshold for subsequent referral for UGI endoscopy across the 11 health boards included in this study. The introduction of standardised national Scottish guidelines following capsule sponge testing would help to minimise this variation in future prospective work. Additionally, most patients with a TFF3 negative result have not undergone UGI endoscopy, reflecting the real-world setting of this study. Therefore, it cannot be said that these are true negative results and the definite "missed" pathology rate is only 0.9% from capsule sponge testing. Patients were

not routinely followed up with endoscopy as this was a real-world implementation study triggered by the COVID-19 pandemic to test the utility of this triage tool in managing the endoscopy waiting list for low-risk patients. The intention was for low-risk patients to be discharged from secondary care, therefore routinely performing UGI endoscopy on this cohort would mean that the purpose of the CytoSCOT programme had not been met. Furthermore, 15/124 patients with positive biomarkers on capsule sponge testing (12.1%) did not have endoscopic biopsies taken as the UGI tract appeared macroscopically normal: pathology (especially focal IM) may also have been missed within this group. Although 70.3% of patients were discharged from secondary care after capsule sponge testing, the median follow-up time in this study is only 20 months at present: longer term follow-up will be required to assess long-term outcomes within this "discharged" cohort to ensure additional pathology has not been missed, with ongoing evaluation of this cohort planned at regular time intervals in the future to mitigate this risk.

In conclusion, this is the first prospective cohort study in clinical practice to demonstrate capsule sponge testing is a promising diagnostic triage tool to UGI endoscopy in symptomatic GORD patients beyond the trial setting. Capsule sponge biomarker testing for TFF3, atypia and p53 is successfully identifying the cohort of patients requiring further investigation with endoscopy. However, clinical assessment remains fundamental to assess suitability for discharge after capsule sponge testing: caution should be exercised where suspicion of gastric malignancy is present and judicious follow-up of the discharged group will be required in the longer term.

Chapter 8 Combination of capsule sponge result with presence of systemic inflammation to assess for Barrett's oesophagus

While capsule sponge testing using TFF3 has proven utility in the diagnosis of Barrett's oesophagus within the trial setting (Fitzgerald et al., 2020), the data presented in Chapter 7 has effectively demonstrated that these results are also applicable within real-world clinical practice (Chien, Glen, Bryce, et al., 2024). However, new technologies require buy-in and support from clinicians. This chapter therefore aims to explore additional methods which may enhance clinicians' confidence in using capsule sponge testing as a diagnostic triage tool. The presence of systemic inflammation has shown a positive correlation with survival in oesophagogastric cancer. Therefore, this chapter aims to establish whether presence of IM at endoscopy demonstrates a positive association with systemic inflammation, and therefore whether combining TFF3 result on capsule sponge testing and serum markers of systemic inflammation can effectively rule out Barrett's oesophagus within the symptomatic reflux population.

8.1 Introduction

Innovation in healthcare has the potential to drastically improve service quality and patient care but is reliant on the willingness of clinicians and policy makers to embrace change. Implementation of new technology challenges existing clinical systems, forces specialists to acquire new skills and frequently requires considerable financial investment (Flessa & Huebner, 2021), as well as the development of robust clinical pathways. The COVID-19 pandemic was a major global health crisis: it considerably challenged the fundamental infrastructure of the NHS and saw the temporary cessation of many standard clinical pathways (including endoscopy services), resulting in significant delays to diagnosis and treatment. This disruption to usual services had a detrimental effect on cancer diagnosis, treatment and survival (Helsper et al., 2020; The Lancet Oncology, 2020; Watt, Sullivan, & Aggarwal, 2022). As discussed in Chapter 1, recent literature has demonstrated the impact on patients with OG cancer in Scotland: during the COVID-19 pandemic, patients presented later with more advanced disease, resulting in a shift towards palliation rather than curative treatment, thereby negatively affecting survival rates (Baxter et al., 2023).

It is evident that early detection of oesophageal cancer improves patient outcomes and long-term survival (Fitzgerald et al., 2022), emphasising the importance of the Barrett's surveillance programme in reducing progression to invasive OAC. However, screening at population level with UGI endoscopy for Barrett's oesophagus remains unfeasible, given this procedure is extremely resource-intensive yet low-yield for concerning pathology within the symptomatic reflux population (Fitzgerald et al., 2020; Lin et al., 2019). Capsule sponge testing using TFF3 to detect presence of IM has shown promise as an alternative diagnostic triage tool, as discussed in previous chapters. However, following the resumption of endoscopy services, the delay in restructuring clinical pathways with appropriate referral and onward management mechanisms, coupled with reluctance from some healthcare professionals to engage in change, has presented barriers to the national standardisation of capsule sponge testing beyond the COVID-19 pandemic.

This chapter therefore explores the possibility of enhancing current pathways to improve clinicians' confidence in capsule sponge testing in the context of the

symptomatic reflux population. Systemic inflammation is intrinsically linked to survival in oesophagogastric cancer (Crumley et al., 2006; Huang et al., 2023; Li et al., 2022; Shi, Wang, & Yan, 2023). The neutrophil-lymphocyte ratio (NLR) is a validated prognostic score for the presence of systemic inflammation (Dolan, Lim, et al., 2017; Dolan, McSorley, et al., 2017; Dolan et al., 2018). Raised NLR is a risk factor for dysplastic progression in Barrett's oesophagus (Campos et al., 2020; Peleg et al., 2021), however its relevance in non-dysplastic Barrett's oesophagus is yet to be established. Additionally, previous literature has demonstrated a positive correlation between prior anaemia and oesophageal cancer (Hippisley-Cox et al., 2023). This chapter aims to determine whether combining capsule sponge testing and full blood count (FBC) can effectively rule out a diagnosis of Barrett's oesophagus within the symptomatic reflux population.

8.2 Methods

Prospectively maintained local databases from 11 Scottish health boards were collated to form a national retrospective Excel database including all consecutive patients who underwent capsule sponge testing following referral to secondary care for investigation of reflux symptoms in the absence of red-flag symptoms (i.e. dysphagia, weight loss, anaemia). Capsule sponge testing was performed using the Cytosponge™ capsule sponge device (Medtronic, Minneapolis, Minnesota, USA) in all cases. Tests undertaken from 14 September 2020 to 30 April 2023 were included in the analysis.

All capsule sponge tests underwent centralised laboratory processing and reporting (Cyted Ltd, Cambridge, UK) as previously discussed (Chien, Glen, Penman, Bryce, et al., 2024; Fitzgerald et al., 2020; Pilonis et al., 2022), with processed reports returned to the parent health board. As in Chapter 7, ongoing clinical management and decision to proceed to UGI endoscopy was subject to clinicians' discretion at local health board level. In addition to capsule sponge testing, all patients underwent clinical assessment either by UGI specialist nurses or consultants in secondary care to aid decision-making and ongoing management: this was performed either virtually via telephone consultation or face-to-face in the outpatient clinic setting.

Individual patient records were interrogated using online clinical systems, including Clinical Portal, SCI Store and SCI Gateway. Baseline patient demographics and capsule sponge result were recorded. This study focused primarily on the presence of TFF3 on capsule sponge testing to aid the diagnosis of Barrett's oesophagus. Helicobacter pylori antigen status was recorded from either serum or faecal samples obtained within 3 months of capsule sponge test. Additional information, including endoscopic histopathology result and ongoing clinical management, was collected for those patients who underwent subsequent UGI endoscopy after capsule sponge testing. Patients were dichotomised into groups, dependent on whether IM was present on endoscopic biopsies or not.

Patients who had available FBC results from within 3 months of capsule sponge testing (inclusive of the 3 months before and after) were included in this analysis. The NLR and platelet-lymphocyte ratio (PLR) are both validated prognostic scores for the presence of systemic inflammation that are derived from FBC results. NLR was calculated by dividing neutrophil count ($x10^9$/L) by lymphocyte count ($x10^9$/L); PLR was calculated by dividing platelet count ($x10^9$/L) by lymphocyte count ($x10^9$/L). Systemic inflammation was deemed present if NLR was >3 or PLR was >150 (Dolan, Lim, et al., 2017; Dolan, McSorley, et al., 2017; Dolan et al., 2018). Patients were defined as anaemic based on WHO guidelines (i.e., haemoglobin <130 mg/L in males and <120 mg/L in females) (World Health Organization, 2011). Patients were defined as iron deficient (i.e. ferritin <15 μg/L) based on BSG guidelines (Snook et al., 2021). Mean corpuscular volume (MCV) is used to sub-categorise anaemia: microcytic anaemia was defined as MCV <80; normocytic anaemia defined as MCV 80-100; macrocytic anaemia defined as MCV >100 (Schop et al., 2021).

Exclusion criteria included all capsule sponge tests for Barrett's surveillance and missing FBC result within 3 months of capsule sponge test. Patients were not actively involved in study design, data collection or analysis. All patients signed an NHS consent form prior to undergoing capsule sponge testing or UGI endoscopy. Ethical approval was obtained via local information governance teams in each health board, in addition to national PBPP approval from the Caldicott guardian. This was approved with the need for individual patient

consent waived due to the study's retrospective nature and for the purposes of service development.

Continuous parameters were presented as median and IQR and categorical data as counts and percentages. The Mann-Whitney U test was performed for comparison of continuous data, and the Chi-squared test was performed for comparison of categorical variables, where appropriate. A p value of ≤ 0.05 was considered statistically significant. Logistic regression was performed to determine univariate relationships between clinical factors and the presence of IM at endoscopic biopsy: selected variables found to have a significant impact on presence of IM from the Chi-squared analysis were carried into the regression analysis to allow calculation of OR and 95% CI. Multivariate logistic regression analysis, including all statistically significant covariables at a p value of ≤ 0.10, was performed by a stepwise backward procedure to derive a final model of the variables that had a statistically significant ($p \leq 0.05$) relationship with the presence of IM on endoscopic biopsies. Statistical analysis was performed using SPSS software version 28.0 (SPSS Inc., Chicago, Illinois, USA).

8.3 Results

During the study period, 1385 capsule sponge tests were performed for reflux symptoms, with a median follow-up time of 20 months (IQR 12-27). 358/1385 tests (25.8%) proceeded to undergo UGI endoscopy at any time point after capsule sponge testing. The median time to endoscopy after capsule sponge testing was 5 months (IQR 2-10). 6 patients (0.4%) had invasive malignancy at endoscopy: 3/6 had gastric adenocarcinoma; 1/6 had OAC; 1/6 had gastric lymphoma; 1/6 had neuroendocrine carcinoma of the oesophagus. 4/1385 patients (0.3%) died because of oesophagogastric cancer within this cohort.

57/1385 patients (4.1%) were confirmed to have IM on endoscopic biopsies. 46 of these 57 patients (80.7%) had a TFF3 positive capsule sponge result: this rose to 85.2% when insufficient tests were excluded. This group included 1 patient with low grade dysplasia (LGD), 1 patient with neuroendocrine carcinoma and 1 patient with gastric lymphoma. There were no other cases of Barrett's dysplasia within the cohort. Although 1 patient had a diagnosis of OAC, review of all

subsequent endoscopic biopsies did not demonstrate the presence of IM, therefore this patient was not included within the IM group.

Table 8.1 compares demographics of those diagnosed with IM compared to those who were not. Patients with IM were significantly more likely to be older (median age 61 vs. 56 years; p=0.004) and male (57.9% vs. 41.7%; p=0.016). The IM group were also significantly more likely to have positive biomarkers for TFF3, atypia and/or p53 positivity on capsule sponge testing (82.5% vs. 7.1%; p<0.001) when compared to the control group. TFF3 status was significantly more likely to be positive in the IM group (85.2% vs. 6.5%; p<0.001). With the 142 insufficient capsule sponge tests excluded, TFF3 positive capsule sponge testing demonstrated sensitivity 85.2%, specificity 93.5%, NPV 99.3%, and PPV 37.4% for the diagnosis of IM at endoscopy in the symptomatic reflux cohort. The number of UGI endoscopies (number needed to scope, NNS) that would have to be performed to diagnose one case of IM in the TFF3 positive capsule sponge cohort was 3. On multivariate analysis, TFF3 positive capsule sponge test retained significance as an independent predictor of IM at endoscopy (OR 70.035; 95% CI 23.426 – 209.383; p<0.001) (Table 8.2).

Table 8.1 Comparison between patients diagnosed with IM with those who were not

[a] Data missing for 411 patients. [b] Data missing for 1182 patients. [c] Data missing for 26 patients. [d] Data missing for 142 patients. This includes patients who also demonstrated atypia and/or p53 positivity on capsule sponge testing. [e] Data missing for 592 patients. [f] Data missing for 1100 patients.

		IM present		P value
		Yes (n=57)	No (n=1328)	
Age (years)	Median (IQR)	61 (53 – 70)	56 (46 – 65)	0.004
	<55	17 (29.8%)	621 (46.8%)	0.027
	≥55 – 74	33 (57.9%)	616 (46.4%)	
	≥75	7 (12.3%)	91 (6.8%)	
Sex	Male	33 (57.9%)	554 (41.7%)	0.016
	Female	24 (42.1%)	774 (58.3%)	
Body mass index [a]	Median (IQR)	28.0 (25.0 – 31.0)	28.1 (25.0 – 32.6)	0.622
PPI use	Yes	47 (82.5%)	1174 (88.4%)	0.174
	No	10 (17.5%)	154 (11.6%)	
Helicobacter pylori status [b]	Positive	0 (0%)	14 (7.1%)	0.464
	Negative	7 (100%)	182 (92.9%)	
Smoking history [c]	Current smoker	6 (10.5%)	145 (11.1%)	0.130
	Previous smoker	22 (38.6%)	346 (26.6%)	
	Never smoker	29 (50.9%)	811 (62.3%)	
Capsule sponge result (by worst pathology)	TFF3 negative	7 (12.3%)	1096 (82.5%)	<0.001
	TFF3 positive only	42 (73.7%)	74 (5.6%)	
	Atypia positive only	3 (5.3%)	17 (1.3%)	
	p53 positive only	0 (0%)	1 (0.1%)	
	Atypia and p53 positive	2 (3.5%)	1 (0.1%)	
	Insufficient	3 (5.3%)	139 (10.4%)	
TFF3 status [d]	Negative	8 (14.8%)	1112 (93.5%)	<0.001
	Positive	46 (85.2%)	77 (6.5%)	
Anaemia [e]	No	32 (94.1%)	704 (92.8%)	0.763
	Yes	2 (5.9%)	55 (7.2%)	
Iron deficiency [f]	No	13 (92.9%)	261 (96.3%)	0.513
	Yes	1 (7.1%)	10 (3.7%)	
Anaemia and MCV [e]	Not anaemic	32 (94.1%)	704 (92.8%)	0.962
	Macrocytic anaemia	0 (0%)	2 (0.3%)	
	Normocytic anaemia	2 (5.9%)	49 (6.4%)	
	Microcytic anaemia	0 (0%)	4 (0.5%)	
NLR [e]	>3	9 (26.5%)	103 (13.6%)	0.035
	≤3	25 (73.5%)	656 (86.4%)	
PLR [e]	>150	14 (41.2%)	285 (37.5%)	0.669
	≤150	20 (58.8%)	474 (62.5%)	

Table 8.2 Univariate and multivariate binary logistic regression analysis of factors impacting on likelihood of presence of IM at endoscopy
[a] Data missing for 142 patients. [b] Data missing for 592 patients.

	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age ≥55 (years)	2.067	1.160 – 3.682	0.014	1.680	0.604 – 4.673	0.320
Male sex	1.921	1.123 – 3.287	0.017	1.454	0.621 – 3.407	0.389
TFF3 positive sponge result [a]	83.039	37.856 – 182.149	<0.001	70.035	23.426 – 209.383	<0.001
NLR >3 [b]	2.293	1.041 – 5.051	0.039	1.882	0.703 – 5.040	0.208

FBC results within 3 months of capsule sponge test were available in 793 cases (57.3%) and were therefore included in this analysis: 34/793 (4.3%) were in the IM group; 759/793 (95.7%) did not have an endoscopic diagnosis of IM. There was a significant correlation between presence of systemic inflammation in the context of raised NLR >3 and presence of IM when compared to the control group (26.5% vs. 13.6%; p=0.035). However, this was not the case when applied to PLR (41.2% vs. 37.5%; p=0.669) (Table 8.1). Figure 8.1 demonstrates the receiver-operator characteristics (ROC) curve to test the correlation of NLR with diagnosis of IM within the symptomatic reflux population (area under the curve = 0.606). There was no significant association between presence of anaemia, type of anaemia, or iron deficiency with the presence of IM at endoscopy.

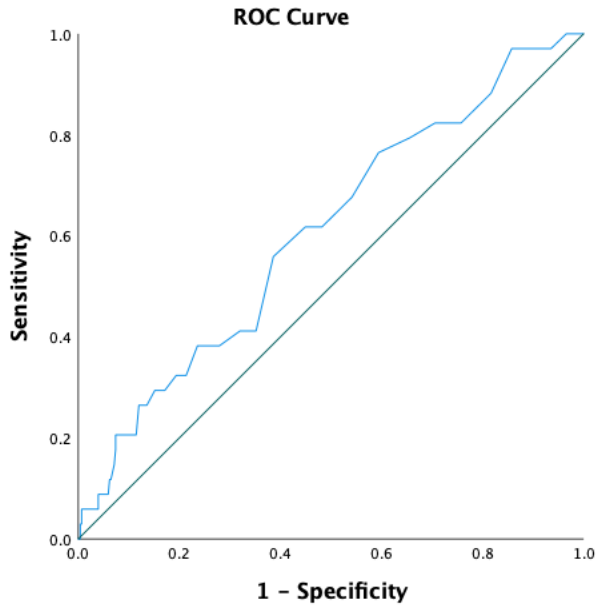

Figure 8.1 ROC curve to test the correlation of NLR with presence of IM within the symptomatic reflux population

As described above, TFF3 positive capsule sponge test and raised NLR were associated with a subsequent diagnosis of IM at endoscopy and were therefore next combined. A total of 704 of 1385 patients (50.8%) in this study had both a valid capsule sponge test result and FBC. Data were missing for 24/57 patients (42.1%) in the IM group. Combining capsule sponge test and NLR, 536 patients had a TFF3 negative sponge and normal NLR; 81 patients had a TFF3 negative sponge and raised NLR; 69 patients had a TFF3 positive sponge and normal NLR; 18 patients had a TFF3 positive sponge and raised NLR. Table 8.3 shows a comparison between these four groups of patients when capsule sponge result and NLR were combined. 2/536 patients (0.4%) with a TFF3 negative capsule sponge and normal NLR had a diagnosis of IM at endoscopy: of these 2 patients, 1 had a very short segment of Barrett's (C0M1 on Prague classification) and 1 patient had urgent endoscopy triggered by a capsule sponge result demonstrating atypia. 3/536 patients (0.6%) within the TFF3 negative sponge and normal NLR group had a cancer diagnosis but no IM present at endoscopy: this included 1 patient with OAC and 2 with gastric adenocarcinoma. Combining TFF3 capsule sponge result with the presence or absence of systemic inflammation in the context of raised NLR resulted in sensitivity 93.9%, specificity 79.6%, NPV 99.6% and PPV 18.5% for the diagnosis of IM at endoscopy (NNS is 5).

Table 8.3 Comparison by combined capsule sponge test result and NLR for all patients with both a valid capsule sponge result and FBC (n=704)

		TFF3 -ve capsule sponge + normal NLR	TFF3 -ve capsule sponge + raised NLR	TFF3 +ve capsule sponge + normal NLR	TFF3 +ve capsule sponge + raised NLR	P value
		N=536	N=81	N=69	N=18	
Age (years)	Median (IQR)	56 (48 – 65)	60 (48 – 70)	63 (58 – 71)	67 (52 – 74)	<0.001
	<55	235 (43.8%)	32 (39.5%)	14 (20.3%)	5 (27.8%)	0.003
	≥55-74	269 (50.2%)	39 (48.1%)	46 (66.7%)	11 (61.1%)	
	≥75	32 (6.0%)	10 (12.3%)	9 (13.0%)	2 (11.1%)	
Sex	Male	212 (39.6%)	30 (37.0%)	22 (31.9%)	8 (44.4%)	0.602
	Female	324 (60.4%)	51 (63.0%)	47 (68.1%)	10 (55.6%)	
Presence of IM at endoscopy		2 (0.4%)	2 (2.5%)	22 (31.9%)	7 (38.9%)	<0.001

8.4 Discussion

Population-wide screening for Barrett's oesophagus within the symptomatic reflux population remains unattainable: UGI endoscopy is a resource-intensive, invasive procedure and therefore not a viable option, particularly given current system pressures in the COVID-19 pandemic recovery era (Fitzgerald et al., 2014; Lin et al., 2019). However, it has been postulated that capsule sponge testing may be an alternative screening test for Barrett's oesophagus and OAC: the BEST 4 trial aims to establish this association in the future (National Institute for Health and Care Research, 2022). At present, real-world evidence is sparse, although initial results from the CytoSCOT programme have demonstrated that capsule sponge testing has a promising role in case-finding and diagnosis of Barrett's oesophagus in clinical practice, as presented in Chapter 7 ((Chien, Glen, Bryce, et al., 2024).

Over recent years, qFIT has been integrated into bowel cancer screening programmes (Li et al., 2021; Moss et al., 2017) and has demonstrated proficiency as a triage tool in urgent suspected cancer pathways (Ayling & Machesney, 2021;

Farrugia et al., 2020; Khan, Klimovskij, & Harshen, 2020; McSorley et al., 2021; Mowat et al., 2021; Nicholson et al., 2020; Pin Vieito, Zarraquiños, & Cubiella, 2019; Westwood, Corro Ramos, et al., 2017; Westwood, Lang, et al., 2017). In addition, a recent study has established the combination of a normal qFIT and FBC can reliably rule out colorectal cancer in symptomatic patient referral pathways (Johnstone et al., 2022), enabling prioritisation of scarce colonoscopy resources.

This study therefore aimed to determine whether this finding could be extrapolated to the symptomatic reflux population undergoing capsule sponge testing. It was hypothesised that, if this association could be successfully demonstrated, this may be of diagnostic value if capsule sponge testing was applied as a screening tool for Barrett's oesophagus in the symptomatic reflux population in the future and could provide clinicians with additional reassurance to safely discharge patients at low risk of this pre-malignant condition.

While the sensitivity and specificity of TFF3 on capsule sponge testing for the presence of IM has previously been demonstrated (Ross-Innes et al., 2015), there remains a small rate of false negative results, which could lead to missed Barrett's oesophagus diagnoses. Although only TFF3 positivity remained an independent predictor of IM on multivariate analysis, this study is the first to demonstrate an association between raised NLR and the presence of IM on endoscopic biopsies: previous work has only established a correlation between raised NLR and dysplastic Barrett's oesophagus (Campos et al., 2020; Peleg et al., 2021). The present study has demonstrated that combining TFF3 status and presence or absence of systemic inflammation in the context of raised NLR increased sensitivity for IM from 85.2% to 93.9% and NPV from 99.3% to 99.6%. The corresponding specificity and PPV decreased from 93.5% to 79.6% and 37.4% to 18.5% respectively, while the number needed to scope to diagnose one case of IM increased from 3 to 5, which was felt to be acceptable. Overall, combining a negative TFF3 result with a normal NLR was able to effectively exclude IM in 99.6% of cases, which should provide clinicians with excellent reassurance when utilising capsule sponge testing. In those with available capsule sponge and FBC results, patients with a TFF3 negative capsule sponge result and normal NLR represented 76.1% of the total cohort. With appropriate safety netting in place, these patients can be reassured. Of note, all patients in the cohort underwent

consultation with an UGI specialist in secondary care pre and post capsule sponge testing to ensure resolution of symptoms prior to discharge. This is important when considering the 3 patients (0.6%) who were subsequently diagnosed with OG cancer within this group: these patients were referred for endoscopy when the presence of red flag symptoms was highlighted. It is also worth noting that capsule sponge testing is designed to identify oesophageal pathology only (Fitzgerald et al., 2020): it does not have a role in the diagnosis of gastric cancer.

Previous literature has suggested that capsule sponge testing is approximately £127 cheaper per patient than UGI endoscopy (National Institute for Health and Care Excellence, 2020). The cost to process one FBC result is approximately £2.65 (Akhtar & Chung, 2014; Mughal et al., 2016), therefore combining capsule sponge result with FBC remains a cheaper alternative to UGI endoscopy as a first-line investigation. Whilst testing for Helicobacter pylori is advised in national guidelines for symptomatic reflux disease, routine FBC is not within the current treatment algorithm (National Institute for Health and Care Excellence, 2014). It is proposed that FBC should be integrated into referral pathways to secondary care for all patients with chronic reflux symptoms to aid decision-making with regards to onward investigation. This is also crucial in the context of the known association between anaemia and OG cancer (Hippisley-Cox et al., 2023), as older patients with dyspepsia and anaemia should be referred direct to scope (National Institute for Health and Care Excellence, 2014).

This study has several limitations. The retrospective nature of this work meant that a large proportion of the overall cohort were missing FBC results so were excluded from the blood test analysis, resulting in small numbers included in this dataset. Furthermore, NLR did not retain significance as a prognostic indicator for IM on multivariate analysis: it is advised that NLR is utilised only as an adjunct alongside TFF3 capsule sponge testing (which did retain significance), rather than a stand-alone prognostic indicator. There was clinical heterogeneity in clinicians' decision to refer for endoscopy after capsule sponge testing as this was decided at local health board level, rather than within national guidance integrated into the pilot programme for capsule sponge testing. Additionally, a large proportion of patients within the TFF3 negative group did not undergo endoscopy therefore it cannot be accurately determined if these are true

negative results for IM. This reflects the real-world retrospective nature of the study design.

In conclusion, combining a TFF3 negative capsule sponge result with a normal NLR excluded the endoscopic diagnosis of IM in 99.6% of cases in the symptomatic reflux population: these patients can be safely reassured and discharged with appropriate safety netting advice. This facilitates appropriate prioritisation of scarce endoscopy resources and minimises the need for invasive procedures in selected patients with chronic reflux symptoms.

Chapter 9 Discussion and Conclusions

9.1 Overview of work

Barrett's oesophagus is a pre-malignant condition that predisposes to the development of OAC. This thesis focuses on the use of capsule sponge testing in the real-world setting as an alternative risk-stratification tool to UGI endoscopy for patients with known Barrett's and those referred through secondary care with symptomatic reflux disease, the latter of whom are an at-risk population for developing Barrett's oesophagus.

Chapter 1 presented an overview of the existing literature, predominantly focusing on the background of Barrett's oesophagus and OAC. While the current recommendations for endoscopic diagnosis and surveillance of the disease were explored in detail, this chapter also analysed the evidence base for alternative Barrett's surveillance strategies, such as capsule sponge testing. Chapter 1 also demonstrated how high-quality targeted surveillance programmes present the potential to transform outcomes in OAC through earlier diagnosis and endoscopic intervention. This was further reinforced in Chapter 3, which highlighted that timely surveillance of Barrett's oesophagus remains important, as delayed surveillance negatively impacts histopathology pattern and leads to increased detection of dysplasia and cancer.

Chapter 2 confirmed the hypothesis that capsule sponge testing is a safe alternative to UGI endoscopy in Barrett's surveillance patients by analysing capsule sponge biomarker results and comparing them with endoscopic histopathology biopsies. Chapter 2 was the first body of work to present these findings in the surveillance cohort in clinical practice, compared to previous studies that have focused on screening populations recruited for clinical trials. These promising results were augmented by the findings of Chapters 4 and 5, which focused on the impact of capsule sponge testing on the endoscopy service in NHS GGC. Chapter 4 demonstrated that detection of HGD, IMC and invasive cancer was similar in those undergoing endoscopic surveillance versus capsule sponge testing, providing clinicians with additional reassurance when utilising

the capsule sponge device. Chapter 5 highlighted that capsule sponge testing has reduced the number of surveillance endoscopies performed in the health board overall, thereby enabling prioritisation of scarce endoscopic resources. Chapter 3 also considered the positive impact of the introduction of capsule sponge testing on service delivery, demonstrating that the CytoSCOT programme has reduced delays to Barrett's surveillance, thereby reducing the burden on endoscopy services.

Chapter 6 focused on the longer-term follow-up of Barrett's surveillance patients and presents reassuring results that cases of dysplasia and cancer were unlikely to have been misdiagnosed at first capsule sponge test. Chapter 6 also presented the first evidence supporting the use of the EndoSign® versus the Cytosponge™ device in clinical practice, with EndoSign® appearing to demonstrate improved detection of TFF3 positivity compared to the latter, although there remain several confounding factors in this analysis and further work is required to validate these claims.

Chapters 7 and 8 then turned attention to the symptomatic reflux population, who have previously been the focus of large clinical trials that provided the initial evidence base for capsule sponge testing. Chapter 7 confirmed that capsule sponge testing is a safe and effective triage tool to endoscopy within this case-finding cohort but did highlight the importance of judicious clinical assessment to ensure gastric pathology is not missed, as the capsule sponge device is designed to detect oesophageal pathology only. Chapter 8 demonstrated that adding a serum FBC to patients' initial investigations to calculate NLR (implying presence of systemic inflammation) could provide additional reassurance for clinicians looking to discharge symptomatic reflux patients, as the combination of TFF3 negativity on capsule sponge testing and normal NLR excluded IM in 99.6% of cases.

9.2 Future work

9.2.1 Barrett's surveillance cohort

While the work presented in this thesis and resultant publications has undoubtedly added to the existing clinical evidence for use of capsule sponge

testing in the Barrett's surveillance population, there is scope for future research to focus predominantly on those patients identified as high-risk on initial capsule sponge testing. A significant proportion of these patients (211/300; 70.3%) had not progressed to dysplasia at UGI endoscopy, despite capsule sponge biomarkers demonstrating atypia and/or p53 positivity. These patients require stringent long-term follow-up, possibly with increased frequency of surveillance, as it is hypothesised that this cohort may be more likely to progress to Barrett's dysplasia or neoplasia compared to those within the lower risk groups. The follow-up data within this high-risk group have yet to be analysed but would be of significant clinical interest.

Anecdotally, local histopathologists have now begun staining for p53 on endoscopic biopsies on a routine basis for those patients identified as high-risk on capsule sponge testing. Previous Barrett's oesophagus biopsies from this high-risk group (inclusive of those who have already developed dysplasia) could be obtained retrospectively and stained for p53, with a particular focus on long-term outcomes of those patients demonstrating p53 overexpression on historical biopsies, as well as capsule sponge testing. Not only could this potentially demonstrate that these patients possess pre-existing biological and molecular changes that predict progression to dysplasia, but analysis could be undertaken to determine the timepoint at which dysplasia first presents within endoscopic biopsies, as this data is currently lacking within existing literature. Improved knowledge of the time frame during which dysplasia tends to develop in Barrett's oesophagus could lead to the revision of recommended surveillance intervals in current societal guidelines, which presently lack a robust evidence base behind their recommendations (Fitzgerald et al., 2014).

At present, the current BSG guidelines are under review: given that the ACG and ESGE guidelines have recently been updated to include statements advocating the use of capsule sponge testing for screening purposes (Shaheen et al., 2022; Weusten et al., 2023), it will be of interest to see if this is incorporated into the British guidelines. As discussed, the lack of formal societal recommendations and clinical guidelines has resulted in clinical confusion as to which surveillance method should be recommended to patients moving forward. The recent publication from Tan et al. also provides further evidence to support the revision of clinical pathways for Barrett's surveillance dependent on risk group (Tan et

al., 2025), which again will hopefully improve clinicians' confidence in utilising the device in clinical practice. The evidence base for capsule sponge testing will continue to expand as further data become available from the DELTA and NHSE implementation studies.

In hindsight, it may have been useful to devise a set of recommendations for suggested time intervals and method of next follow-up specifically for those patients undertaking capsule sponge testing within the CytoSCOT programme to reduce heterogeneity in clinical decision-making across different health boards. However, the emergent nature of the COVID-19 pandemic and rapid roll-out of the service without real-world data to support its use precluded this from happening at the outset of the programme. The expected imminent publication of the updated BSG guidelines for Barrett's surveillance is likely to provide impetus to formalise recommendations for these patients moving forward: this will be critical for the future of the CytoSCOT programme.

The development of the national CytoSCOT database was a laborious process, requiring extensive initial data collection across multiple health boards and different electronic record systems. This was performed using an Excel spreadsheet initially. However, in hindsight, data collection using a web-based online database (such as REDCap) would have been a preferential option in the first instance to ensure data were stored securely and readily available for future work. Administrators within the CytoSCOT programme are currently working to develop such a REDCap database to facilitate cohesive prospective data collection across different health boards. However, utilising this platform from the outset would certainly have been of benefit.

To ensure follow-up was performed regularly and cancers were not missed as the programme expanded, the database was manually cross-checked at 6-monthly intervals. This method of stringent follow-up was no longer feasible as the programme expanded beyond 5000 tests performed in Scotland. While work continues to ensure data is collected nationally for prospective tests performed, it would be beneficial for the CytoSCOT database to be linked to Scottish MDT networks, in liaison with Public Health Scotland, to rapidly identify those cases subsequently diagnosed with HGD and cancer after capsule sponge testing. This would improve follow-up methodology in the future without the need to employ

a full-time research fellow, whilst maintaining high standards of clinical governance and safety.

While the national CytoSCOT database is robust, as discussed in previous chapters, lack of access to national endoscopic data due to ethical restrictions has limited comparison of outcomes in the Barrett's surveillance cohort undergoing capsule sponge testing versus traditional endoscopy to a single health board. The development of a national Scottish Barrett's registry inclusive of all patients undergoing surveillance by both modalities would be highly valuable moving forward to undertake meaningful analysis of both cohorts with a larger number of patients. Again, it would be beneficial if REDCap could be used as this platform, for the reasons described previously. This could provide scope for future work.

9.2.2 Symptomatic reflux population

This thesis validates the trial findings of Fitzgerald et al. in the symptomatic reflux group (Fitzgerald et al., 2020), confirming the capsule sponge device has proven utility in this cohort within clinical practice. However, many cases of Barrett's-related OAC continue to present de novo at an advanced stage and without a prior diagnosis of Barrett's oesophagus (Dulai et al., 2002; El-Serag et al., 2016; Visrodia, Singh, et al., 2016b), therefore forgoing the opportunity for early intervention through the surveillance programme. This highlights the relevance of population screening for Barrett's oesophagus, targeting individuals at higher risk for recruitment into screening programmes.

As discussed in Chapter 8, within the context of colorectal cancer, qFIT testing has recently been integrated into bowel cancer screening programmes (BCSP) (Li et al., 2021; Moss et al., 2017) and is an excellent example of how population screening can dramatically enhance patient pathways. This non-invasive stool sample test detects occult blood in faeces, which may be an indicator of colorectal cancer. Due to the extensive research demonstrating its proficiency as a triage tool for colorectal cancer, qFIT testing has become the national standard of care for both BCSP and investigation of red-flag colorectal symptoms (Ayling & Machesney, 2021; Farrugia et al., 2020; Khan, Klimovskij, & Harshen, 2020; McSorley et al., 2021; Mowat et al., 2021; Nicholson et al., 2020; Pin

Vieito, Zarraquiños, & Cubiella, 2019; Westwood, Corro Ramos, et al., 2017; Westwood, Lang, et al., 2017). Furthermore, the combination of a normal qFIT and FBC can reliably rule out colorectal cancer in patients referred with lower gastrointestinal symptoms through urgent suspected cancer pathways (Johnstone et al., 2022). In a similar means to capsule sponge testing, utilising qFIT as a triage tool has facilitated prioritisation of colonoscopy resources, with yield of colorectal cancer diagnosed at colonoscopy directly linked to qFIT result (McSorley et al., 2021).

As discussed in Chapter 1, within the context of Barrett's oesophagus and OAC, capsule sponge testing is an ideal modality to deliver a similar screening programme, given that this investigation is non-invasive, non-endoscopic, more cost-effective and requires less clinical resources compared to traditional UGI endoscopy. There have been suggestions that capsule sponge testing could function in a similar manner for OAC as qFIT does for colorectal cancer and the national BCSP, however the evidence is lacking at present to support this hypothesis.

The implementation of a quantitative threshold of qFIT for colorectal cancers has resulted in optimised biomarker performance and reduced over-diagnosis (Clackett et al., 2021; MacDonald et al., 2022). It has been hypothesised that this principle may also be applicable to TFF3 on capsule sponge testing for Barrett's oesophagus screening. While TFF3 has classically been interpreted in a binary fashion on capsule sponge testing (i.e. positive or negative), there can be significant variation in the number of TFF3 positive gland groups identified on each slide. A recently published study analysing samples from the BEST 2 and 3 trials has demonstrated that a positive correlation exists between quantitative assessment of TFF3 and length of Barrett's segment (i.e. patients with clinically relevant Barrett's oesophagus had a higher mean TFF3 gland count compared to those with focal IM only at endoscopy). This implies that TFF3 gland count could potentially be used to set thresholds to trigger confirmatory endoscopy to reduce over-diagnosis of focal IM cases with very low cancer-associated risk in the context of Barrett's screening (Berman et al., 2022). While this work is continuing to evolve, it is an interesting concept to consider when hypothesising how a real-world Barrett's screening programme could function in clinical

practice to reduce the number of negative endoscopies performed and prioritise resources towards those with clinically relevant pathology.

Recent work has focused on targeting an optimal population to screen for Barrett's oesophagus and early OAC, with the aim of maximising diagnostic yield and minimising harm from overdiagnosis, by undertaking a post-hoc analysis from the BEST 3 RCT (Tan et al., 2024). Moreover, the BEST 4 trial is currently ongoing and aims to directly address this issue. As previously discussed, this large RCT is currently recruiting patients with symptomatic reflux disease (i.e. males aged 55-79 years and females aged 65-79 years requiring at least 6 months of acid-suppressant medication use in the previous 12 months). Its primary outcome is to evaluate whether capsule sponge testing is a useful screening tool to reduce OAC-related mortality (National Institute for Health and Care Research, 2022). Ultimately, the development of a formal Barrett's screening programme within the next decade utilising capsule sponge testing (potentially incorporating clinical assessment and routine blood tests) targeted at the above suggested population would be the goal. While the evidence presented in Chapters 7 and 8 adds to the existing literature for the use of capsule sponge testing in the symptomatic reflux cohort, it is simply not feasible for an established screening programme to be developed until the results of the BEST 4 trial are available. The outcomes from this trial undoubtedly have the potential to change clinical management moving forward and will be critical to the future expansion of capsule sponge testing as an established UGI investigation long-term.

Furthermore, the Scottish Health Technologies Group (SHTG) presented preliminary base case results of a budget impact analysis comparing capsule sponge testing with usual care in patients with chronic reflux symptoms referred for endoscopy. This national evaluation predicted cost savings of £700,000 in the first year and £3.3 million over five years (Scottish Health Technologies Group, 2023). However, a formal health economics assessment for capsule sponge testing has yet to be undertaken. Collaboration with experts in this field provides exciting scope for future work in both the symptomatic reflux and Barrett's surveillance cohorts.

9.3 Wider clinical and research implications

Following the publication of work presented in Chapters 2 and 7, data have subsequently become available from the NHS England implementation studies, as discussed earlier in this thesis. Application of the previously published risk-stratification tool for Barrett's surveillance has been demonstrated to enrich for dysplasia within the real-world setting (Tan et al., 2025), validating the findings presented Chapter 2. In the context of capsule sponge testing for reflux symptoms, data from the NHS England prospective real-world study have also demonstrated capsule sponge testing to be safe and effective at identifying those likely to have pathology at endoscopy, thereby reducing burden on endoscopy services and increasing diagnostic yield of Barrett's oesophagus cases (Gourgiotis et al., 2025). Furthermore, a recent single-site study prospectively evaluating the real-world pathway for routine reflux investigation with capsule sponge testing published identical biomarker results to the Scottish pilot programme, demonstrating results were robust in both cohorts. Analysis of endoscopic biopsy results in this study also demonstrated that positive biomarkers on capsule sponge testing enriched the proportion of major findings at endoscopy, compared to the normal capsule sponge test control group (Angel et al., 2026). These recent publications also externally validate the findings from the CytoSCOT programme presented in Chapter 7 and provide clinicians with additional reassurance that this pathway is safe and feasible in clinical practice.

In Scotland, the Accelerated National Innovation Adoption (ANIA) collaborative commissioned the SHTG to produce a report evaluating the clinical effectiveness, cost effectiveness, safety and patient experience of using capsule sponge testing for Barrett's surveillance and chronic reflux symptoms in 2023 (Scottish Health Technologies Group, 2023). This report was published at a juncture when the full funding provided for the CytoSCOT capsule sponge service from the Scottish government was coming to an end and its ongoing use was being evaluated at local health board level. This report was based on preliminary data available from the CytoSCOT database, using data collected during the generation of this thesis (Appendix 1). This extensive report also included a provisional budget impact analysis and concluded that "the Cytosponge™ and EndoSign® devices will be considered for national rollout in

NHS Scotland" due to the positive initial results of the CytoSCOT programme (Scottish Health Technologies Group, 2023). This highlights the impact of the work conducted during this thesis on clinical practice, consequently resulting in change in practice in several Scottish health boards.

9.4 Conclusion

Overall, this thesis has demonstrated that capsule sponge testing is a highly promising new technique, with the evidence presented in this body of work supportive of its ongoing use in clinical practice and successfully addressing the aims laid out in Chapter 1. The papers presented in this thesis are also the first to provide external validation for the data published by the Fitzgerald group from the University of Cambridge in support of capsule sponge testing. However, ongoing work is fundamental before capsule sponge testing can be incorporated into formal clinical guidelines and pathways for Barrett's surveillance and screening, and it cannot be justified as a definitive replacement for UGI endoscopy at this juncture. Results of the BEST 4 trial will be critical to this progress moving forward.

Appendix 1

Healthcare Improvement Scotland

Edinburgh office
Gyle Square
1 South Gyle
Crescent
Edinburgh
EH12 9EB

Glasgow office
Delta House
50 West Nile
Street
Glasgow
G1 2NP

0141 225 6999
www.healthcareimprovementscotland.scot

Date: 10/10/2025

To Whom It May Concern,

Assessment of capsule sponge technologies

In December 2023, the Scottish Health Technologies Group (SHTG), part of Healthcare Improvement Scotland, published an assessment of capsule sponge technologies for the detection of Barrett's oesophagus and early-stage oesophageal cancer.

SHTG was asked to evaluate the use of capsule sponge devices to detect Barrett's oesophagus and early-stage oesophageal cancer. We considered clinical effectiveness, cost effectiveness, safety, and the patient experience. This work was requested by the Accelerated National Innovation Adoption (ANIA) collaborative.

Dr Siobhan Chien, Clinical Research Fellow, NHS Golden Jubilee at the time when the work was being completed, contributed to our work on this topic by undertaking data collection and taking part in the peer review process.

Dr Chein's contribution was extremely valuable and was acknowledged in the final publication.

Kind regards

Edward Clifton

Unit Head - Scottish Health Technologies Group (SHTG)

NHS SCOTLAND

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