Detection and characterisation of dysplasia within the colon

Subtitle: Detection and characterisation of colonic dysplasia for patients with Inflammatory Bowel disease

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I confirm that the work submitted is my own, except where work which has formed part of jointly authored publications has been included. The contribution by myself and the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within the thesis where reference has been made to the work of others. The contributions of co-authors are stated below.

Chapter 2: Are Random Biopsies still required during IBD surveillance: A systematic review and meta-analysis

- **Lord R**, Khan A, Burr NE, Subramanian V. The first author conceptualised the project, performed data analysis and wrote the manuscript. RL, AK, NEB performed literature search and data extraction. VS assisted with data extraction, assisted with data analysis and review of manuscript.

Chapter 3: Chromoendoscopy with 0.03% indigo carmine delivered via a foot pump compared with 0.2% indigo carmine delivered via spray catheter for detecting dysplasia in patients undergoing surveillance in inflammatory bowel disease. A randomised controlled trial.

- **Lord R**, Sood R, Mohammed N, Burr NE, Subramanian V. RL and VS conceptualised the study. RL, RS, VS performed procedures and collected data within the study. Data analysis and manuscript writing was performed by RL. VS reviewed data analysis. Review of manuscript was performed by all authors.

Chapter 4: Colonic lesion characterization in IBD: A Systematic Review and Meta-analysis

- **Lord R**, Burr NE, Mohammed N, Subramanian V. RL and VS conceptualised the study. Literature search and data extraction was performed by RL and NEB. RL performed data analysis and writing of manuscript. Review of data analysis and manuscript was performed by VS.

Chapter 5: Accuracy of Real Time In-vivo Lesion Assessment in IBD-colitis

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Abstract

Introduction

Patients with Inflammatory bowel disease (IBD)-colitis have an increased risk of developing colorectal cancer (CRC). CRC associated with IBD-colitis is understood to evolve along the inflammation-dysplasia-cancer pathway. Optimising the detection and characterisation of dysplasia is paramount for delivering effective surveillance, in order to prevent progression of dysplasia to cancer.

Aims

- Are random biopsies during surveillance colonoscopy in patients with IBD colitis still required
- To determine the optimal endoscopic method for the detection of dysplasia in patients with IBD colitis during surveillance and is there a subpopulation in which invisible dysplasia exists
- To determine the accuracy of in-vivo lesion characterisation in real-life practice

Methods

Two systematic reviews/meta-analyses were conducted 1) To determine what proportion of patients with dysplasia during surveillance colonoscopy are identified by random biopsy alone and if the proportion detected is influenced by the cohort's perceived risk for dysplasia 2) The diagnostic accuracy of optical imaging techniques for in-vivo lesion characterization in colonic IBD. A randomised controlled trial was then performed to determine whether 1) The concentration of dye (0.03% versus 0.2% indigo carmine) impacts on the detection of dysplastic lesions in patients with colitis 2) Understand the optical diagnostic accuracy of in-vivo lesion characterisation for colonic lesions detected 3) Predictors for invisible dysplasia.

Results

On pooling proportions, 13.05% (95% CI 7.28 – 19.87%) of patients with dysplasia had this identified by random biopsies alone. Pooled proportion of patients with dysplasia identified by random biopsy alone within the high-risk group was more than double, 14.19% (95% CI 7.43 – 22.29%), compared to the low-risk group, 6.42 (95% CI 0.04 – 18.45%). The lesion characterisation meta-analysis showed Confocal Laser Endomicroscopy (CLE) as the most accurate technology, with a sensitivity of 87% (95%

CI 71%-95%), specificity of 94% (95% CI 87%-97%), area under the SROC curve of 0.96 (95% CI 0.94-0.97).

For the RCT, 300 procedures were randomised into 0.2% (n=150) and 0.03% (n=150) indigo carmine concentrations. Targeted neoplasia was detected in 32 (21.3%) procedures in the 0.2% arm and 26 (17.3%) procedures in the 0.03% arm; p=0.465. Nine procedures (3%) had random biopsy only dysplasia, with 88.9% in the BSG high risk group. Overall sensitivity, specificity, positive and negative predictive values for dysplasia optical diagnosis were 84.7%, 82.3%, 64.3% and 93.5%. Accuracy further improved using the 0.2% dye concentration.

Conclusion

Dye-based Chromoendoscopy has a high yield for detecting targeted dysplasia regardless of the concentration used, although numerically favours the 0.2% indigo carmine using the spray catheter. Accuracy of in-vivo lesion characterisation also favours the more concentrated dye solution. When using high definition chromoendoscopy, random biopsies for invisible dysplasia are not required when patients are risk stratified within the BSG low and intermediate risk groups.

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List of abbreviations

AFI	Autofluorescence
AI	Artifical intelligence
ALM	Adenoma like mass
APC	Adenomatous polyposis gene
ASGE	American Society Gastroenterology
AUSROC	Area under the receiver operator curve
BBPS	Boston Bowel preparation score
BSG	British Society of Gastroenterology
CD	Crohn's disease
CE	Chromoendoscopy
CI	Confidence intervals
CLE	Confocal Laser Endomicroscopy
CONSORT	Consolidated Standards of Reporting Trials
CRC	Colorectal cancer
CSI	Chromosomal Instability
DALM	Dysplasia associated lesion or mass
DBC	Dye-based chromoendoscopy
DNA	Deoxyribonucleic Acid
DOR	Diagnostic Odds Ratio
EMR	Endoscopic mucosal resection
ESD	Endoscopic submucosal dissection
FICE	Fujinon intelligent image enhancement
FN	False negative
FP	False positive

FUSE	Full-spectrum endoscopy
GETAID	Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives
GI	Gastrointestinal
HD	High definition
HDCE	High definition chromoendoscopy
HDWL	High definition white-light
HGD	High grade dysplasia
HR	Hazard Ratio
IBD	Inflammatory Bowel Disease
IC	Indigo carmine
ICV	Ileo-caecal valve
IL	Interleukin
IQRs	Interquartile range
JAG	Joint advisory group
JNET	Japan NBI expert team
LGD	Low grade dysplasia
LHR	Likelihood Ratios
LST G	Lateral spreading tumour Granular
NBI	Narrow Band Imaging
NHS	National Health Service
NICE	NBI International Colorectal Endoscopic
NNT	Number needed to treat
NPV	Negative predictive value
OR	Odds Ratio
PCCRC	Post colonoscoy colorectal cancer
PP	Pit pattern
PPV	Positive predictive value

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSC	Primary Sclerosing Cholangitis
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RCT	Randomised Controlled Trial
SDCE	Standard definition chromoendoscopy
SDWL	Standard Definition white-light
SES – CD	Simple endoscoic score for Crohn's disease
SIR	Standardised incidence ratios
SM	Submucosa
SROC	Summary receiver operator curve
SSL	Sessile serrated lesion
TN	True negative
TNF	Tumour necrosis factor
TP	True positive
UC	Ulcerative colitis
VCE	Virtual chromoendoscopy
VOCs	Volatile organic compounds
WHO	World health organisation

Chapter 1
Introduction

1.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing autoimmune condition involving the gastro-intestinal tract (GI tract), believed to be triggered by certain environmental factors within those genetically primed individuals(1). Such individuals are understood to have an altered gut microbiome permitting immune dysregulation, culminating in a compromised intestinal mucosal barrier, which ultimately activates an inflammatory response by the body, that progresses to inflammation of the colon, known as colitis(2).

IBD embodies two disease subtypes: Ulcerative colitis (UC) and Crohn's disease (CD). Although both UC and CD are categorised under the bracket of IBD, they each hold well recognised differences(3). Patients with UC have inflammation predominantly affecting the superficial layer (mucosa) of the colon, characterised by a continuous pattern, starting at the anorectal margin and progressing proximally to varying degrees towards the caecum(4). CD, on the other hand, can cause transmural inflammation, generally in a non-continuous pattern, occurring anywhere within the GI tract. The area within the GI tract most commonly affected is the ileocaecal region, although it can have a similar distribution and potentially mimic that of UC. CD's transmural inflammatory nature results in a proportion of patients developing complications such as strictures, abscesses and fistulae within the GI tract, contributing to significant patient morbidity(5). The disease course in both of these conditions generally follows a relapsing/remitting course, exposing the bowel to inflammatory insults over many years (see Figure 1).

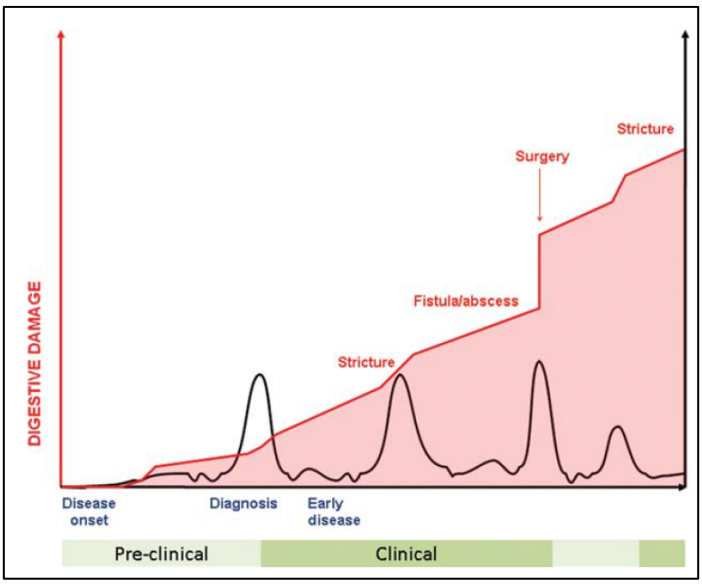


Figure 1 The theoretical damage of patients with IBD as a result of ongoing inflammation(6)

Initially thought to be a disease inflicting on a westernized society, research now shows IBD as a global issue, with increasing incidence in newly industrialised countries. Epidemiological data now suggests IBD is plateauing in westernized society, but the peak has yet to occur in these newly industrialised countries(7). Currently, there are over 1.5 million people that suffer from the disease in North America and around 2 million in Europe with a worldwide prevalence currently exceeding 0.3%. It has a bimodal incidence distribution with a main first peaking affecting those between the ages of 15-30 and a second smaller peak affecting those between 50-70 years of age(8).

As described in Figure 1, the relapsing and remitting disease process exposes the bowel to cumulative damage over many years. However, in this fight against repeated bowel damage, we have now accumulated a vast array of medical treatments giving patients treatment options, providing the opportunity for patients to attain deep remission (endoscopic and histological remission), halting its progressive nature and thereby preventing future complications. This armamentarium includes medications such as 5-aminosalicyates, steroids, immunosuppressive oral drugs, biologic drugs and the recently introduced Janus Kinase inhibitors, although none of these medications provide patients with a cure currently (9, 10). For UC however, surgery in the form of a colectomy offers cure for patients with disease refractory to medical treatments or with acute severe refractory colitis, perforation, strictures or cancer/dysplasia of the colon that is not amenable to endoscopic treatment(11). Studies have shown that colectomy is associated with an improved quality of life, although is attached with some morbidity, including a proportion becoming infertile. Therefore, for most patients surgery is still a last resort treatment option when medical treatment has failed(12).

1.2 Increased risk of colorectal cancer in IBD

Discovery of the association of colorectal cancer (CRC) and IBD dates back to 1925 and historically accounted for around 15-20% of the deaths in patients with IBD(13, 14). Over the last several decades many studies have endeavored to quantify this risk. One of the earliest landmark meta-analyses looking at CRC risk in patients with UC showed a cumulative risk of 2% at 10 years, 8% at 20 years and 18% at 30 years of disease(15). However, this study was thought to have overestimated the risk due to a significant proportion of studies involving cohorts from tertiary referral centres. Such cohorts are known to inherit subpopulations with more aggressive disease phenotypes and thereby higher risk of developing CRC. A decade on, and in 2012 a meta-analysis looking purely at population-based cohorts revealed the cumulative risk was lower than previously thought, although the trend still increased with length of time with the disease. Results demonstrated a cumulative incidence of 0.4% at 10 years and 1.1%-5.3% at 20 years. Overall there was a 2.4-fold (95% CI 2.1 – 2.7) higher risk of developing CRC for patients with UC compared with that of the general population(16). This increased risk of CRC isn't only unique to UC but is also translated to patients with Crohn's colitis. A meta-analysis in 2013 involving more contemporary studies, showed a cumulative incidence of 1% at 10 years, 2% at 20 years, and 5% at > 20 years of disease duration, echoing comparable estimates to those patients with UC(17).

1.3 Colorectal cancer in IBD

This increase risk of CRC in IBD colitis is linked to the chronic inflammatory process within the colon. Damage to the mucosa resulting from chronic mucosal inflammation has shown to result in increased epithelial cell turnover as a consequence of higher rates of mitosis, in an attempt to repair the mucosa. Release of proinflammatory mediators, such as tumour-necrosis-factor alpha (TNF) and interleukin-6 (IL-6), with the addition of reactive oxygen and nitrogen species results in an environment for DNA damage, such as p53 gene and DNA mismatch repair genes(18). Repetition of this process, through either longstanding or consecutive bouts of inflammation, increase the risk of DNA damage. If inflammation is controlled then proliferation should subside however, proliferating cells that have sustained DNA damage are at risk of continued proliferation. These genetic changes have the potential to progress along a pathway of low-grade

dysplasia to high grade dysplasia through to cancer. This transition through to the development of cancer is known as the “inflammation-dysplasia-cancer pathway”. As a consequence of this aberrant pathway, several differences are known to exist when comparing sporadic CRC to that of colitis associated CRC.

1.3.1 Differences between colitis associated and sporadic CRC

CRC in IBD does share some similarities to sporadic CRC, specifically encompassing its molecular and genetic mutations, although there are well known established differences. These differences include the order in which these molecular events unravel, the morphology of these lesions and the fact that they tend to be synchronous within the colon.

1.3.2 Molecular pathways

Transition in sporadic CRC tends to progress along the well-defined adenoma-carcinoma pathway. This involves loss of the adenomatous polyposis gene (APC), a tumour suppressor gene, usually acting as the first insult within this pathway. Finally, loss of the p53 gene (another tumour suppressor gene) completes this transformation.

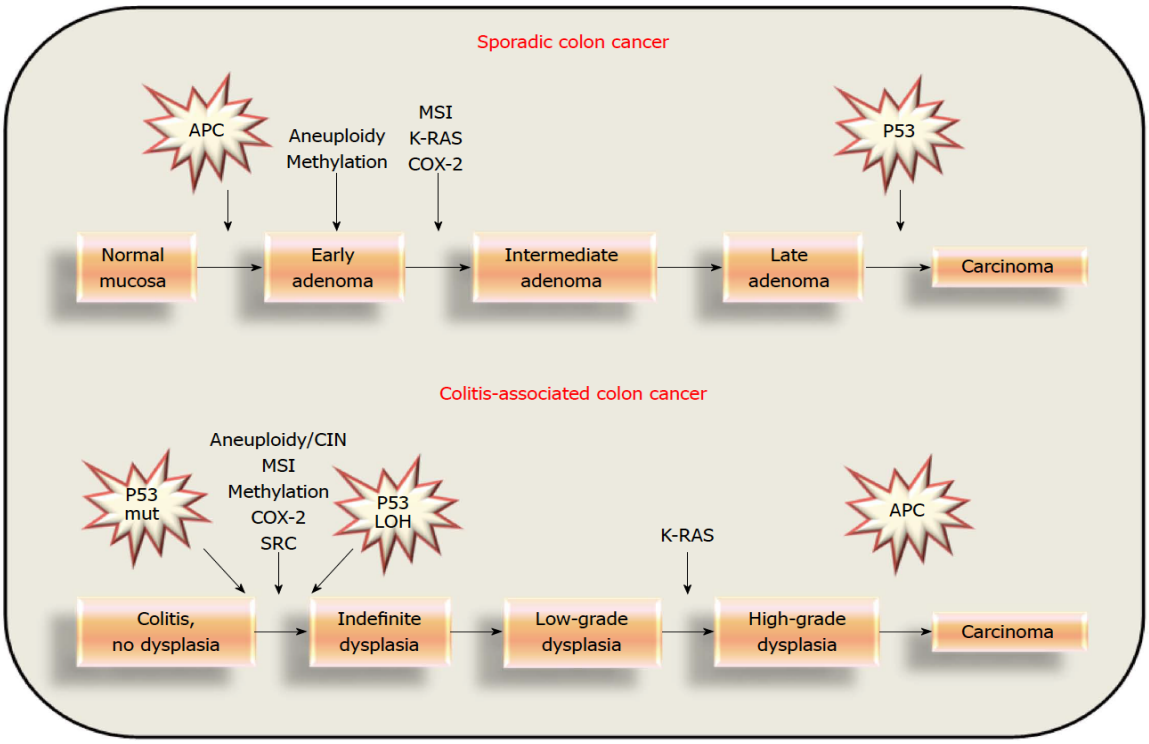


Figure 2 Difference in the order of mutations within sporadic and colitis associated colorectal cancer. This picture illustrates the differences regarding the order of mutations between sporadic colorectal cancer and colitis-associated cancer. Image courtesy of Scarpa et al(19)

Transition to CRC within IBD, as previously described, is thought to progress along an inflammation-dysplasia-cancer pathway, although progression may potentially be accelerated, when compared with sporadic CRC, and may not always occur following this stepwise manner (see Figure 2). The chromosomal instability (CIN) in IBD seems to be reversed with the p53 mutation occurring very early on and loss of APC gene function potentially occurring much later within this pathway(20). These mutations are thought to occur by exposure of the colonic epithelial cell DNA to oxidative stress brought about by chronic inflammation within the colon(21).

A further molecular pathway that is thought to account for between 15-30% of CRC in non-IBD patients, involves serrated lesions. Histologically these serrated lesions have a saw-toothed appearance of the crypt epithelium and include hyperplastic polyps, sessile serrated lesions with or without dysplasia and traditional serrated adenomas. Their pathway to CRC normally starts with a BRAF mutation (rarely KRAS mutation) followed by aberrant methylation involving the cytosine nucleotide at the CpG dinucleotide. This aberrant DNA methylation results in silencing of tumour suppressor genes, potentially resulting in neoplastic growth(174).

1.3.3 Morphology

Sporadic CRC cancer most commonly develops from a dysplastic lesion known as a tubular adenoma. These sporadic lesions tend to be polypoid, with a clear distinction between abnormal and normal mucosa, providing a border that is well demarcated. In contrast, a significant proportion of IBD colitis associated dysplastic lesions tend to be morphologically flatter with a less clearly demarcated border, making distinction of what is normal and abnormal sometimes very challenging (see Figure 3). This can be further compounded by surrounding inflammation which distorts the mucosal patterns.

These differences are thought to arise by the fact that sporadic dysplastic polyps develop from transformation of cells at the top of the crypts within the lumen, whereas those associated with IBD-colitis are thought to develop from cells transforming within the base of the crypts(22).

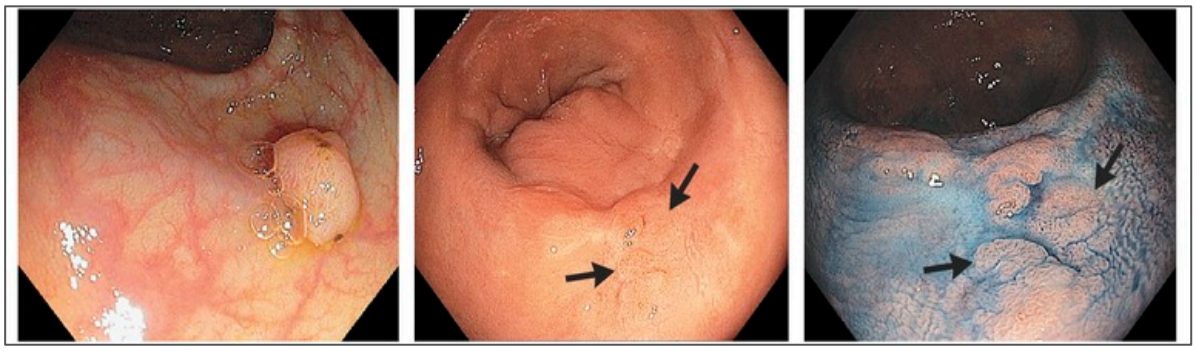


Figure 3 The image on the left shows a sporadic colorectal polyp with its clearly demarcated border. The central and image to the left show a dysplastic lesion associated with IBD-colitis with unclear demarcation and flatter appearance(21)

1.3.4 Synchronous and metachronous lesions

Within IBD colitis there is an increased risk of developing synchronous and metachronous cancers compared with non-colitic CRC's(23, 24). Such findings have also been seen in other organs were cancer develops within an area of inflammation. This has led to a hypothesis known as the "field effect", whereby molecular changes occur throughout the inflammatory-affected colonic mucosa even before dysplasia develops (25, 26). The theory being that chronic inflammation exposes the mucosa to inflammatory cytokines potentially resulting in widespread cellular aberrations, such as point mutations, telomere shortening and aneuploidy. These clonal molecular abnormalities occur despite histological analysis of colonic tissue appearing normal (27). This "field effect" or "field cancerisation" hypothesis allows an opportunity to search for new biomarkers within colonic mucosal tissue even before dysplastic changes have developed. This could potentially provide objective markers for patients at future risk of developing dysplasia, offering a more individualised risk stratification, and allow greater

resource allocation for those deemed higher risk. New biomarkers may also grant less invasive ways for determining a patient's risk. A single rectal sample maybe all that is required. Discovery of new biomarkers for high risk patients may also provide a greater window of opportunity to halt the "inflammatory driver" which is known to significantly increase the risk of dysplasia and CRC developing.

1.4 Risk Factors

Risk of progression to dysplasia / CRC is not uniform within the IBD population. Certain risk factors have been extrapolated from the data rendering certain subpopulations at higher risk. In response to these findings, gastroenterological societies now vary their surveillance frequency according known risk factors in an attempt to make it more individualized and to prioritize resources to those deemed at highest risk. However, this is still very generalized, and currently imprecise at risk stratifying, with more objective ways of assessing individual risk still required. The current BSG guidelines are now over a decade old but risk stratify patients into three categories based on certain criteria. Low risk individuals are offered a colonoscopy every five years, medium risk every three years and high risk with annual colonoscopy (see Figure 4)(28).

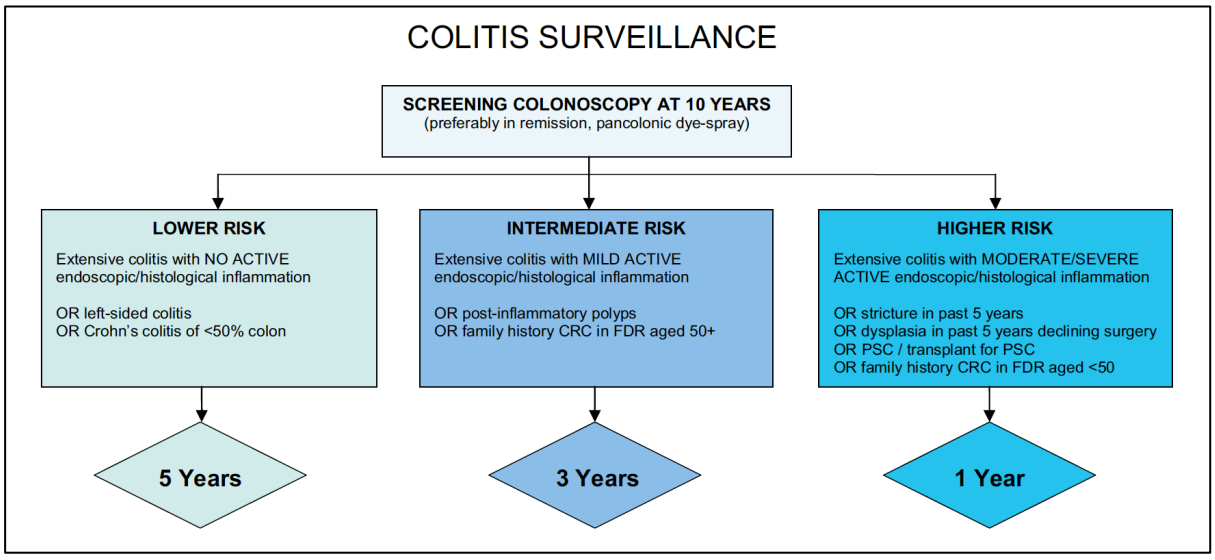


Figure 4 BSG 2010 IBD surveillance guidelines. The frequency of surveillance is dependent on certain risk factors displayed in each box(28).

1.4.1 Length of time of colitis

As depicted earlier within this chapter, increasing length of time of colitis is strongly associated with the increasing risk for developing CRC(15-17). This is why current surveillance guidelines suggest commencing surveillance at 8-10 years of duration when risk is known to increase(28-30). Intuitively this would make sense, as the colonic mucosa is potentially exposed to more years of inflammation, increasing the risk of progression along this "inflammation-dysplasia-cancer" pathway. However, a retrospective study has suggested there is a significant risk of developing CRC before the recommended index colonoscopy time frame. Using the old BSG guidelines of commencing surveillance from date of diagnosis at 8 years for extensive colitis and 15 years for left-sided colitis, 22% of patients developed cancer before the 8- or 15-year starting points of surveillance. By using the onset of symptoms to calculate the time interval, 17% would present with cancer prior to the recommended surveillance starting points (31). Although one of the flaws highlighted in this paper, was that this study contained data from a tertiary centre thereby making it more likely to inherit severe phenotypes of disease and therefore a higher risk population. As well, the true onset of symptoms is difficult to calculate due to the retrospective nature, however it does highlight symptom onset rather than diagnosis should be used when planning the timing of surveillance. Despite this, it would seem the excess risk only occurs after the first decade of disease(17).

1.4.2 Disease extent

Disease extent is another significant risk factor for CRC. Patients with more extensive colitis are at an increased risk. To quantify disease extent, studies and societies generally divide inflammatory extent into three categories: proctitis (inflammation only involving the rectum), left sided colitis (inflammation extending up to the splenic flexure) or extensive colitis (inflammation extending beyond the splenic flexure). Data several decades old, from a population based cohort, showed that disease extent affects risk of developing CRC: those with proctitis had a standardized incidence ratio of 1.7 (95% CI 0.8-3.2, left sided colitis was 2.8 (95% CI 1.6-4.4) and for pancolitis 14.8 (95% CI 11.4-18.9)(32). More recent data, including a meta-analysis showed that patients with extensive colitis had a standardized incidence ratio (SIR) of 6.9 (95% CI 1.9-11.9) for developing CRC when looking at purely population-based cohorts compared with1.7 (95% CI 0.6-4.5) for left sided and 1.0 (95% CI 0.5-1.6) for proctitis(17). Proctitis would seem to consistently confer no additional risk and therefore such patients do not generally undertake surveillance.

1.4.3 Severity of inflammation

Studies have also revealed an association for the increased risk of developing CRC with the increasing severity of inflammation(33, 34). A case-control study showed a 3-fold increase risk for developing CRC if severe inflammation was present (OR 3.38 95% CI 1.41-10.38)(35). The same study also showed an increase risk with the presence of pseudopolyps (OR 2.14 95% CI 1.24-3.70) and strictures (OR 4.22 95% CI 1.08-15.54) and these are incorporated into the national risk stratification guidelines. Pseudopolyps themselves are not thought to transition to cancer, however they signify previous significant inflammation and theoretically make it more challenging to identify dysplastic lesions, especially when the colonic mucosa is “carpeted” with such lesions.

1.4.4 Previous dysplasia

Previous dysplasia is also a very strong risk factor for developing CRC. A recent meta – analysis showed that previous low-grade dysplasia (LGD) resulted in 19.1% of patients developing subsequent high-grade dysplasia (HGD) or CRC over a median of 48 months, with univariate analysis showing association with multifocal dysplasia (HR 3.9; 95% CI 1.9 – 3.8) and metachronous dysplasia (HR 3.5; 95% CI 1.6 – 7.5) (38). A further recently published meta – analysis has shown that previous LGD increases the risk of developing advanced colonic neoplasia around 10 fold(36). This may be explained by the “field effect”, as described earlier, or by incomplete previous dysplastic resections or missed synchronous pathology.

1.4.5 Primary Sclerosing Cholangitis (PSC)

PSC is another autoimmune related disease resulting in inflammation and fibrosis of the bile ducts, which may eventually progress to cirrhosis in a significant number of patients. Around 60 – 83% of patients with PSC also have IBD, most commonly being UC(37). Those with UC tend to have rectal sparing with disease being worse on the right side of the colon, sometimes with backwash ileitis (inflammation involving the terminal ileum just within the ileo-caecal valve). It is now well-established that PSC is a significant risk factor for colonic dysplasia and CRC. This is especially true for right sided lesions, with a risk of nearly five times when compared to patients with colitis without having PSC (39). When comparing patients with UC with PSC against those with UC only, one meta – analysis has shown that the risk of developing CRC is increased by four times (OR 4.09; 95% CI 2.89 – 5.76)(38). Further, a population – based cohort showed a 10 – fold increase risk of CRC in PSC colitic patients compared to those without PSC(39). Studies have also shown that patients with PSC colitis are more likely to develop invisible dysplasia and when dysplasia develops it seems to progress at a more advanced rate(40). Because of this, patients with PSC currently undergo annual surveillance.

1.4.6 Family History of CRC

Family history of CRC is a well – established risk factor for patients without colitis, with a doubling of a patients future risk(41). Patients with IBD – colitis and a family history of CRC have additional risks. A recent population – based study looking at the risk of patients with IBD colitis (both UC and CD) with a first degree relative with a history of CRC, had double the risk of developing CRC when compared to patients with IBD – colitis and no family history of CRC (relative risk 1.88; 95% CI, 0.8–4.3)(42). Although this didn't reach statistical significance due to small numbers included within the study. A slightly more historical study showed those with IBD – colitis, and having a first – degree relative diagnosed with CRC before the age of 50, had a nine fold increased risk than those without(43).

1.4.7 Strictures

Patients with IBD colitis are at risk of developing colonic strictures (narrowing of the lumen) related to ongoing severe inflammation. This seems to be more common in Crohn's colitis, due to its transmural nature, with up to 10% of patients at some point in time being at risk of this(44). In UC colitis, risk of stricture formation is significantly lower, with a recent population – based study showing a prevalence of around 1%(45).

Strictures are known to have an increased risk of dysplasia and CRC in patients with IBD – colitis. Current BSG guidelines place patients with colonic strictures within the high – risk group, requiring annual surveillance. This risk however, is extremely variable when looking at the literature studying this outcome, due to varied studied populations, selection bias and duration of cohort follow – up. The risk of dysplasia/CRC within strictures has been shown to be higher in UC patients than those with CD. However, the risk range from several published studies, ranges from 2 – 90%(45-47). More recent data suggests the risk is lower, with 2% of patient with a colonic stricture having HGD/CRC, and were 14.3 [5.8–30.2]) times more likely to have HGD/CRC than patients without a stricturing phenotype(45, 48).

1.5 Surveillance

For surveillance to be effective several principles were proposed by Wilson and Junger in a paper for the World Health Organization (WHO) in 1968(49). In essence, the condition should be an important problem within a target population, with a clear understanding of the natural progression of disease. There should be a recognized early phase for which there is a valid test that is acceptable to the population in question.

Treatment needs to be effective, safe but also cost-effective. Finally, case-finding should be a continuous process.

As previously described, with colonic IBD there is at least a doubling of risk for CRC when compared with the general population, thereby representing a target population. For colonic IBD, surveillance has been recommended by numerous gastroenterological societies throughout the world, including the UK, which adheres to the British Society of Gastroenterolgy (BSG) guidance(28, 29). The aim of surveillance currently within the IBD population is to detect dysplastic (pre-malignant) lesions early within the molecular pathway, before cancer has developed; this signifying the early phase. By detecting these less advanced lesions, treatment is more likely to be successful and amenable to less invasive curative methods, such as endoscopic rather than surgical resection.

Case-controlled studies do provide evidence that surveillance seems to be effective. Studies have shown that patients participating in a surveillance programme have less advanced lesions and improved survival compared to controls(50, 51). Sceptics may argue that this data is less robust and subject to selection and lead time bias, but for ethical reasons no randomised controlled trials have been performed and are unlikely to be in the future(52).

However, with surveillance comes additional cost to an already stretched healthcare service and with an increased number of endoscopic procedures, this carries a small but not insignificant risk for procedural complications. Therefore, optimal methods for detecting dysplasia and being able to differentiate dysplastic lesions from non-dysplastic lesions (characterization), as well as allocating more intense surveillance for those deemed highest risk (risk stratification) is key for an effective, cost efficient, surveillance programme.

1.6 Surveillance practice and techniques

The backbone of current surveillance practice for IBD colitis is to perform colonoscopy. In order for this procedure to provide useful information, it should be undertaken when the patient is in remission. Any ensuing inflammation can make it almost impossible for the endoscopist to detect flat subtle lesions which could harbor dysplasia. Biopsies taken from the colonic mucosa whilst inflammation is present, may also provide confusion for the histopathologist, providing significant challenges when trying to differentiate inflammation from dysplasia(30, 53).

The bowel preparation ideally should be meticulous and certainly should not be undertaken if poor quality. High quality bowel preparation improves the visualization of the entire colonic mucosa and has shown to improve the endoscopist's ability to complete the test and increase dysplasia detection(54). During intubation to the caecum, the bowel should be washed, using a foot – pump activated water – jet and suctioned any pools of fluid in order to remove any luminal residue and further improve mucosal visualization during withdrawal.

Once at the caecum (end of the large bowel), this should be confirmed by identification of the appendiceal orifice, ileo-caecal valve (ICV), tri-radiate folds and attempt at ileal intubation should be performed. Inspection and assessment of the colonic mucosa should then take place on withdrawal. For standard colonoscopy, guidelines suggest a withdrawal time of 6 – 10 minutes, with increasing adenoma detection rate correlating with more time spent on inspection during withdrawal, with this effect plateauing after 10 minutes(54, 55). No study has performed such metrics during IBD surveillance, but inspection and withdrawal is likely to be longer due to the intricate nature of lesions within IBD colitis and the associated techniques to detect dysplasia.

1.7 Dysplasia detection

Historically, dysplasia in IBD colitis was always thought to be largely invisible. As a consequence, detection of dysplasia was initially performed by taking extensive colonic random mucosal biopsies, usually 4 biopsies every 10cms on withdrawal from the caecum. Each procedure typically yielded on average 32 biopsies, sampling around 0.05% of the colonic mucosa (18, 56). Unfortunately, this was time consuming for both the endoscopist and the pathologist, had a low yield for detecting dysplasia (around 0.1% of random biopsies being positive for dysplasia) and generally poorly adhered to by gastroenterologists(57). Pooled analysis has shown that random biopsies only detect around 20% of all type dysplasia when using standard definition endoscopes, thereby missing a large number of dysplastic areas if this was solely to be relied upon (58). With such a diminutive representation of the colonic mucosa this technique risked a high false negative rate for dysplasia detection.

The evidence supporting dysplasia detection using random biopsies during surveillance was largely obtained from older studies using fiberoptic and early standard definition colonoscopes, which provide image signals of lower pixel density as compared to their high-definition counterparts. Image resolution was significantly poorer than today's

endoscopes, hindering the endoscopist's ability to visualize the mucosa and better differentiate normal from abnormal mucosa.

In patients without colitis, neoplastic lesions or polyps tend to be easily visible, more polypoid and well circumscribed. In contrast, lesions within IBD are thought to be flatter and less easily discriminated from the surrounding mucosa, especially if inflammation is present, making them much more challenging to detect. This led to the exploration and adoption of complimentary endoscopic techniques to detect these subtle dysplastic lesions. As technology and technique improved, endoscopist's gained a greater appreciation and understanding, with dysplasia predominantly thought to be macroscopically visible, in the form of visible lesions or polyps in the majority of cases(59, 60).

1.7.1 Dye-based Chromoendoscopy

These new techniques led to the practice of dye spray, also known as chromoendoscopy (CE), as a means of dysplasia detection(59). During CE various dyes are sprayed onto the colonic mucosa on withdrawal from the caecum. This is applied following the SURFACE guidelines(61). Two main contrast agents are used during surveillance colonoscopy. The first, indigo carmine (IC), is a non – absorbed contrast dye which covers the mucosa and pools within crevices, enhances mucosal pit patterns, helps to accentuate any irregularities or lesions and also improves delineation of their borders(62). Methylene blue is another contrast which is an absorptive agent, being less well absorbed by inflammatory or dysplastic mucosa. However, there were concerns over potential DNA damage as a result of methylene blue therefore studies now predominantly apply indigo carmine as the contrast of choice(63).

Studies initially looked to compare standard definition white-light (SDWL) colonoscopy versus standard definition chromoendoscopy (SDCE) for the detection of dysplasia. This consistently showed improved detection of dysplasia favouring SDCE (64-68). Several meta-analyses have further confirmed the above findings(69, 70). One showed a pooled incremental yield for dysplasia detection on a per patient basis of 7% supporting SDCE, with a number needed to treat (NNT) to detect one extra patient with dysplasia or cancer of 14(69).

More recently high definition (HD) colonoscopies have been studied. They contain a higher pixel density, of over 1 million pixels per square inch, allowing a clearer, more well-defined image (71). Cohort studies have shown a higher yield for dysplasia from HD compared to SDWL colonoscopic surveillance (72). Current societal recommendations agree that HD colonoscopes should be used over their older counterparts(30).

Current guidelines suggest HD chromoendoscopy (HDCE) is preferable to HD white-light (HDWL) endoscopy for dysplasia detection, but the strength of this recommendation is conditional, based on low-quality data at the time of writing this guidance(30). More data has become available since the publication of the guidelines, but the data has led to some ambiguity regarding the clear benefits for dysplasia detection. One RCT in 2015, presented as abstract, showed HDCE to be significantly better at detecting dysplastic lesions when compared to HDWL (22% vs 9.4%)(73). A second study showed numerically superior but non-statistically significant results favouring HDCE (20.6% vs 12%) but this difference was less impressive when concerned with targeted biopsies within a colitic segment (3.9% versus 1.8%)(74). However, this was within a population known to be at low risk for dysplasia. A third study looking at HDWL versus virtual chromoenodsocpy (VCE) and HDCE, showed non-inferiority of HDWL for dysplasia detection (75). The most recent single-centred RCT, published in 2020, showed significantly more patients to have dysplasia detected in the HDCE group when compared to the HDWL group (11% vs 5%; p=0.032) and this was true for the total number of dysplastic lesions detected (24 vs 7; p=.029)(76). In summary, apart from one study, numerically more dysplasia would seem to be detected using HDCE over HDWL, but its benefit would seem to be less obvious than when using standard definition endoscopes. The heterogeneity of the studies described above, specifically the concentration and modality of the dye used, may explain some of the inconsistencies in the outcomes, making interpretation of the results challenging.

1.7.2 Virtual chromoendoscopy

Despite the higher dysplasia detection associated with DCE techniques, there is still reluctance for all endoscopist's to undertake this during surveillance colonoscopy. Barriers in performing DCE are likely reflect the additional time required during withdrawal, inadequate training and absence of validated standards. Continued advancements in endoscopic technology have led to the creation of dye-less chromoendoscopy, also known as virtual chromoendoscopy (VCE).

There are different types of VCE technology depending on the brand of the endoscope and its processor. Olympus use an end of scope filter, known as Narrow Band Imaging (NBI) whereas companies like Pentax or Fuji use post-processing technology (i-scan or Fujjinon intelligent image enhancement FICE). These work by increasing mucosal detail and enhancement by the switch of a button on the endoscope or processor.

Currently few studies have compared both HD DCE with that of VCE and those that have tended to be small and performed in expert centres. There are also many variables to consider when looking to compare HD DCE with that of VCE. The type and concentration of the dye has not been examined previously and also what VCE technology is better.

This makes direct comparisons challenging. For now, current guidance recommends HD DCE.

1.7.3 Random biopsies

Ongoing debates regarding whether random biopsies are still required during surveillance chromoendoscopy is still a hot topic. Panelists, from the most recent Scenic guidelines 2015, could not reach a consensus on whether random biopsies should still be taken if HDWL or chromoendoscopy is used as the technique(30). It is clear that when SD colonoscopy is performed, random biopsies have a higher yield for detecting the proportion of patients with dysplasia, being 20%, whereas this is halved using other modalities. Data from a meta-analysis has shown that if random biopsies weren't taken, around 1 – 1.5% of all patients undergoing surveillance would not have dysplasia detected, thereby potentially down staging their surveillance intensity. A counter argument for random biopsies is that only 1 in 1000 biopsies identify dysplasia making this an inefficient, expensive and labour intensive process. It may also deter the endoscopist from actually looking for pathology, giving them a false sense of security.

Intriguingly, a multi-centre study involving the GETAID group performed a prospective study looking at the number of patients with dysplasia detected by random biopsy only(77). They concluded that 12.8% of patients with dysplasia were detected by random biopsy alone. Using a multivariable regression analysis, they found that primary sclerosing cholangitis (PSC), a personal history of neoplasia and a "tubular colon" to be independently associated with detection of random biopsy only dysplasia. Other studies have also looked at cohorts exclusively involving PSC patients and found a high proportion of patients with PSC having random biopsy dysplasia only (78, 79). It's possible the dogma for invisible dysplasia holds true within a subpopulation of patients. More data is clearly required when using HDCE.

1.8 Current practice of chromoendoscopy

Once endoscopic confirmation that the caecum has been reached, withdrawal then commences using dye-spray followed by careful inspection of each segment looking for any mucosal irregularities or lesions. Different concentrations of IC are used throughout the world, generally applied through the colonoscope. The North Americans tend to use a more dilute concentration, 0.03% IC, delivered via a foot-pump, spraying the dye via the water-jet channel within the colonoscope. The water-jet containing dye is aimed at the non-dependent side of the colonic lumen in order to achieve circumferential

coverage. The European and Japanese centres tend to use a more concentrated solution, with a median dye concentration of 0.2% IC, using a spray catheter inserted via the accessory channel(30). The spray catheter tip should protrude around 2cms from the tip of the scope and is then connected to a syringe (containing the dye) placed within a spray gun which sprays the dye under pressure, aimed within the centre of the lumen, providing 360 degrees of mucosal coverage, whilst the colonoscope is withdrawn. Once sprayed along each segment of the colon, inspection takes place looking for any abnormalities. Inspection involves re-examining the proximal 20-30cm of colonic mucosa that has been sprayed, with suctioning out any pools of fluid, to assist in mucosal visualization.

If any lesions are seen, careful inspection of the area followed by targeted biopsies or endoscopic resection is performed depending on the endoscopist's opinion that complete lesion resection can take place. Biopsies around the lesion should also be taken to confirm complete resection(58). Random segmental biopsies are also required to look for inflammatory activity under the microscope.

Currently there remains an important clinical question over the concentration of the dye and its mode of application. This has never been addressed or compared in previous studies and whether this makes a real difference regarding dysplasia detection. This has been highlighted as an area of further research in current guidelines(30).

1.9 Lesion characterization

Once a lesion has been detected, endoscopic interrogation should take place. The aim of this interrogation is to help the endoscopist differentiate neoplastic from non-neoplastic lesions. There are several ways to help determine this in-vivo. Initially the polyp should be washed to allow clear visualization before assessment begins.

1.9.1 Morphology

Morphological description of the lesion is usually the first way to assess and characterize lesions. The most ubiquitous classification for gastrointestinal lesions is the Paris classification system(80). These were based on the original work from Japan, with Type 0 representing superficial neoplastic lesions (mucosal or submucosal). This can then be furthered divided into Type 0-I or 0-II if protruding or non-protruding lesions. Protruding lesions can be further sub classified into Ip (pedunculated), Isp (subpedunculated) or Is (sessile). Non-protruding lesions include IIa (superficial elevated), IIb (flat) or IIc (flat with superficial depression). Non-polypoid and excavated lesions are type III. Additionally

some lesions can be mixed type, displaying more than one subtype such as a IIa+IIc lesion. Figure 5 shows a further sub division of the Paris type 0 superficial lesions.

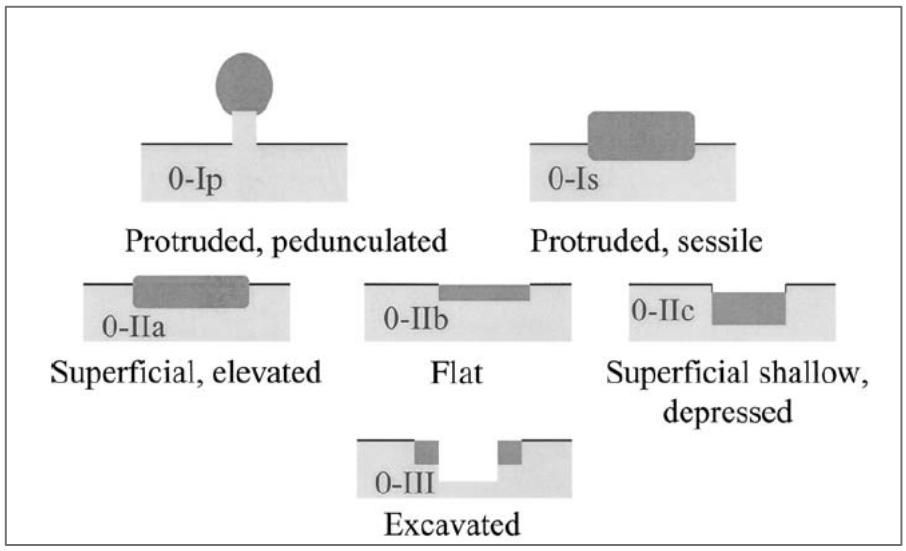


Figure 5 Paris classification of type 0 gastrointestinal lesions. Polypoid lesions include Ip and Is. Non-polypoid lesions include IIa, IIb and IIc. Non-polypoid and excavated lesions III(80).

It is useful to have such a classification system in order to standardize practice internationally and also provides information regarding the risk of submucosal invasion. The morphology therefore provides predictive value for risk of neoplastic invasion and therefore can determine if endoscopic treatment is going to be curative and appropriate. Studies have looked at the risk of submucosal (SM) invasion according to the Paris classification with one study showing low risk with Is and IIa lesions (7.5% and 4.1%), whereas high risk for IIa+IIc lesions (31.8%) (81).

1.9.2 Size

Following on from the morphological assessment, the size of the lesion should be determined. This can be challenging as there is no accurate way of calculating this whilst in – situ. However, instruments placed down the accessory channel can assist. Closed biopsy forceps have a diameter of around 2.5mm, whilst open biopsy forceps have a diameter of 8mm. When placed against a polyp these can give the endoscopist a relatively good estimation of the size. Snares also provide information to help determine the size. Snares come in various sizes and are sized according to their diameter. Therefore, placement of a snare over a lesion again can give a relatively accurate lesion size.

Determining the size of a polyp is important for several reasons. Most importantly size can be a predictor for submucosal invasion. A prospective registry looked at 11,188 adenomas and found that adenomas <5mm had no invasive cancer, whereas as the size increased so did the risk, with those >35mm had an 75.8% risk of invasive cancer(82). However, this data is from non – IBD cohorts and it is unclear if the same can be said for lesions within colitis. Also the size can be used to determine the type of resection, with sizes greater than 20mm more likely to require piecemeal resection due to higher chance of perforation if large lesions are removed in one piece. As well, the larger the lesion the more technically challenging the resection becomes and may indicate that an advanced endoscopist should resect the lesion.

1.9.3 Pit Patterns

More careful inspection then begins, concentrating on the surface pattern of the lesion. It is now over a quarter of a century since recognition of the structure of the crypts has been used to assist in determining whether a lesion is neoplastic versus non-neoplastic(62). Interrogating the mucosal surface architecture and vessel pattern, by using magnification and with the assistance of dye, can assist the endoscopist in making an in-vivo diagnosis regarding the nature of the lesion, with high accuracy(83).

Kudo first made the recognition that determining “pit patterns” can be applied to help differentiate neoplastic from non-neoplastic colonic lesions. Kudo grouped type I (roundish pits and type II (stellar) into non-neoplastic pattern and grouped type III (small or tubular pits), type IV (gyrus pits) and type V (non-structured pits) into neoplastic group (See Figure 6). Further, pit patterns can be used to predict the invasion depth of a lesion. Type V pit patterns can be further subclassified into type Vi, describing irregular crypts suggestive of superficial submucosal infiltration, and type Vn, describing non-structured pits more indicative of deep submucosal invasion. The overall accuracy of using pit patterns to differentiate superficial submucosal invasion from that of deep submucosal invasion in one paper was 88.6% in sessile lesions and 99.2% in flat lesions(84). However, Kudo pit patterns were not created to characterize lesions within colitic patients and therefore the accuracy in IBD is still unclear.

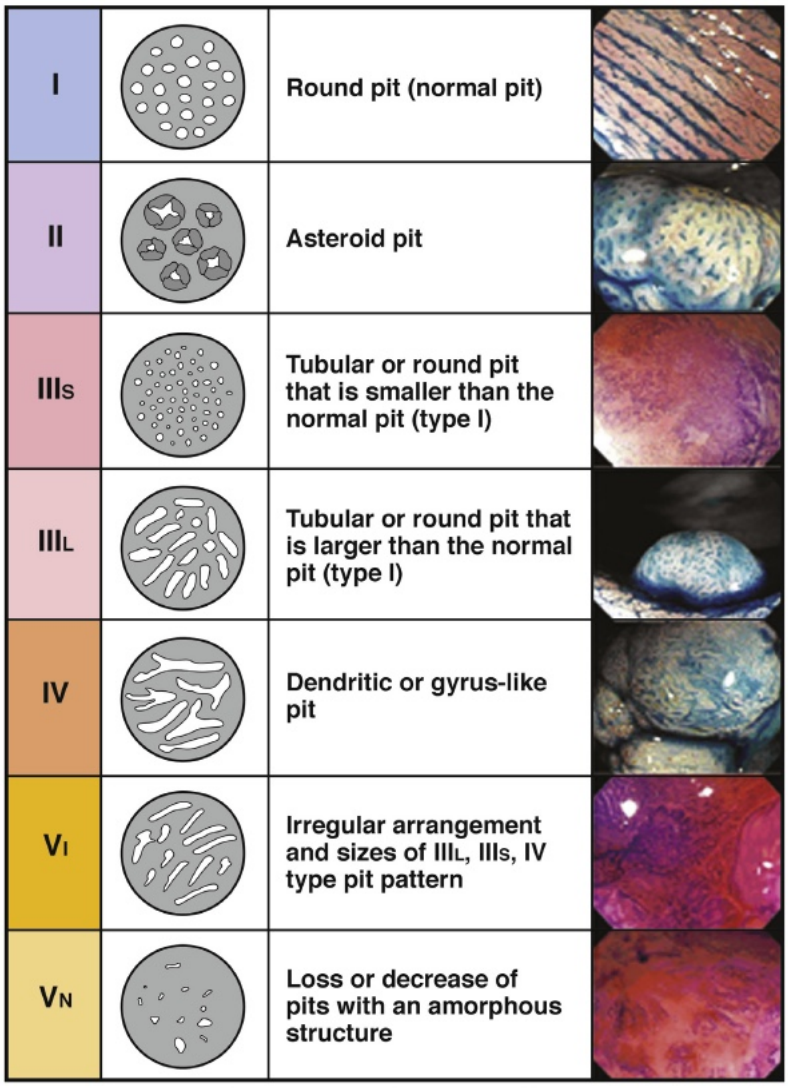


Figure 6 Kudo pit pattern classification(85)

1.9.4 Image Enhancement Technology

Virtual chromoendoscopy can also assist in lesion characterisation. The most studied endoscopic virtual technology is NBI. Several endoscopic classifications exist when applying NBI for lesion characterization. These include NICE (NBI International Colorectal Endoscopic), Sano and JNET (Japan NBI Expert Team). These classification systems focus predominantly on the surface and vascular patterns of lesions, each having their own slight nuance. The NICE classification system is simple to use and is based around describing lesions according to their colour, vasculature and surface pattern, differentiating lesions into hyperplastic, neoplastic or malignant (see Figure 7). The additional benefit of the NICE classification is that it can be used in both magnified and non-magnified endoscopy.

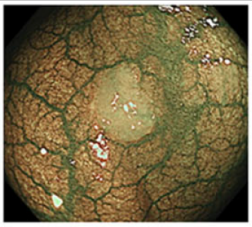
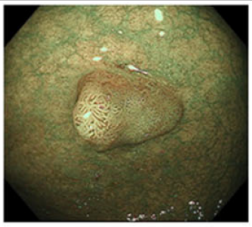
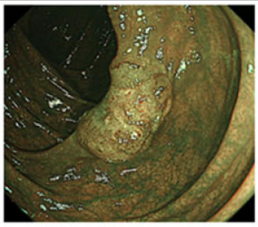
	Type 1	Type 2	Type 3
Color	Same or lighter than background	Browner relative to background (verify color arises from vessels)	Brown to dark brown relative to background; sometimes patchy whiter areas
Vessels	None, or isolated lacy vessels may be present coursing across the lesion	Brown vessels surrounding white structures**	Has area(s) of disrupted or missing vessels
Surface pattern	Dark or white spots of uniform size, or homogeneous absence of pattern	Oval, tubular or branched white structures** surrounded by brown vessels	Amorphous or absent surface pattern
Most likely pathology	**Hyperplastic & sessile serrated polyp (SSP) *** **	**Adenoma**** **	**Deep submucosal invasive cancer**
Endoscopic image			

Figure 7 NBI International Colorectal Endoscopic (NICE) classification (86)

However, using the NICE classification system serrated lesions maybe misclassified as purely non-neoplastic. In order to circumnavigate this, an additional classification system has been created specifically to assist in identifying such lesions. This is known as the WASP (Workgroup serrated polyp and Polyposis) classification system(175). When using this classification system, initially NICE is used to determine Type I from Type II lesions. Following this, the lesion is inspected for characteristics such as, a cloudy surface, indistinct border, irregular shape, or dark spots inside the crypts. If the lesion has two or more of these features, then it is sufficient to name this a sessile serrated lesion.

In studies using populations without colitis, virtual chromoendoscopy without magnification for in-vivo lesion characterization, seems to be accurate in the hands of expert endoscopists however, outside expert centres the results cannot be replicated and fall outside of current international society guidance(87, 88). Results looking at lesion characterization within a population consisting of colitis is likely to be even harder to accurately characterize.

This is an excellent discriminatory tool in patients without colitis, but in patients with colitis this has been less well studied and concerns over accuracy exist because of associated surrounding inflammation obscuring the mucosal pit patterns. There is currently no robust evidence regarding non-magnified conventional or virtual chromoendoscopy being able to accurately differentiate between neoplastic and non-neoplastic lesions in colitis(89).

1.9.5 Resectability

Once these assessments have taken place, the question posed will then be is it endoscopically resectable. Several additional questions require answering before the endoscopist considers resection. Assuming that the lesion is no deeper than the superficial submucosa, the next question is if there is active inflammation present and is the lesion well circumscribed, i.e. a clear border between normal mucosa and lesion. Active inflammation may make the lesion harder to resect as it does not always lift well when submucosal injection is applied and therefore this may sway the endoscopist regarding technique for removing this. Only if the lesion has a clear border should an attempt at endoscopic removal be performed. Unclear margins would make it unlikely that the lesion will be fully removed. Once the decision to remove the lesion has been made, currently guidelines recommend biopsies adjacent to the resection defect to help confirm complete resection of the polyp has taken place(30).

1.10 Efficacy of surveillance and missed CRC

The efficacy of surveillance has been contested. No high-quality randomized control trial has ever been performed comparing those patients undergoing IBD colitis surveillance to that of a control group which do not. This would clearly be deemed unethical. Thereby, we rely on lower quality observational studies comparing patients who have undergone surveillance to those that haven't. A recent Cochrane meta-analysis review looking at observational studies revealed a reduced incidence of cancer detection (OR 0.58; 95% CI 0.42-0.80) and death from CRC (OR 0.36; 95% CI 0.19-0.69) in favour of those undergoing surveillance colonoscopy(90). This is likely to be explained by earlier intervention in the pre-cancerous stage or detection of early stage cancer, allowing treatment by endoscopic or surgical resection.

Unfortunately, no diagnostic test is 100% accurate, with pathology being missed and this holds true regarding colonoscopy. Currently a lot of interest has been placed on missed cancers following colonoscopy. Those patients that develop a cancer following a colonoscopy were no cancer was found, is coined the term post colonoscopy colorectal cancer (PCCRC). This can be the result of a missed cancer at the time of the procedure or due to a missed or inadequately treated neoplastic lesions. An agreed timescale to correctly identify a PCCRC is when a negative colonoscopy has occurred 6 - 36 month prior to a colonic cancer diagnosis. An interval cancer represents a further subdivision for patients undergoing surveillance procedures, were no CRC has been detected at the

time of surveillance and then develops a CRC before the date of the next recommended procedure(91).

A study looking at a standardized way for calculating PCCRC found that within the NHS the risk of PCCRC was 8.6%, however there was a trend show a declining rate over a 6 - year period(92). A more recent population based cohort study within the NHS also confirmed declining rates of PCCRC, falling from 9.0% in 2005 to 6.5% in 2013(93). Further, within a subgroup of advanced endoscopists, whom have gained additional accreditation and perform colonoscopies to a high standard, this rate can be reduced further to 3.6%. These findings suggest improvements can be made if guidelines are followed but also likely attributed to improved bowel preparation, better technology (HDWL), better training and wider awareness of what constitutes a good colonoscopy resulting from tighter regulations by the governing bodies of endoscopy, the Joint Advisory Committee (JAG).

However, disappointingly these findings may not be transferred to patients with IBD – colitis. Within the same study, the subgroup analysis looking at this group showed a 3 year PCCRC rates of 38.3% in 2005 and 35.5% in 2013(93). This seems disappointing and remains constant over an 8-year period. Another Swedish population-based cohort study displayed similar results with a risk of PCCRC in CD patients of 28.3% and 41% in UC compared with non-IBD showing 6.3% PCCRC rates. This equated to a relative risk increase of 3.82 in CD and 5.89 in UC(94).

Although these findings are extremely disconcerting, the standard of these colonoscopies are unlikely to reflect current surveillance practice. The most recent IBD guidelines were implemented in 2010, and as described earlier, the adoption of guidelines can be extremely slow and poorly adhered to. Also discussed earlier, these higher rates can be explained by ongoing inflammation making lesion detection harder and the differing biology of lesions within IBD-colitis. This was reflected in a retrospective tertiary single-centred study that looked causes of PCCRC, in which only 11.1% had chromoendoscopy performed(95).

This raises questions regarding the need for optimizing neoplasia detection, better lesion characterization and treatment of lesions and also are we able to better risk stratify patients in order to concentrate surveillance on patients with a higher risk for developing dysplasia and neoplasia.

1.11 Aims and objectives of the research

1.11.1 Aims and Objectives

The main aims of this research project were to determine optimal ways of detecting dysplasia within IBD-colitis and the ability to characterise in-vivo lesions. This will involve systematic reviews/meta-analyses of studies so far, followed by a randomised controlled trial looking to explore both dysplasia detection and lesion characterisation.

Hypotheses being tested:

- Are random biopsies during surveillance colonoscopy in patients with IBD colitis still required
- To determine the optimal endoscopic method for the detection of dysplasia in patients with IBD colitis during surveillance and is there a subpopulation in which invisible dysplasia exists
- To determine the accuracy of in-vivo lesion characterisation in real-life practice

The following chapters will attempt to answer the above hypotheses.

Chapter 2: Are Random biopsies still required – Meta-analysis

Not only was I interested in the ability of detecting visible dysplasia but I also wanted to obtain a better understanding for which subpopulations invisible dysplasia was more likely to be present. In order to investigate this, a meta-analysis will be performed to determine the magnitude of invisible dysplasia throughout studies and also the likelihood of invisible dysplasia being present according to a cohorts perceived risk.

Chapter 3: RCT on Dysplasia detection

An RCT will be performed to look at optimal ways of detecting visible dysplasia and secondary outcomes will look at which patients invisible dysplasia occurs and testing this against the current BSG risk stratification.

Chapter 4: Lesion characterisation – Meta-analysis

A meta-analysis will be performed to determine the current accuracy of in-vivo lesion characterisation in patients with colitis, analysed according to the different technologies used.

Chapter 5: RCT lesion Characterisation

Finally, real-time optical lesion characterisation will be assessed in all lesions detected during the RCT. The endoscopist will provide an in-vivo lesion diagnosis, split according to whether the lesion if felt to be dysplastic or non-dysplastic.

Chapter 2

Are Random Biopsies still required during IBD surveillance: A systematic review and meta-analysis

2.1 Background

Numerous studies and meta-analyses have firmly established that colonic inflammatory bowel disease (IBD) predisposes patients to a heightened risk for developing colorectal cancer (CRC)(16, 96, 97). In response, gastroenterological societies have established surveillance guidelines recommending a colonoscopy, usually commencing 8-10 years from disease onset, with an aim to detect precursor dysplastic lesions or early cancer (28-30). However, there still remains a degree of ambiguity even amongst experts over the most effective surveillance techniques for dysplasia detection. This has resulted in inconsistencies and often confusion over surveillance adoption, especially within non-expert centres, and a growing concern over the association of higher rates of PCCRC when compared to patients without colitis(57, 92).

Colorectal cancer within IBD colitis is thought to progress along an inflammation-dysplasia-cancer pathway(98). By detecting dysplasia early, it allows a window of opportunity to potentially prevent progression to cancer. Initial opinions when using older generation fibreoptic endoscopes centred around dysplasia being predominantly invisible or flat, resulting in traditional surveillance practice involving quadrantic random biopsies taken every 10 cm on withdrawal from the caecum(28). On average this equated to 32 biopsies per procedure. However, only 1 in 1000 biopsies (0.1%) detected dysplasia, reflecting an inefficient, time consuming, expensive, not to mention labour intensive practice(30).

With advancements in endoscopic technology, most notably high-definition colonoscopy, opinion regarding dysplasia detection changed. Dysplasia was now thought to be macroscopically visible, resulting in a paradigm shift, with targeted rather than the aforementioned random biopsies. Morphologically these dysplastic lesions tended to be much flatter than non-colitic dysplastic lesions. This led to the adoption of dye-based colonoscopy (chromoendoscopy), with numerous randomised studies and meta-analysis displaying an increased dysplastic yield over white-light endoscopy(69).

In 2015 an international group of leading experts in the field of IBD surveillance, reviewed the literature and developed evidence-based consensus recommendations on how

surveillance colonoscopy should be performed in order to optimise dysplasia detection. With 84% agreement, their recommendation was in favour of high-definition chromoendoscopy with targeted biopsies, although evidence was deemed low quality for selecting high-definition chromoendoscopy over high-definition while-light endoscopy(30). However, uncertainty was highlighted on the continuation of obtaining random biopsies. No consensus could be reached, with agreement amongst experts varying dependent on the type of endoscopic technology used.

Interestingly, a recent multi-centre study involving the GETAID group performed a prospective study looking at the number of patients with dysplasia detected by random biopsy only. They found that 12.8% of patients with dysplasia were detected by random biopsy alone. Further, using multivariable regression analysis they described PSC, a personal history of neoplasia and a “tubular colon” to be independently associated with detection of random biopsy only dysplasia. Other studies have also looked at cohorts exclusively involving PSC patients and found a high proportion of patients with PSC having random biopsy dysplasia only(78, 79). It’s possible the dogma for invisible dysplasia holds true within a subpopulation of patients.

Up until recently the significance of finding invisible dysplasia was unknown. However a recent systematic review and meta-analysis involving cohort studies in patients undergoing IBD surveillance showed that the pooled incidence ratio for developing advanced neoplasia with nonvisible LGD was 6.1 per 100 patient-year follow- up (95% CI, 0.9–11.4), comparing with endoscopically visible dysplasia, which was 1.0 per 100 patient-year follow-up (95% CI, 0–2.1)(99). This suggests that patients with invisible LGD are more likely to progress to advanced neoplasia than patients with visible neoplasia, making it imperative that we are able to identify these patients.

Our aim for this meta-analysis was to determine what proportion of patients with dysplasia during surveillance colonoscopy are identified by random biopsy alone and to determine if the proportion detected is influenced by the type of endoscopic technology used or the cohort’s perceived risk for acquiring dysplasia.

2.2 Methods

2.2.1 Search strategy and study selection

We conducted a meta-analysis in concordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines(100). A literature search was performed (by RL and AK) utilising the Healthcare Databases Advanced Search

(HDAS) system, specifically using MEDLINE (from 1947 to July 2018) and EMBASE (from 1947 to July 2018). Search terms included: ((((IBD).ti,ab OR (coliti*).ti,ab OR ("inflammatory bowel disease").ti,ab) AND ((dysplasi*).ti,ab OR (neoplasi*).ti,ab OR (CRC).ti,ab OR ("colorectal cancer").ti,ab)) AND ((surveillance*).ti,ab OR (detection).ti,ab)) AND (("random biopsies").ti,ab OR ("chromoendoscopy").ti,ab OR ("narrow band imaging").ti,ab OR ("FICE").ti,ab OR (“i-scan”).ti,ab OR ("high definition").ti,ab OR ("standard definition").ti,ab). Study references of relevant articles were also reviewed as a safety check for any potentially studies missed using the above search criteria.

Study selection was conducted by two independent investigators (RL and NEB). Study inclusion required adult patients (≥18 years old) undergoing surveillance colonoscopy for IBD colitis, were random biopsies had been taken and adequate information could be obtained in order to answer the outcomes. Randomised controlled trials (RCTs), prospective or retrospective cohort studies were included. There were no language restrictions. Abstracts were included if sufficient data could be obtained and satisfaction the study was of sufficient quality. Any discrepancies over study inclusion was clarified by a third investigator (VS). Case studies and series were excluded from the analysis.

2.2.2 Outcomes

The primary outcome was the pooled proportion of patients with dysplasia who were identified by random biopsy only (number of patients with dysplasia only on random biopsy divided by the total number of patients with all type dysplasia (targeted +/- random biopsy)) and the pooled proportion of random biopsies positive for dysplasia (number of random biopsies positive for dysplasia divided by the total number of random biopsies taken).

A subgroup analysis was performed using the primary outcomes stated above, with studies being pooled according to the different endoscopic technologies used and according to the perceived studies risk for developing invisible dysplasia.

2.2.3 Data extraction

Data was extracted independently by two investigators, (RL and AK), into a Microsoft Excel spreadsheet (Microsoft Excel version 16.9; Microsoft Corp, Redmond, WA, USA). Any disagreements between investigators was clarified by a third investigator (VS). The following data was extracted: study authors, country of study origin, year of study, number of centres, type of study, technology used, proportion of cohort with high risk features (including percentage with PSC, previous dysplasia and extent of colitis), number of patients, number of patients with dysplasia, number of patients detected by

random dysplasia only, number of random biopsies, number of random biopsies positive for dysplasia.

Dysplasia was defined as low grade, high grade or CRC. Indefinite for dysplasia was not defined as dysplasia for this meta-analysis, and therefore not included when determining the proportions. For missing data corresponding authors were contacted via email.

Endoscopic technologies was stratified into standard definition white-light (SDWL), standard definition chromoendoscopy (SDCE), high definition white-light (HDWL), high definition chromoendoscopy (HDCE), virtual chromoendoscopy (VCE), autofluorescence (AF) and full-spectrum endoscopy (FUSE) technologies.

Stratifying according to the perceived risk of each study population was based on emerging risk factors for invisible dysplasia. High-risk studies were defined if the cohorts had at least one of the following risks: only included extensive colitis (beyond splenic flexure), ≥ 9% of the cohort had PSC or the cohort had ≥ 20% of previous colonic dysplasia. If the study had none of these it was deemed a low risk cohort. We used figures of 9% as a cut-off for PSC and 20% cut-off for previous dysplasia as this was around the median value from the included cohorts.

Individual studies using a parallel study design were split into two or more separate technology groupings if random biopsies were taken in the different arms, or an individual arm of a study was excluded if no random biopsies were taken. For risk stratification analysis using studies with a parallel design, if the risk between the two or more arms was provided and was contrasting, these were split. Otherwise study cohorts of the two or more arms were combined if risks were similar. When extracting data for cross-over or back-to-back studies, arms were split according to the technology and only the proportions differed, but when this study design was used to define risk, the study was grouped as a single study because risk was the same.

2.2.4 Statistical analysis

Data analysis was performed using STATA version 13 (Stata Corp, Texas, USA)(101), using the command Metaprop(102). This command is used specifically for proportionate meta-analyses, building on the other command Metan, used for pooled binomial data. Metaprop allows pooling when proportions are at their margins when the normal approximation procedure usually falters, thereby preventing exclusion of studies with proportions equal to 0 or 1. With Metaprop, a random-effects model was used for the pooled proportions, allowing for study heterogeneity (i.e. study design, study population etc.). Overall pooled proportions were displayed using forest plots with their associated 95% confidence intervals (95% CI).

Heterogeneity across studies was assessed using the I^2 statistic(103). The I^2 statistic is expressed as a percentage between 0% and 100%. Values of 25% to < 50%, 50% to < 75% and >75% are said to represent low, moderate and high risk for variation across studies that is due to heterogeneity rather than chance.

2.2.5 Publication bias

Publication bias was assessed using funnel plots with the intervention effect estimate (log of proportion) against the standard error. Asymmetry was determined using Egger's test(104).

2.2.6 Quality assessment and risk of bias

The risk of study bias was assessed by two independent investigators (RL and VS) using the QUADAS 2 tool (Quality Assessment of Diagnostic Accuracy Studies)(105). This looks at the risk of bias and applicability regarding four domains: patient selection, index test, reference standard, flow and timing. Risk of bias (involved all four domains) and applicability (involved three domains) is scored using low risk, high risk or unclear. Any indifference on risk scoring between the two investigators was resolved by discussion.

2.3 Results

The literature search identified 469 citations, with 3 additional studies being identified from searching article references. Of these,150 studies underwent full manuscript review, with 36 studies deemed suitable and making inclusion into this meta-analysis (Figure 8). Description of these studies can be seen in Table 1 below.

Author	Country	Publication Type	No. of centres	Study design	Technology	Endoscopic equipment	No. of patients	Cohort inclusion (all surveillance colonoscopy)	Percentage of cohort with PSC	Percentage of cohort with previous dysplasia
Jaramillo et al. (1996)[59]	Sweden	Article	Single centre	Prospective, single arm study	SDWL	Fujinon EC7-HL2, EC7-HM2 Olympus CF-200HL, CF-200Z	85	Extensive UC only	/	/
Kiesslich et al. (2003)(64)	Germany	Article	Single centre	RCT, parallel design	SDWL vs SDCE	Pentax EC-3839FK, EC-3831FZ Olympus CF-Q160ZI	165	General UC	11.5% (n=11)	/
Rutter et al. (2004)[106]	UK	Article	Single centre	Prospective, tandem design	SDWL vs SDCE	Olympus CF 240	100	Extensive UC	/	/
Rutter et al. (2004)[106]	UK	Article	Single centre	Retrospective	SDWL	/	525	Extensive UC	/	/
Dekker et al. (2007)[107]	Netherlands	Article	Single centre	RCT, cross-over design	SDWL vs First generation NBI	Olympus CF-140, CF-160, CF-Q240	42	Extensive UC	29% (n=12)	/
Kiesslich et al. (2007)[67]	Germany	Article	Single centre	RCT, parallel design	SDWL vs SDCE/CLE	Pentax EC-3830FK, eCLE	153	General UC	9.8% (n=15)	/
Blonski et al. (2008)[108]	USA	Article	Single centre	Retrospective	SDWL	/	49	General All patients included had dysplasia	/	/
Marion et al. (2008)[68]	USA	Article	Single centre	Prospective, tandem design	SDWL vs SDCE	Olympus PCF	102	General IBD	/	38.2% (n=39)
Van den Broek et al. (2008)[107]	Netherlands	Article	Single centre	RCT, tandem design	SDWL vs AFI	Olympus XCF-H240FZL	50	Extensive UC	14% (n=7)	14% (n=7)

Viennot et al. (2011){Viennot, 2011}	French	Abstract	Multi - centre	Prospective, tandem design	SDWL vs SDCE vs NBI	/	51	General IBD	11.8% (n=6)	/
Hlavaty et al. (2011)[109]	Slovakia	Article	Single centre	Prospective, tandem design	SDWL vs SDCE	Pentax EC-3870CIFK	45	General IBD	2.2% (n=1)	/
Gunther et al. (2011)[110]	Germany	Article	Single centre	Retrospective	SDWL vs SDCE vs CLE	Pentax EC-3870CIFK	50	General IBD	/	/
Chiorean et al. (2012){Chiorean, 2012}	USA	Abstract	Single centre	Prospective, tandem design	SDWL vs SDCE	/	63	General IBD	25% (n=16)	44% (n=28)
Subramanian et al. (2013)[93]	UK	Article	Single centre	Retrospective, two arm study	SDWL vs HDWL	Olympus CF-230L, CF-240L, CF-240AL, CF 240DL, CF-260DL, CF-Q260DL	353	General IBD	/	/
Van den Broek et al. (2014)[111]	Netherlands	Article	Single centre	Retrospective, single arm study	SDWL	CF-Q140, CF-Q160, CF-Q180	167	General UC	14% (n=31)	9.6% (n=16)
Mooiweer et al. (2015)[112]	Netherlands	Article	Multi - centre	Retrospective, two arm study	SDWL vs SDCE	/	772	General IBD	9% (n=68)	/
Navaneethan et al. (2013)[78]	USA	Article	Single centre	Retrospective, single arm	SDWL	/	71	UC patients with PSC	100% (n=71)	/
Freire et al. (2014)[113]	Portugal	Article	Two centres	Randomised, parallel design	SDWL vs SDCE	Pentax EC-3870CIFK	73	General IBD (excluded patients with PSC or previous dysplasia)	0	0
Marion et al. (2016)[114]	USA	Article	Single centre	Prospective, tandem design	SDWL vs SDCE	Olympus PCF-160, PCF-180	68	General IBD	/	/
Gasia et al. (2016)[115]	Canada	Article	Single centre	Retrospective, seven arms	SDWL vs HDWL vs VCE(iSCAN) vs HDCE	Pentax EC-3940Fi	454	General IBD	7.9% (n=36)	9.3% (n=42)

Matsumoto et al. (2003)[65]	Japan	Article	Single centre	Prospective, single arm	SDCE	Olympus CF-200, CF-230, CF-240, CF-240Z	57	Extensive UC	5.3% (n=3)	/
Van den Broek et al. (2011)[116]	Netherlands	Article	Single centre	RCT, cross-over design	NBI vs HDWL	Olympus CF-H260	48	Extensive UC	16.7% (n=8)	37.5% (n=18)
Ignjatovic et al. (2012){Ignjatovic, 2012}	UK	Article	Multi - centre	RCT, parallel design	NBI vs HDWL	Olympus XCF-H240FZL, CF-H260AZL	112	General UC	3.6% (n=4)	8% (n=9)
Picco et al. (2013)[117]	USA	Article	Multi - centre	Prospective, tandem design	HDWL vs HDCE	/	75	Extensive UC	/	/
Leifeld et al. (2015)[118]	Germany	Article	Multi - centre	Randomised, tandem design	HDWL vs NBI	Olympus CF-H260	159	General UC	6.9% (n=11)	/
Cassinotti et al. (2015)[119]	Italy	Abstract	Single centre	RCT, parallel design	FICE vs HDWL	/	91	General UC	/	/
Mohammed et al. (2015)[73]	UK	Abstract	Single centre	RCT, parallel design	HDWL vs HDCE	Olympus CF-H290	100			
Dlugosz et al. (2016)[79]	Sweden	Article	Single centre	Prospective, tandem design	HDWL vs HDCE	Olympus CF-H180	69	IBD with PSC	100% (n=69)	10% (n=7)
Bopanna et al. (2016)[120]	India	Article	Single centre	Prospective, single arm study	HDWL	Olympus CF-H180	28	UC with mainly extensive disease	7.1% (n=2)	/
Watanabe et al. (2016)[121]	Japan	Article	Multi - centre	RCT, parallel design	HDWL random vs targeted	Majority HD colonoscopes	107	General UC	0%	/
Efthymiou et al. (2013)[122]	Australia	Article	Single centre	Prospective, tandem design	HDCE vs NBI	Olympus CF-H180/PCF	44	General IBD	5% (n=2)	18.2% (n=8)
Leong et al. (2017)[123]	Australia	Article	Single centre	Randomised, back-to-back cross-over study	HDWL vs FUSE	FUSE Endochoice Olympus CF H180,190/PCF	52	General IBD	5.8% (n=3)	13.5 (n=7)

Vleugels et al. (2018)[124]	International	Article	Multi - centre	Randomised, parallel design	HDCE vs AFI	Olympus CF-H240AZL/I	210	General UC	18% (n=38)	16.2% (n=34)
Carballal et al. (2016)[125]	Spain	Article	Multi - centre	Prospective, tandem design	WL vs CE (SD +HD)	Olympus CF-H180, CF-H190, CF-Q160L, CF-Q165L Fujinon EC-390LI, Pentax EC-590WL, EC-590ZW, EC-380LKP	350	General IBD	6.6% (n=23)	/
Moussata et al. (2017)[77]	French	Article	Multi - centre	Prospective	CE targeted vs random biopsies	All definition endoscopes/different companies	1000	General IBD	9% (n=85)	10% (n=103)
Lord et al. (2018)[126]	UK	Abstract Interim analysis	Single centre	RCT, parallel design	HDCE 0.2% vs 0.03% IC	Olympus CF-H290	150	Extensive IBD	18% (n=27)	12% (n=18)

Table 1 Description of all studies included within the meta-analysis. Randomised control trial (RCT), standard definition white-light (SDWL), standard definition chromoendoscopy (SDCE), high definition white-light (HDWL), high definition chromoendoscopy (HDCE), narrow-band imaging (NBI), virtual chromoendoscopy (VCE), autoflourescence (AFI), full-spectrum endoscopy(FUSE).

There were 14 RCTs (38.9%), 9 of which were parallel-study design (of which 3 were abstracts) and 5 being tandem/cross-over studies. A further 14 studies (38.9%) were prospective cohort studies (of which 2 were abstracts), whilst 8 (22.2%) were retrospective studies.

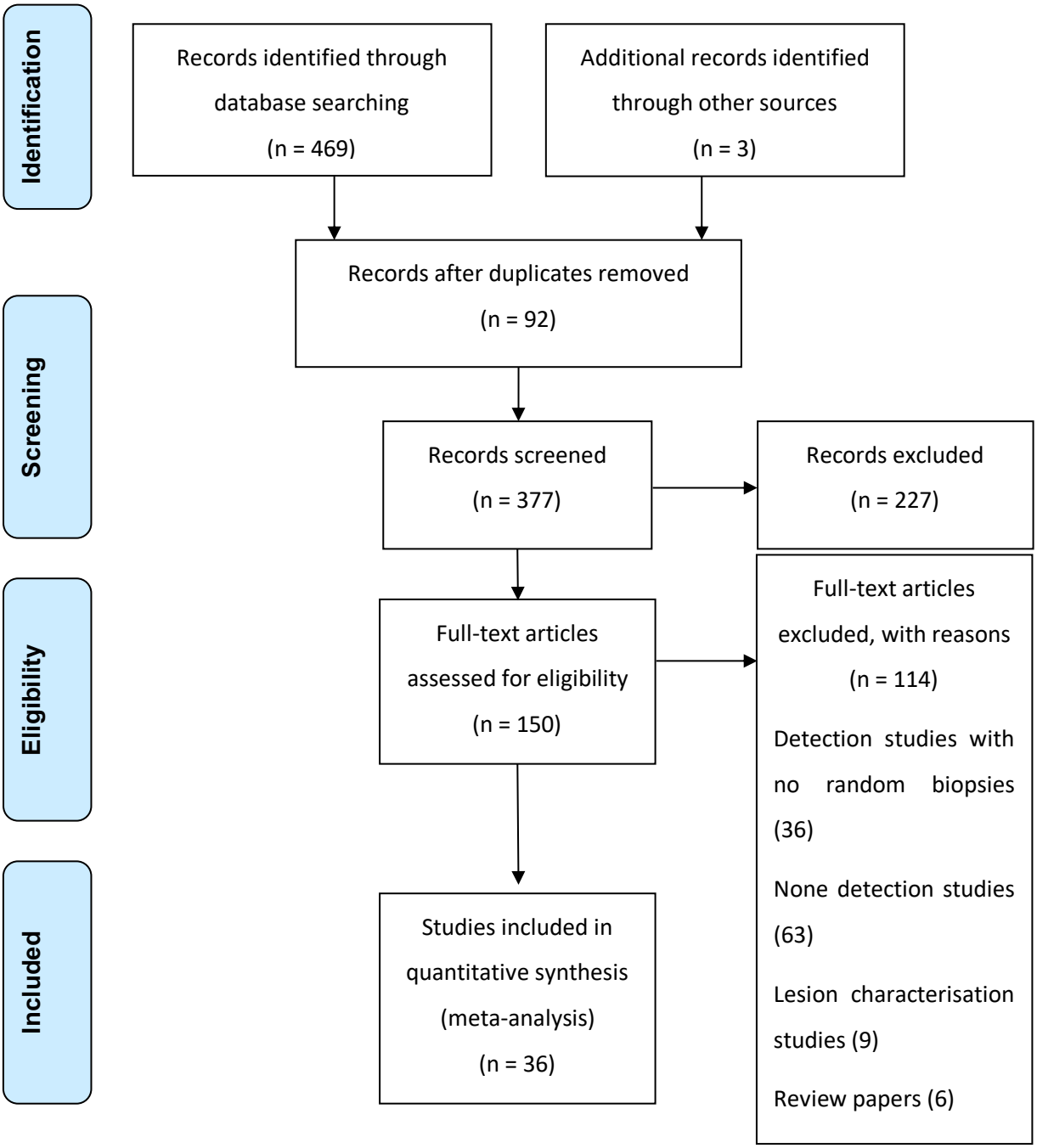


Figure 8. PRISMA flow diagram

2.3.1 Overall pooled proportions

Proportion of patients with dysplasia who had dysplasia identified by random biopsy only

We identified 30 studies that reported on the proportion of patients with dysplasia who had dysplasia identified by random biopsy alone. Six studies did not provide data. On pooling proportions, 13.05% (95% CI 7.28 – 19.87%) of patients with dysplasia had this identified by random biopsies alone (Figure 9). There was a high degree of heterogeneity with studies included, I^2 = 81.19% (p < 0.001).

Proportion of random biopsies positive for dysplasia

There were 33 studies which provided data on the overall proportion of random biopsies that were positive for dysplasia. In 3 studies no data was provided. The pooled proportion for random biopsies positive for dysplasia was 0.13% (95% CI 0.08 – 0.18%), with heterogeneity high at I^2 = 83.28% (p < 0.001).

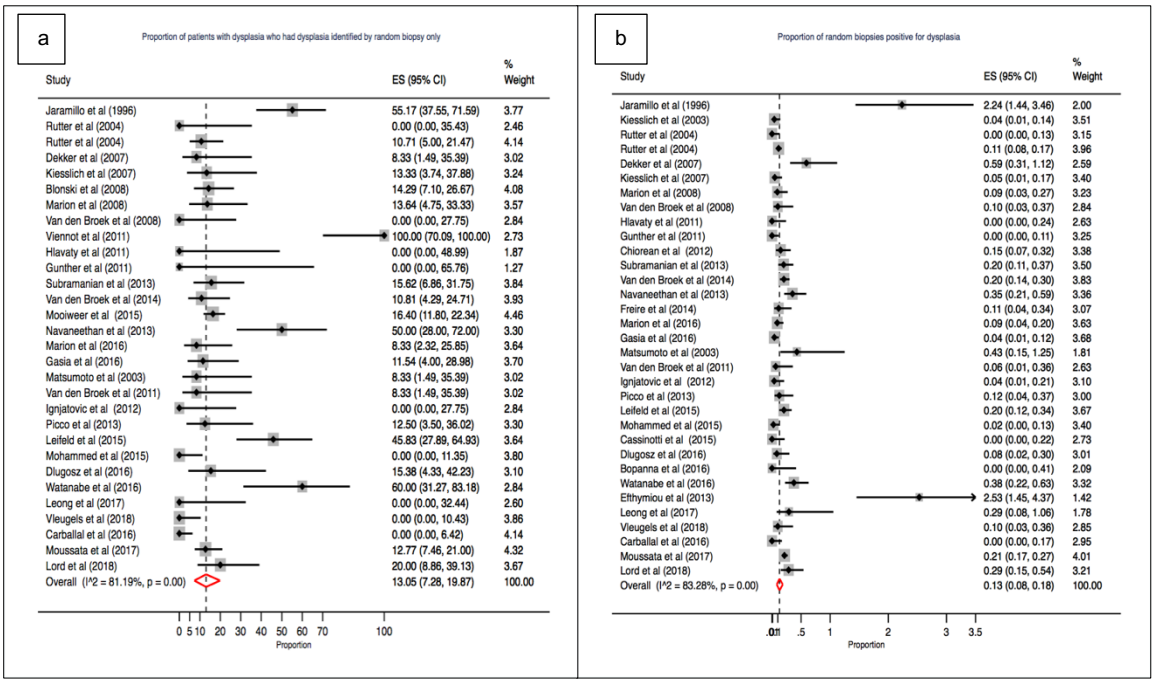


Figure 9 Forest plot including all studies showing the pooled proportion of patients with dysplasia identified by random biopsy only b. Forest plot including all studies showing the pooled proportion of random biopsies positive for dysplasia.

2.3.2 Pooled proportions categorised according to endoscopic technology

Technology was categorised into SDWL, SDCE, HDWL, HDCE, VCE (NBI, FICE), AFI and FUSE in order to determine whether the type of endoscopic technology for dysplasia detection, accounted for any differences with the proportion of dysplasia detected by random biopsies alone. In order to focus any differences for random dysplasia being attributed to the technology used solely, rather than other variables such as disparity

between the number of biopsies per technology, we calculated the mean number of random biopsies taken per patient after pooling studies according to the technology used (See Table 2).

Colonoscopy technology	Number of studies	Mean number of random biopsies/technology with associated standard deviation
SDWL	18	29.7 ± 9.5
SDCE	9	28.8 ± 12.1
HDWL	11	29.4 ± 9.4
HDCE	7	27.2 ± 11.9
VCE	7	21.8 ± 10.2
AFI	2	24.8 ± 21.5
FUSE	1	13

Table 2 Mean number of random biopsies taken per patient after pooling data according to the endoscopic technology used. Standard definition white-light (SDWL), standard definition chromoendoscopy (SDCE), high definition white-light (HDWL), high definition chromoendoscopy (HDCE), virtual chromoendosocpy (VCE), autoflourenscence (AFI), and full-spectrum endoscopy (FUSE).

Proportion of patients with dysplasia who had dysplasia identified by random biopsy only, grouped according to the endoscopic technology used

For the SDWL group we were able to extract data from 16 studies. The pooled proportion for patients with random biopsy only dysplasia using SDWL endoscopy was 20.39% (95% CI 10.70 – 31.79%) (See Figure 10). This represents almost double the overall proportion when compared with the overall pooled proportions, yielding the highest proportion of people with random biopsy only dysplasia. However, the 95% confidence intervals are wide and have extremely marked heterogeneity between the studies, I^2 = 80.81% ($p < 0.001$).

Data was obtained from 9 studies for SDCE technology. Using SDCE, the pooled proportion for patients with random biopsy alone dysplasia was 9.20% (95% CI 0.00 – 31.93%). Despite representing half the proportion when compared with SDWL, the confidence intervals are extremely wide and clearly overlap. Again, high levels of heterogeneity were identified, I^2 = 83.41% ($p < 0.001$).

HDWL endoscopy included 9 studies with a pooled proportion of 13.68% (95% CI 5.18 – 25.10%) for patients with random biopsy alone dysplasia. Heterogeneity was classed as moderate, I^2 56.67% (p = 0.02).

HDCE included 7 studies with extractable data. This technology had one of the smallest proportion of patients with random biopsy only dysplasia at 4.94% (95% CI 0.00 – 16.10%) and represented the lowest heterogeneity figure classed as moderate, I^2 55.88% (p = 0.03).

VCE technology had 6 studies and included NBI and i-scan studies. The pooled proportion for patients with random biopsy alone dysplasia was 16.42% (95% CI 0.00 – 53.14%). The confidence intervals are representative of the studies included which had smaller cohorts. Large heterogeneity was seen, I^2 88.42% (p < 0.001).

AFI included 2 studies. The pooled proportion for patients with random biopsy alone dysplasia was 0.00% (95% CI 0.00 – 8.24%). FUSE only had 1 study. The proportion for patients with random biopsy alone dysplasia was 0.00% (95% CI 0.00-32.44%). Heterogeneity was not calculated due to the limited studies included. The mean number of random biopsies was around half that of other studies, possibly accounting for the low detection rate.

Proportion of random biopsies positive for dysplasia

Interestingly, the highest yield for random biopsy positive for dysplasia was seen in the VCE technology, 0.26% (95% CI 0.03 – 0.68%), followed by HDCE with 0.20% (95% CI 0.00 – 0.58) (Fig.3).

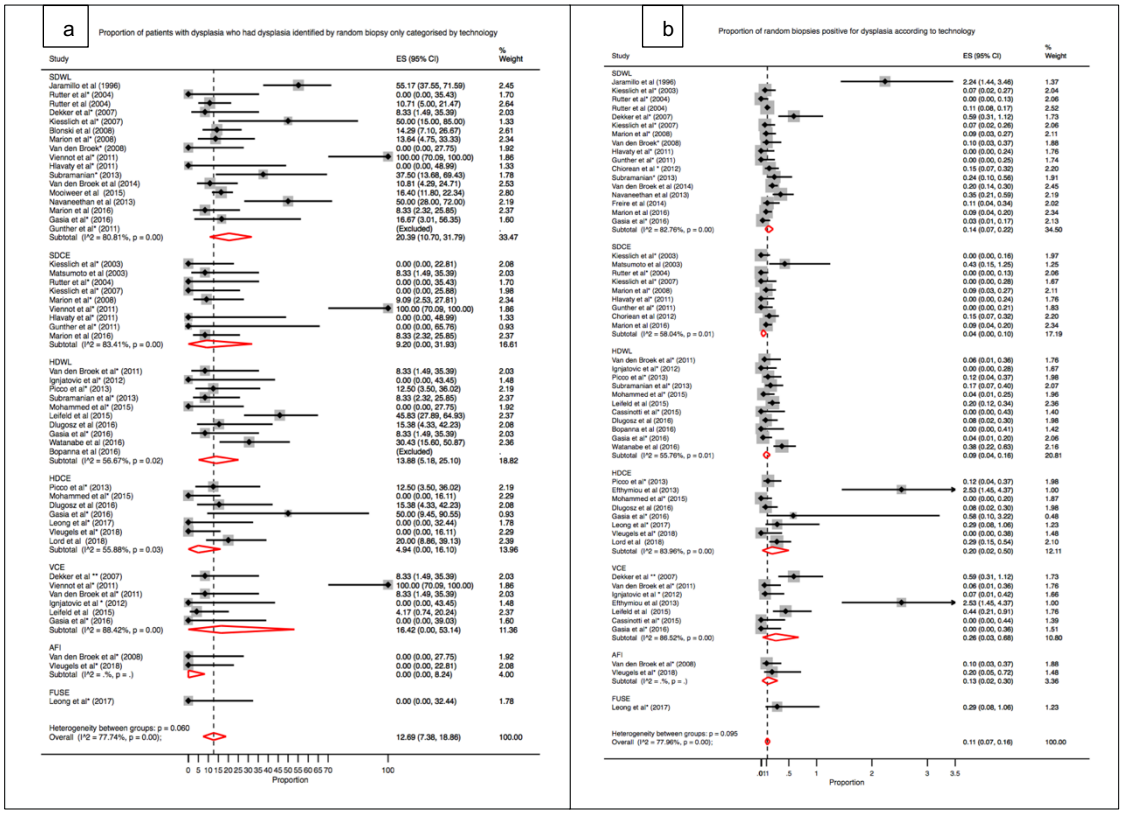


Figure 10. a. Forest plot showing the pooled proportion of patients with dysplasia who had dysplasia identified by random biopsy only according to endoscopic technology b. Forest plot showing the pooled proportion of random biopsies positive for dysplasia according to endoscopic technology. Standard definition white-light (SDWL), standard definition chromoendoscopy (SDCE), high definition white-light

2.3.3 Pooled proportions according to risk

Studies were grouped into low-risk or high-risk cohorts depending on the proportion of several predetermined cohort risk factors. Studies including patients with extensive colitis only, ≥ 9% of the study population having PSC and/or ≥ 20% of the cohort having previous dysplasia (any type), we considered these cohorts to be high-risk for dysplasia.

Proportion of patients with dysplasia who had dysplasia identified by random biopsy only grouped according to risk

The high-risk cohort included 22 studies, whilst the low-risk cohort included 11 studies (Figure 11). The pooled proportion of patients with dysplasia identified by random biopsy alone within the high-risk group was more than double, 14.19% (95% CI 7.43 – 22.29%),

when compared with that of the low-risk group, 6.42 (95% CI 0.04 – 18.45%). However, there is clear overlap of confidence intervals and heterogeneity was high in both groups.

Proportion of random biopsies positive for dysplasia according to risk

Similar yield was obtained between the high-risk and low-risk cohorts. High-risk group contained 21 studies with a pooled proportion of 0.13% (95% CI 0.08 – 0.20%), whilst the low risk cohort consisted of 15 studies with a pooled proportion of 0.10 (95% CI 0.03 – 0.21%).

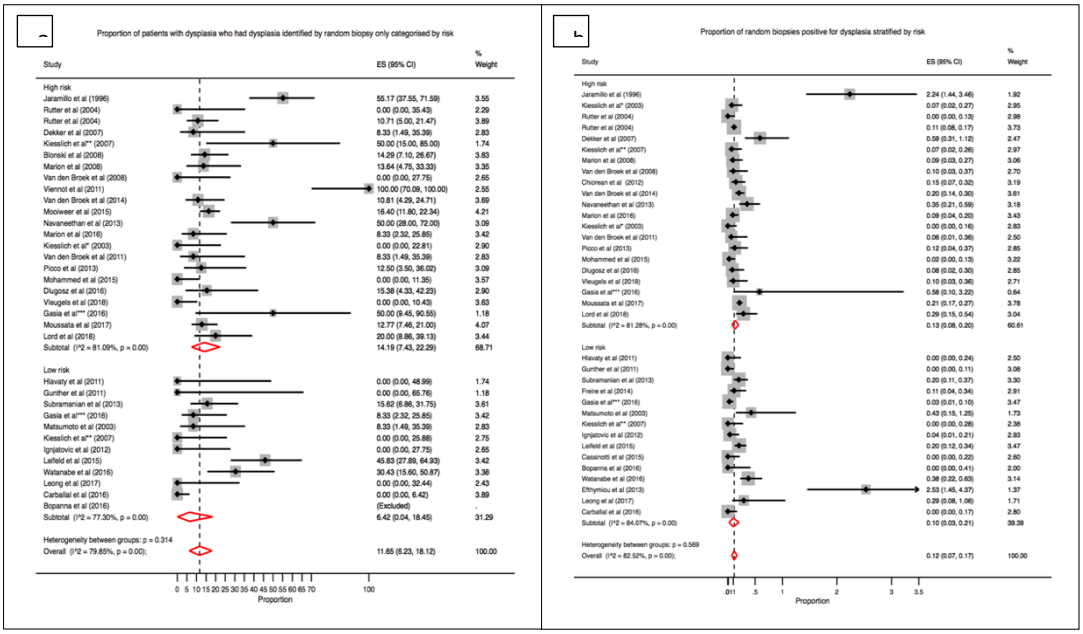


Figure 11 Forest plot showing the pooled proportion of patients with dysplasia who had dysplasia identified by random biopsy only according to the cohort's risk b. Forest plot showing the pooled proportion of random biopsies positive for dysplasia according to the cohort's risk. High – risk defined as study cohort with either ≥ 9% with primary sclerosing cholangitis, ≥ 20% with previous dysplasia or only included extensive colitis. If a cohort had none of these risks, they were defined as low risk

2.3.4 Risk of study bias

Please see the QUADAS 2 tool table below.

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Study 1 (Jaramillo 1996)(59)	?	☺	?	☺	?	☺	?
Study 2 (Kiesslich 2003)(64)	☺	☺	☺	☺	☺	☺	☺
Study 3 (Rutter 2004)(106)	☺	☺	☺	☺	☺	☺	☺
Study 4 (Rutter 2004)(106)	☹	☺	☺	☺	☹	☺	☺
Study 5 (Dekker 2007){	☺	☺	☺	☺	☺	☺	☺
Study 6 (Kiesslich 2007)(67)	☺	☺	☺	☺	☺	☺	☺
Study 7 (Blonski 2008)(108)	☹	☺	?	?	☹	☺	?
Study 8 (Marion 2008)(68)	☺	☺	☺	☺	☺	☺	☺
Study 9 (van den broek 2008)(107)	☺	☺	☺	☺	☺	☺	☺
Study 10 (viennot 2011){Viennot, 2011 #37}	?	☺	?	☺	?	☺	?
Study 11 (Hlavaty 2011)(109)	☺	☺	☺	☺	☺	☺	☺
Study 12 (Gunther 2011)(110)	☹	☺	☺	☺	☹	☺	☺
Study 13 (Chiorean 2012)	☹	☺	?	☺	☹	☺	?
Study 14 (Subramanian 2013)(72)	☹	☺	☺	☺	☹	☺	☺

Study 15 (Van den broek 2014)[111]	☹	☺	☺	☺	☹	☺	☺
Study 16 (Mooiweer 2015)[112]	☹	☺	?	☺	☹	☺	?
Study 17 (Navaneethan 2013)[78]	☹	☺	☺	☺	☹	☺	☺
Study 18 (Freire 2014)[113]	☺	☺	☺	☺	☺	☺	☺
Study 19 (Marion 2016)[114]	☺	☺	☺	☺	☺	☺	☺
Study 20 (Gasia 2016)[115]	☹	☺	?	☺	☹	☺	?
Study 21 (Matsumoto 2003)[65]	☹	☺	☺	☺	☹	☺	☺
Study 22 (Van den broek 2011)[116]	☺	☺	☺	☺	☺	☺	☺
Study 23 (Ignjatovic 2012)[23]	☺	☺	☺	☺	☺	☺	☺
Study 24 (Picco 2013)[117]	☹	☺	☺	☺	☹	☺	☺
Study 25 (Leifeld 2015)[118]	☺	☺	☺	☺	☺	☺	☺
Study 26 (Cassinotti 2015)[119]	☺	☹	?	☺	☺	☹	?
Study 27 (Mohammed 2015)[73]	☺	☺	☺	☺	☺	☺	☺
Study 28 (Dlugosz 2016)[79]	☺	☹	☺	☺	☺	☹	☺
Study 29 (Bopanna 2016)[120]	☹	?	☺	☺	☹	?	☺
Study 30 (Watanabe 2016)[121]	☺	☺	☺	☺	☺	☺	☺
Study 31 (Efthymiou 2013)[122]	☺	☺	☺	☺	☺	☺	☺
Study 32	☺	☺	☺	☺	☺	☺	☺

(Leong 2017)[123] Study 33	☺	☺	☺	☺	☺	☺	☺
(Vleugels 2018)[124] Study 34	☺	☺	☺	☺	☺	☺	☺
(Carballal 2016)[125] Study 35	☺	☺	☺	☺	☺	☺	☺
(Moussata 2017)[77] Study 36	☺	☺	☺	☺	☺	☺	☺
(Lord 2018)[126]							

Table 3 QUADAS 2 of studies included. ☺Low Risk ☹High Risk ? Unclear Risk

2.3.5 Publication Bias

Figure 12 shows the funnel plots for the overall proportion and when stratified according to risk. Nearly all of the studies stray outside the 95% confidence intervals and there is clearly some asymmetry present. The likeliest explanation for this is the large heterogeneity between the studies such as the varying risk of the study populations included within individual studies and the study design.

The Egger test for overall proportion had a coefficient of -7.76 (95% CI -23.32 – 7.79) with a P = 0.32. Egger test for high risk proportion had a coefficient of -8.39 (95% CI -24.03 – 7.23) with a P = 7.28. The Egger test for low risk had a coefficient of 2.20 (95% CI -35.71 – 40.11) with a P = 0.90. Although Egger test has shown non-significant p values, this is likely to represent poor detection of bias of the test when between study heterogeneity is large.

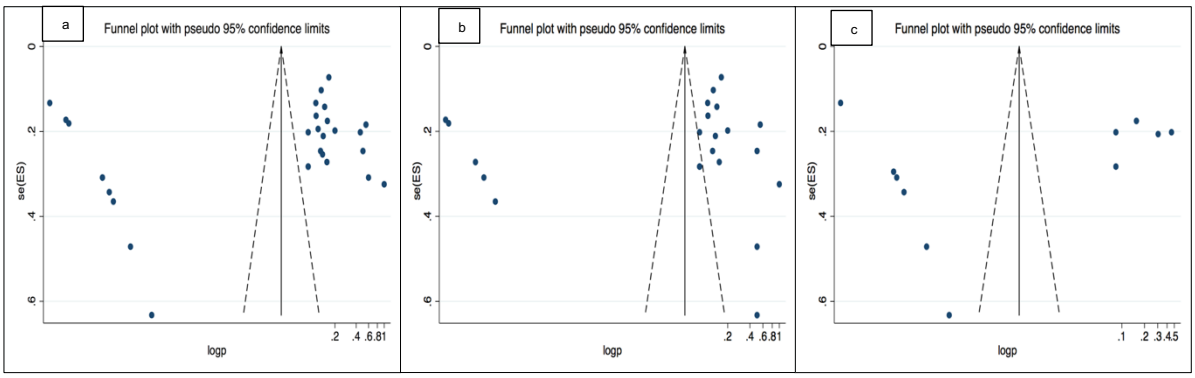


Figure 12 a is the funnel plot for overall proportion. b is high risk papers. C low risk papers

2.4 Discussion

Continued debate regarding the current requirement of random biopsies during surveillance colonoscopy ensues. Despite most dysplasia being visible, invisible dysplasia does exists and can be substantiated by the pathophysiological evidence that dysplasia within IBD seems to develop from the crypt bases in a “bottom-to-top” growth pattern(127). Recent published literature suggests there may still be a role for obtaining random biopsies within a certain subpopulation. The prospective paper by the GETAID group pronounced that an additional 15% of patients with dysplasia are detected by random biopsy alone and would thereby miss a significant proportion of patients had random biopsies not been performed (77). Further, a contemporary meta-analysis suggests that patients with invisible LGD are more likely to develop advanced neoplasia than their visible counterparts (99).

On pooling data, our meta-analysis confirms that just over one in ten patients with dysplasia are identified only if random biopsies are performed. Without obtaining random biopsies a substantial proportion could have their risk for developing advanced dysplasia or CRC incorrectly down staged. A counter argument would be the low yield when taking multiple random biopsies in every patient. We showed that only 0.13% of random biopsies being taken are positive for dysplasia. In addition to targeted biopsies, this would amount to a very labour-intensive and expensive way of detecting additional dysplasia. We therefore performed a subgroup analysis to determine if the type of surveillance, or the risk of the cohort undergoing surveillance could impact the yield of patients with random biopsy only dysplasia, allowing a smaller, more defined population for which to perform random biopsies.

When pooling the studies according to the type of technology being used, HDCE yielded a low proportion of random only dysplasia accounting for only 5% or 1 in 20 patients with dysplasia. Converse to this, SDWL colonoscopy found 20% or 1 in 5 patients with dysplasia having this detected solely by random biopsies only, accounting for a significant proportion of patients potentially having dysplasia missed if random biopsies are not performed. A credible argument for this difference could be that fewer biopsies were taken for the HDCE studies thereby artificially lowering the yield. However, the mean number of biopsies were not significantly different between the two modalities, HDCE with 27.2 $\pm$11.9 versus SDWL with 29.7 $\pm$9.5, ($p = .609$, independent t-test). Interestingly the yield for dysplasia when taking random biopsies was 0.20% for HDCE compared with 0.14% when using SDWL. Despite the difference being small (and with

overlapping confidence intervals), this would seem contrary to the findings above. One theory to explain this could be that visible dysplasia is detected alongside invisible dysplasia, increasing the number of random biopsies positive for dysplasia yet not increasing the proportion of patients with random only dysplasia. Another reason for this paradox could be that when invisible dysplasia is detected it could in fact be within multi-focal areas within the same patient, again only affecting the proportion of random biopsies. These findings would look to support current evidence and guidance that HDCE is the optimal method for detecting dysplasia using targeted biopsies and provides a degree of reassurance when random biopsies have not taken that few patients will be incorrectly down staged to low-risk.

Despite both AFI and FUSE showing promising results with no patients having random biopsy only dysplasia, we are unable to interpret these findings accurately due to the limited number of studies for each technology and the suspected inherent bias when few expert endoscopists perform the procedures. Furthermore, the number of random biopsies within the FUSE study was half the number when compared with the average taken with the other technology groups, thereby less random biopsies taken is likely to equate less chance of detecting invisible dysplasia.

When studies were classified as high-risk cohorts according to our predetermined criteria, the chance of having random biopsy only dysplasia more than doubled. Proportions within the high-risk cohort (14.19%) were found similar to that of the GETAID group, with the risk declining to 6.42% when stratified to the low-risk group. From this we postulate that within the high-risk cohort studies, more patients have higher risk factors for invisible dysplasia and therefore patients whom have random biopsy dysplasia alone are statistically more likely to be patients with these high-risk features.

The are several drawbacks to this study. Firstly, we divided studies into high or low risk according to having at least one of the two previously identified risk factors for random biopsy only dysplasia (PSC or previous dysplasia) or having extensive colitis, which is a well-established risk factor for dysplasia. These were selected because of the association with invisible dysplasia and because such data was extractable retrospectively. However, we were limited to which variables to test, as we were dependent on the specific data provided by the studies. When defining high and low risk groups we used the median value of all studies within this meta-analysis for the proportion of patients with PSC and previous dysplasia. Clearly determining what is high or low risk according to this is arbitrary but provided the most standardised way to define the two categories.

Secondly, few studies provided exact figures on proportions relating to risk factors for patients with random biopsy alone dysplasia, compared to those with visible dysplasia

or even those with no dysplasia. This was because most studies looked at random biopsy dysplasia as a secondary outcome. To circumnavigate this issue, we used the cohort's overall risk as a surrogate marker. We therefore cannot draw strong conclusions that these risk factors are associated with a higher chance of having invisible dysplasia. However, it is highly plausible that those patients with random only dysplasia, within these high-risk cohorts, are individually more likely to have these risk factors.

Thirdly, all the pooled proportions have overlapping confidence intervals reducing the validity of our findings. This is likely to be explained by the small sample sizes of some studies and also the proportion of patients with dysplasia found within detection studies. Further, high levels of heterogeneity existed within the pooled proportions as seen by the I^2 test (>50%). This likely reflects the differing study design and quality, the contrasting populations included and the varying dysplasia risk between studies. Thereby it is difficult to draw strong conclusions from the results, but it does provide further weight towards the evidence that a significant proportion of patients with dysplasia have random biopsy alone dysplasia with proportions being influenced by the technology and a cohort's perceived risk.

In summary this is the first meta-analysis to look at potential factors influencing the chance of detecting invisible dysplasia and searching for risk factors that may help identify patients at risk. Bearing in mind this study's limitations, it would seem that when performing HDCE, invisible dysplasia in isolation is less likely to occur however, random biopsies may still be required in high risk populations regardless of the endoscopic technologies.

To gain a greater understanding of factors predicting invisible dysplasia, we propose that future prospective detection studies require multiple segmental random biopsies along with standard targeted biopsies, and to provide data regarding known risk factors between those with and without invisible dysplasia. We can then start to define a subpopulation for which random biopsies may still be required.

Chapter 3
Chromoendoscopy with 0.03% indigo carmine delivered via a foot pump compared with 0.2% indigo carmine delivered via spray catheter for detecting dysplasia in patients undergoing surveillance in inflammatory bowel disease. A randomised controlled trial.

3.1 Background

As previously described, patients with IBD-colitis have an increased risk of developing CRC (16, 96, 128). CRC associated with IBD colitis is understood to evolve along the inflammation-dysplasia-cancer pathway (98). Several established risk factors enhance an individual's risk of progression along this pathway including, length of time of disease, extent and severity of inflammation, family history of CRC, and the coexistence of PSC (29, 129, 130). The premise for surveillance in IBD is to ascertain those at greatest risk of developing dysplasia, optimise its detection, then deliver an effective intervention, such as endoscopic resection, thereby halting the progressive pathway to cancer. Within UK practice clinicians adhere to the BSG guidance which provides stratification of individual patients according these established risk factors, resulting in colonoscopy being performed 5-yearly for low-risk individuals, 3-yearly for intermediate-risk or annually for those deemed at highest-risk (128).

Optimising the detection of dysplasia is paramount for delivering effective surveillance. Our understanding of colonic dysplasia has evolved alongside advances in technology and endoscopic techniques. Historically, colonic dysplasia was believed to be predominantly invisible supporting the rational for obtaining random biopsies every 10 cm on withdrawal from the caecum. Unfortunately, this resulted in low biopsy yield for dysplasia, whilst placing a huge burden on histopathology resources and finances (111). Alongside advancements in endoscopic image resolution, opinions regarding dysplasia transformed with the majority thought to be visible as lesions (121). Furthermore, the morphology of these dysplastic lesions tended to be flatter and thereby more challenging to detect when compared to lesions in patients without colitis. This led to the adoption of dye-based chromoendoscopy (DBC) with numerous meta-analyses revealing an

enhanced dysplastic lesion detection when compared to standard definition white-light endoscopy (69, 70, 131).

With the adoption of DBC, the most frequently used dye is indigo carmine due to its known safety profile (63). This blue dye, when applied to the colonic mucosa, pools around mucosal irregularities and crevices, highlighting subtle lesions, and assisting in the visual detection and lesion border demarcation (58). On closer inspection the dye fills mucosal crypts, which accentuates crypt patterns, and thought to assist in in-vivo lesion characterization, helping to differentiate neoplastic from non-neoplastic lesions (132). Despite expert consensus statements advocating DBC there is an absence of data concerning the optimal mode of delivery and concentrations of indigo carmine when used for identifying dysplasia. In North America chromoendoscopy is generally performed with a foot pump utilising the water channel, applying a more dilute concentration of indigo carmine. In Europe and Japan, chromoendoscopy tends to be executed using a spray-catheter placed down the working channel, attached to a syringe placed within a dilatation gun, spraying a more concentrated solution. To date, no study has compared these two different dye concentrations and modes of delivery to determine the impact on dysplasia detection within IBD surveillance.

Present guidelines state that targeted biopsies should now be performed when using high definition endoscopes ideally with chromoendoscopy, although random biopsies are still required to determine ongoing microscopic inflammation (128). However, opinion still holds that biopsies for invisible dysplasia should be performed in a subgroup of patients with IBD colitis. Invisible dysplasia is likely to develop as a result of a phenomenon known as “field cancerization” (27). Field cancerization is a process whereby normal colonic mucosal cells undergo widespread replacement by pre-dysplastic clonal cells that have acquired molecular alterations within histologically normal appearing tissue (26). These pre-dysplastic fields provide the potential to progress to dysplasia and cancer and provide an explanation why dysplasia can be widespread and sometimes invisible. A recent multi-centered prospective study found that 12.8% of patients with all type dysplasia were detected by random biopsy alone and certain individual risk factors were independently associated with the detection of random biopsy only dysplasia (77).

Therefore, in this randomised controlled trial we wanted to determine whether the concentration of dye impacts on the detection of dysplastic lesions in patients with colitis. Secondary outcomes included comparing withdrawal time and number of ampoules of indigo carmine used between the two arms and predictors for invisible dysplasia.

3.2 Methods

In this randomised controlled study, we compared dysplasia detection using 0.2% indigo carmine versus 0.03% indigo carmine at a single large tertiary centre at Leeds Teaching Hospitals, United Kingdom (UK). Leeds Teaching Hospitals serves a large IBD population in the north of England and homes one of the six liver transplant centres within the UK. The IBD surveillance service is well established within the gastroenterology department at Leeds with dedicated chromoendoscopy lists. For this trial we gained National Research Ethics Committee approval (17/EM/0033) and local NHS R&D approval, along with registration at ClinicalTrials.gov NCT03250780. This study was constructed using the CONSORT guidelines for randomised controlled trials.

Consecutive eligible patients undergoing IBD surveillance were enrolled from March 2017 through to March 2019. Eligibility for enrolment included patients aged ≥18 years, had at least 8-10 years of IBD-colitis or coexistent PSC, extensive colitis (Montreal classification E3), or if Crohn's colitis >50% of the colon affected. Exclusion criteria included patient with only left sided colitis, proctitis or in the case of Crohn's colitis <50% of the colon affected, poor bowel preparation, active disease rendering examination difficult, pregnancy, extensive colonic surgery, inability to complete the colonoscopy or did not consent to enrolment in the trial.

Patients were enrolled into the trial following an informed discussion either during IBD clinic consultation or on the day of the procedure at the time of consent, along with providing a patient information leaflet. Patients were randomised at the start of the procedure by an independent coordinator blinded to the patient's history. Patients would be randomised into one of two indigo carmine concentrations according to a computer-generated random number sequence, with even numbers assigned to the 0.2% concentration with spray catheter and odd numbers assigned to 0.03% concentration using the foot pump.

3.2.1 The procedure

Colonoscopy was performed by three JAG accredited doctors with varying degrees of chromoendoscopy experience (two consultants and one senior registrar; VS, RS and RL), all of whom had training in chromoendoscopy technique and colonic lesion characterisation, including narrow band imaging (NBI). All procedures were performed using the Olympus CF-HQ290L/I or PCF-H290 colonoscopes with the CV-90 Evis Lucera Elite Processors (Olympus, Tokyo, Japan). Standard bowel preparation was used, which at Leeds Teaching Hospitals was PEG solution (Moviprep, Norgine) with split dosing. Bowel preparation was assessed using the Boston Bowel Preparation Score (BBPS) (133). Procedures were performed with patients either opting for Entonox, conscious

sedation (fentanyl +/- midazolam) or both, which is standard practice at Leeds. The degree of active inflammation was assessed using the Mayo endoscopic subscore for patients with UC and the Simple endoscopic score for Crohn's disease (SES-CD) score for patients with Crohn's colitis (134, 135). Patients with evidence of moderate or severe endoscopic activity (Mayo endoscopic subscore ≥2 or SES-CD >5) involving at least one colonic segment were not enrolled into the trial.

Individual patient data was collected and recorded including evidence of previous dysplasia (invisible or targeted), patient's BSG risk stratification for dysplasia going into the study (low, intermediate or high risk), presence of PSC, previous liver transplant and family history of colorectal cancer. Additional data collected during the procedure included the number of segments containing pseudopolyps and the presence of any strictures.

Standard intubation to the caecum was achieved with minimal air insufflation and meticulous washing in order to achieve optimal views before withdrawal. On reaching the caecum chromoendoscopy was commenced with the corresponding dye that was assigned as per the computer-generated random number sequence. For the 0.2% arm, one ampoule (5mls of 0.8% indigo carmine) was diluted with 15mls of sterile water (normally at least four aliquots prepared) and placed in a 50ml syringe and inserted into a dilator gun and attached to a spray catheter (MTW, Endoskopie Manufaktur, Germany). The catheter was placed through the biopsy channel and the dye sprayed circumferentially on withdrawal. Any pooling of dye was suctioned before careful inspection of the segment for any mucosal abnormalities and lesions. The process was repeated for each segment. The 0.03% concentration involved mixing two ampoules of 0.8% indigo carmine (total of 10mls) with 250mls of water in a bottle and connecting this to the foot pump at the time of reaching the caecum, exchanging with the normal water bottle used as a jet wash during insertion. The jet of water containing the dye was aimed at the opposing wall to that of the gravity dependent side, allowing circumferential coverage. Again, suction of any dye pooling was performed before each segment was carefully inspected.

The time of the procedure started from initial insertion of the colonoscope at the anus until final withdrawal at the anus. Time of withdrawal started from the time of initial spray at the caecum again until withdrawal at the anus. These were recorded using a stopwatch. The exact number of ampoules used to spray the colon was recorded at the end of the procedure.

3.2.2 Biopsy protocol and histology

Segmental biopsies were taken to look for histological evidence of inflammation, performed on withdrawal following inspection after dye spray. This is a requirement as part of BSG guidelines to assess for microscopic inflammation and to help determine correct surveillance intervals (128). Histological classification was determined according to the revised Vienna classifications into non-neoplastic or neoplastic described as indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, or invasive neoplasia. However, histology defined as “indefinite for dysplasia” was not considered neoplastic for trial purposes (136). Biopsies were processed as standard procedure and reviewed by an expert tertiary centred gastrointestinal (GI) histopathologist based locally, who was blinded to the randomisation. Any dysplasia detected was reviewed by a second GI histopathologist as standard practice.

3.2.3 Outcomes

The primary outcome of this study was to determine the number of procedures detecting targeted dysplasia, comparing the more concentrated 0.2% indigo carmine dye using a spray catheter, to that of the dilute 0.03% indigo carmine using the foot pump. Secondary outcomes included number of dysplastic lesions, withdrawal time, the amount of indigo carmine used between the two arms, and predictors for invisible dysplasia.

3.2.4 Sample size calculation and Statistical analysis

This study was powered as a superiority study. We expect about 20% of patients to be excluded due to bad bowel prep, active disease or incomplete colonoscopy. We therefore planned to include 150 patients in each arm. Based on our previous randomized controlled trial, we have shown that chromoendoscopy with 0.2% dye spray and high definition equipment has a 22% yield (73). High definition alone had a 9.4% yield of dysplasia. Assuming that dilute indigo carmine will do no better than high definition white-light endoscopy we would get a sample size of 121 patients per arm from the primary outcomes of the study using a 1-sided McNemar z test for paired proportions with an 80% power and a Type 1 error rate of 5%.

Quantitative variables are expressed as means ±SD for normally distributed data and medians with IQRs or ranges for non-normally distributed data. Categorical variables are expressed as a total numbers and frequencies (%). Quantitative variables were analysed by independent t-test or Mann Whitney U test. Categorical variables were analysed using Chi-square test or Fishers exact test. Univariate analysis to test for association were performed by binary logistic regression using Chi-square tests of significance. P value $\leq$ 0.05 were considered statistically significant. For the detection of dysplasia on a per procedure basis the Chi-square test was used with the confidence intervals (CI) for the relative difference based on the standard error of the log relative risk. For the number of

lesions detected the data did not follow a Poisson distribution because of over-dispersion (variance greater than the mean) and therefore a negative binomial regression was used to compare the means in this group. All statistical analysis was performed using SPSS version 24 (IBM Corp).

3.3 Results

We enrolled 342 colonoscopic procedures into this trial from March 2017 to March 2019. However, 42 procedures were excluded as they did not meet the inclusion criteria (see study flow diagram (Figure 13). Therefore 300 colonoscopies were included within this study, involving 276 patients. Following randomisation, 150 procedures (144 patients) were performed in the 0.2% indigo carmine arm, and 150 procedures (146 patients) in the 0.03% indigo carmine arm.

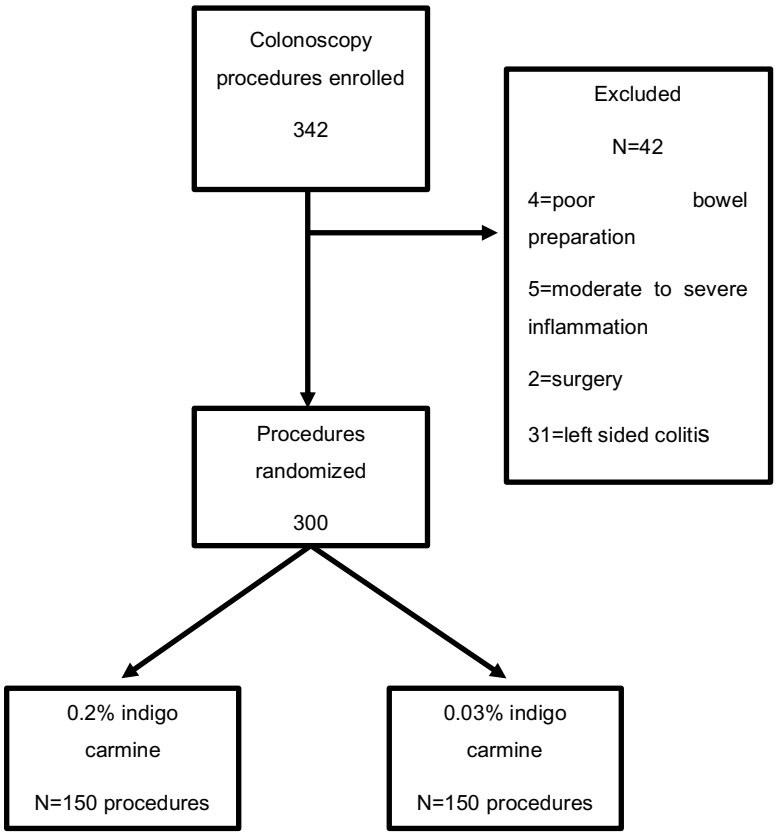


Figure 13 Study flowchart displaying enrolment and randomisation

The mean age of the cohort was 52 (±15.69) years old, with 92.3% being Caucasian and 56.7% being male. The mean duration of colitis was 19.39 (±10.94) years with the majority having ulcerative colitis (71.3%). According to BSG risk stratification, 29.3% of the cohort were classified as high risk, with 19% having PSC. Table 4 compares baseline characteristics of patients within both arms, overall being well-matched and showing no significant difference between the groups. No patients enrolled within this study had any adverse events, such as bleeding, perforation or death.

	0.2% IC (n=150)	**0.03% IC (n=150)**	***P* value**
Age	50.93 ±16.05	53.09 ±15.31	0.234
Duration of colitis	18.41 ±9.75	20.38 ±11.98	0.121
BSG risk group			0.549
Low	82 (54.7%)	79 (52.7%)	
Medium	22 (14.7%)	29 (19.3%)	
High	46 (30.6%)	42 (28%)	
First – degree relative with CRC diagnosis			0.409
No	148	146	
Yes	2	4	
Patients with previous dysplasia	27 (18%)	28 (18.7%)	0.881
Patients with PSC	28 (18.6%)	29 (19.3%)	0.883
Pseudopolyps			0.128
No	96	114	
1 segment	20	11	
2 segments	14	9	
>2 segments	20	16	
BBPS	8 (IQR 7 – 9)	8 (IQR 7 – 9)	0.542
Mayo score	0 (IQR 0 – 1)	0 (IQR 0 – 1)	0.560
SES - CD	0 (IQR 0 – 3)	0 (IQR 0 – 3)	0.870

Table 4 Comparison of baseline characteristics between the 0.2% dye concentration cohort and the 0.03% dye concentration cohort. IC, indigo carmine. CRC, colorectal cancer. PSC, primary sclerosing cholangitis. BBPS, Boston bowel preparation score. SES – CD, simple endoscopic score for Crohn's disease

3.3.1 Per procedure analysis

A total of 67 (22.3%) procedures detected all type neoplasia; 35 (23.3%) in the 0.2% indigo carmine arm and 32 (21.3%) in the 0.03% arm; p=0.677. Targeted neoplasia was detected in 58 (19.3%) procedures within the study, including 32 (21.3%) procedures in the 0.2% arm and 26 (17.3%) procedures in the 0.03% arm; p=0.465. These findings show that 0.2% indigo carmine using a spray catheter was numerically but not statistically superior at detecting visible neoplasia when compared to 0.03% indigo carmine using a foot pump. See Table 5.

A total of 85 neoplastic lesions were detected, of which 49 were found in the 0.2% arm and 36 in the 0.03% arm; p=0.373. Of the 49 neoplastic lesions in the 0.2% arm all had LGD, 5 of which were sessile serrated lesions (SSL) with dysplasia. In the 0.03% arm, 36 neoplastic lesions were detected, of which 35 lesions had LGD (one being an SSL) and one lesion having HGD. The mean number of neoplastic lesions per procedure again numerically favoured the 0.2% arm but was not found to be statistically significant; 0.33 ±.709 vs 0.24 ±.576, p=0.278. No adenocarcinoma was found within the study.

	0.2% IC (n=150)	**0.03% IC (n=150)**	***P* value**
Procedures with neoplasia (%)			
All type neoplasia	35 (23.3)	32 (21.3)	0.677
Targeted neoplasia	32 (21.3)	26 (17.3)	0.465
Random dysplasia	6 (4.0)	9 (6.0)	0.427
Random dysplasia only	3 (2.0)	6 (4.0)	0.501
Total number of neoplastic lesions	49	36	0.373
Low grade dysplasia (LGD)	49	35	-
High grade dysplasia	0	1	-
Mean number of neoplastic lesions per procedures	0.33 ±.790	0.24 ±.576	0.278

Table 5 Diagnostic yield comparing the number of procedures with all type neoplasia (including targeted and random only), the total number of neoplastic lesions in each arm and the mean number of neoplastic lesions per procedure, according to dye concentration. IC, indigo carmine.

3.3.2 Random biopsy dysplasia

A total of 5,592 random biopsies were taken during the study, corresponding to an average of 19 random biopsies per procedure; see Table 6. The overall total yield of random biopsies for dysplasia was 0.44%, showing no statistical significance when split between the two arms.

	0.2% IC	0.03% IC	*P* value
Median number of random biopsies per procedure	20 (IQR 15 – 22))	20 (IQR 16 – 22)	0.856
Total number of random biopsies per arm	2769	2823	-
Proportion of random biopsies positive for dysplasia	0.54%	0.35%	0.453

Table 6 The median number of random biopsies per procedure, total number of biopsies and proportion of random biopsies positive for dysplasia in each arm. IC, indigo carmine.

A total of 15 procedures (5%) were found to have random biopsy dysplasia; 6 procedures in the 0.2% arm and 9 in the 0.03% arm; p=0.427. Of the 15 procedures with random biopsy dysplasia, 23 biopsy samples were found to harbour LGD (13 procedures) and two samples had HGD (2 procedures). The two procedures with HGD also had targeted dysplasia detected. Four procedures with random LGD had multifocal random biopsy dysplasia and of these 2 procedures also had targeted dysplasia. Overall, 110 sites of all type neoplasia (random or targeted) were identified, of which 22.7% was identified by random biopsies, with the rest being identified by targeted biopsy (77.3%).

Of these 15 procedures with random biopsy dysplasia, 9 procedures (8 patients) had dysplasia found only in random biopsies (3 procedures in the 0.2% arm and 6 in the 0.03% arm; p=0.501). The proportion of procedures with all type dysplasia, who were identified by random biopsy only was 13.4%. Univariate analysis suggests that being in the BSG high risk group (OR 21.10 (2.6 – 171.4); p=0.004), the presence of PSC (OR 9.41 (2.3 – 38.9); p=0.002), previous dysplasia (OR 7.39 (1.9 – 28.6); p=0.004), and having had a liver transplant (OR 8.9 (1.6 – 49.2); p=0.038) were associated with having random biopsy only dysplasia (see Table 7). The median number of biopsies taken when comparing the presence of these risk factors versus procedures without these risks showed no numerical difference (median 20 biopsies). A multi-variable binary logistic regression could not be performed due to marked collinearity between variables.

	Odds ratio (95% CI)	***P* value**
BSG high risk group	21.10 (2.6 - 171.4)	0.004
Presence of PSC	9.41 (2.3 – 38.9)	0.002
Previous dysplasia within a colitic segment	7.39 (1.9 – 28.6)	0.004
Liver transplant	8.9 (1.6 – 49.2)	0.038

Table 7 Univariable analysis evaluating association between risk factors and the detection of random biopsy only dysplasia. PSC, primary sclerosing cholangitis.

For the nine procedures in which random biopsy only dysplasia was detected, 8 of the procedures were performed in patients within the BSG high risk group (see Table 8). The 2x2 table shows a high sensitivity, 88.9% (95% CI 51.3 – 99.4%), and an excellent NPV, 99.5% (95% CI 97.9 – 100%), for detecting random biopsy only dysplasia when stratified according to this.

	Random biopsy only dysplasia positive		
BSG high risk group	Yes	No	
Yes	8	80	PPV = 9.1% (95% CI 5.2 – 10.2%)
No	1	211	NPV = 99.5% (95% CI 97.9 – 100%)
	Sensitivity = 88.9 (95% CI 51.3 - 99.4%)	Specificity = 72.5 (95% CI 71.3 – 72.8%)	

Table 8 A 2x2 table showing the accuracy for detecting random biopsy only dysplasia stratified according to the BSG high risk group versus non – high risk group (low and intermediate groups).

3.3.3 Withdrawal time and amount of dye used

The median withdrawal time displayed statistical significance in favour of the 0.03% indigo carmine group using the foot pump, with on average being 6 minutes faster than applying; 15 (IQR 12 – 20) vs 21 (IQR 18 – 24) minutes, p<0.001 (seeTable 9). The number of ampoules used was also significantly less within the 0.03% group, with a median of 1 (IQR 1 – 2) ampoule when compared with the 0.2% dye spray, 4 (IQR 3 – 5) ampoules; p<0.001.

	0.2% IC	0.03% IC	*P* value
Median procedure time	38 (IQR 31 – 45)	31 (IQR 26 – 38)	<0.001
Median withdrawal time	21 (IQR 18 – 24)	15 (IQR 12 – 20)	<0.001
Average number of ampoules per procedure	4 (IQR 3 – 5)	1 (IQR 1 – 2)	<0.001

Table 9 Median procedure time, withdrawal time and average number of ampoules of indigo carmine in each arm. IC, indigo carmine.

3.4 Discussion

To our knowledge this is the first randomized controlled trial comparing dye concentrations and techniques used for detecting targeted dysplasia during IBD surveillance. The results display a numerical, but a non-statistical significant difference favouring the more concentrated 0.2% indigo carmine dye using a spray catheter when compared with the more dilute 0.03% concentration using a foot pump. The mean number of neoplastic lesions per procedure also numerically favoured the more concentrated dye, although again not being statistically significant.

It has been widely agreed that the optimal way for detecting dysplasia in patients with IBD-colitis is by the use of dye-based chromoendoscopy along with high definition technology (137). However, there is no general consensus concerning optimal concentrations or delivery systems for applying dye-based chromoendoscopy due to an absence of studies specifically examining this. The North Americans utilise the more dilute concentrations delivered by a foot pump, whilst the Europeans and Japanese favour a more concentrated dye delivered through a spray catheter. It has therefore remained a relevant question, in order to further optimise and standardise dysplasia detection during surveillance procedures.

Our findings show an incremental yield of 4% (95% CI -4.9 – 12.9%) for detecting targeted dysplasia favouring the more concentrated 0.2% indigo carmine application. Although this did not reach statistical significance, if this 4% represented the true population this would surely represent a clinically significant difference. The 0.03% indigo carmine did however outperform the original assumption, which stated it would do no better than HDWL endoscopy (73). Therefore, if we infer the results with that of a previous study at Leeds comparing HDCE versus HDWL, even a very dilute concentration such as 0.03% indigo carmine would seem to provide additional yield for detecting dysplastic lesions when compared to HDWL.

When considering the practicalities of applying these two methods, deploying the 0.03% concentration via a foot pump was on average six minutes faster than the spray catheter and although not measured, generally felt a less laborious task. Whether these six minutes saved per procedure becomes clinically significant is questionable. At Leeds we allocate four chromoendoscopy procedures per list in the morning or three chromoendoscopy procedures plus an additional diagnostic gastroscopy or flexible sigmoidoscopy in the afternoon. The eighteen to twenty four minutes saved per list when using the 0.03% dye could potentially allow an additional diagnostic procedure to be added. Though this maybe pleasing to the departments business managers, for the endoscopist it maybe an additional encumbrance to an already technically challenging list. An additional observation, was the dye from the foot pump was ejected as a stream rather than a circumferential spray, as seen with the spray catheter, resulting in more pooling of fluid along the gravity dependent wall, requiring more suctioning to visualise the underlying mucosa. Whether the time spent suctioning this fluid discourages the endoscopist from spending time examining the rest of the mucosa for lesions is difficult to determine. However this did not significantly reduce the number of dysplastic lesions detected.

Preneoplastic fields are thought to exists in patients with IBD colitis, also referred to as the “field effect” (138). This term denotes widespread replacement of normal colonic mucosal cells by clonal mutant non-dysplastic cells, comprising molecular abnormalities, which have occurred before the presence of any detectable dysplasia. Such aberrations encompass TP53 mutations, aneuploidy, mitochondrial dysfunction, telomere shortening and epigenetic alterations (27, 139). These changes are thought to be brought about by oxidative stress following long-term colonic inflammation. This concept provides an explanation for why more synchronous and metachronous lesions are discovered in patients with IBD-colitis (23).

Histologically dysplastic transformation in non-colitic CRC begins at the luminal surface of crypts, whilst in contrast, IBD-colitis displays a tendency to start at the crypt bases (140). This would provide logical explanation to why invisible dysplasia endures despite advances in endoscopic technology. Furthermore, a recent systematic review and meta-analysis of cohorts undergoing IBD surveillance showed that the pooled incidence ratio for developing advanced neoplasia regarding nonvisible LGD was higher than with visible dysplasia, suggesting the detection of invisible dysplasia is clinically relevant (99). Likewise, a multi-centred prospective study looking at the number of patients with dysplasia detected by random biopsy only, found that 12.8% of patients with dysplasia were detected by random biopsy alone, and by using multivariable regression analysis they described PSC, a personal history of neoplasia and a “tubular colon” to be

independently associated with detection of random biopsy only dysplasia (77). Therefore, the debate regarding obtaining random biopsies and in whom continues.

Within this study, we obtained on median of 20 biopsies per procedure, taken according to BSG recommendations that segmental biopsies should be taken to establish any underlying microscopic inflammatory activity. In total, 9 (3%) procedures were found to harbour dysplasia detected by random biopsies only. Additionally, the proportion of patients with any type dysplasia, having this detected by random biopsy alone, was 13.4%. The 3% of procedures that detected random biopsy only dysplasia within this study echoes similar results seen in the meta-analysis in chapter 2, where the pooled subgroup analysis for HDCE technology detected 4.94% of patients.

The yield of dysplasia by random biopsies was 0.44% per biopsy. In the meta-analysis in the previous chapter, the pooled yield of dysplasia per biopsy for HDCE was 0.2%. This would suggest our findings are more than double the average. One explanation for the high yield of dysplasia per random biopsy when compared with aforementioned studies is likely rationalised by the fact that 30% of the cohort lie within the BSG high-risk category, with a significant proportion with PSC, known to harbour invisible dysplasia, of which can be multi-focal. Multi-focal invisible dysplasia may thereby inflate the yield of dysplasia per biopsy, though will not increase the expected number of procedures with invisible dysplasia only procedures. Additionally, in such a high-risk cohort, targeted dysplasia occurred alongside invisible dysplasia, reducing the proportion of patients with invisible dysplasia alone, but still contributing to the per biopsy analysis. Experts may further question this high yield of random biopsy dysplasia as a surrogate for missed dysplastic lesions. However, to counter this argument, targeted dysplasia was detected in 19.3% of procedures which is similar to other studies using HDCE, suggesting visible dysplasia was not missed.

This is the first randomised controlled trial assessing BSG risk against the risk of harbouring invisible dysplasia. Interestingly, when stratifying patients with random biopsy only dysplasia according to BSG high-risk versus non-high-risk groups (intermediate and low), we discovered a sensitivity of 90% and a NPV of 99.5%. By extrapolating these results, taking no random biopsies in patients of low and intermediate BSG risk (apart from those pertaining to detecting microscopic inflammation) is perhaps acceptable, and segmental random biopsies only warranted for patients within the BSG high-risk group. Pending superior ways to identify patients whom have developed this “field effect” or enhanced technology in detecting invisible dysplasia, one recommendation could be that patients in the high-risk group still undergo targeted biopsies, with the addition of random biopsies (four biopsies every 10cm) during high definition chromoendoscopy. Those patients outside this group would only require targeted biopsies.

The expense of indigo carmine also needs to be taken into consideration. The 0.2% indigo carmine arm averaged three ampoules more per procedure. Performing two hundred chromoendoscopy procedures annually at Leeds Teaching Hospitals, the cost of dye utilizing the 0.2% concentration would average £24,000 per year, compared with £6,000 using the 0.03% arm, making savings in the region of £18,000 if the foot pump protocol was adopted. Additional to this is the cost of the spray catheter, totalling a supplementary £8,500 per year. Savings in the order of £26,500 per year could have significant financial implications for individual endoscopy units. Both the time and expense of each procedure may influence an endoscopists decision regarding the concentration and technique utilised.

There are several limitations to our study. Firstly, when powering this study, we assumed the 0.03% indigo carmine would perform no better HDWL, based on the fact that at this concentration the dye is extremely dilute and therefore hypothesized no better than HDWL. A detection rate of 9.4% was used when performing the power calculation for the 0.03% indigo carmine arm founded by a recent randomised study at Leeds (73). In fact, the 0.03% indigo carmine arm performed considerably better, almost doubling the presumed yield. With the power calculation being based on these figures it may explain why the primary outcome didn't achieve statistical significance. Having set a smaller effect size however, would have resulted in an extremely large trial which potentially would not have been feasible within the timescale.

Secondly, outcomes between both arms didn't solely compare differing dye concentrations but also the mode of dye delivery varied correspondingly. Applying the more dilute dye we used the foot pump, ejecting the dye as a stream and therefore it was very important to target the antigravity wall to allow circumferential application. The more concentrated dye was delivered via a spray catheter providing easier circumferential coverage. This unaccounted variable may have been as important regarding dysplasia detection as the concentration of dye used. However, we attempted to nullify this by providing full circumferential coverage of each segment with the dye and with the addition of suctioning of any pooling of fluid. Application of the 0.2% concentration using the foot pump alternatively would have resulted in appreciably more ampoules of indigo carmine per procedure making it not financially viable. Whilst delivering the 0.03% concentration via a spray catheter would have negated one of its major benefits, time of withdrawal and ease of use. Therefore, we adopted a pragmatic approach and mirrored real-world use.

A further limitation to the study is that it was performed at a single, tertiary regional transplant centre, potentially reducing the studies generalisability. Evidently patients within this cohort represent a higher risk for developing dysplasia than those IBD patients

within the general population. Nevertheless, this should not affect the interpretation of the primary outcome, with only the yield of dysplasia potentially being inflated in both arms. Additionally, the nature of the procedure made it impossible to blind the endoscopist as to which arm the patient was allocated however, patients were randomised by an independent coordinator blinded to the patient's history. Being a tertiary centre, histology was analysed by expert histopathologists whom also were blinded to the arm the patient was assigned.

Despite being undertaken at a single centre, a major strength of this study was that three endoscopists of varying experience, all being trained in chromoendoscopy and lesion interpretation, participated within this study. This is unusual for detection studies, with most studies having one or two extremely advanced endoscopists undertaking all of the procedures, reducing its generalisability and general acceptance to adopt within practice. We have provided evidence that high detection rates can be achieved with skilled endoscopists, although with diverse experience. This hopefully provides reassurance that surveillance chromoendoscopy can be undertaken in non-expert centres provided that the service is focused and with lists planned purely for this purpose.

In conclusion, our randomised trial has demonstrated that both the concentrated and dilute forms of indigo carmine have excellent detection rates regarding detecting targeted dysplasia and the mean number of dysplastic lesions per procedure, although numerically favours the 0.2% indigo carmine using the spray catheter. Using high definition chromoendoscopy, random biopsies for invisible dysplasia are not required when patients are risk stratified within the BSG low and intermediate risk groups, but should strongly be considered for patients in the high-risk group.

Future studies may look at enhanced modes of delivering the dye spray, such as an externally placed cap on the tip of the scope with spraying capabilities, utilizing both the circumferential delivery of a spray catheter whilst reducing the exchange time down the accessory channel. Additionally, the ability to identify preneoplastic fields will provide the optimal way of risk stratification and allowing the ability to personalise surveillance procedures.

Chapter 4

Colonic lesion characterization in IBD: A Systematic Review and Meta-analysis

4.1 Introduction

As revealed throughout the previous two chapters, the majority of dysplasia found within IBD-colitis is visible and the vast majority being detected endoscopically due to recent technological advancements(30). Evidence now supports DBC as the most sensitive way to detect such pathology (30, 128, 141).

However, once dysplasia is detected, endoscopic interrogation should take place in order to determine if the lesion is dysplastic or non-dysplastic. An international consensus group in 2015 recommended that dysplastic polypoid or non-polypoid lesions within a colitic segment should all treated as significant and that well circumscribed lesions with no endoscopic features of submucosal invasion can now be resected(30). The risk for developing CRC following complete endoscopic resection is now thought to be lower than previous studies suggested(142).

Novel technologies, including Narrow Band Imaging (NBI), Fujinon Intelligence Chromoendoscopy (FICE), i-scan, magnification endoscopy and Confocal Laser Endomicroscopy (CLE), have been studied to obtain an in-vivo optical diagnosis of colorectal lesions. DBC using contrast agents, such as indigo-carmine, or absorptive agents, like methylene blue, are customarily applied via a spray catheter to provide mucosal enhancement. Virtual chromoendoscopy (NBI, FICE, i-scan) are dye-less enhancement technologies that are built into the colonoscope or processor. NBI uses optical filter enhancement at the distal end of the endoscope, narrowing the light bandwidth, thereby improving visualization of the mucosa. FICE and i-scan use digital post-processing technology with spectral estimation to achieve mucosal enhancement. Magnification endoscopy possesses a variable lens, providing magnification up to 150-fold, permitting detailed examination of the mucosal pit patterns. Whilst CLE technology involves focusing laser light onto the mucosa and the reflected light is returned via a pinhole. This filters out non-focused light, giving a highly magnified, real-time histological diagnosis. CLE can either be integrated (iCLE) within the endoscope or via a probe (pCLE), which can be passed through the biopsy channel.

In patients without colitis, multiple studies have looked at in-vivo optical diagnosis of colorectal lesions using these technologies, allowing differentiation between neoplastic

and non-neoplastic lesions. The hope that this would be cost-effective, reduce risk associated with polypectomy and provide instant determination of polyp surveillance intervals for the patient. A recent meta-analysis by the ASGE group looked at novel technologies to allow a “diagnose and leave” and “resect and discard” strategy (143). To achieve a “diagnose and leave” strategy, (a decision to leave in-situ diminutive rectosigmoid polyps), the technology had to achieve >90% NPV for adenomatous histology. To achieve a “resect and discard” strategy, (remove diminutive adenomatous polyps without histological assessment), the technology should provide >90% agreement in post-polypectomy surveillance intervals. The meta-analysis showed that this could only be achieved with NBI technology, in endoscopists that were experienced and that the assessment of the polyp was made with high confidence. Recently a large multicenter prospective study evaluated the use of NBI assisted optical diagnosis in non-expert endoscopists for small colonic polyps and was found to not achieve the above criteria(87).

Accuracy of these technologies during surveillance colonoscopy in colonic IBD is unclear with the majority of studies being small and assessed as secondary outcomes. With additional hurdles to overcome in patients with colitis, such as active inflammation and the fact that lesions tend to be morphologically different (flatter rather than polypoid), how precise are we at characterizing lesions in IBD with the current technologies available. Our objective was to perform the first systematic review and meta-analysis for the diagnostic accuracy of optical imaging techniques for in-vivo lesion characterization in colonic IBD. We aimed to calculate the pooled estimated sensitivities, specificities, positive and negative likelihood ratios (+LHR, -LHR), diagnostic odd ratios (DOR), and Area Under Summary Receiver-Operator Characteristic (AUSROC) curve for each technology type, with histopathology as the reference standard. We also planned to perform a subgroup analysis looking at the accuracy of studies using real-time non-magnified Kudo pit pattern and real-time CLE [39].

4.2 Methods

4.2.1 Information sources and search strategy

We performed a meta-analysis in concordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines(144). RL searched Medline (from 1946 to May 2017) and Embase (from 1974 to May 2017), using the Healthcare Databases Advanced Search (HDAS) system. The search terms used included: ((((("high definition").ti,ab OR (HD).ti,ab OR ("white light").ti,ab OR (WL).ti,ab

OR (chromoendoscop*).ti,ab OR (CE).ti,ab OR (NBI).ti,ab OR ("narrow band").ti,ab OR (FICE).ti,ab OR ("fujinon intelligent chromoendoscopy").ti,ab OR ("I-scan").ti,ab OR (AFI).ti,ab OR (autofluorescence).ti,ab OR (CLE).ti,ab OR ("confocal laser").ti,ab OR ("real time histology").ti,ab) AND (("colon imag*").ti,ab OR ("intestinal imag*").ti,ab OR (colonoscop*).ti,ab)) AND (("inflammatory bowel disease").ti,ab OR (IBD).ti,ab OR (coliti*).ti,ab OR (uc).ti,ab OR ("ulcerative coliti*").ti,ab OR ("crohns coliti*").ti,ab OR ("crohn's coliti*").ti,ab)) AND ((lesion*).ti,ab OR (polyp*).ti,ab OR (dysplas*).ti,ab OR (neoplas*).ti,ab)". A Cochrane Library search for any systematic reviews relevant to this area was also performed. No language restrictions were used. The results for each database were combined and any duplicates removed.

4.2.2 Inclusion and exclusion criteria

4.2.2.1 Study inclusion and exclusion was determined by predefined criteria.

Inclusion criteria: 1) Studies using novel technologies to provide in-vivo optical characterisation of lesions in patients with colonic IBD during colonoscopy; 2) Characterised lesions into neoplastic and non-neoplastic using histology as the reference standard; 3) Able to extract data to obtain a 2x2 contingency table to calculate the true positive (TP), false positive (FP), false negative (FN) and true negative (TN); 4) Real-time characterisation or retrospective image-review.

4.2.2.2 Exclusion criteria:

Exclusion criteria: 1) Case studies or case series; 2) Studies not involving patients with colonic IBD; 3) Inability to construct a 2x2 contingency table from the data given; 4) Inability to differentiate detection from characterization studies; 5) Not used histology as reference standard; 5) Children

4.2.3 Study selection and Data extraction

RL and NB identified study eligibility using the above inclusion and exclusion criteria. We searched the combined list of results for relevant studies, looking at the abstract or if supplementary information required, the full article. Reference lists of selected papers were also checked for potential missed articles. Abstract or articles for clinical trials or observational studies were eligible for inclusion if characterization of lesions by NBI, FICE, i-scan, DBC, magnification endoscopy or CLE, differentiated neoplastic from non-neoplastic lesions in colonic IBD, using histopathology as the gold standard. From this, data was extracted using a 2x2 contingency table. If exact figures for the true positive (TP), false positive (FP), false negative (FN) and true negative (TN) were not represented in the articles, it was calculated from the documented sensitivity, specificity, accuracy,

positive predictive value (PPV) or negative predictive value (NPV). RL and VS performed data ascertainment and calculations. If TP, FP, FN and TN proved challenging to calculate from the article data, attempts were made to contact relevant authors by email for clarification of figures.

4.2.4 Risk of study Bias

As studies included were diagnostic, RL and NM used the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool to independently assess the degree of study validity(105). This looks at the risk of bias and applicability regarding four domains: patient selection, index test, reference standard, flow and timing. Risk of bias (involved all four domains) and applicability (involved three domains) is scored using low risk, high risk or unclear. Any indifference on determining risk between RL and NM was discussed and clarified with VS, who made the final decision.

4.2.5 Statistical Analysis of Data

In performing a systematic review for diagnostic studies, a bivariate meta-analysis using a random effects model was performed, allowing for the assumption of heterogeneity between the studies(145). A random effects model was the preferred in order to provide a more conservative result due to differences between study methods such as endoscopic expertise, classification model, study type and the population studied. We obtained summary estimates for sensitivity, specificity, +LHR, -LHR and DOR, with their 95% confidence intervals. A hierarchical summary receiver-operator characteristic (ROC) curve was plotted, with its summary point estimate, and a dashed line around representing its 95% confidence interval. The area under the SROC curve (AUROC) served as a marker of test accuracy. Forest plots were also calculated to demonstrate study sensitivity and specificity.

Heterogeneity between studies was assessed using the Cochrane *Q* and I^2 tests. Cochrane *Q* is established upon the chi-squared test, providing a weighted sum of the squared differences of each study estimate from the overall pooled estimate. *P* valves are given. I^2 describes the percentage of variation between studies that is due to heterogeneity rather than chance and is not dependent on the number of studies included. I^2 quantifies the impact of heterogeneity on the meta-analysis rather than just the extent of heterogeneity. Results range from 0-100%: 0% means there is no heterogeneity between the studies, whereas scores >50% equate to moderate heterogeneity and >75% high heterogeneity.

To help determine factors that may account for heterogeneity, we performed a subgroup analysis concentrating on real-time mucosal characterization, dividing into two groups: non-magnified Kudo pit pattern (using VCE and DBC) and CLE. We also pooled results

for all studies (real-time and retrospective image-capture) looking at non-magnified Kudo pit pattern.

Publication bias was assessed by the Deeks et al funnel plot(146). This uses regression of diagnostic log odds ratio against1/sqrt (effective sample size), weighting by sample size with a P <0.10 for the slope coefficient as an indicator of substantial asymmetry.

All data analysis was done using Stata version 13 (Stata Corp, Texas, USA) using the user written command Midas (Dwamena, 2009) [38,14]

4.3 Results

4.3.1 Study selection

One hundred and seventy-two abstracts and articles were obtained following the initial keyword search, following removal of duplicates (Figure 14). 21 studies were excluded following screening of the title, leaving 151 citations. A further 63 studies were excluded following review of the abstract, leaving 88 citations. 66 more studies were excluded following review of papers as a result of: 35 being detection studies, 25 were review articles, 2 involved patients without colonic IBD and 4 we were unable to construct a 2x2 contingency table.

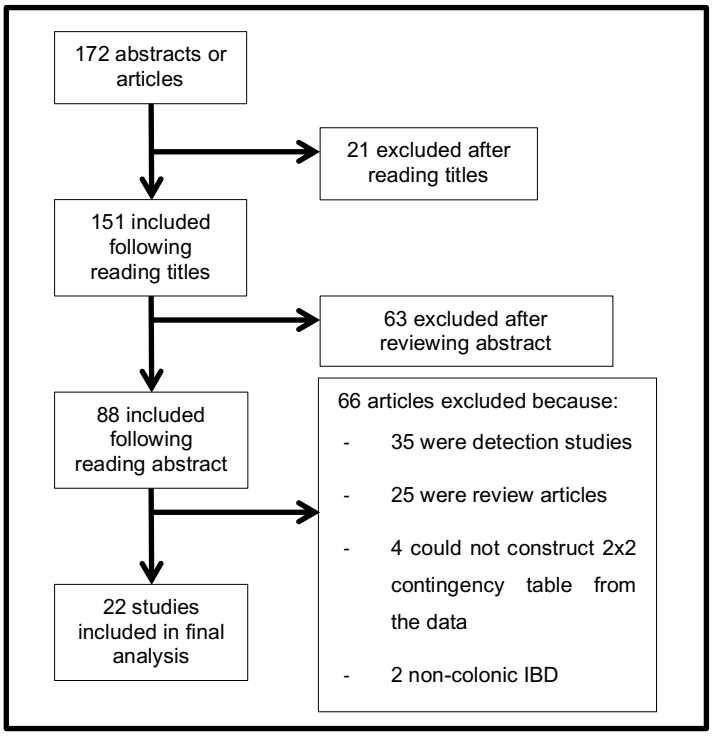


Figure 14 Study flow chart

4.3.2 Study characteristics

The characteristics of the 22 studies included are presented in Table 10. Twenty-one studies included 1491 patients, with one study not reporting the number of patients included, and 4674 lesions, of which 539 (11.5%) were neoplastic.

The VCE group consisted of five studies, with one study looking at i-scan technology, two studies involved NBI and a further two used FICE. Three of the papers were abstracts and two being articles. All of these studies used endoscopic real-time diagnosis of lesions.

The DBC group entailed six studies, using either indigo-carmine (0.2-0.4%) or methylene blue (0.1%) as the contrast agent. One of these studies performed endoscopic lesion diagnosis using a retrospective image-captured questionnaire, whilst the others used real-time diagnosis. Two were abstracts with the others being articles.

The CLE group comprised of nine studies; four studies used iCLE and five studies used pCLE. Three studies were retrospective image based, with the remaining being real-time studies. Two were abstracts and the others being articles.

The magnification endoscopy group consisted of five studies, four of which being used in conjunction with NBI and one used with DBC. One study was retrospective image-based, with the others being real-time diagnosis. All were articles.

For the subgroup analysis, real-time non-magnified Kudo pit pattern involved ten studies and real-time CLE involved six studies. The “all study” Kudo pit pattern included twelve studies of which two were retrospective image-based abstracts.

Authors	Year	Abstract/article	Technology	Number of Endoscopists	Study Design	Real time vs Image review	No. of Patients	No. of Polyps	Mucosal classification method
Virtual Chromoendoscopy									
Cassinotti et al [147]	2016	Abstract	i-scan HD	/	Single centre / Prospective cohort	Real time	40	287	Kudo PP + other endoscopic features
Efthymiou et al [122]	2013	Article	NBI HD	2	Single centre / Prospective cohort	Real time	44	121	Kudo PP + low level magnification
Van den broek et al [116]	2011	Article	NBI HD	4	Single centre / Randomised cross-over	Real time	48	153	Kudo PP
Cassinotti et al [119]	2015	Abstract	FICE HD	1	Single centre / Randomised parallel	Real time	41	261	Kudo PP
Cassinotti et al [119]	2015	Abstract	FICE HD	1	Single centre / Prospective cohort	Real time	59	205	Kudo PP
Dye-based Chromoendoscopy									
Carballal et al [148]	2016	Article	IC 0.4% SD/HD	15	Multi-centre / Prospective cohort	Real time	350	595	Kudo PP + 10 other items
Buchner et al [149] *	2016	Abstract	MB 0.1% HD	/	Prospective cohort	Real time	22	21	/
Wanders et al [150] **	2016	Article	MB 0.1% SD	>1	Multi-centre / Prospective cohort	Real time	61	66	Kudo PP
Munoz et al [151]	2016	Abstract	IC 0.2%-0.4% HD	>1	Multi-centre / Retrospective cohort	Real time	243	953	Kudo PP
Wanders et al [152]	2015	Article	MB 0.1% or IC 0.3%	17	Multi-centre / Retrospective questionnaire	Image review	/	30	/
Hlavaty et al [109] ***	2011	Article	IC 0.4% SD	2	Single centre / Prospective cohort	Real time	30	100	Kudo PP
Confocal Laser Endomicroscopy									
Wanders et al [150] **	2016	Article	iCLE	>1	Multi-centre / Prospective cohort	Real time	61	60	Mainz criteria
Dlugosz et al [79]	2016	Article	pCLE	1 endoscopist (2 reviewed images)	Single centre / Retrospective cohort	Image review	69	644	Crypt + vessel architecture
Buchner et al [149] *	2016	Abstract	pCLE	/	Prospective cohort	Real time	22	20	Miami classification
Freire et al [113]	2014	Article	iCLE	1	Single centre / Randomised trial	Real time	72	104	Mainz criteria
Rispo et al [153]	2012	Article	pCLE	1	Single centre / Prospective cohort	Real time	51	15	De Palma classification
Shahid et al [154]	2011	Abstract	pCLE	3 reviewed images	Single centre / Retrospective cohort	Image review	25	61	/
Hlavaty et al [109] ***	2011	Article	iCLE	2	Single centre / Prospective cohort	Real time	30	68	Mainz classification
Van den broek et al [116] ****	2011	Article	pCLE	4 endoscopists (2 reviewing images)	Single centre / Retrospective cohort	Image review	22	48	Crypt + vessel architecture
Keisslich et al [67]	2007	Article	iCLE	/	Single centre / Randomised trial	Real time	80	134	Mainz classification
Magnification endoscopy									
Nishiyama et al [155]	2016	Article	NBI	5 reviewed images	Single centre / Retrospective cohort	Image review	27	33	Surface + vessel patterns
Van den broek et al [116] ****	2011	Article	NBI	4	Single centre / Prospective cohort	Real time	22	48	Kudo PP + vascular patterns
Van den broek et al [107]	2008	Article	NBI	3	Single centre / Randomised trial	Real time	50	98	Kudo PP

Matsumoto et al [156]	2007	Article	NBI	1	Single centre / Prospective cohort	Real time	46	296	Surface structure
Keisslich et al [64]	2003	Article	MB 0.1%	1	Single centre / Randomised trial	Real time	84	118	Kudo PP
Studies using combined technologies									
Bisschops et al [157]	2013	Abstract	Dye-based chromo/NBI	10 reviewed images	Multi-centre / Retrospective cohort	Image review	27	50	Kudo PP

Table 10 Study characteristics. List of studies included in meta-analysis and displayed according to technology type. PP, pit pattern; /, data missing; *Two different technologies from same abstract; ** Two different technologies from same article; Two different technologies from same article; ** Two different technologies from the same article**

4.3.3 Quality of assessment

The results for the study quality assessment using the QUADAS 2 tool are presented using stacked bar charts (Figure 15 and Figure 16), displaying risk of bias and applicability. Individual study quality assessment can be seen in Table 11. Results varied across the twenty-two studies. Abstracts predominantly scored “unclear” for domains associated with “risk of bias”, due to lack of in-depth information within the abstract. However, studies also scored “unclear” for “risk of bias” with regards “reference standard” if it did not clearly state if the histopathologist was blinded to the endoscopic diagnosis. Papers scoring “high” for “patient selection”, “index test” and “flow and timing” for “risk of bias”, were generally associated with retrospective image-captured studies which selected and reviewed only clear images of lesions, thereby introducing attrition bias. All studies scored “low” for all three domains with regards “applicability”.

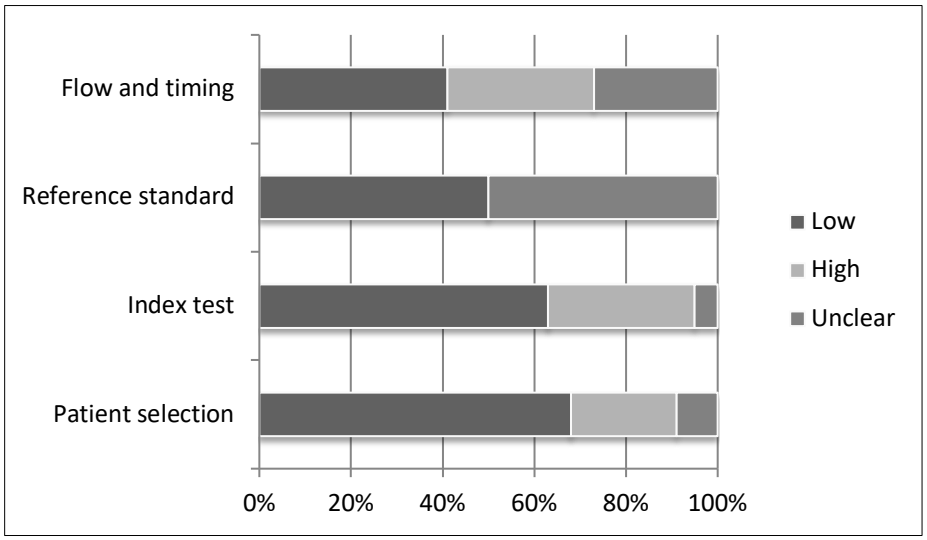


Figure 15 Stacked bar charts showing proportion of studies with low, high or unclear risk of bias. Vertical axis represents the four domains of the QUADAS 2

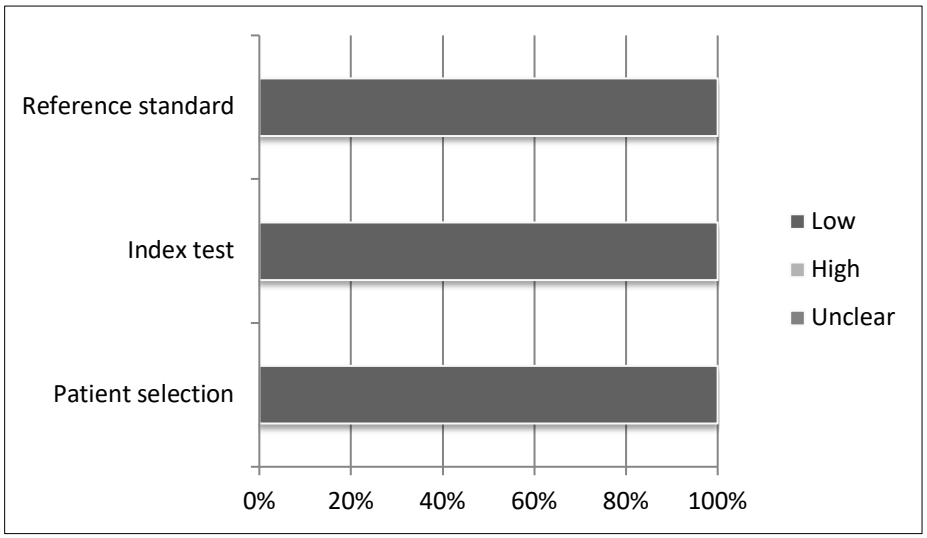


Figure 16 Stacked bar charts showing proportion of studies with low, high or unclear applicability. Vertical axis represents the three domains of the QUADAS 2.

	Cassinotti et al 2016 [22]	Efthymiou et al 2013 [21]	Van den broek et al 2011 [23]	Cassinotti et al 2015 [24]	Cassinotti et al 2015 [25]	Carballel et al 2016 [26]	Buchner et al 2016 [27]	Wanders et al 2016 [20]	Munoz et al 2016 [28]	Wanders et al 2015 [29]	Hlavaty et al 2011 [18]	Dlugosz et al 2016 [30]	Freire et al 2014 [31]	Rispo et al 2012 [32]	Shahid et al 2011 [33]	Van den broek et al 2011 [19]
DOMAIN 1 **Patient selection**																
Risk of bias																
Could selection of patients introduced bias?	L	L	L	L	L	L	U	L	L	H	U	L	L	L	H	H
Concerns regarding applicability																
Concern included patients don't match review question?	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
DOMAIN 2 **Index test**																
Risk of bias																
Conduct or interpretation of index test introduced bias?	L	L	L	L	L	L	L	L	U	H	H	H	L	L	H	H
Concerns regarding applicability																
Concern index test, its conduct or interpretation differs from review question?	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
DOMAIN 3 **Reference standard**																
Risk of bias																
Could reference standard, conduct or interpretation have introduced bias?	U	U	L	U	U	U	U	L	U	U	L	L	L	L	U	L
Concerns regarding applicability																
Concern target condition as defined by reference standard not match review question?	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
DOMAIN 4 **Flow and timing**																
Risk of bias																
Could patient flow introduced bias?	U	L	H	U	U	L	U	L	U	H	H	U	L	L	H	H

	Keisslich et al 2007 [34]	Nishiyama et al 2016 [35]	Van den Broek et al 2008 [36]	Matsumoto et al 2007 [37]	Keisslich et al 2003 [38]	Bisschops et al 2013 [39]
DOMAIN 1 **Patient selection**						
Risk of bias						
Could selection of patients introduced bias?	L	H	L	L	L	H
Concerns regarding applicability						
Concern included patients don't match review question?	L	L	L	L	L	L
DOMAIN 2 **Index test**						
Risk of bias						
Conduct or interpretation of index test introduced bias?	L	H	L	L	L	H
Concerns regarding applicability						
Concern index test, its conduct or interpretation differs from review question?	L	L	L	L	L	L
DOMAIN 3 **Reference standard**						
Risk of bias						
Could reference standard, conduct or interpretation have introduced bias?	L	U	L	L	L	U
Concerns regarding applicability						
Concern target condition as defined by reference standard not match review question?	L	L	L	L	L	L
DOMAIN 4 **Flow and timing**						
Risk of bias						
Could patient flow introduced bias?	L	H	L	L	L	H

Table 11 QUADAS 2 for each study. L, Low risk; H, High risk; U, Unclear

4.3.4 Pooled diagnostic accuracy results

A summary for the pooled diagnostic accuracy estimates for the different technologies and for the subgroup analysis are outlined in Table 12.

The meta-analysis for the five studies involving VCE showed it was fairly accurate at differentiating neoplastic from non-neoplastic lesions with a pooled sensitivity of 86% (95% CI 62%-95%), specificity of 87% (95% CI 72%-95%), and the area under the SROC curve was 0.93 (95% CI 0.90-0.95).

Pooled results of the six studies for DBC revealed the least accurate results for lesion characterisation, with a sensitivity of 67% (95% CI 44%-84%), specificity of 86% (95% CI 72%-94%) and an area under the SROC curve was 0.84 (95% CI 0.81-0.87). Most of the studies within this group were multi-centre with more than one endoscopist.

Results of the five studies for magnification endoscopy showed a pooled sensitivity of 90% (95% CI 77%-96%), specificity of 87% (95% CI 81%-91%), and an area under the SROC curve was 0.93 (95% CI 0.91-0.95). The results are similar to those of VCE. However, these were mainly single centre, single expert endoscopist studies.

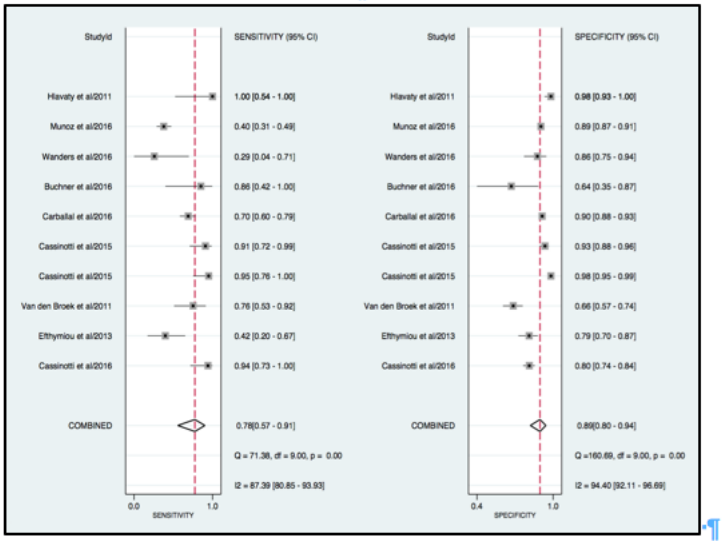
Meta-analysis of nine studies for CLE showed a sensitivity of 87% (95% CI 71%-95%), specificity of 94% (95% CI 87%-97%), with an area under the SROC curve of 0.96 (95% CI 0.94-0.97). Again, these are all single centre, single expert endoscopist studies.

Analysis Groups	No. of Studies	Pooled estimates (95% CI)		Likelihood Ratios (95% CI)		Diagnostic Odds Ratio (95% CI)	Area under SROC curve (95% CI)
		Sensitivity	**Specificity**	**LHR+**	**LHR-**	**DOR**	
All							
VCE	5	0.86 (0.62-0.95)	0.87 (0.72-0.95)	6.7 (2.6-17.8)	0.17 (0.05-0.53)	41 (6-297)	0.93 (0.90-0.95)
DBC	6	0.67 (0.44-0.84)	0.86 (0.72-0.94)	4.9 (2.1-11.3)	0.38 (0.20-0.73)	13 (3-48)	0.84 (0.81-0.87)
Magnification	5	0.90 (0.77-0.96)	0.87 (0.81-0.91)	7.0 (4.6-10.7)	0.11 (0.05-0.28)	62 (18-209)	0.93 (0.91-0.95)
CLE	9	0.87 (0.71-0.95)	0.94 (0.87-0.97)	14.0 (6.1-32.4)	0.14 (0.06-0.33)	101 (23-442)	0.96 (0.94-0.97)
Real-time							
Kudo PP	10	0.78 (0.57-0.91)	0.89 (0.80-0.94)	6.9 (3.5-13.5)	0.24 (0.11-0.55)	28 (7-110)	0.91 (0.89-0.94)
CLE	6	0.91 (0.66-0.98)	0.97 (0.94-0.98)	28.4 (13.6-59.1)	0.09 (0.02-0.43)	322 (41-2529)	0.98 (0.97-0.99)
All Kudo PP	12	0.78 (0.61-0.88)	0.86 (0.76-0.92)	5.5 (2.9-10.1)	0.26 (0.14-0.50)	21 (7-66)	0.89 (0.86-0.92)

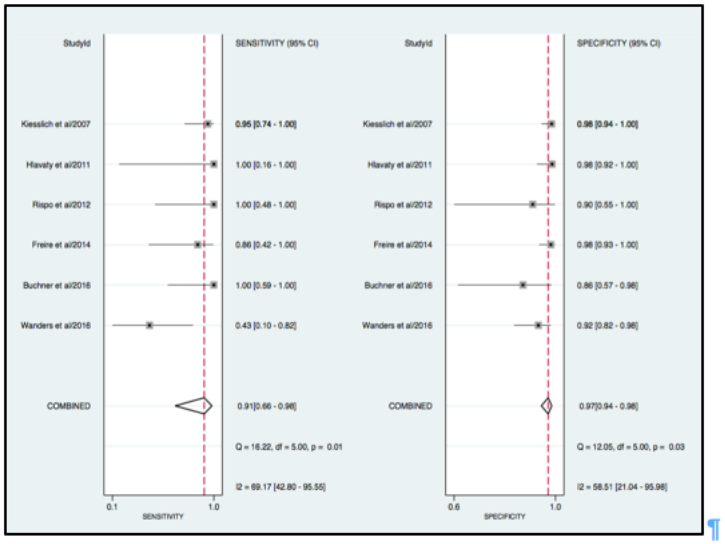
Table 12 Accuracy of the different technologies. All, using both real-time and image based studies for the different technologies. Real-time, sub-group analysis with studies using only real time Kudo pit pattern (both VCE and DBC) and real-time CLE. All Kudo pit pattern includes all studies using Kudo pit pattern (real-time and image-based).

A subgroup analysis was performed involving studies using real-time endoscopic mucosal characterisation of lesions, divided into real-time non-magnified Kudo pit pattern (with VCE and DBC) and real-time CLE. Both the forest plots and SROC curves for real-time non-magnified Kudo pit pattern and real-time CLE are given in Figure 17 and Figure 18. The subgroup for real-time Kudo pit pattern included ten studies, with a pooled estimate sensitivity of 78% (95% CI 57%-91%), specificity of 89% (95% CI 80%-94%), with an area under the SROC of 0.91 (95% CI 0.89-0.94). The subgroup analysis looking at real-time CLE included 6 studies. The pooled estimated sensitivity was 91% (95% CI 66%-98%), specificity was 97% (95% CI 94%-98%), and the area under the AUSROC was 0.98 (0.97-0.99).

A further subgroup analysis was performed looking at all (real-time and image review) non-magnified Kudo pit pattern. This included twelve studies. The pooled estimate sensitivity was 78% (95% CI 61%-88%), specificity of 86% (95% CI 76%-92%), and an area under the SROC of 0.89 (95% CI 0.86-0.92). This clearly shows some limitations when using kudo pit pattern classification within the IBD population, as the kudo classification was never designed to assess lesions within colitic patients, especially in the presence of inflammation or regenerative hyperplasia.



(a)



(b)

Figure 17 Forest plot for Real-time Kudo pit pattern; b, Forest plot for Real-time CLE

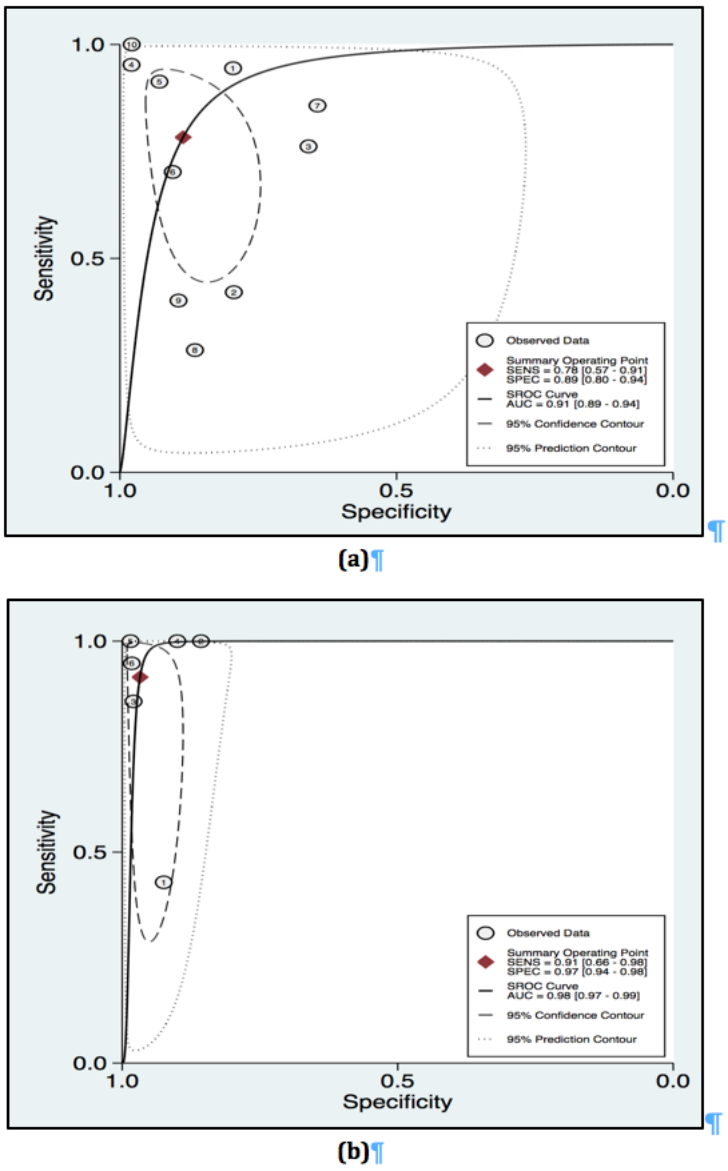


Figure 18 Area Under SROC curve for Real-time Kudo pit pattern; b, Area Under SROC curve for Real-time CLE

4.3.5 Test for heterogeneity

I^2 and Cochrane Q were used to test for heterogeneity. Heterogeneity for VCE was moderate to high with an I^2 = 63% (95% CI 16%-100%) and Q = 5.347 (p=0.034). DBC showed extremely high levels of heterogeneity between studies with an I^2 = 89% (95% CI 78%-100%) and Q = 18.573 (p=0.00). Magnification ((I^2 = 0 (95% CI 0%-100%) and Q = 0.607 (p=0.369)) and CLE ((I^2 = 40% (95% CI 0%-100%) and Q = 3.335 (p=0.094))) represented low levels of heterogeneity between studies, however had very broad 95% confidence intervals.

Real-time non-magnified Kudo pit pattern had an I^2 = 96% (95% CI 92-99) and Q = 45.575 (p<0.001) showing exceptionally high levels of heterogeneity. Real-time CLE studies had low levels of heterogeneity, with an I^2 = 0 (95% CI 0%-100%) and Q = 1.697 (p=0.214).

4.3.6 Publication Bias

Deeks et al [15] funnel plot, seen in Figure 19, was used to assess publication bias. The funnel plot has slope coefficient of 9.84 (P=0.194). The non-significant P valve would suggest a low likelihood of publication bias in this meta-analysis.

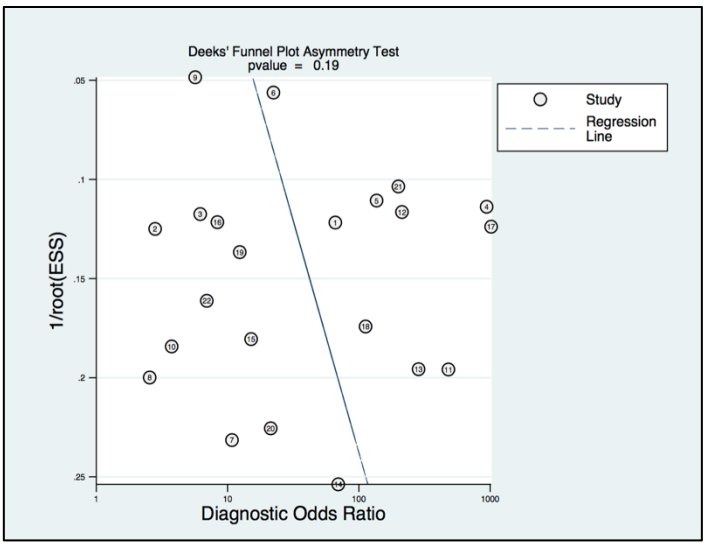


Figure 19 Funnel plot with superimposed regression line looking for publication bias

4.4 Discussion

Our meta-analysis illustrates that real-time CLE currently appears to be the best performing technology in performing in-vivo lesion characterisation in patients with colonic IBD, with an impressive AUSROC of 0.98 (95% CI 0.97-0.99). It demonstrates an extremely high specificity, 97% (95% CI 94%-98%), and sensitivity, 91% (95% CI 66%-98%), in differentiating neoplastic from non-neoplastic lesions. Using all study types (real-time and image capture) CLE again out-performs the other technologies, with an area under SROC cure of 0.96 (95% CI 0.94-0.97). Magnification and VCE technologies

also show a good accuracy with a SROC of 0.93 (95% CI 0.91-0.95) and 0.93 (95% CI 0.90-0.95), respectively.

Despite CLE being a highly accurate technology in lesion characterisation, there are several concerns with regards applicability. Most of the studies in our meta-analysis for CLE involved a single endoscopy operator within a single centre. They were vastly experienced in IBD surveillance endoscopy and in using CLE technology. Studies in which inexperienced operators used this technology, they themselves did not make real-time lesion diagnosis. Instead, people trained in the interpretation of the histology reviewed the images retrospectively. This is because CLE is not a routinely used modality. It requires expertise in handling, positioning of the colonoscope/probe onto the lesion and in analysing/interpreting in-vivo histology. Bowel preparation has to be meticulous, as any faecal material can interfere with image capture and lesion interrogation. This is unlikely to be achieved consistently during “real-life” surveillance lists. In one study, 32% of lesions were not accessible to CLE evaluation (109) and a second study, 1.5% of lesions the histology was not visualised by CLE (116). These unclassified lesions aren’t accounted for in the final results, contributing to attrition bias in the observed results. In addition, IV fluorescein injection is required before lesion analysis, further adding to procedure time. One study showed the mean additional time per procedure being 20 minutes. (150) Adoption of this technology in throughout less experienced centres is doubtful. It would demand vast resources for training, education and require new guidance for endoscopic competence.

A further concern with CLE was equipment failure. In one multi-centred study, four of the five centres had to send the equipment back to the manufactures as the lens on the endomicroscope broke. Repair took the teams months to address, significantly affecting recruitment, resulting in the study being underpowered. [150] With concerns over equipment failure, costs of purchasing the technology and repairs, CLE could in fact be a financial burden, negating any benefit obtained from the reduction in polypectomies and histological analysis. Therefore, questions still remain unanswered with regards practicalities and applicability for this technology.

VCE showed relatively good accuracy although fell short of reaching the 90% mark for sensitivity and specificity. One major limitation for this technology was the small number of studies for VCE technology. We therefore combined the NBI, FICE and i-scan to obtain pooled results. Although the technologies have been grouped as one, there are obvious differences in the way they achieve the modified image and the modes used with that technology. NBI endoscopes contain a rotating filter in front of the light source at the end of the endoscope, allowing a narrow wavelength of light to strike the mucosa resulting in image enhancement, whereas both FICE and i-scan use a post-processing technology

built within the processer to provide a coloured-enhanced image. There were several other drawbacks with the VCE group analysis. One study in our meta-analysis used the first generation NBI technology, resulting in images being less bright, undoubtedly having an impact on lesion characterisation when compared with newer generation technology(122). Three of the five studies for VCE were abstracts making critical analysis for the quality of these studies difficult to determine. From our results we cannot currently recommend using VCE solely as an accurate technology for lesion characterisation in IBD. However, with newer generation endoscopes, further evaluation is clearly warranted as these technologies continue to improve. In comparison with CLE, VCE is potentially less complicated to use, more robust, economical as they are almost universal in newer endoscope processors, and training is more likely to be attainable.

Magnification endoscopy achieves similar accuracy to VCE technology. However, in the majority of these studies magnification was used in combination with NBI, predominantly using older NBI technology. This makes it challenging to differentiate the two technologies. With new colonoscopes delivering digital magnification, like “near focus” technology, it is questionable the additional information optical magnification will provide. A threshold may be reached at which further magnification provides no additional benefit for differentiating neoplastic from non-neoplastic pit patterns. However, this meta-analysis cannot necessarily address that question.

DBC pooled results were suboptimal for lesion characterisation. However more than half of the studies used standard-definition colonoscopies, reducing image resolution, and therefore impacting on lesion interpretation. With most centres now using high-definition colonoscopies accuracy is likely to improve. Another confounding factor was that the majority of the studies were multi-centred, with multiple operators, undoubtedly accounting for a diverse range of endoscopic experience and therefore skill at lesion classification.

A subgroup analysis was performed in order to look for potential sources of heterogeneity and to determine whether it was the type of mucosal classification used that influenced the accuracy rather than the technology. Real-time studies were used as this provided the most clinically authentic evaluation of lesions and minimises bias as a result of photographic selection and time for analysis. Most studies used Kudo pit pattern or a variation on the Kudo pit pattern (Kudo pit pattern plus additional features) and therefore we pooled the results for both real-time VCE and DBC. Real-time Kudo pit pattern had an area under the SROC curve of 0.91 (95% CI 0.89-0.94), with a reasonable specificity of 89% (95% CI 80%-94%) but a poor sensitivity of 78% (95% CI 57%-91%). The poor sensitivity likely reflects inclusion of the DBC group with the majority involving standard-definition scopes. The use of Kudo pit pattern and Kudo pit pattern plus did not seem to

influence the accuracy of lesion characterisation, independent of the technology. Caution has to be noted for combining DBC and VCE using Kudo pit pattern as a mucosal classification system. Studies have shown a lack of pit pattern agreement between chromoendoscopy and NBI. [40] This has led to the adoption of new classification systems, such as NICE for NBI. [41] Further mucosal classification systems may need to be studied, especially for i-scan and FICE. However, determining the ideal post-processing mode for these software systems could be challenging as these technologies have multiple combination options of modes.

Another important issue that wasn't clearly stated for studies in this meta-analysis was the degree of mucosal inflammation in which the lesions resided. Varying degrees of mucosal inflammation unquestionably contribute to difficulties in pit pattern and vasculature interpretation and therefore diagnostic accuracy. Future studies looking at in-vivo lesion diagnostic accuracy could stratify patients depending on the degree of inflammation surrounding the lesions.

As with any meta-analysis there are limitations. The number of studies for each technology group was fairly limited, except for the CLE group. Seven of the twenty-two studies were abstracts introducing concerns with regards data extraction and interrogation for study validity.

Despite an extensive literature review, no papers had direct head-to-head studies, comparing the different technologies against each other. However, this would require a very large cohort looking specifically at lesion characterisation and all endoscopists participating being familiar with the different technologies. Endoscopic familiarity with certain technologies in such a study could potentially confound the accuracy of lesion interpretation.

In the majority of studies, lesion characterisation was a secondary outcome, therefore in some studies the number of lesions being characterised was small. Some studies didn't clearly state the TP, FP, FN and TN, therefore calculations had to be performed in order to achieve this.

There was also a large degree of heterogeneity within the VCE and DBC groups that was further increased when we performed real-time Kudo pit pattern assessment. Further areas of subgroup classification that were not explored within this meta-analysis were the number of endoscopists performing the procedures in each study and also whether it was a single centre or multi-centred study. This undoubtedly will have an impact on the accuracy of the technology being used. Single-centre, single endoscopist studies are more likely to achieve better results.

Suggested avenues to explore in future studies looking at in-vivo lesion characterization in colonic IBD include: accuracy according to varying endoscopic experience, accuracy dependent on the degree of surrounding mucosal inflammation, whether the endoscopist confidence (high or low) in lesion characterization impacts accuracy and exploring new mucosal lesion classification for different technologies.

In summary**, r**eal-time CLE appears to be currently the best commercially available technology at differentiating neoplastic from non-neoplastic lesions in patients with colonic IBD, with an area under the SROC of 0.98 (95% CI 97%-99%). However, most CLE studies were single centered and single expert users, which could significantly confound the results, and some studies not reporting non-interpretable images, contributing to attrition bias. Clinical applicability for this technology is likely to be a challenge. VCE technology performed well but currently cannot be recommended for in-vivo lesion characterization in such a high-risk group. However, with improved endoscopes and newer generation technologies further studies are required to assess their real-time performance in clinical settings with trained colonoscopist.

Chapter 5
Accuracy of Real Time In-vivo Lesion Assessment in IBD-colitis

5.1 Background

The current gold standard regarding dysplasia detection in patients with IBD-colitis is dye based chromoendoscopy (DBC)(30). The vast majority of dysplasia detected during surveillance colonoscopy is visible, therefore we are able to deliver targeted treatment to prevent progression along the pathway to cancer(106). Historically, dysplasia detected within patients with IBD-colitis resulted in colectomy for the vast majority of cases(158). Although this may offer a cure for patients with UC, it carries a small mortality risk and additionally can carry a much higher risk of short and long-term morbidity, despite advances in laparoscopic surgical techniques(159, 160). However, as diagnostic technology improved, alongside greater knowledge and understanding surrounding the management of dysplastic lesion in IBD-colitis, the paradigm shift moved towards endoscopic resection(161). For this to be effective, optical in-vivo lesion characterisation carries greater significance.

Present-day guidelines advocate endoscopic resection of lesions if they contain a clear circumferential border (clear demarcation between lesion and normal mucosa), and the endoscopist doesn't feel the lesion has advanced features, such as deep submucosal invasion(30). Additionally, dysplastic lesions can be more problematic to remove in patients with colitis. This is because lesions within colitic segments are known to have an appreciable risk of submucosal fibrosis, as a consequence of previous or ongoing inflammation, making lifting of the lesion by submucosal injection more challenging(162). Thereby, expert endoscopists skilled at therapeutic resection are normally best suited to tackle such lesions in patients with inflammatory bowel disease (IBD). Depending on the size and morphology of the lesion, advanced therapeutic options include endoscopic mucosal resection (EMR) or more advanced techniques such as endoscopic submucosal dissection (ESD)(163).

Once resection has been achieved, most GI societal guidelines recommend biopsies to be taken from around the defect to confirm no surrounding dysplasia has been left behind, which would confirm or refute successful resection (30). However, there is an ongoing debate amongst experts regarding the necessity of such biopsies in an era of high-definition endoscopic technology. A recent retrospective study looked specifically

at the proportion of biopsies showing dysplasia from surrounding dysplastic lesions. This study showed very low rates, with only 5% of all biopsies harbouring dysplasia. There were no adverse clinical consequences due to dysplasia positive biopsies from around the lesion in any of these patients(164). Further a retrospective single centre study showed dysplasia in adjacent biopsies in only 2 patients (0.7%) and was endoscopically visible on both occasions(165). This raises a pertinent question on whether surrounding biopsies are still required when using high definition chromoendoscopy.

Close endoscopic follow-up is strongly advocated following endoscopic resection of dysplasia and the intensity of this, certainly initially, is determined by the lesions risk and the technique deployed to remove this, with the ESD technique having a reduced risk of recurrence when compared with piecemeal EMR(162, 163). Contemporary data is supportive of endoscopic resection over colectomy for endoscopic resectable dysplasia. A recent meta-analysis looking at the subsequent incidence of CRC and/or HGD in patients who have undergone endoscopic resection of dysplasia showed the pooled risk was only 2 per 1000 person-years of follow- up and the risk of any type of dysplastic lesion being 43 per 1000 person-years follow-up(166). This data currently supports the resect and surveillance guidance set by international governing bodies(167).

Previous nomenclature described dysplastic lesions into two types: adenoma-like mass (ALM) or dysplasia-associated lesion or mass (DALM). These terms were based on historical data, whereby DALM was an atypical looking lesion, not clearly demarcated and different to its sporadic counterparts and were highly thought to contain cancer, therefore requiring surgical treatment(168). It was thought that ALM's represented sporadic dysplasia but within a colitic segment, with a similar morphology to that seen within non-colitic patients. These terms, however, were ambiguous, with no clear unifying definition (both endoscopically and histologically), resulting in confusion on how best to manage these lesions. This unclear, binary terminology was abandoned in 2015 and lesions are now classified according to if they are clearly demarcated or not(30, 167).

Following the relinquishment of such terminology, lesions should now be described in a standardised manner, similar to lesions in patients without colitis. This should include the morphology, size, location, pit patterns and if it's clearly demarcated. Ensuing this uniform approach, detailed interrogation will assist the endoscopist in making an in-vivo diagnosis to determine if the lesion is dysplastic or non-dysplastic and it's endoscopic resectability.

The morphology of a lesion has been standardised using the Paris classification(80). This classification system divides lesions into polypoid and non-polypoid, with further subclassifications, providing a standardised why of describing lesions and is informative in determining the risk of submucosal invasion(169, 170). Assessment of size is also an

important predictor of cancer being present, with larger lesions at more risk of harbouring adverse pathology(82). Larger size can also make endoscopic resection more challenging and may reduce the likelihood that it can be resected en bloc.

Kudo pit patterns are used to help differentiate dysplastic or non-dysplastic lesions with good accuracy using magnification endoscopy(62). These pit patterns are further enhanced by the application of contrast agents such as indigo carmine (IC). Type I pits display round pits; Type II pits are stellar pits; Type III-s pits are small roundish, tubular pits and Type III-L are tubular/elongated pits; Type IV pits appear as branch-like and Type V pits appear as non-structured pits. Type I and II represent benign or hyperplastic polyps, whereas pit pattern classes III-V are considered to show dysplasia, with 56% of Type V showing malignant changes(81). However, the assessment of these patterns can be challenging if inflammation present and its usefulness in patients with IBD-colitis is debateable.

Additionally, new technologies such as NBI provide optical enhancement by allowing better visualisation of blood vessels and mucosal patterns. This is achieved by a light filter placed at the distal end of the scope, allowing only a narrow wavelength of light to be emitted, executed by the press of a button on the endoscope. This narrow wavelength of light doesn't penetrate as deep and is also absorbed by the superficial blood vessel, permitting high detail of mucosal vessels and crypt patterns. Several classification systems have been studied in order to characterise lesions in-vivo to obtain an optical diagnosis when using NBI technology. One of the first and most frequently used system is called the NBI International Colorectal Endoscopic (NICE) classification(171). This allows description of the lesion colour, surface and vessels to differentiate lesions into hyperplastic, dysplastic or malignant and is used without the assistance of magnification. It has been shown to be highly accurate for in-vivo lesion characterisation in patients without IBD-colitis. However, its accuracy in real time optical diagnosis in colitis hasn't been well studied.

Therefore, as part of a randomised controlled study looking at detection of dysplasia in patients with colitis, we wished to determine the optical diagnostic accuracy of in-vivo lesion characterisation for any colonic lesions detected.

5.2 Methods

This was part of a randomised controlled study comparing dysplasia detection using 0.2% indigo carmine versus 0.03% indigo carmine at a single large tertiary centre at Leeds Teaching Hospitals, United Kingdom (UK). This trial received National Research

Ethics Committee approval (17/EM/0033) and local NHS R&D approval, along with registration at ClinicalTrials.gov NCT03250780.

Consecutive eligible patients undergoing IBD surveillance were enrolled from March 2017 through to March 2019. Eligibility for enrolment included patients aged ≥18 years, had at least 8-10 years of IBD-colitis or coexistent PSC, extensive colitis (Montreal classification E3), or if Crohn's colitis >50% of the colon affected. Exclusion criteria included patients with only left sided colitis, proctitis or in the case of Crohn's colitis <50% of the colon affected, poor bowel preparation, active disease rendering examination difficult, pregnancy, extensive colonic surgery, inability to complete the colonoscopy or didn't consent to enrolment in the trial.

Patients were enrolled into the trial following an informed discussion either during IBD clinic consultation or on the day of the procedure at the time of consent, along with providing a patient information leaflet. Patients were randomised at the start of the procedure by an independent coordinator blinded to the patient's history. Patients would be randomised into one of two indigo carmine concentrations according to a computer-generated number sequence, with even numbers equating to the 0.2% concentration with spray catheter or odd numbers representing the 0.03% concentration using the foot pump.

5.2.1 The Procedure

Colonoscopy was performed by three JAG accredited doctors with varying degrees of chromoendoscopy experience (two consultants and one senior registrar; VS, RS and RL), all of whom had training in chromoendoscopy technique and colonic lesion characterisation, including narrow band imaging (NBI). All procedures were performed using the Olympus CF-HQ290L/I or PCF-H290 colonoscopes with the CV-90 Evis Lucera Elite Processors (Olympus, Tokyo, Japan). Standard bowel preparation was used, which at Leeds Teaching Hospitals was PEG solution (Moviprep, Norgine) with split dosing. Bowel preparation was assessed using the Boston Bowel Preparation Score (BBPS)(133). Procedures were performed with patients either opting for Entonox, conscious sedation (fentanyl +/- midazolam) or both, which is standard practice at Leeds.

Standard intubation to the caecum was achieved with minimal air insufflation and meticulous washing in order to achieve optimal views before withdrawal. On reaching the caecum chromoendoscopy would commence with the corresponding dye. For the 0.2% arm, one ampoule (5mls of 0.8% indigo carmine) would be diluted with 15mls of sterile water (normally at least four aliquots prepared) and placed in a 50ml syringe and inserted into a dilator gun and attached to a spray catheter (MTW, Endoskopie Manufaktur, Germany) and the dye sprayed circumferentially on withdrawal. The 0.03%

concentration would involve mixing two ampoules of 0.8% indigo carmine (total of 10mls) with 250mls of water in a bottle and connecting this to the foot pump at the time of reaching the caecum, exchanging with the normal water bottle used to wash during insertion, and then aimed at the opposing wall to that of the gravity dependent side, allowing circumferential coverage.

5.2.2 Endoscopic characterisation of lesions

Following segmental dye application, any lesions seen were endoscopically scrutinised in-vivo by the endoscopist performing the procedure. Descriptions of all lesions were recorded on a data sheet which included: lesion size (mm), segmental location, the morphology using the Paris classification and if the lesion was circumscribed (23). Lesions would contain a covering of IC, with a dye concentration corresponding to the arm which they had been randomised into; 0.2% or 0.03%. Optical magnification was then used to assess Kudo pit patterns and recorded. Lesions were then subdivided into a binary classification of either non-neoplastic (type I and II crypts) or neoplastic (type III-V crypts) and recorded(62). NICE classification (colour, vessels, surface pattern), using Narrow band imaging (NBI), was also recorded to differentiate lesions into non-neoplastic (type 1), neoplastic (type 2) and invasive cancer (type 3)(171). Any serrated lesions were classified into non-neoplastic if they had Kudo pit patterns I and II or NICE type I and neoplastic if they had some features consistent with Kudo pit pattern III-V or NICE type 2. At the time of the procedure, the endoscopist was asked to make a prediction on whether they deemed the lesion to be non-neoplastic or neoplastic based on the above criteria. These predictions were compared with the final histology report (gold standard) to determine accuracy. The type of endoscopic treatment was recorded which included biopsy, cold snare or EMR. Surrounding biopsies were also obtained, and results recorded to determine if any dysplasia was detected in the adjacent mucosa.

5.2.3 Histology

All specimens were fixed in 10% formalin and embedded in paraffin, serially sectioned, and stained with haematoxylin and eosin (H&E). Histological classification was determined according to the revised Vienna classifications into non-neoplastic or neoplastic described as indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, or invasive neoplasia(136). However, histology defined as “indefinite for dysplasia” was not considered neoplastic for trial purposes. Tissue samples were processed as standard procedure and reviewed by an expert tertiary centred gastrointestinal histopathologist based locally, who was blinded to the randomisation. Any dysplasia detected would be reviewed by a second expert GI histopathologist as standard clinical practice.

5.2.4 Outcomes

The primary outcome of this study was to determine the real-time optical accuracy using kudo pit pattern and NICE classification, for differentiating dysplastic from non-dysplastic lesions, in patients with IBD-colitis. For this a 2x2 contingency table looking at sensitivity, specificity, NPV and PPV comparing endoscopic real-time diagnosis with that of the gold standard, histopathological diagnosis. A subgroup analysis was performed to determine whether the concentration of indigo-carmine dye used affected it accuracy of lesion characterisation.

5.2.5 Statistical Analysis

Continuous variables were summarised by using median values and associated inter-quartal range (IQR). Categorical variables were summarised using frequency and percentages. Contingency tables were used to determine the sensitivity, specificity, and accuracy of Kudo pit pattern and NICE classification for characterising dysplasia by comparing the assessment of optical assessment and histopathologic biopsies taken from the same location. All statistical analysis was performed using SPSS version 24 (IBM Corp).

5.3 Results

A total of 300 patients were included in the study, 150 patients randomised into the 0.2% IC spray arm and 150 patients in the 0.03% foot-pump arm. Targeted neoplasia was detected in 58 (19.3%) procedures within the study, including 32 (21.3%) procedures in the 0.2% arm and 26 (17.3%) procedures in the 0.03% arm; p=0.465. A total of 311 lesions were detected and interrogated. Eighty-five of the 311 lesions harboured dysplasia resulting in a dysplasia detection yield of 27.3%. Forty-nine were found in the 0.2% arm and 36 in the 0.03% arm; p=0.373. Full descriptions of lesion morphology, colonic location and size, according to dye concentration, can be seen within Table 13. Of the 49 neoplastic lesions in the 0.2% arm all had LGD, 5 of which were sessile serrated lesions (SSL) with dysplasia. In the 0.03% arm, 36 neoplastic lesions were detected, of which 35 lesions had LGD (one being an SSL) and one lesion having HGD. Additionally, within each arm there was no difference regarding the degree of background inflammation, as seen by the Mayo endoscopic score and SES-CD.

	0.2% IC (n=49)	**0.03% IC (n=36)**
Paris classification of dysplastic lesions		
Ip	1 (2.0%)	0
Isp	3 (6.1%)	3 (8.3%)
Is	30 (61.1%)	18 (50.0%)
IIa	14 (28.6%)	14 (38.9%)
LST G	1 (2.0%)	1 (2.8%)
Location of dysplastic lesions		
Left sided	11 (22.4%)	17 (47.2%)
Transverse	13 (26.5%)	13 (36.1)
Right sided	25 (51.1%)	6 (16.7%)
Median size of dysplastic lesions (range)	4 mm (2 – 30 mm)	3 mm (1 – 25 mm)
Mayo score	0 (IQR 0 – 1)	0 (IQR 0 – 1)
SES-CD	0 (IQR 0 – 3)	0 (IQR 0 – 3)

Table 13 Baseline endoscopic descriptions of dysplastic lesions detected differentiated according to the dye concentration. LST G, laterally spreading tumours granular

5.3.1 Accuracy using optical diagnosis

When using both kudo pit pattern and NBI (using the NICE classification), investigators accurately classified 72/85 neoplastic lesions, achieving a sensitivity of 84.7% (95% CI 76.5 – 90.8%) (see Table 14). For non-neoplastic lesions, endoscopist's accurately classified 186/226 lesions; specificity of 82.3% (95% CI 79.2 – 84.6%). The negative predictive value was high at 93.5% (95% CI 90.0 – 96.1%).

	Histological diagnosis		
Endoscopic diagnosis	Neoplastic	Non-neoplastic	
Neoplastic	72	40	PPV = 64.3% (95% CI 58.1 – 68.9%)
Non-neoplastic	13	186	NPV = 93.5% (95% CI 90.0 – 96.1%)
	Sensitivity = 84.7% (95% CI 76.5 – 90.8%)	Specificity = 82.3% (95% CI 79.2 – 84.6%)	

Table 14 A 2x2 table displaying the combined (both 0.2% and 0.03% IC arms) overall accuracy of in-vivo endoscopic diagnosis using kudo pit pattern and NICE classification, compared with the gold standard histological diagnosis. PPV, positive predictive value. NPV, negative predictive value.

When looking at in-vivo lesion diagnosis comparing the two dye concentrations applied, the sensitivity when using 0.2% indigo carmine was 89.8% (95% CI 76.9 – 96.2%), compared with 77.8% (95% CI 60.4 – 89.3%), using 0.03% IC. Specificity also favoured the 0.2% arm showing an 84.4% (95% CI 77.4 – 89.6%) versus 77.1% (95% CI 65.3 – 86.0%) difference. (Table 15 and Table 16). A high NPV was also superior in the lesions assessed by the 0.2% IC; 96.3% (95% CI 91.1 – 98.6%) versus 87.1% (95% CI 75.6 – 93.9%). Although all results had overlapping confidence intervals.

	Histological diagnosis		
Endoscopic diagnosis	Neoplastic	Non-neoplastic	
Neoplastic	44	24	PPV = 65.0% (95% CI 52.1 – 75.6%)
Non-neoplastic	5	130	NPV = 96.3% (95% CI 91.1 – 98.6%)
	Sensitivity = 89.8% (95% CI 76.9 – 96.2%)	Specificity = 84.4% (95% CI 77.4 – 89.6%)	

Table 15 A 2x2 table displaying the accuracy using 0.2% indigo carmine for in-vivo endoscopic diagnosis using kudo pit pattern and NICE classification, compared with the gold standard histological diagnosis. PPV, positive predictive value. NPV, negative predictive value.

	Histological diagnosis		
Endoscopic diagnosis	Neoplastic	Non-neoplastic	
Neoplastic	28	16	PPV = 63.6% (95% CI 47.7 – 77.2%)
Non-neoplastic	8	54	NPV = 87.1% (95% CI 75.6 – 93.9%)
	Sensitivity = 77.8% (95% CI 60.4 – 89.3%)	Specificity 77.1% (95% CI 65.3 – 86.0%)	

Table 16 A 2x2 table displaying the accuracy using 0.03% indigo carmine for in-vivo endoscopic diagnosis using kudo pit pattern and NICE classification, compared with the gold standard histological diagnosis. PPV, positive predictive value. NPV, negative predictive value.

We then assessed the biopsies of the surrounding mucosa which were taken when any lesion was resected. A total of 643 biopsies were taken from surrounding areas of lesions resected endoscopically. Of the 204 biopsies taken from the mucosa surrounding dysplastic lesions only, following endoscopic resection, none of the biopsies contained any grade of dysplasia.

5.4 Discussion

Numerous studies have attempted to translate the success of in-vivo lesion characterisation seen in patients without colitis, to those lesions found within colitis. The ability to discriminate between neoplastic and non-neoplastic provides additional challenges within patients with colitis, as a consequence of overlying inflammation disrupting interpretation of mucosal pit and vascular patterns. A recent meta-analysis looking at in-vivo lesion characterization using different technologies showed that virtual chromoendoscopy (VCE) (pooling NBI, FICE and I-scan technologies) had a sensitivity of 86% and specificity of 87%, whilst dye-based chromoendoscopy (DBC) had a sensitivity of 67% and specificity of 86%(126). Subgroup analysis when looking purely at real-time non-magnified Kudo pit pattern showed a sensitivity of 78% and specificity of 89%. However, the DBC group and real-time kudo pit pattern also included groups with standard-definition as well as high-definition technology, likely accounting for the reduced levels of accuracy.

Within this study we used high-definition endoscopy with optical magnification to assess Kudo pit patterns assisted by the corresponding dye concentration, combined with NBI

using NICE classification in order to give an in-vivo lesion diagnosis of neoplastic versus non-neoplastic. The diagnostic accuracy for in-vivo lesion characterisation when looking at the whole cohort showed a sensitivity of 84.7% (95% CI 76.5 – 90.8%) and specificity of 82.3% (95% CI 79.2 – 84.6%), similar to findings within the meta-analysis for pooled VCE (126). Interestingly, when the accuracy of in-vivo lesion characterisation was split according to the dye concentration used, accuracy favoured the more concentrated 0.2% indigo carmine compared with 0.03% concentration; sensitivity 89.8% vs 77.8% and specificity 84.4% vs 77.1%.

An explanation for why greater accuracy was achieved when using the 0.2% dye compared with the 0.03%, is likely related to the more concentrated dye assisting in Kudo pit pattern interpretation. Of course, when Kudo initially identified pit patterns with the use of magnified endoscopy and dye application, they used concentrations of 0.4% indigo carmine(62). The dye pools within the lesions crypts and therefore delineates the type of pit pattern more clearly, providing greater contrast with that of the normal surrounding mucosal patterns, assisting in its interpretation. Therefore, the only different variable between these two arms was the concentration of dye applied. This evidence would seem to favour applying more concentrated IC when attempting to accurately classify lesions in colitic segments. It also suggests that dual modality assessment of lesions provides superior accuracy to that of solely using NBI NICE classification. Furthermore, when looking at the background inflammation within the two arms, there was no difference between the two, with scores being comparable, excluding this as a confounding variable for this disparity.

A recent meta-analysis by the ASGE group looked at novel technologies to allow a “diagnose and leave” strategy for diminutive (<5mm) recto-sigmoid polyps(143). To achieve a “diagnose and leave” strategy, (a decision to leave in-situ diminutive rectosigmoid polyps), the technology had to achieve >90% NPV for adenomatous histology. Our study achieved an overall NPV value of 93.5% and when using the 0.2% concentration, this increased further to 96.3%. Although, the results in our study incorporated all size and location of lesions throughout the colon, not just left sided diminutive (<5mm) lesions. This data would further evidence to the current AGA recommendations for “diagnose and leave” however, in such a high-risk population, this approach wouldn’t be without additional risk. The small proportion of lesions incorrectly classified as non-dysplastic could potentially lead to placement in the wrong risk stratification group, with extended surveillance intervals. In theory, incorrectly characterised dysplastic lesions could undergo an accelerated transformation to CRC, as recognised in patients with colitis. This delayed subsequent surveillance could result in detrimental consequences for the individual.

There is an ongoing debate amongst experts regarding whether biopsies surrounding the resection margin of lesions are still warranted in an era of high-definition endoscopy. When these biopsies are being performed, ambiguity exists amongst endoscopists about how many should be taken and how close to the resection margin these should be. Data from recent studies, pertaining to adjacent biopsies surrounding the resection margin of lesions, would suggest the yield for detecting dysplasia is extremely low(164, 165). Our study further adds weight to the argument that biopsies adjacent to lesions may not be required, with no patients having any surrounding biopsies positive for dysplasia. However, all the lesions that were resected in this study were well circumscribed and clearly demarcated, making pre-resection risk for surrounding dysplasia exceedingly unlikely. Adjacent biopsies may still have a role in those lesions were a clearly demarcated border is uncertain, in order to confirm complete resection has taken place. Future prospective studies may look at the yield of taking surrounding biopsies of resected dysplastic lesions, comparing if the endoscopist has high or low confidence regarding complete resection and whether the lesion is clearly demarcated. It might be possible that in lesions were the endoscopist has low confidence (highly likely in those lesions which are poorly demarcated), that surrounding dysplasia may still be found and therefore biopsies would be warranted in these specific cases only.

There are several limitations concerning this study. Firstly, this is a single centre study design at a tertiary centre and therefore its external validity, regarding extrapolation of findings to other centres, will always be under scrutiny. However, our findings are in keeping with other contemporary studies investigating similar outcomes, and if anything, more robust than a lot of these studies looking at similar outcomes; as this study was part of a large randomised controlled study, rather than being purely observational. We also followed strict international guidelines on performing surveillance colonoscopy and used world renowned endoscopic classification systems in order to classify lesions.

Despite being a large study, with a high yield of dysplastic lesions detected and characterised, it will not capture all the diverse types of dysplastic lesions seen within colitic patients. Within this study only one lesion contained HGD and no CRC was detected. This may be explained by previous good quality surveillance in our centre with endoscopic resection of all dysplastic lesions early before they have a chance to become advanced. Therefore, the vast majority of dysplastic lesions contained LGD only. A drawback when looking at characterising lesions using the NICE classification is its ability to discriminate between LGD and HGD/submucosal invasion. A more apt classification system for doing this is the JNET classifications which allows the endoscopist the ability for further subclassify dysplastic lesions into LGD or HGD. In future studies this is likely to replace the NICE classification system, as being able to differentiate the two can alter the management of such lesions, with ESD a better option for lesions showing HGD.

Future studies should look at the accuracy of subclassifying dysplastic lesions using the JNET system in patients with colitis. Another shortcoming of the NICE classification is its inability to accurately classify serrated lesions. SSLs (with and without dysplasia) and hyperplastic polyps were found in both arms within this study. Of which, two patients fulfilled the criteria for the Serrated polyposis syndrome. Within this study, SSLs without kudo pit pattern and NICE features of dysplasia were classified as non-dysplastic lesions, downplaying their significance. Such lesions are not necessarily benign entities and should be differentiated from hyperplastic polyps. In retrospect, the WASP classification would have been a useful additional tool to better characterise such lesions and determine the accuracy in patients with IBD-colitis.

Our study included three endoscopists with differing experience, ranging from a senior gastroenterology consultant, a newly assigned consultant and a senior registrar. All had experience in lesion characterisation and the use of kudo pit pattern interpretation and NBI prior to commencing this study. Therefore, I believe this is a real strength rather than a limitation that high accuracy of in-vivo lesion characterisation can be achieved in endoscopists with a varying array of experience. This provides confidence for endoscopists who perform surveillance procedures, providing they've been trained in kudo pit pattern recognition and NBI, that they can achieve high levels of accuracy, rather than studies that contain single world leading experts as seen in most other previous studies looking at lesion classification.

As already discussed, we have shown that optical characterisation of lesions can be attained with high accuracy but highlighted potential consequences of miss classification. This data provides further evidence that a trained endoscopist in lesion characterisation can identify dysplastic lesions with excellent accuracy, allowing the correct modality regarding en bloc resection (EMR or ESD) for those lesions identified as dysplastic. Furthermore, in patients with multiple pseudopolyps, attempted biopsy of all such lesions is time consuming and costly. With this data revealing a high NPV, such lesions can be optically diagnosed as benign with high accuracy and therefore no biopsies are required.

Currently, studies are now looking at artificial intelligence (AI) for both the detection and characterisation of colonic lesions. AI uses deep learning neural networks to provide in-vivo real-time diagnosis of colonic lesions. Multiple different AI technologies are being investigated, with the aim of delivering further assistance to the endoscopist in order to provide a quick and highly accurate ability to classify lesions. Currently these devices are being trialled in bowel cancer screening and not in patients with inflammatory bowel disease. However, it is likely some time before this is adopted for patients with colitis due to the aforementioned nuances.

In summary, in-vivo lesion optical characterisation used by trained endoscopists in patients with quiescent colitis, using a combination of both Kudo pit pattern and NICE classification, has good accuracy which is further enhanced on applying a more concentrated dye (0.2% IC). For lesions that are well demarcated, surrounding biopsies are not required due to their very low dysplasia yield. In the future, the addition of advanced technologies such as AI have the potential to further improve the accuracy of optical diagnosis however, the adoption of “diagnose and leave” or “resect and discard” in such high risk populations would be risky practice and may not sit comfortably with the endoscopist or the patient. Further studies should be done to assess the level of risk that is acceptable to patients and endoscopists who will be performing these procedures before these are adopted in clinical practice.

Chapter 6
Discussion

The risk of CRC is increased in patients with colonic IBD(16, 96). As with other chronic inflammatory conditions, this is believed to arise along an inflammation-dysplasia-cancer pathway. Therefore detecting dysplasia is vital in order to prevent progression to CRC and the reason why patients are recommended to undertake regular surveillance colonoscopy. The majority of dysplasia is endoscopically visible and current guidelines recommend DBC as the prime technique. However, no studies have compared the differing concentrations of dye applied as to whether this alters the yield of dysplasia detected, and if random biopsies are still required when using such advanced technology. This was one of the future research questions proposed by the SCENIC group(30).

Initially a systematic review and meta-analysis was performed in order to determine what proportion of patients had invisible dysplasia only and looked to see if this was influenced by the type of technology used or the perceived cohort risk for dysplasia. Overall, I found that 13.05% (95% CI 7.28 – 19.87%) of patients with dysplasia had this identified by random biopsies alone, confirming that random biopsies are still important. However, the pooled proportion for random biopsies positive for dysplasia was 0.13% (95% CI 0.08 – 0.18%), indicating that a lot of biopsies are required to identify a very small proportion of invisible dysplasia. When using HDCE this proportion was much smaller, suggesting that when using other technologies, what was labelled as invisible dysplasia, may have in fact been missed visible dysplasia. Further, a subgroup analysis was performed to attempt to identify high risk cohorts (extensive colitis, PSC and/or previous dysplasia) known previously to inherit a higher yield for invisible dysplasia. The pooled proportion of patients with dysplasia identified by random biopsy alone within the high-risk group was more than double when compared with its perceived low-risk group. It is highly plausible that within the high risk cohorts, patients with invisible dysplasia had these known associated risk factors however, prospective data was required to confirm this.

A second systematic review and meta-analysis looked at in-vivo lesion characterisation using different technologies in patients with IBD-colitis. On pooling data, CLE was superior however this is costly, time consuming and performed solely by experts trained in such technology. Therefore this is not transferable outside expert centres. DBC was the least accurate but this may be related to the use of older endoscopic systems, non-expert endoscopists and also the differing classification systems used when assessing lesions. Clearly a contemporary study looking to address this was required.

Chapters 3 and 5 have attempted to answer these questions. Chapter 3 describes the results from a large single-centred RCT comparing dysplasia detection for the two most commonly used dye concentrations of IC (0.03% via a foot pump and 0.2% via a spray catheter), using different delivery modalities. Around 4% more procedures detected visible dysplasia using the more concentrated 0.2% dye, showing a clear numerical and potential clinical advantage, although this did not reach statistical significance. Applying 0.2% indigo carmine dye when assessing lesions, using the Kudo pit pattern along with the NICE classification system, greater accuracy was achieved at characterising dysplastic from non-dysplastic lesions, compared to the 0.03% dye. The likely explanation relates to the concentrated dye, which delineates Kudo pit patterns more clearly. To summarise these findings, it would seem using the 0.2% IC delivered by a spray catheter is likely better for both detection and characterisation, although its drawbacks include being more time consuming and more expensive (greater dye volume used and additional costs of the spray catheter).

Additionally, 3% of patients in the entire cohort were found to have invisible dysplasia only. In order to further identify such patients, I looked at assessing the three BSG risk categories against the risk of harbouring invisible dysplasia. Interestingly, when stratifying patients with random biopsy only dysplasia according to BSG high-risk versus non-high-risk groups (intermediate and low), I discovered a sensitivity of 90% and a NPV of 99.5% in predicting patients who had endoscopically invisible dysplasia. Thereby, it could be assumed that taking no random biopsies for invisible dysplasia, in patients of low and intermediate BSG risk, is perhaps clinically acceptable due to the high NPV for detecting endoscopically inviable dysplasia in this patient cohort. Further, these results would suggest that biopsies for invisible dysplasia are potentially most indicated for patients classed as high-risk by current BSG guidelines, even when using high definition, dye-based technologies for surveillance colonoscopy in this patient cohort.

6.1 Future Research

Within the next decade, studies looking at artificial intelligence (AI) are likely to be rolled out, assessing the accuracy of detection and characterisation of dysplasia within IBD-colitis. Multiple studies have been performed in non IBD cohorts and have been shown to be highly accurate(172, 173, 176). However, this will be significantly more challenging for patients with IBD-colitis due to the presence of background inflammation, the differing morphology of lesions and also the ability to detect invisible dysplasia.

However, I believe future research should aim to focus on ways to accurately identify patients that are predisposed to developing dysplasia. This would provide a more

individualised risk stratification that would enable higher intensity surveillance to patients who are at higher risk. Benefits would also include patients having a clearer understanding of their own risk, allowing a more informed decision on whether they wish to undertake surveillance. Endoscopic resources could be then targeted at those identified as at higher risk and theoretically may lead to earlier diagnosis with better outcomes. Complications in those with no risk would be significantly reduced. From a societal point of view, it should result in reduced costs as a consequence of better utilisation of resources.

Areas of development for an individualised risk stratification approach would include searching for potential tissue biomarkers. Identification of patients with the “field effect” maybe one method. Taking biopsies in an attempt to look for molecular changes within cells that are compatible with this pre-dysplastic “field cancerisation” is one possible way(177). Less invasive ways to risk stratify patients are being explored. Future studies may look at biomarkers such as classifying specific colonic gut microbiota, identifying specific “dysbiosis” which may predict the risk or presence of dysplasia(178). This may provide an individual “faecal microbial signature” with regards risk. Volatile organic compounds (VOCs) are gases released from biological tissue and can be found within faeces. Certain VOCs in the future may be associated with IBD-colitis dysplasia, further providing another non-invasive way of risk stratifying individuals(179).

In summary, DBC with high definition colonoscopy is an accurate way of identifying dysplasia within IBD-colitis, with its ability to detect and characterise lesions numerically favouring the more concentrated dye. Endoscopically invisible dysplasia does exist, with the vast majority residing in those stratified as the BSG high risk group. Future studies should look to concentrate on precise personalised risk profiling, likely using non-invasive multivariable models, allowing better utilisation of resources and providing accurate identification of those individuals at greatest risk.

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Appendix

A. Appendix: Documentation of ethical approval for RCT. Also included are consent form, patient invitation letter, GP letter and patient information leaflet.

NHS
Health Research Authority

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16 March 2017

Dear Dr Subramanian

Letter of HRA Approval

Study title:	**Chromoendoscopy with 0.03% indigo-carmine delivered via a foot pump compared with 0.2% indigo-carmine delivered via spray catheter for detecting dysplasia in patients undergoing surveillance in inflammatory bowel disease. A randomized control trial.**
IRAS project ID:	**218555**
REC reference:	**17/EM/0033**
Sponsor	**Research and Innovation Department**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

IRAS project ID	218555

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators"*, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the ***After Ethical Review*** document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

IRAS project ID	218555

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **218555**. Please quote this on all correspondence.

Yours sincerely

Dr Claire Cole
Senior Assessor

Email: hra.approval@nhs.net

Copy to: *Anne Gowing, Research and Innovation Department*

IRAS project ID	218555

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	*Version*	*Date*
Covering letter on headed paper [Cover sheet]	1.0	20 December 2016
Covering letter on headed paper [Cover sheet]	version 2.0	20 January 2017
GP/consultant information sheets or letters [GP information sheet]	version 1.0	19 December 2016
IRAS Application Form [IRAS_Form_22122016]		22 December 2016
Letters of invitation to participant [Patient invitation letter:patient copy]	version 2.0	15 January 2017
Letters of invitation to participant [Patient invitation: site notes]	version 2.0	15 January 2017
Letters of invitation to participant [Patient invitation: medical notes]	version 2.0	15 January 2017
Participant consent form [Consent form: medical notes]	version 2.0	15 January 2017
Participant consent form [Consent form: patient copy]	version2.0	15 January 2017
Participant consent form [Consent form: site notes]	version 2.0	15 January 2017
Participant information sheet (PIS) [Patient information leaflet: patient copy]	version 2.0	15 January 2017
Participant information sheet (PIS) [Patient information sheet: medical notes]	version 2.0	15 January 2017
Research protocol or project proposal [Research protocol]	version 2.0	15 January 2017
Summary CV for Chief Investigator (CI) [Chief investigator's CV]	1.0	20 December 2016
Summary CV for student [Principal investigator CV]	1.0	20 December 2016
Summary CV for student [Dr Noor Mohammed CV]	1.0	20 December 2016
Summary CV for student [Dr Nick Burr CV]	1.0	20 December 2016
Summary CV for supervisor (student research) [Dr Peter Culmer CV]	1.0	20 December 2016
Summary CV for supervisor (student research) [Prof Animesh Jha]	1.0	20 December 2016

IRAS project ID	218555

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards?	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	No agreement is expected as the participating NHS organisation is also the study sponsor.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	No funding transfer arrangements are expected.
5.1	Compliance with the Data Protection Act and data	Yes	No comments

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Section	HRA Assessment Criteria	Compliant with Standards?	Comments
	security issues assessed		
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is one site in this study which is also the study sponsor. All study activities will take place at site. The Chief Investigator should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website. If Chief Investigators, sponsors or Principal Investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the Chief Investigator, sponsor or Principal Investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

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Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.
This is a single site study sponsored by the site. The R&D office will confirm to the CI when the study can start.

Principal Investigator Suitability

This confirms whether the sponsor's position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A PI is expected for this study and this will be the CI. GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken.
Local staff who have a contractual relationship with the organisation will undertake the expected activities, therefore no honorary research contracts or letters of access are expected for this study.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England in study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

Researchers: Dr Venkat Subramanian
Dr Richard Lord
Dr Noor Mohammed
Dr Nick Burr

CONSENT FORM

Chromoendoscopy with 0.03% indigo-carmine delivered via a foot pump compared with 0.2% indigo-carmine delivered via spray catheter in detecting dysplasia for patients undergoing surveillance in Inflammatory Bowel Disease.

Name: _____ **D.O.B** _____

Patient's Identification No.: _____

Please initial to confirm

1. I have read the information sheet for the above study. _____
2. I understand that relevant sections of medical records/data collected during the study may be looked at by individuals from the University of Leeds, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. _____
3. I have had the opportunity to ask questions about the study, and to discuss it with family and friends. _____
4. I understand the purpose of the study, and how I will be involved. _____
5. I understand, and accept, that if I take part in the study I will not gain any direct, personal benefit from it. _____
6. I understand, and accept, that as is explained in the information sheet the procedures, which will be carried out, may possibly have some side effects. _____
7. I understand that all information collected in the study will be held in confidence and that, if it is presented or published, all my personal details will be removed. _____
8. I understand that my samples will be stored anonymously, identifiable only to the research team and my usual clinical team using a coded sequence, and may be used in future studies. No information regarding my personal details will be stored. _____
9. I confirm that I will be taking part in this study of my own free will, and I understand that I may withdraw from it, at any time and for any reason, without my medical care or my legal rights being affected. _____

Please tick appropriate box

10. I give permission for my GP to be informed about my inclusion in this study. Yes ☐ No ☐
11. I agree to take part in the above study. Yes ☐ No ☐

Signed	Date
Person taking consent	Date
Researcher (if different from above)	Date

IRAS project ID: 218555
Version: 2.0
Date: 15/01/2017

Copy 3
Medical notes

The Leeds Teaching Hospitals NHS

Dear Patient,

We are inviting you to join a research study here at Leeds Teaching Hospitals NHS Trust entitled " 0.03% versus 0.2% indigo-carmine in chromoendoscopy for inflammatory bowel surveillance".

As you are likely to be aware, having colitis puts you at an increased risk of having colorectal cancer. Hence why it's recommended that you have regular colonoscopies at set intervals to check the health of your bowel, and to detect any abnormal patches. We now use a harmless dye to improve the detection of abnormal patches, called chromoendoscopy and you may have received this before.

We would like to invite you, by means of this letter to take part in a research study.

Our study involves comparing two different concentrations of dye that is sprayed on the inside of your bowel at the time of colonoscopy. Taking part in the study will not compromise your care in anyway. All patients will have the recommended set of samples taken for analysis, plus we will take an additional two from the lower end of the bowel. This should not place you at any additional risk.

Please note you have the option to opt-in to the study. **Taking part in the study is entirely voluntary**.

Please find the enclosed Patient Information Leaflet, which will explain the study in more detail. Please take your time to read it and you will be able to ask further questions before you decide whether to take part.

Best wishes,

Dr Richard Lord

Endoscopy Fellow and Specialist Registrar in Gastroenterology

richard.lord2@nhs.net

IRAS Project ID: 218555
Version 2.0
15/01/2017

Copy 3
Medical notes

GP name
GP address

Dear GP,

Re:

I am writing to inform you that your patient has agreed to participate in a randomized clinical trial at Leeds Teaching Hospital. The study is titled:

"Chromoendoscopy with 0.03% indigo-carmine delivered via a foot pump compared with 0.2% indigo-carmine delivered via spray catheter detecting dysplasia in patients undergoing surveillance in Inflammatory Bowel Disease. A randomized trial."

Briefly, we are looking at the detection rate of neoplastic polyps during chromoendoscopy for IBD surveillance using two concentrations of indigo-carmine. Indigo-carmine is a contrast agent used to enhance lesion detection. Chromoendoscopy is currently the national standard during IBD surveillance; however the concentration of indigo-carmine used varies nationally and internationally. We would like to know if there is any difference between the two concentrations.

Any polyps seen will be analysed endoscopically, following spray with indigo-carmine, looking at its morphological characteristics as part of our normal standard of care. We will also further analyse the lesion using the WavSTAT optical biopsy forceps, which uses laser light induced fluorescence to analyse the lesion. There has been no safety concerns using the biopsy forceps in a recent trial performed at Leeds Teaching Hospitals. It only takes a few seconds to use and does not increase the time of the procedure.

Finally we will take two additional rectal biopsies and freeze them in liquid nitrogen to be analysed for rectal biomarkers for dysplasia. This again will take around 20 seconds to perform and not place the patient at any additional risk.

If you have any questions about this, please do not hesitate to contact myself.

Yours sincerely,

Dr Richard Lord

Endoscopy Research Fellow and Gastroenterology Specialist

richard.lord2@nhs.net

IRAS Project ID: 218555
Version 1.0
1/12/16

Copy 1
Patient copy

The Leeds Teaching Hospitals NHS

Patient Information Leaflet

Chromoendoscopy with 0.03% indigo-carmine versus 0.2% for detecting dysplasia in patients undergoing surveillance in Inflammatory Bowel disease

You are being invited to participate in a research study. Before you decide whether to take part, it is important that you understand why the research is being done and what it involves. *Please take your time to read this information sheet carefully* and you can discuss it with friends, relatives or your GP if you wish. If there is anything unclear or would like further information then you can also contact us directly (see final page of leaflet). There will also be a good opportunity for questions in the endoscopy department before the procedure, if you wish to participate.

Take your time and thank you for reading this.

What is the purpose of this study?

We know that people with colitis (both Ulcerative and Crohn's) have a higher risk of developing bowel cancer than people without this condition. For this reason it is recommended that you undergo regular checks on the health of your bowel, called surveillance colonoscopy. Often bowel cancer in patients with colitis develops from small patches of bowel lining, which over time become abnormal and unstable. The aim of surveillance colonoscopy is to detect these patches early and remove them before they have the chance to become cancerous.

The best test we have for this currently is colonoscopy and spraying a blue dye, called indigo-carmine, through the colonoscope during removal of the instrument. This is called chromoendoscopy. This technique has been proven to enhance detection of these abnormal patches. What has not been investigated before is the concentration of the dye that is sprayed. Both the concentrations used in this study are used internationally. However, we want to compare the two most commonly used concentrations of dye (0.2% vs 0.03%) and see if there are any differences in detection of these abnormal patches, between the two concentrations.

If any abnormal patches are seen, standard of care is to look at these areas very closely through the lens of the colonoscope to see if we can characterise them any further. The options then are to leave (if not concerned), biopsy or remove (called a polypectomy).
Additionally in this study, whilst withdrawing the colonoscope, we will use a special optical fibre, which is built into standard biopsy forceps, placed down a channel in the colonoscope and simply held against any patches for a few seconds and a reading is obtained on a screen. You will not feel this. This will hopefully help characterising the patches further. We will then manage the patches as stated above, therefore not deviating from standard practice.

During the same colonoscopy national current practice is to takes biopsies from the lining of the bowel. This is painless and the reason for doing this is to determine if your bowel is inflamed under the microscope. In this study we would *additionally* like

to take two small biopsy samples from the tail end of the bowel during the same test. Again this will take no longer than 20 seconds to do and will be painless.
On obtaining these two small tissue samples, we will freeze them and analyse the tissue characteristics. In doing this we hope to find new ways of determining which patients with colitis are more likely to develop colon cancer in the future.

Why have I been chosen?

We are currently inviting all patients with colitis involving more than half of their bowel that are scheduled to have routine surveillance colonoscopy.

Do I have to take part?

No. Participation is entirely voluntary and completely separate from your usual care. If you decide to take part you will be asked to sign an extra consent form at the time of signing your standard colonoscopy consent form.
If you decide to take part, you are still free to withdraw at any time without having to give a reason. **This will not affect your usual medical care or quality of colonoscopy**. If you decide **not** to take part, this will not affect you in any way.

What will happen if a participant agrees to take part in the study?

You may have been invited to participate in this study during your IBD clinic appointment or invited via letter in the post. You will have opportunity before attending the colonoscopy to ask any questions, via our contact details below, if any questions need answering before the day of your colonoscopy. On the day, you will have further opportunity to ask questions you have about the study, to the study doctor who will be performing your colonoscopy. At this time, if you are willing to participate, you will be asked to sign a consent form.

By participating in this study you will only require one procedure as you would if you were not participating in the trial, therefore no additional appointment will be required.

You will have the colonoscopy in the same way and offered the usual choice of painkillers and sedatives. The colonoscopy tube will be inserted in the usual manner to the end of your large bowel.

Just before we start to bring the colonoscope slowly out of the bowel, we will randomly decide which concentration of dye spray we will use. This decision will be made using a special computer code. The concentration of dye spray you will be exposed to will either be 0.2% or 0.03% indigo-carmine. The concentration basically means how much additional water we will add to the dye (indigo-carmine). The use of dye (indigo-carmine) is recommended by the national societies but the optimal concentration is not yet known. Therefore using this dye is standard of practice and participating in this trial will not deviate from this. Therefore you will **not receive any**

inferior care. We will spray short segments of the colon with the dye spray through a channel in the colonoscope, then inspect the lining to look for any abnormalities.

Additionally, if abnormalities are seen we will use the biopsy forceps with a special optical fibre, placed down a channel during the colonoscopy, and take a very quick reading by pressing a foot peddle. You will not feel this and not be aware of this. This is a very quick *additional part* of the study, which we hope in the future be able to characterise these abnormal patches more efficiently and hopefully reduce the future number of biopsies required during a procedure. There are no additional risks with this optical fibre. We will then either biopsy the abnormality or remove it as standard of care.

As previously mentioned, we take several biopsies of the lining of the bowel as we pull the scope back to look for on going inflammation under the microscope. This is routine and standard practice. Additional for this study, during the same procedure, we will take two further samples from the lower end of the large bowel, which again is painless and adds no significant risk to the procedure. Once out of your body, we will freeze these two samples and store them in a secure laboratory. We will then use new techniques to look at the tissue characteristics, which hopefully in the future help us define patients that are at higher risk of developing these abnormal patches.

At the end of the procedure you will receive the same care you would usually receive after the colonoscopy and allowed to go home shortly after the procedure.

Your involvement in the study will then be over and no further tests required. Your clinician who referred you for the test will review your results.

Is there anything else I have to do?

No, this study involves no extra visits to hospital either before or after the colonoscopy. All the above takes place during the one colonoscopy.

What are the possible benefits of taking part in the study?

There will be no direct benefit to you from participating in this study. However, seeing if there is a more effective concentration of dye for detecting abnormal patches will help improve our understanding for the best way of looking for these abnormalities. By using the optical fibre biopsy forceps in this study, we may be able to get information that will mean less biopsies for patients undergoing colonoscopy in the future. Taking the two extra large bowel biopsies might allow us to find markers which categorise patients more accurately about their future risk of cancer.

Are there any risks in taking part?

The risk of having a colonoscopy test for any reason carries a very small risk of making a hole in the bowel. This risk is less than 1 in 1000. The use of dye spray does not carry any additional risk and the varying concentrations are being used by

international gastroenterology societies. There is a small chance that the dye may make your stools or urine go blue for a short period of time; this is harmless. The optical biopsy forceps carry no additional risk. Biopsies are generally taken during this procedure and therefore two additional biopsies are unlikely to increase the risk of this procedure.

What is something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to negligence, then you may have grounds for a legal action but you may have to pay for it.
If you have any concerns about any aspect of the study, you should ask to speak to the researchers who will do their best to answer your questions [contact Dr Richard Lord (075452139030].
If you remain unhappy and wish to complain formally, you can do so through the NHS Complaints Procedure or PALS. Details can be obtained from either your doctor or the Leeds Teaching Hospitals NHS Trust PALS centre at Tel: 0113 2067168.

Will my taking part in the study be kept confidential?
If you consent to take part in the research, your own GP will be notified. This means that other doctors in the same practice may be aware of your participation in the study. If you give permission, your medical records may be looked at by responsible people involved in this research. All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name, address and other identifiers removed so that you cannot be recognised from it.

Samples will be stored in a secure laboratory for a period of time. *Only research teams and your usual doctors will be able to link your tissue samples to your personal and medical details.*

We will aim to publish the results of the study in a specialist research journal so that other health care professionals can see the results. You will not be identified in such a report.

What will happen to my samples after the study is complete?

Samples will be stored in a secure facility at the Leeds Teaching Hospitals NHS Trust and University of Leeds, known as the research tissue bank. The samples maybe used for further projects after successful application for permission has been made from the ethics committees for specific projects. Personal information will not be stored therefore you will not be identified in any future projects.

Who has reviewed the study?

The way this study will be conducted has been reviewed by the Ethics Committee for Research, to protect your interests. It has been reviewed and given favourable opinion.

Who to contact for further information

If you have any questions, please ask the study doctor when you come for your colonoscopy. Alternatively, please contact either:

Dr Richard Lord, Specialist Registrar in Gastroenterology, Leeds Teaching Hospitals on 07545213903

Dr Venkat Subramanian, Consultant Gastroenterologist, Leeds Teaching Hospitals on 0113 2062288 (secretary)

If you decide to participate in the study, you will be given a copy of this information sheet along with a signed consent form to keep. Finally, we would like to thank you for reading this information sheet and considering participation in our study.

Yours sincerely,

Dr Richard Lord

Dr Venkat Subramanian

Dr Nick Burr

Dr Noor Mohammed