Title

Role of image-enhanced endoscopy in the assessment of inflammatory bowel disease.

Title page

Title: Role of image-enhanced endoscopy in the assessment of inflammatory bowel disease.

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Contributions and publication statements

I confirm that the work submitted is my own, except where the work is co-authored with other collaborators. The contribution of co-authors has been explicitly detailed below.

Chapter 1: Introduction

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- N Mohammed wrote the manuscript.
- Dr Subramanian provided critical review.

Chapter 2: Can standard white light endoscopy predict a flare in Ulcerative colitis?

- N Mohammed collected the data, performed statistical analysis and wrote the manuscript.
- Dr Subramanian conceptualised the study.
- All authors provided critical review.

Chapter 3: Can faecal calprotectin predict a relapse in IBD: a meta-analysis

- N Mohammed –collected the data, performed statistical analysis and wrote the chapter.
- E Telakis- Helped in data collection.
- V Subramanian-Conceptualising the study and critically reviewed the paper.

Chapter 4: Does newer generations of NBI quantitatively improve contrast enhancement of endoscopic images?

- N. Mohammed- Data collection, analysis and writing up the chapter
- E. Telakis-Helped in taking endoscopic images.
- J. Ariyarathnam-Helped in data collection.
- V. Subramanian-Designing of study and critical review.

Chapter 5: Use of NBI in predicting disease outcomes in UC-a prospective observational study

- N Mohammed wrote the study protocol, obtained approval from R&D and NREC, recruited patients for trial, performed endoscopic procedures, analysis and write up of the chapter.
- Drs O Rotimi and P Prasad performed the histological analysis of the biopsies independently
- Dr V Subramanian recruited patients and performed endoscopic procedures. Critically reviewed the chapter.

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- First author performed all spectroscopy related experiments along with Professor A Jha. He also contributed in writing manuscript on spectroscopic perspective.
- N Mohammed obtained R&D and NREC approval for the study extension, performed endoscopic procedures, supplied biopsies for spectroscopic experiments and wrote the endoscopic part of the manuscript.
- Dr O Rotimi performed histological assessments of the biopsies.
- Statistical analysis was performed by Dr V Subramanian.
- All co-authors critically reviewed the manuscript.

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- Dr N Mohammed wrote the abstract, presented in conference, performed statistical analysis and wrote the chapter
- Dr V Subramanian conceptualised and designed the study.
- All authors provided critical review of the paper.

Chapter 8: High definition white light endoscopy versus High definition chromoendoscopy in detecting dysplasia in UC

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- N Mohammed wrote the study protocol, obtained R&D and NREC approval, designed the patient information leaflets and GP letters. Recruited patients for study, performed the endoscopic procedures and wrote the chapter.
- Dr P Kant helped draft the protocol.
- Drs O Rotimi and P Prasad provided histopathological analysis of the samples.
- Dr Subramanian conceptualised and designed the study, performed endoscopic procedures and provided critical review of paper.

Abstract

Introduction

Ulcerative colitis (UC) which is a form of inflammatory bowel disease (IBD) is characterised by a relapsing and remitting disease course. Clinical disease activity indices (DAIs) are used to assess the severity of the disease activity relying solely on the clinical symptomatology of the patients. Non-invasive biomarkers help in assessment and possibly predicting the disease relapse. Although faecal calprotectin (FCP) is one such biomarker that is extensively researched, its accuracy in assessment and prediction of relapse is only modest. Similarly endoscopy in IBD with white light examination (WLE) alone is not accurate in either the assessment of disease activity or the prediction of disease course. Narrow band imaging (NBI) allows examination of the vasculature and pit pattern of the mucosa in greater detail than WLE. Patients with colonic IBD also have a higher risk of developing dysplasia or colorectal cancer (CRC). Chromoendoscopy (CE) provides a contrast enhancement and aids in highlighting the dysplastic areas.

Aims

Primary aim of the research is to assess the role of advanced endoscopy, NBI and Chromoendoscopy (CE) in assessment of disease activity and dysplasia detection respectively in UC. The secondary aim is to assess the role of DAIs in assessment of disease activity, their correlation with endoscopic & histological markers and overall outcomes during the follow up period.

Methods

We performed two different experiments using advanced endoscopic techniques for this research project; one is in assessment of inflammatory activity and second is in detection of dysplasia in UC.

We performed retrospective analysis of our practice to identify if white light alone predicts relapse in patients with quiescent UC. Based on our findings we devised a prospective observational study to look at the effect of adding NBI to WLE in assessment of disease activity in patients with UC of varying grades of severity. As newer generation of NBI (H290 series of Olympus KeyMed®) endoscopes were being introduced into the UK market at the time of the study, we compared the effect of NBI in three generations of endoscope (Q240, H260 and H290 series). We also assessed the use of Raman spectroscopy in endoscopic

and histological assessment of inflammation in UC.

In another retrospective study we looked at the uptake of chromoendoscopy in surveillance colonoscopies in UC. A randomised controlled study (RCT) was also designed to compare high definition WLE (HDWLE) to high definition CE (HDCE) in detecting dysplasia in UC surveillance. As part of relapse-prediction work we also conducted a meta-analysis of published RCTs on FCP to analyse its predictive capability in IBD.

Results

In the retrospective analysis, we found that the presence of either Mayo Endoscopic Subscore >1 or Geboes score ≥2.1, increases the risk of relapse up to 6 times in the subsequent twelve months period. In our comparative study of NBI in three different generations of endoscopes, we demonstrated that NBI is superior to WLE in the assessment of the presence of blood. We also noticed a significant improvement in NBI in the newer generation of endoscopes (H290 and H260) compared to the earlier endoscopes (Q240).

From the meta-analysis of RCTs we found that the FCP can predict disease flare with an accuracy of up to 75% only. In the observational study we determined that addition of NBI to WLE did not provide additional value in either assessment of disease activity or predicting relapse. Among the clinical disease activity indices (DAIs), the simple clinical colitis index or Walmsley index with score of ≥3 correlated well with endoscopy and histological findings. From the Raman spectroscopy study we identified the intensities of peaks (carotenoid and the phospholipids) that were statistically significantly different between the Raman spectra of the inflamed and quiescent colonic tissue.

In our second retrospective analysis CE was found to be superior to WLE in detecting all dysplastic lesions and the detection of endoscopically visible flat non-polypoid lesions. However CE was performed only in one third of the study population. In the RCT we found that HDCE has an incremental yield of about 12.7% with a NNT of about 8, suggesting that HDCE would detect one additional patient with a dysplastic lesion for every 8 patients on whom this procedure is done.

Conclusion

The thesis has shown that endoscopic biomarkers and FCP do not reliably predict relapse in UC. Addition of NBI does not confer added benefit in assessment of disease activity. HDCE is superior to HDWLE and should be adapted as a standard practice in surveillance of dysplasia in UC.

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List of abbreviations

AE	Adverse Event
CE	Chromoendoscopy
CRF	Case Report Form
DAI	Disease activity index
EAI	Endoscopic activity index
EOT	End of Trial
FCP	Faecal calprotectin
GCP	Good Clinical Practice
HD	High definition
ICF	Informed Consent Form
IBD	Inflammatory bowel disease
LTHT	Leeds Teaching Hospitals Trust
MH	Mucosal Healing
NBI	Narrow Band Imaging
NHS	National Health Service
P/GIS	Parent / Guardian Information Sheet
PI	Principal Investigator at a local centre

PIS	Participant Information Sheet
REC	Research Ethics Committee
R&D	Research and Development department
UCEIS	Ulcerative colitis endoscopic index of severity
WLE	White light examination

Author abbreviations

NM	Noor Mohammed
VS	Venkataraman Subramanian
OR	Olorunda Rotimi
PP	Padmini Prasad
ET	Emmanuel Telakis

1 Introduction

Ulcerative colitis

Ulcerative colitis (UC) is a chronic inflammatory condition of unknown aetiology, characterized by diffuse and confluent mucosal inflammation of the colon starting from the rectum with a characteristic relapsing and remitting course(1). Conventional endoscopy was thought to be a reliable parameter for assessment of disease activity(2), but microscopic inflammation can persist despite normal mucosal findings(3). Histologically detectable inflammation is thought to be associated with a greater risk of subsequent relapse(4, 5). A flare in UC activity is difficult to predict, but a simple, easily measured biological marker of relapse would be important in guiding the most appropriate and cost-effective therapy.

Approximately 25% of patients with UC experience acute exacerbation of their disease activity during the course of their disease(6). Colectomy rate increases with more than one hospital admissions with acute severe UC, reaching up to 40% after two admissions (7). Therefore the treatment goals in UC must focus on keeping the disease in remission and a colectomy-free survival.

Mucosal healing in UC

Although there is no consensus definition of mucosal healing, ‘International Organisation of Inflammatory Bowel Disease’ proposed the following criteria to define mucosal healing: absence of friability, blood, erosions and ulcers in all visualised segments of the gut mucosa(8). Essentially disappearance of endoscopic lesions such as erosions and ulcers is called as mucosal healing. Drugs such as 5-aminosalicylates (5 ASA) (delayed release and multimatrix mesalamine), Immunomodulators like azathioprine, methotrexate and infliximab are used in induction and maintenance of MH in UC (9-15). MH is associated with favourable short and long-term clinical outcomes like reduced hospitalisation due to flares decreased colectomy rates and lower incidence of colorectal cancers(16-20). MH is increasingly recognised as a therapeutic endpoint not only in clinical trials but also in routine clinical practice.

Assessment of disease activity in UC

Assessment of disease activity in UC is performed by clinical, biochemical and endoscopic measures. Each of these parameters is described in detail below.

Clinical assessment of disease activity

Clinical activity indices help physicians to assess the severity of the disease to optimise the treatment based on patients' symptoms alone. Various assessment tools are available in clinical practice; some use clinical variables alone and others include a combination of clinical and biochemical markers. Truelove and Witts severity index (TWSI)(21) described in 1955 as a clinical assessment tool for disease activity in UC has been the widely used in clinical trials. The variables used are discriminative enough to assess the severity of disease and to be used in clinical practice. However, the major limitation of this scoring system is that it has never been validated externally. Stool frequency and presence of blood in the stools are the variables along with temperature, pulse rate and Erythrocyte sedimentation rate (ESR). A score of <4 is mild disease and >6 is considered as a severe disease. The variables are less clear for a moderate disease which is something in between mild and severe disease scores, and fulminant colitis is diagnosed if there is continuous blood in the stools necessitating blood transfusion.

Clinical activity index (CAI) also known as Rachmilewitz index(22) includes biochemical markers along with clinical parameters in the assessment tool. Additional subjective assessment of patient's symptoms by the physician was allowed along with incorporating extra-intestinal manifestations of UC. This was subsequently validated in one study(23) in which a CAI score of ≤4 corresponded to clinical remission.

Physician global assessment (PGA)(8) is another non-validated, arbitrary measure of disease activity which ranges from 1 to 6. It is helpful in assessing response to therapeutic interventions from the baseline but lacks objective evidence to the scores described. One similar measure is Investigator global evaluation which allows physicians to rate patients' symptoms on their objective assessment.

Lichtiger et al(24) described a modified Truelove and Witts index for assessment of severity. This was an 8 point scoring system as opposed to 5 in TWSI, incorporating subjective assessment scores of the treating physician. Clinical remission was defined as a score less than ≤3 and clinical response to treatment was defined as a reduction of ≤10 points.

Walmsley et al(25) described a scoring system which was termed as 'Simple clinical colitis index' (SCCI). This score was an adaptation of clinical features from the Powell-Tuck index of severity and the general wellbeing component from the Harvey-Bradshaw index for Crohn's' colitis. The score was derived using various regression analyses; however, the original paper does not describe any cut off values for remission or relapse. This score has not been validated in clinical trials. Higgins et al in a prospective study described in 2005 that a score of <2.5 corresponds to patient defined clinical remission(26).

More recently an 'Ulcerative Colitis Clinical Score' was proposed by Feagan et al(27). Stool frequency and rectal bleeding were the markers for objective evidence of disease activity. Symptoms such as abdominal pain, nocturnal diarrhoea were not considered in the score; however, subjective assessments and overall scores from both physician's and patient's perspective were included

In an attempt to standardise the available clinical and endoscopic scores for UC, a study conducted by Japanese researchers evaluated the use of these scores in the previous one hundred clinical trials(28). They found that Rachmilewitz score, Sutherland index (also known as disease activity index-DAI), TLWSI, Mayo clinical score and Lichtiger score were the commonly used clinical indices in decreasing order of frequency. These representative scores were then used to grade the disease prospectively before and after the treatment over 2, 4 and 8 weeks on the seventy-four recruited patients. Their results suggested that these scores were equally effective in assessing disease activity. However, this claim has not been verified by further studies.

Endoscopic assessment of disease activity:

Endoscopy is essential to establish a diagnosis of inflammatory bowel disease and also to distinguish UC from Crohn's disease. Direct mucosal visualisation and obtaining biopsies for histological analysis is the advantage of endoscopy over other modes of assessment of disease activity. Endoscopic examination helps physicians to assess the extent & severity of the disease. In addition to this, it is a useful tool to identify and resect dysplastic lesions during surveillance for colorectal cancer and dysplasia.

There are at least ten endoscopic scores designed to assess the disease activity in UC since the development of first such score by Baron et al in 1964(29) (Tables 1-5). Table 1 contains the different disease activity indices with only endoscopic variables and Table 2 contains the indices with non-endoscopic variables. These scores use clinical, biochemical and endoscopic components in an attempt to grade the disease activity. Endoscopic parameters of assessment include mucosal vascular pattern (MVP), friability and mucosal damage.

Mayo endoscopic subscore is an endoscopic component of full Mayo score(30). Both Modified Baron score and Mayo endoscopic subscore have been used in clinical trials; however, these scores have not been validated rigorously(8).

Table 1 Disease activity indices with endoscopic component alone.

Disease activity index	Endoscopic variables
Baron score(29) **1964**	Bleeding MVP
Rachmilewitz endoscopic index(22) **1989**	Granulation MVP Mucosal vulnerability Mucosal damage
UC colonoscopic index of severity (UCCIS)(31) **2013**	MVP Granularity Ulceration Bleeding Segmental assessment of endoscopic severity Global assessment of endoscopic severity
UC endoscopic index of severity (UCEIS)(32) **2013**	MVP Bleeding Erosions Ulcers

MVP= mucosal vascular pattern

Table 2 Disease activity indices with endoscopic and non-endoscopic components

Disease activity index	Endoscopic variables	Non-endoscopic variable
Powell-Tuck score(2) 1982	Bleeding	Wellbeing Abdominal pain Stool frequency & consistency Bleeding Anorexia nausea & vomiting EIM Temperature
Sutherland index(33) 1987	Friability Bleeding	Stool frequency Bleeding Physician's rating of disease activity
Mayo score(30) 1987	Erythema MVP Friability Erosions Ulcers spontaneous bleeding	Stool frequency Bleeding PGA
Improvement based on individual symptom scores(34) 2002	Mucosal oedema MVP Granularity Friability Petechiae Ulceration Spontaneous bleeding	Rectal bleeding Stool frequency Abdominal pain PFA PGA

EIM=Extra-intestinal manifestations, PFA=Patient functional assessment, PGA=Physicians global assessment.

Mayo endoscopic subscore is widely used in the endoscopic assessment of the inflamed colon. Osada et al compared four endoscopic indices between expert and non-expert endoscopists (28). In the inter-observer analysis, kappa values for expert-endoscopist was found to be very good for Mayo endoscopic subscore. It is easy to use and intuitive to grade the inflammation; however, we felt there is little manoeuvrability in Grade 3 for grading superficial and deep ulcerations (Table 3). This was later addressed by the UCEIS which further categorises the endoscopic markers of severity in details. Whether this grading of inflammation will correlate with the disease outcomes is not known.

Table 3 Mayo Endoscopic subscore

Score	Description
0	Normal / inactive disease
1	Mild disease (Erythema, Decreased vascular pattern, Mild friability)
2	Moderate disease (Marked erythema, absent vascular pattern, friability, erosions)
3	Severe disease (Spontaneous bleeding, Ulceration)

Assessment of inflammation using Baron Score included scores from 0-3; 0 was normal appearance and 3 was severely inflamed mucosa. The inflammation was graded predominantly using haemorrhage alone as the variable. It did not include mucosal friability, erosions or ulcerations. For the purposes of simplifying and including other endoscopic variables, a Modified Baron Score was proposed (Table 4)

Table 4 Modified Barons index

Score	Description
0	Normal, smooth, glistening mucosa with visible vascular pattern. No friability
1	Granular mucosa, Obscure vascular pattern, erythema; no friability
2	Score 1 + friability of mucosa. No spontaneous bleeding
3	Score 2 but with spontaneous bleeding
4	Score 3 but with ulceration and denuded mucosa

Recently Travis et al designed and validated a new scoring system using endoscopic 'descriptors' called ulcerative colitis endoscopic index of severity (UCEIS) (32, 35) (Table 5). Ten Inflammatory bowel disease (IBD) experts evaluated sigmoidoscopic videos of varying degree of endoscopic inflammation seen in UC. Inter and intra-investigator reliability was tested using Kappa statistics. In the validation phase, they report kappa values ranged from 0.34 to 0.65 and 0.30 to 0.45 for inter and intra-investigator reliability respectively. No significant difference was observed when investigators were tested with or without the knowledge of clinical details of subjects. Whether this score can be used as a reliable endoscopic assessment tool in clinical trials and in general practice remains to be established.

Table 5 Ulcerative colitis endoscopic index of severity (UCEIS)

Descriptor	Descriptor
Vascular pattern	Normal (0) Patchy obliteration (1) Obliterated (2)
Bleeding	None (0) Mucosal (1) Luminal mild (2) Luminal moderate or severe (3)
Erosions and ulcers	None (0) Erosions (1) Superficial ulcer (2) Deep ulcer (3)

1.1.1.1 White light endoscopy (WLE)

Endoscopic examination is commonly performed under white light for assessment of disease activity. Mucosal visualisation is improved greatly due to the advent of high definition endoscopes. However, mucosal evaluation varies among endoscopists owing to the lack of hard objective endpoints for variables such as mucosal friability, vulnerability and healing. Another factor such as relative lack of experience among endoscopists in grading the severity of the disease is also common. Moreover, it is known that inflammation persists despite the normal appearance of the mucosa under the white light which may result in subsequent relapse (2, 5, 38). Although white light examination is easy to perform for a quick assessment during relapse, factors such as lack of objective endpoints, variability among assessors and lack of predictive capacity for a relapse necessitates the search for an alternative, more advanced methods of mucosal assessment in IBD.

1.1.1.2 Advanced endoscopic techniques

Data on use of advanced imaging modalities such as autofluorescence imaging (AFI), NBI and magnification chromoendoscopy in the assessment of inflammatory activity in IBD are rare (36-41). Osada et al reported a close correlation between the green component of AFI with endoscopic (Mayo endoscopic subscore) and histological inflammation among UC patients(41). They also noted that as the inflammatory activity increased in the colon the green colour component of AFI decreased. Magnification chromoendoscopy involves topical application of dye onto the mucosa and visualisation of abnormal pit pattern using the zoom feature of the colonoscope. It is time-consuming, involves a steep learning curve and is

cumbersome which has resulted in low uptake despite superior diagnostic yield compared to WLI. Data available from a limited number of studies is encouraging in both detections of inflammation and prediction of relapse (42-45). NBI, in couple of small studies has shown improved diagnostic yield in the assessment of inflammation compared to white light examination alone(38, 46). NBI is easy to use and intuitive to the endoscopist; a button mounted on the endoscope handle is used to switch between WLI and NBI.

Advanced imaging techniques like high-resolution endoscopy, Narrow band imaging, Zoom endoscopy, chromoendoscopy helps in detailed assessment of mucosa and submucosal vasculature; however, the studies are rare and involved a small number of subjects and results are conflicting. The applicability of these techniques into routine clinical practice and value in predicting relapse needs further work.

Biomarkers in the assessment of disease activity

Biomarkers are measurable characteristics that reflect the presence of disease state or its severity. These could be specific cells, molecules, genes, gene products, enzymes, hormones or organ function. They must indicate a change in expression or state of a protein that correlates with the risk or progression of a disease.

An ideal biomarker must be helpful in diagnosing plus monitoring the disease activity and also predicting a relapse. It must also correlate with the susceptibility of the disease to a given treatment. It must be non-invasive, ideal to be used for all age group of patients, quick and easy to perform. Desired characteristics of a biomarker include reliability (high sensitivity and specificity), stability from degradation factors, and independent of physiological, molecular or diurnal changes.

Biomarkers can be serological such as C reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell count (WBC), platelet count, 1-acid glycoprotein, serum amyloid A-protein, 2-globulin, lactoferrin, orosomucoid and thrombopoietin; or they could be faecal markers such as Faecal calprotectin (FCP), Lactoferrin, DNA excretion, Myeloperoxidase, Faecal immunohistochemistry testing (FIT). Commonly used biomarkers in UC include WBC, Platelet count, CRP, ESR and FCP.

The primary application of biomarkers in IBD is to reliably differentiate it from irritable bowel syndrome; others include disease monitoring and reliably predicting a flare. Flare-ups in UC are unpredictable, a real health economic burden, and have a devastating impact on quality of life (QOL) of patients. In this era where physician’s therapeutic armamentarium is

strengthened by the availability of potent drugs like biologic therapies, prediction of flares could help to escalate medications to prevent flare and its implications.

1.1.1.3 C reactive protein (CRP):

C reactive protein (CRP) was first discovered by Tillett and Francis in 1930(47). It was seen reacting to C-polysaccharide of Pneumococcus and hence the name CRP. It is synthesized in the liver in response to macrophages and adipocytes. It is detected in serum from 6 hours of inflammation which peaks at 48 hours (half-life around 19 hours). CRP acts as bactericidal protein by activating complement system and in phagocytosis of bacterial nuclei(48).

CRP has been extensively investigated in diagnosing, monitoring and predicting the disease flare in IBD. In a prospective study to evaluate the usefulness of CRP in adult patients with chronic abdominal pain (n=82), Shine et al(49) found that all the patients subsequently diagnosed as Crohn's disease (19/19) and half the patients with UC (11/22) had elevated CRP. Interestingly none of the 41 patients with functional abdominal pain had elevated CRP. Similar results were found in the paediatric population(50). Moreover, CRP elevation was found to correlate well with clinical, endoscopic and histological inflammation in IBD(51).

CRP is commonly used in monitoring disease activity and to evaluate the response to treatment. A significant correlation between CRP and simple clinical colitis index was noted in prospective studies while monitoring the disease activity in patients with acute flare-up of UC requiring hospitalisation (52, 53). However, the evidence for CRP as a tool to predict flares is conflicting (See 1.1.4.2). CRP is easy to perform, inexpensive, and a reliable test to differentiate patients with IBD from functional gastrointestinal disorders. It is hence widely used as a screening tool both in primary and secondary care services.

1.1.1.4 Faecal calprotectin

Faecal calprotectin (FCP) was first described in 1980. Calprotectin is a calcium and zinc binding protein belonging to the SA100 group of proteins. It is found predominantly in the cytosol of neutrophils and to a lesser extent in monocytes and reactive macrophages. It is available in abundance in body fluids which is resistant to bacterial or enzymatic degradation. Calprotectin constitutes about 60% of the cytosolic protein in the granulocytes and about 5% of total body protein(54). Intestinal inflammation is marked by the influx of inflammatory cell infiltrates in the mucosal lining. Breakdown of cytosolic protein liberates calprotectin in the faeces the concentration of which is found to be proportional to the intestinal inflammation(55) and white cell scanning (56). A spot test of <5gm of faeces is shown to be as reliable as 24-hour stool collection and can remain stable for up to 7 days in room temperature (54). It can be readily quantified using enzyme-linked immunoassay testing (ELISA). FCP is hence classed as a 'damage-associated molecular pattern' protein

and seems to fulfil the desirable qualities of a non-invasive surrogate marker of intestinal inflammation. Elevated FCP levels are seen in patients with non-steroidal anti-inflammatory drug (NSAID) intake, and other non-IBD causes such as infective enteritis, untreated coeliac disease, diverticulitis, intestinal bleeding and malignancies(57-59). Other drugs which could potentially influence FCP levels, but lacks evidence, are proton pump inhibitors (PPIs) and Nicorandil (which is known to cause mucosal ulcerations in the gastrointestinal tract).

1.1.1.5 Role of faecal calprotectin in IBD:

FCP levels are used in discriminating between irritable bowel syndrome (IBS) from IBD with a specificity of 97-100% and a negative predictive value of 100% (Table 6). There is no convincing evidence that FCP levels can differentiate between subtypes of IBD; however, their levels correlate well significantly with endoscopic disease activity in IBD(60). This simple test appears useful in clinical practice for non-invasive assessment of disease in activity and in remission. In a meta-analysis of thirty prospective trials including 5983 subjects, FCP levels were found to be elevated in IBD population compared to normal subjects with a sensitivity of 95% and specificity of 91%. In the same study, a non-significant elevation of FCP was observed with a sensitivity and specificity of 36 % and 41% respectively. This makes FCP unreliable tool for colorectal cancer screening(59). Another meta-analysis of 13 prospective studies including 670 subjects evaluated the diagnostic accuracy of FCP. In these studies FCP when used as a screening tool resulted in reduction of 67% of endoscopic examinations(61).

Table 6 Studies on role of Calprotectin in differentiating IBD from IBS

Study design	Study characteristics	Findings
Tibble et al(62) Prospective study	220 subjects with abdominal symptoms. Radiological/Histological diagnosis of CD. IBS-ROME criteria (CD / IBS. UC excluded)	FCP >30mg/L was 100% sensitive and 97% specific in discriminating IBS v/s IBD
Dolwani et al(63) Prospective	73 patients undergoing SMFT for ? IBD. 25 IBS & 25 normal controls.	FCP >60mcg/gm predicted abnormal small bowel radiology. 100% NPV.
Sydora et al(64) Prospective	50 participants. Diagnosed UC, CD, IBS and normal volunteers.	FCP levels were high in CD/UC compared to IBS & normal volunteers. 100% specific for IBD pts.

IBS= Irritable bowel syndrome, IBD=Inflammatory bowel disease, NPV=Negative predictive value, UC=Ulcerative colitis, CD=Crohn's disease

1.1.1.6 Calprotectin in disease monitoring

FCP is used in monitoring response to treatment in acute severe colitis and in those on maintenance therapy. Significantly high levels of FCP were found in patients admitted with acute severe colitis requiring colectomy and in those who were non-responders to corticosteroid or infliximab therapy(65). Normalisation of FCP levels correlated well with disease remission (clinical, endoscopic and histological) in a small study where the maintenance therapy was either with 5-aminosalicylates (5ASA) or azathioprine (66). However results from studies looking at monitoring disease activity with FCP in patients on Infliximab therapy vary widely. Tursi et al(67) reported that FCP is better at predicting persistence of inflammation than complete remission (Positive predictive value of 96.2% versus 41.8%). On the other hand de Vos et al reported that rapid decline in FCP levels induced by infliximab resulted in lasting disease remission(68). The data on FCP levels in post-operative patients with Crohn's disease and risk of recurrence is conflicting (69-71).

Histological assessment of disease activity

Histology is the gold standard in the assessment of the mucosal activity in UC. Various histological indices are available for grading the inflammatory activity (3, 20, 21, 72-77). There are at least 18 scoring systems available for histological assessment in UC, however none of these are validated externally(78).

Researchers have tried to compare the endoscopic findings to the histological inflammation seen(74, 75), however to the best of our knowledge there is no study comparing these score head-to-head in accuracy of assessment. A recent study has shown some correlation between the endoscopic findings and histological activity only in the extremes of the disease(79).

Histological findings have also been used to predict clinical outcomes. Bitton et al in 2001 demonstrated histological markers of predicting a flare in quiescent UC. There are growing numbers of publications exploring this further. These are discussed in detail in subsequent chapters.

The three scores used in our research studies are as below

Riley index

Acute inflammatory cell infiltrates (Polymorphonuclear cells in Lamina propria)
1-None, 2-Mild, 3-Moderate, 4-Severe

Crypt Abscesses:
1-None, 2-Mild, 3-Moderate, 4-Severe

Mucin Depletion:
1-None, 2-Mild, 3-Moderate, 4-Severe

Surface epithelial Integrity:
1-None, 2-Mild, 3-Moderate, 4-Severe

Chronic inflammatory infiltrate:
1-None, 2-Mild, 3-Moderate, 4-Severe

Crypt architectural irregularities:
1-None, 2-Mild, 3-Moderate, 4-Severe

Histologic Inflammatory activity (HIA) Score: (Rubin Score)

Scores & description.

0 Normal (completely uninvolved, no architectural distortion, no infiltrates

1 Quiescent (architectural distortion, increased lamina propria lymphocytes, but no activity)

2 Increased lamina propria granulocytes without definite intraepithelial granulocytes

3 Intraepithelial granulocytes (e.g. cryptitis) without crypt abscesses

4 Crypt abscesses in less than 50% of crypts

5 Crypt abscesses in greater than 50% of crypts, or erosion/ulceration

Geboes score

Grade 0 Structural (architectural change)

0.0 No abnormality

0.1 Mild abnormality

0.2 Mild or moderate diffuse or multifocal abnormalities

0.3 Severe diffuse or multifocal abnormalities

Grade 1 Chronic inflammatory infiltrate

1.0 No increase

1.1 Mild but unequivocal increase

1.2 Moderate increase

1.3 Marked increase

Grade 2 Lamina propria neutrophils and eosinophils

2A Eosinophils	**2B Neutrophils**
2A. 0 No increase.	2B.0 None
2A.1 Mild but unequivocal increase	2B.1 Mild but unequivocal increase
2A.2 Moderate increase	2B.2 Moderate increase
2A.3 Marked increase	2B.3 Marked increase

Grade 3 Neutrophils in epithelium

3.0 None

3.1 < 5% crypts involved

3.2 < 50% crypts involved

3.3 > 50% crypts involved

Grade 4 Crypt destruction

4.0 None

4.1 Probable—local excess of neutrophils in part of crypt

4.2 Probable—marked attenuation

4.3 Unequivocal crypt destruction

Grade 5 Erosion or ulceration

5.0 No erosion, ulceration, or granulation tissue

5.1 Recovering epithelium and adjacent inflammation

5.2 Probable erosion—focally stripped

5.3 Unequivocal erosion

5.4 Ulcer or granulation tissue

Prediction of relapse in UC

Various clinical activity indices, serum, faecal, endoscopic and histological markers have all been used in the clinical trial to predict short and long-term outcomes in IBD(4, 40, 71, 80-83). The results are conflicting and at times confusing owing to the use of differences in activity indices, biomarkers and definitions of relapse used in these studies.

Can disease activity indices predict relapse in UC?

Approximately ten disease activity indices are available for use in UC as mentioned above. Some have been more commonly used than the others in clinical trials. Studies looking at the outcomes in using clinical activity indices alone are rare. They are usually used in conjunction with other parameters such as serum/faecal biomarkers, endoscopic or histological variables(80, 84). Data from these studies suggest that clinical activity indices have a reasonable role to play in the assessment of inflammatory activity but have little in the way of predicting outcomes.

Can biomarkers predict relapse?

1.1.1.7 C-Reactive Protein (CRP)

Bitton et al(4) evaluated the clinical, biochemical, endoscopic and histological predictors of relapse in UC patients. 74 adult patients with quiescent colitis were followed up for a year or until an episode of relapse. Younger age, multiple relapses and histological finding of basal plasmacytosis were found to be predictors of relapse within 12 months but not CRP. On the other hand, Consigny et al(85) reported a CRP>20 in association with ESR >15 mm/hour predicted flare up within 12-18 months in patients with IBD in clinical remission (sensitivity 89% and specificity 43%).

1.1.1.8 Faecal calprotectin

Extension of the role of FCP in the prediction of a flare-up in IBD has drawn a lot of attention recently. The results in the published literature are variable. A meta-analysis involving six studies and 672 IBD patients found that elevated FCP levels predicted flare-up of disease in the following twelve months with a sensitivity of 78% and a specificity of 73%(86). Whilst FCP seems to have a role in predicting the course of IBD the sensitivity and specificity found in this meta-analysis were not as high as expected. Serial measurements of FCP are found to be more helpful than a baseline reading to improve the accuracy of prediction. Further research is required to substantiate this finding.

1.1.1.9 Histological markers

Acute inflammatory infiltrates, crypt abscesses, mucin depletion are associated with increased risk of relapse of up to two to three-fold in subsequent twelve months (5).

Presence of increased amount of plasma cells in the lower third of the mucosa is termed as 'basal Plasmacytosis'-which was the feature associated with up to 4.5 fold increased risk of flare-up within 12 months' time (4, 82). Feagin et al noted that basal lymphoplasmacytosis, basally located lymphoid aggregates and markers of more severe inflammation such as erosions and ulcerations were all associated with risk of relapse(16). On the other hand complete mucosal healing is associated with lesser relapse rates and lower colectomy rates (87).

Can advanced endoscopy predict relapse?

Advances in endoscopy such as High definition white light endoscopy (HD-WLE), Narrow Band Imaging (NBI), Autofluorescence imaging (AFI), Chromoendoscopy (CE), and Magnification Chromoendoscopy have all been investigated to assess the accuracy in assessment and prediction of clinical outcomes. Kudo et al(38) included 30 patients with quiescent colitis in their study in which they examined the mucosal vascular pattern of colon with standard white light endoscopy (WLE) and NBI. For assessment of disease activity the examination was performed by arbitrarily dividing the colon into segments. They reported that with the use of NBI the segments that were identified as 'abnormal' by the WLE, were further characterised into either 'clear' or 'obscure' (WLE- 60 normal & 97 abnormal segments, NBI-60 normal & 44-clear, 53-obscure). This was further corroborated with histological findings where the obscure segments showed raised acute inflammatory infiltrates, goblet cells and basal plasmacytosis. In another study Jauregui-Amezaga et al(40) reported that use of NBI did predict flare up in quiescent UC patients in 12 months follow up period.

Magnification colonoscopy with chromoendoscopy in one study from Japan(42) has shown similar benefits when they examined the pit pattern of rectal mucosa of quiescent patients. They found that the grade of pit-pattern irregularity/disruption correlated with the severity of histological inflammation and also predicted the flare.

Unfortunately all these studies are limited by the low number of recruits. The other limitation is that the endoscopies were performed by expert and hence results might not equate to findings by endoscopists with no such advanced skills. There is also a steep learning curve to master the techniques of optical diagnosis.

Narrow Band Imaging

Narrow band imaging (NBI) is also called ‘virtual chromoendoscopy’. By digitally enhancing the optical image NBI helps better visualisation of the blood vessels and mucosal surface pattern. This is developed to help endoscopist identify lesions by live contrast enhancement. In addition to the components of the conventional endoscopes, NBI system has a special image processor and an image filter. The system is activated and deactivated by pressing a button placed on the handle of an endoscope.

Principles of Narrow Band Imaging

Light is an electromagnetic wave, and the peak to peak distance is called as “wavelength” which is measured in nanometres (nm). Wavelengths of light visible to the human eye as white colour are between 400-700nm, in which 400nm is seen as blue, 550nm is seen as green and 700 is seen as red colour. Light when illuminated on objects undergoes absorption, reflection and scattering. Light with a wavelength between 400-550nm gets absorbed more as it has less penetration and scatter. And the light between 550-700nm penetrates deeper and gets reflected more. The reflected light helps us to perceive the colour of the given object. Narrow band imaging is the technology which relies on the principle of depth of penetration of the light to visualise the tissue in wavelengths between 400-550 nm. NBI filters decrease the wavelengths of the lights illuminated; the blue light is centred at 415nm and the green lights at 540 nm. The blue filter is designed to correspond to the spectrum at which haemoglobin absorption is at its peak. Submucosal vessel vascular structures are enhanced by light at 540nm. Therefore these dual wavelengths of light have less penetration and scatter into the deep submucosal tissue. These, in turn, are absorbed strongly by the haemoglobin and hence capillaries on the mucosa are optically enhanced on NBI. The reflected image is captured by a charged coupled device chip (CCD). The resultant merged image displayed on the screen thus highlights mucosal vascular pattern with greater enhancement than with white light. Figures 1 and 2 describe these principles schematically (see below).

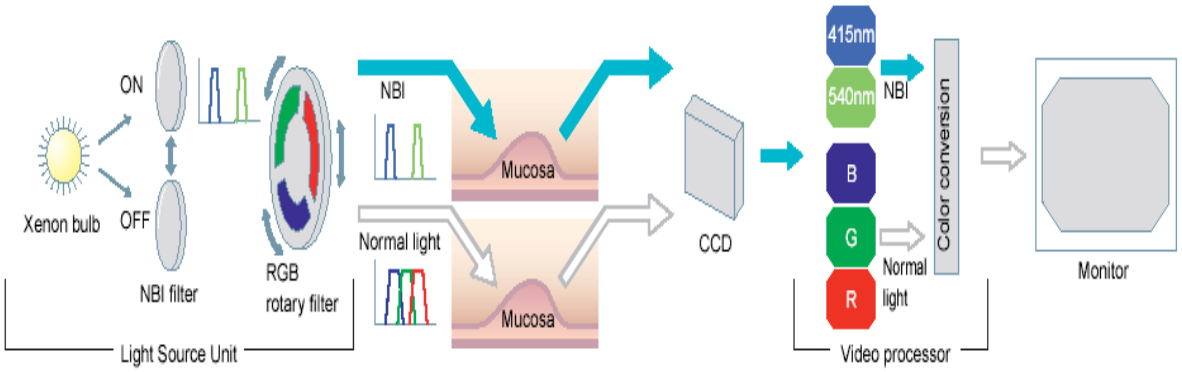

Figure 1 Schematic diagram of NBI filters to decrease wavelengths

The wavelengths of blue and green lights filtered through the RGB filter are centred to 415 and 540 nm.

Image courtesy Olympus Keymed®

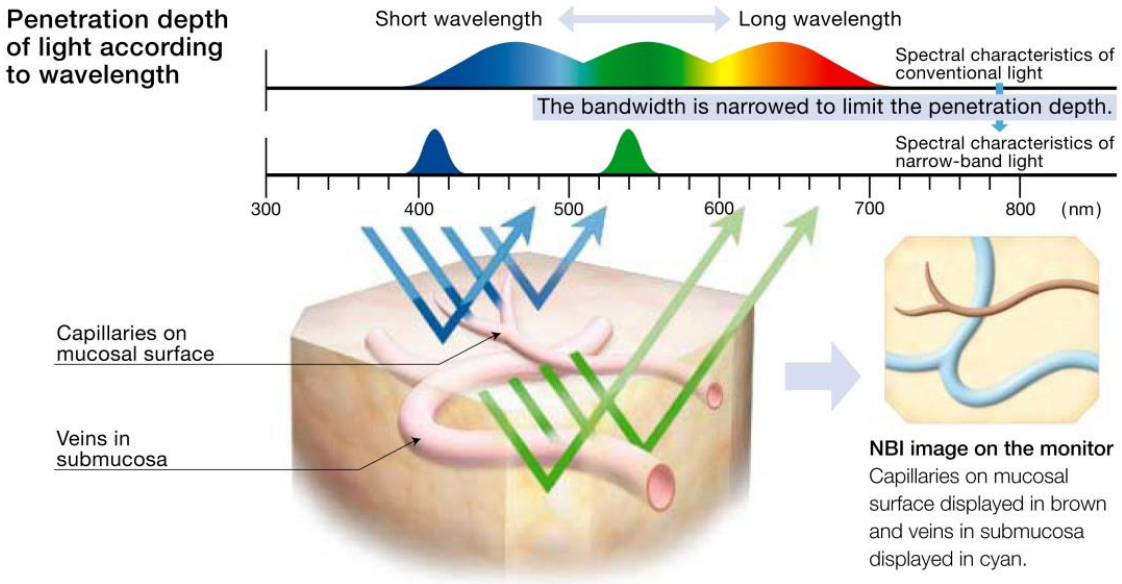

Figure 2 Schematic diagram showing the dispersion of light

This diagram shows the mechanism of penetrance, reflection and absorption of blue and green light**.** The blue light penetrates and scatters less than the others as a result of which superficial mucosa is visualised in great detail. Capillaries on the mucosal surface are highlighted as brown colour as the blue light is readily absorbed by haemoglobin.

Image courtesy Olympus Keymed®

Narrow Band imaging in colorectal diseases.

NBI is extensively studied to assess the accuracy of diagnosing and characterising gastrointestinal lesions. When used with magnification, NBI has high diagnostic yield for high-grade dysplasia and specialised intestinal metaplasia in Barrett's mucosa with sensitivity, specificity and diagnostic odd's ratio of 95%, 65% and 37.53 respectively(88). NBI has the highest diagnostic yield and inter-observer agreement compared to WLE and Autofluorescence imaging (AFI) when used for characterisation of colonic polyps. Furthermore, NBI with magnification is highly accurate in characterising colonic polyps <10mm with a detailed assessment of mucosal pit pattern and vascular pattern. Combination of these factors increases the diagnosis as opposed to using pit pattern alone (sensitivity of 98% p=0.006)(89). NBI International Colorectal Endoscopic (NICE) classification is simple and well-established methods for characterisation of polyps(90). It utilises colour, mucosal pit pattern and vascular architecture to classify colorectal polyps. Advantages of using this in clinical practice include that it does not require magnification colonoscopes, has a high diagnostic accuracy, sensitivity and negative predictive value in differentiating neoplastic from non-neoplastic lesions (89%, 98% and 95% respectively)(91). Whilst use of NBI has clear advantages in the characterisation of polyps in colorectum, the results on adenoma detection are disappointing. NBI did not improve detection of adenomas compared to high definition WLE in a randomised controlled trial in patients at risk of developing colonic adenomas(92). Cochrane review of eleven randomised controlled trials involving 3673 participants did not find evidence to support NBI is superior to WLE in improving colorectal polyp or adenoma detection(93). Use of NBI in long-standing colitis for detection of dysplastic lesions has not been encouraging either. Randomised controlled trials comparing NBI with WLE and CE did not show improvement in detection of dysplastic lesions(94, 95).

Figures 3 shows an area of the colon examined under white light and NBI. The superficial mucosal vasculature looks brown under NBI as the haemoglobin in the capillaries readily absorb the blue light; whereas the submucosal veins appear cyan owing to lesser haemoglobin concentration. Furthermore, the pit pattern on the mucosa looks more prominent under NBI compared to white light (Figures 4)

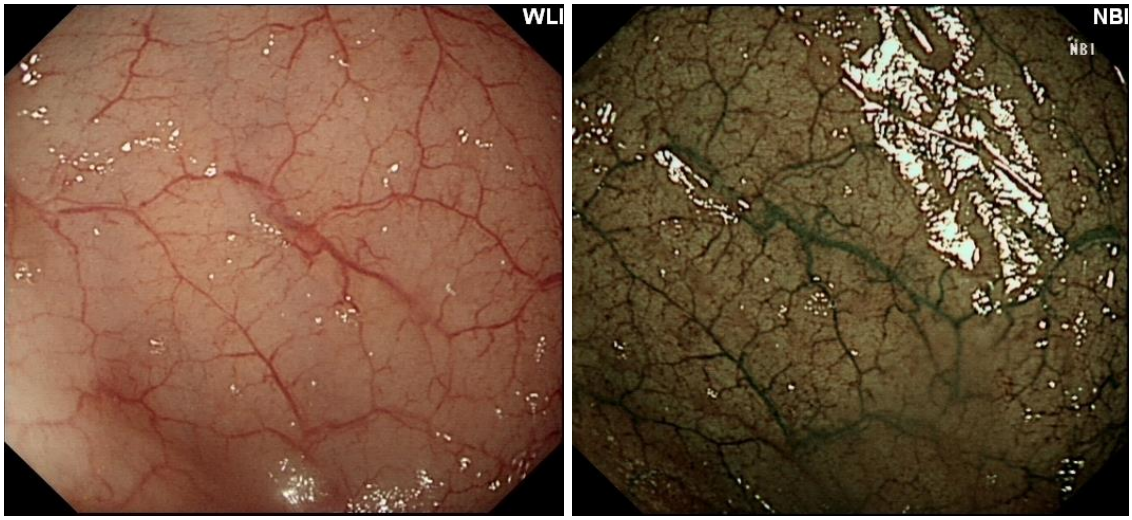

Figure 3 Normal colonic mucosa in white light (left) and in Narrow band imaging (right)

White light and narrow band examination of the normal colonic mucosa. NBI enhances mucosal capillary network (brown). Submucosal vessels are seen with cyan tinge.

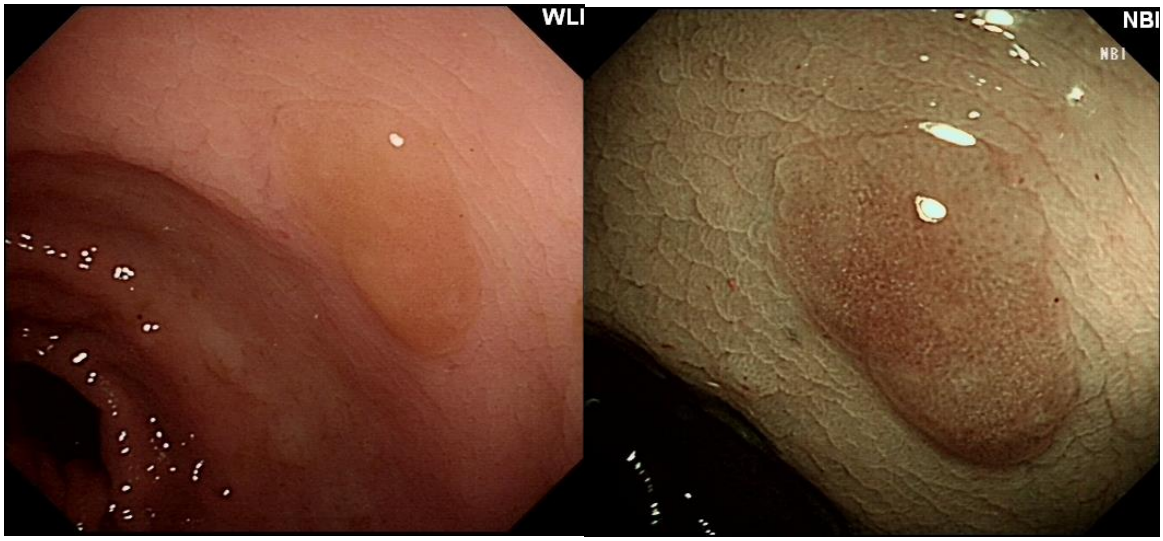

Figure 4 A sessile polyp seen with white light (left) and Narrow band imaging (right)

A sessile polyp in the rectum is examined under white light and narrow band imaging. NBI enhances the surface pit pattern and improves the characterisation of the polyp.

Chromoendoscopy

Adenoma detection at colonoscopy is one of the established quality indicators of colonoscopy outcomes. Dye spraying or 'conventional chromoendoscopy' (CE) is the application of dyes like indigo carmine or methylene blue to the colonic mucosa to improve identification of mucosal abnormalities. Early adenoma detection and resection reduces the development of colorectal cancer (CRC) and related mortality(96, 97). National Institute for clinical excellence (NICE) has recommended routine use of CE in surveillance colonoscopies for long standing Ulcerative colitis and colonic IBD.

Cancer risk in long-standing IBD

Duration, extent, the involvement of terminal ileum (backwash ileitis), primary sclerosing cholangitis, the presence of endoscopic and histological inflammation, family history of CRC are well-known risk factors for the development of colorectal cancer (CRC) in UC patients(20, 98-102). Risk of CRC increases with the extent of the disease ranging from 1.7 fold for proctitis to 14.8 fold rise for pancolitis, compared to the general population(103). The absolute risk of developing CRC in patients with pancolitis after 35 years of the disease is calculated at 30% in the same study. Owing to this risk, surveillance colonoscopy is recommended to detect early dysplastic lesions in long-standing UC patients.

Endoscopic surveillance for dysplasia

Various advanced endoscopic imaging techniques were tried to improve detection of dysplasia in surveillance colonoscopies. First generation NBI did not improve diagnostic ability compared to WLE in an RCT conducted by Dekker et al(104). HDWLE was found equivalent to NBI in detecting dysplastic lesions in two subsequent RCTs. (94, 105). In a randomised cross-over trial, NBI when compared with chromoendoscopy marginally improved withdrawal time (26.87 ± 9.89 minutes vs 15.74 ± 5.62 minutes, $P < .01$) and showed significantly low false positive and false negative rates for dysplasia detection on targeted biopsies ($p<0.001$); however, missed rates for intraepithelial neoplasia were higher with NBI(95). These results questioned the use of NBI in UC surveillance (Table 7).

AFI has been used extensively in the detection of dysplasia in UC. In a randomised trial of tandem colonoscopies, WLE, NBI and AFI (tri-modal imaging) were used for detection and characterisation of dysplastic lesions. In this small study involving 50 patients, AFI was found to improve detection and predict neoplasia in histology(106). In a large multi-centre randomised controlled study, AFI was compared with chromoendoscopy in detecting dysplasia in patients with long standing UC(107). The results suggested that the AFI did not meet the criteria for a larger non-inferiority study and that the technology should not be offered as an alternative in dysplasia surveillance in UC.

Confocal laser endocytoscopy (CLE) is a novel imaging technique which allows *in vivo* analysis of the tissue histology with high accuracy in distinguishing normal, regenerative and neoplastic tissues(108, 109). In a randomised controlled trial, conventional colonoscopy with random biopsies was compared with chromoendoscopy with CLE and targeted biopsies. A 4.75 fold increase in detection of neoplastic lesions was noted in CE+CLE group (p=0.005), although 50% fewer biopsies were required (p=0.008)(110). Although CLE with chromoendoscopy guided targeted biopsy has excellent outcomes, routine use of this technique is not only time consuming and tedious but involves a steep learning curve. Table 7 provides a summary of the studies using advanced endoscopic modalities to improve the detection of dysplasia in long-standing UC.

Role of chromoendoscopy in UC

Due to the increased risk of cancer in long-standing UC population(111) endoscopic follow up is recommended for early detection of dysplastic lesions. Random colonic biopsies (up to 30-40 per patient) have been the mainstay of detecting dysplasia. Chromoendoscopy improves adenoma detection in UC patients by contrast enhancement of dysplastic lesions. Meta-analysis of six randomised controlled studies including 1277 patients comparing the diagnostic yield of dysplastic lesions between WLE and CE clearly demonstrated the superiority of CE. In this study 44% more dysplastic lesions on targeted biopsies [95% Confidence interval (CI) 28.6-59.1] and 27% more flat lesions (95% CI 11.2-41.9) were diagnosed with CE(112). Available data unequivocally demonstrated the usefulness of CE over WLE. The newer generation of colonoscopes equipped with high definition image quality has improved detection of colonic polyps(113). When used in surveillance colonoscopies in long-standing UC similar results were found(114). However, there is no data available comparing HD colonoscopy with or without CE. The British Society of Gastroenterology guidelines recommend using CE for surveillance colonoscopies in patients with colonic IBD(115)

Table 7 Diagnostic value of advanced endoscopic imaging methods in UC.

Author (year)	Study design	Endoscopic modalities	Total number of patients	Number of patients with dysplasia
Kiesslich *et al(116)*. (2003)	Randomized controlled trial	CE v/s WLE	165	32with CE vs 10 with WLE
Hlavaty *et al(117)*. (2011)	Tandem colonoscopy	CE vs WLE	20	7with CE vs 0 with WLE
Dekker *et al(104)*. (2007)	Randomized controlled trial	NBI vs WLE	42	9 with NBI vs 12 with WLE
van den Broek *et al(105)*. (2011)	Randomized controlled trial	NBI vs HDWLE	48	13 with NBI vs 11with HDWLE
van den Broek *et al(106) (2008)*	Randomised controlled trial	AFI vs WLE. Detected lesions analysed by NBI	50	*AFI-first:* 10 with AFI 0 additional lesion detected by WLE *WLE-first:* 3 with WLE v/s 6 additional lesions detected by AFI
Kiesslich *et al(110) (2007)*	Randomised controlled trial	CE+CLE targeted biopsies VS WLE with random biopsies	153	19 with CE+CLE vs 4 with WLE
Vleugels et al(107) (2018)	Randomised controlled trial	AFI versus CE in surveillance of long standing UC	210	Dysplasia was detected in 12% (13 patients) in AFI arm and 19% (20 patients) in the CE arm

CE-chromoendoscopy, NBI-Narrow Band Imaging, WLE-White light endoscopy, HDWLE-High definition white light examination, CLE-Confocal Laser Endomicroscopy, VS-versus, AFI-Autofluorescence Imaging.

Uptake of chromoendoscopy in clinical practice

Chromoendoscopy refers to the application of diluted indigo carmine or methylene blue to the colonic mucosa to highlight subtle mucosal abnormalities. By providing a rim of contrast around the lesions, CE helps in detection, delineation, and characterisation of dysplastic tissue. There is growing evidence to suggest CE is superior in detecting dysplasia in UC surveillance compared with the standard white light examination (WLE). A Meta-analysis including 665 patients from 6 studies in 2013 confirmed that CE detects more dysplastic lesions compared to random biopsies obtained from standard WLE(118). CE is recommended by British Society of Gastroenterology(119) and European Crohn's and Colitis Organisation(120) as a preferred method of surveillance in colonic IBD. Similar to the studies from the UK, a recent physician survey from Canada looked at the practice of surveillance colonoscopy in patients with UC among the academic gastroenterologists (121). This study showed that only 26.5 % of Canadian Gastroenterologists routinely use CE, despite the fact that the majority (71%) of the participants were physicians with IBD as their subspecialty.

The uptake of CE among the colonoscopists has been variable. This may be due to the steep learning curve involved, time constraints on endoscopy lists and the common perception that this is a 'messy' time-consuming procedure.

Aims and Hypothesis

Hypothesis to be tested

- NBI confer additional benefit over standard endoscopic evaluation in the assessment of disease activity and prediction of short and long-term outcomes in UC.

- Spectroscopic assessment of inflamed and non-inflamed tissue in UC correlated well with histological staging.

- High definition chromoendoscopy detects more dysplastic lesions than high definition white light endoscopy alone.

Aim

The main aim of the research project was to assess the role of image-enhanced endoscopy in the assessment of disease activity, prediction of relapse and improvement in detecting dysplastic lesions in patients with Ulcerative colitis.

The objectives of the studies performed are:

- a) To establish if addition of NBI in endoscopic assessment of patients with UC improves the staging of disease activity.

    - To examine if NBI predicts short as well as long-term outcomes in UC.

- To compare the findings of spectroscopy of inflamed and non-inflamed mucosa with histology and endoscopic findings and identify potential metabolic correlates of inflammation.

  To compare the rate of detection of dysplasia in patients with long-standing UC with HD-WLE compared to HD-CE.

Methods and results of each study

2 Can standard white light endoscopy predict a flare?

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) with a characteristic relapsing and remitting disease course. Inflammation in UC is continuous, starts from the rectum and extends proximally. Genetic, immunological and environmental factors have been linked to the mucosal inflammation seen in the UC; however, the exact etiopathogenesis is unknown. Similarly, the pathophysiology of flare-ups is not well understood.

Relapse in UC has associated consequences related to health, social & psychological well-being. It is also a considerable economic burden to patients and the healthcare provider. The aim of treatment in UC is to achieve mucosal healing (MH) and maintain remission. MH is used as a treatment endpoint in clinical trials and has shown to reduce flare related hospital admission and the need for surgical interventions. (87)

Serum biomarkers are widely used to assess and monitor disease activity in UC. In one of the landmark studies, Bitton et al (4) report the predictors of clinical relapse in patients with quiescent disease. 74 patients in clinical and endoscopic remission underwent serum biomarkers and a colonoscopy at induction. They were prospectively followed up every three months for a year, or shorter if they had a relapse. They observed that younger age, multiple previous relapses (in women) and basal plasmacytosis were independent predictors of relapse. However, they found no evidence that serum levels of CRP, ESR and IL-1β, IL-6 and IL-15 predict relapse in UC. However this has been refuted in subsequent studies; in one study an elevated CRP and ESR correlated well with right colonic inflammation(122), and in another study, an eight-fold elevation of a predictive biologic score increased the rate of relapse (85).

Calprotectin (FCP) is a commonly used faecal biomarker with promising results in clinical trials. However, a meta-analysis of six prospective studies in patients with quiescent disease showed that the FCP only predicts flare within 12 months with a sensitivity and specificity of 78% and 73% respectively (123).

Endoscopic assessment is the key to evaluating the extent and severity of the disease activity. However histological inflammation is known to persist despite the normal endoscopic appearance of the mucosa at endoscopy(3, 124). White light endoscopy although routinely used in the assessment of severity of disease, has not shown any value in predicting disease course. Furthermore, results from studies evaluating disease outcomes

using advanced endoscopic features such as high definition endoscopy, narrow band imaging and magnification endoscopy have been conflicting (38, 40, 42, 44).

Presence of histological inflammation is the driving force behind relapses in the short term and dysplasia in the long-standing UC (20). Various histological scores are available to grade inflammation in UC (3, 72, 73). The correlation between the presence of inflammation and subsequent relapse has been suggested by a number of studies (4, 16, 82, 125); however, a recent prospective study did not show any correlation (40)

The available biomarkers are not reliable in predicting flare-ups of the disease. Despite the invasiveness of the endoscopic assessment and its subjective interpretation, there is no substantive replacement available with a reliable non-invasive biomarker. Our retrospective cohort study aimed at assessing the association between endoscopic disease activity assessed with Mayo endoscopic subscore (MES), histological inflammation (Geboes score) and combined endoscopic and histologic disease activity in predicting disease course (clinical relapse in 12 months) in quiescent UC patients.

Methods

We conducted a retrospective review of adult patients in clinical remission who underwent surveillance colonoscopies in our institution from January 2008 to December 2011. This study was conducted as an audit on local practice of surveillance procedure. The study protocol conforms to the ethical guidelines of the World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004. All procedures were done as a part of patient care and no extra tests or procedures were done as a part of the study. Data was collected using electronic records for endoscopy reports and the subsequent clinical care. Demographic details of the patients were recorded along with duration and extent of the disease, medications (current and past), use of oral steroids in the last 6 months, endoscopic assessment of inflammation using MES and follow up data for any disease relapse in the next 12 months. An expert gastrointestinal pathologist (OR) independently assessed the histology and graded the inflammation using Geboes score (72). The pathologist was blinded to the patient's clinical outcomes (occurrence of relapse).

Patients were deemed to have had a relapse if they required steroids or an increase in their medication dose for symptom control in the subsequent 12 months following index colonoscopy.

Inclusion criteria: All adult patients (Age ≥18 years) with histologically confirmed UC on surveillance colonoscopy program were included.

Exclusion criteria: Patients were excluded from the study if they had been diagnosed to have a flare at colonoscopy, underwent colonic resection in the follow up period for dysplasia/cancer, had a diagnosis changed to Crohn's disease either at the colonoscopy or in the follow-on period, failed procedure either due to poor prep or incomplete colonoscopy or with missing information in records/lost to follow up.

Statistical analysis:

The main aims of the study were to assess the association between white light endoscopic disease activity, histological inflammation and combined endoscopic/histological disease activity with the risk of clinical relapse in the next 12 months of follow-up. Results are expressed as means ± standard deviations for continuous variables and as frequencies for categorical variables. P values from ANOVA or chi-square tests were considered statistically significant if ≤ 0.05. Binary Logistic regression analysis was performed using the Enter method to calculate odds ratios (OR) and their 95% confidence intervals. All variables were analysed by univariate analysis and included in the multivariate regression model was used if $p<0.3$. Correlation matrices were used to identity collinearity. When collinearity was detected (rho >0.6) this was minimized by inputting the variable separately in the multivariate analysis. Hosmer-Lemeshow's test was used to test the null hypothesis that there is a linear relationship between the predictor variable and the log odds of the outcome variable. All statistical tests were done using PASW version 20 (IBM Corp, NY).

Results

A total of 406 patients were identified to have undergone surveillance colonoscopy during the study period of which 295 were included in the study. The flow chart of patient selections is shown in Figure 5. 295 patients were included in the final analysis and their demographic characteristics are outlined in Table 8. The mean age of patients was 56.3 years and the mean duration of disease after histological diagnosis was 22.3 years. 181 (61.3%) patients were diagnosed with Pancolitis and 114 (38.6%) with the left sided disease. 95 (32.2%) patients were on various disease-modifying agents such as Azathioprine, Methotrexate, Infliximab or Adalimumab. The rest (n=183, 62%) were on oral 5-Aminosalicylic acid (5-ASA) maintenance therapy alone. 17 patients were not taking any regular treatment for their ulcerative colitis. None of the patients were on topical therapy alone (5-ASA or steroids).

65 (22%) of the 295 patients had a clinical relapse documented in the 12 months following their colonoscopy. The results of the univariate analysis are outlined in Table 9. Factors significantly associated with a greater risk of clinical relapse in the next 12 months included

younger age, shorter disease duration, not on immunomodulator/biologic drugs, endoscopic inflammation (MES>1), histologic inflammation (Geboes > 2.1) and both MES > 1 and Geboes score > 2.1 (Combined Both) and either MES > 1 or Geboes score > 2.1 (Combined Any). On multivariate analysis, every unit increase in age (in years) was significantly associated with a 0.96 fold (95% CI 0.93-0.99) reduction in risk of relapse and immunomodulator/biologic use with a 0.30 fold (014-0.66) reduction in risk of relapse. An MES of > 1 and a Geboes score of > 2.1 was associated with a 4.63 (95% CI 2.39-9.00) fold and 4.90 (2.61-9.18) fold increase in the risk of clinical relapse. Table 10 outlines the results from the binary logistic regression analysis. Table 11 lists the sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of the MES, Geboes score and combinations of these 2 scores in predicting the risk of relapse in the next 12 months. The diagnostic accuracy parameters for these scores are modest with sensitivities ranging from 49.2 to 73.8% and specificity from 68.7 to 83%.

Table 8 Baseline characteristics of the patients included in the study.

Total number of patients	295
Gender: Male (%) Female (%)	157 (53%), 138 (47%)
Age (mean ±SD in years)	56.3 ± 13.2
Duration (mean ±SD in years)	22.3 ± 10.6
Disease extent: Pancolitis (%), Left sided colitis (%)	181 (61.3), 114 (38.7%)
Immunomodulator/biologic drug use (%)	95 (32.2)
5-ASA (aminosalicylate) use (%)	183 (62)
Recent steroid use (within 6 months) n (%)	20 (6.7)
Geboes score >2.1 n (%)	95(32.2)
Mayo score >1 n (%)	96 (32.5)

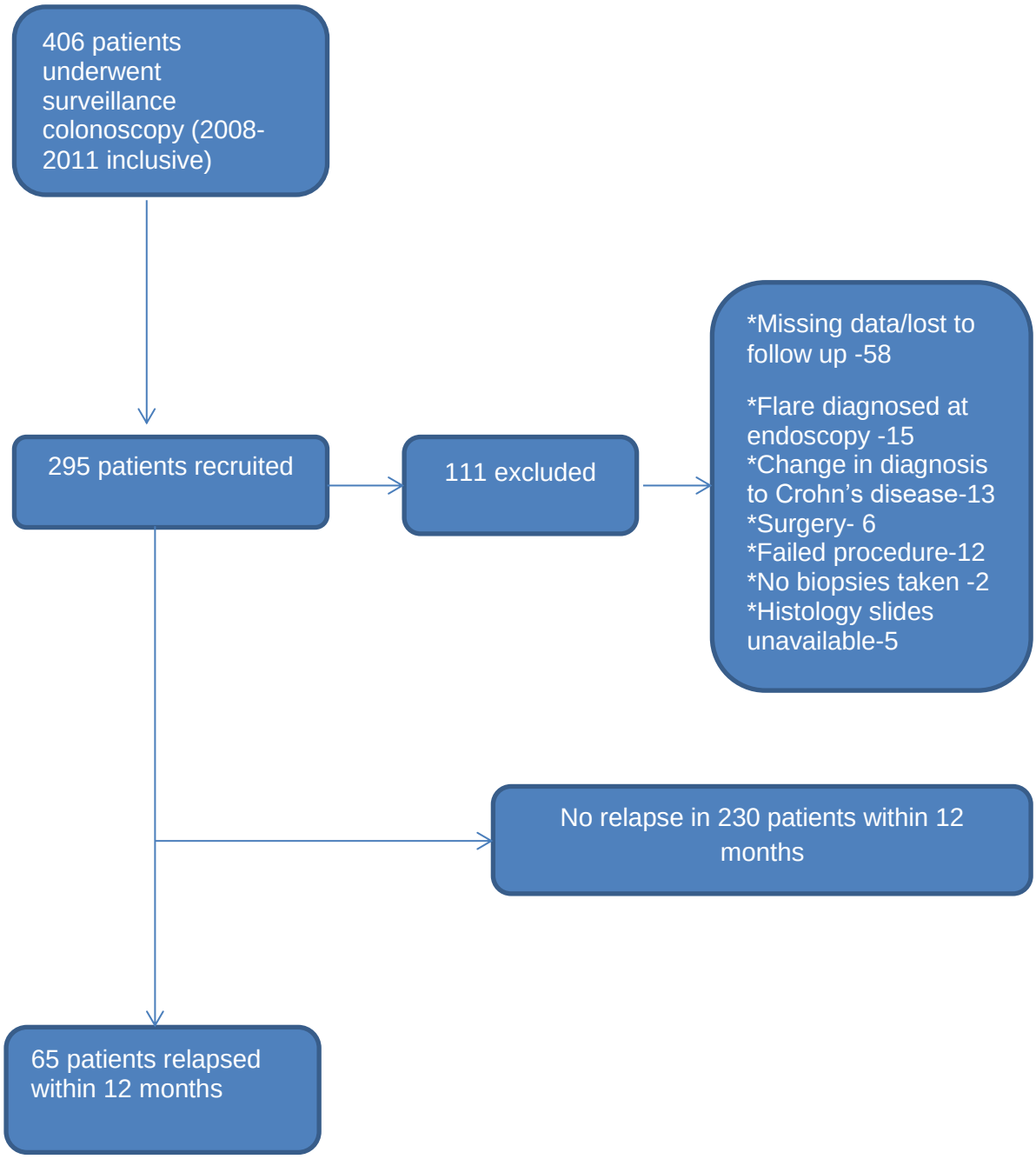

Figure 5 Flowchart of patients included in the study 1

Table 9 Univariate analysis of baseline parameters

	Relapsers (n=65)	**Non-relapsers** (n=230)	**P value**
Age (Mean ± SD)	50.8 ± 14.4	57.89 ± 12.4	0.001
Duration of disease (years) (Mean ±SD)	20 ± 11	23 ± 11	0.043
Gender (male/female)	33/32	124/106	0.67
Extent (left sided/pancolitis)	30/35	84/146	0.19
Immunomodulator or biologic use (yes/no)	13/52	82/148	0.017
Recent steroid use (within 6 months) (yes/no)	8/57	12/218	0.048
Geboes score **≤2.1** **>2.1**	 24 41	 176 54	 <0.001
MES **0** **≥1**	 26 39	 173 57	 <0.001
Combined Both **Either Geboes Score ≤2.1 or MES =0** **Geboes Score > 2.1 and MES ≥1**	 32 33	 191 39	 <0.001
Combined Any **Both Geboes Score ≤2.1 and MES =0** **Either Geboes Score > 2.1 or MES ≥1**	 48 17	 158 72	 <0.001

MES-Mayo endoscopic subscore

Univariate analysis of Mayo endoscopic score (MES) > 1, Geboes histopathological score > 2.1, both MES > 1 and Geboes score > 2.1 (Combined Both) and either MES > 1 or Geboes score > 2.1 (Combined Any) in predicting risk of a clinical flare in the next 12 months adjusted for age, duration of disease, extent of disease, immunomodulator/biologic use and steroid use in the last 6 months.

Table 10 Binary logistic regression analysis

	Odds ratio	95% CI
Age	0.96	0.93-0.99
Duration of disease	1.00	0.97-1.03
Extent (Left sided vs Pancolitis)	0.97	0.51-1.83
Immunomodulator/Biologic use (Yes vs no)	0.30	0.14-0.66
Recent steroid use (Yes vs No)	1.46	0.45-4.73
Geboes score >2.1	4.90	2.61-9.18
MES>1	4.63	2.39-9.00
Combined Both	4.15	2.11-8.14
Combined Any	6.19	3.19-12.00

Binary logistic regression analysis of Mayo endoscopic score (MES) > 1, Geboes histopathological score > 2.1, both MES > 1 and Geboes score > 2.1 (Combined Both) and either MES > 1 or Geboes score > 2.1 (Combined Any) in predicting risk of a clinical flare in the next 12 months adjusted for age, duration of disease, extent of disease, immunomodulator/biologic use and steroid use in the last 6 months.

Table 11 Results

Score	Sensitivity (95%CI)	Specificity (95% CI)	Negative predictive value	Positive predictive value	Overall accuracy (95%CI)
MES > 1	60.0% (48.8-70.3)	75.2% (72.0-78.1)	0.87 (0.83-0.90)	0.41 (0.33-0.48)	71.9% (66.9-76.4)
Geboes score > 2.1	63.1 % (51.9-73.2)	76.5% (73.4-79.4)	0.88 (0.84-0.91)	0.44 (0.36-0.50)	73.6% (68.6-78.0)
Combined Both	49.2% (38.5-59.5)	83% (80.0-86.0)	0.85 (0.82-0.88)	0.45 (0.35-0.55)	75.6%(70.9-80.1)
Combined Any	73.8% (62.7-83.0)	68.7% (65.6-71.3%)	0.90 (0.86-0.94)	0.40 (0.34-0.45)	69.8% (64.9-73.9)

Sensitivity, Specificity, negative likelihood ratio, positive likelihood ratio and overall accuracy of Mayo endoscopic score (MES) > 1, Geboes histopathological score > 2.1, both MES > 1 and Geboes score > 2.1 (Combined Both) and either MES > 1 or Geboes score > 2.1 (Combined Any) in predicting risk of a clinical flare in the next 12 months.

Discussion

Our study demonstrates that MES ≥1 and Geboes score ≥2.1, both individually and combined are predictors of disease relapse in patients with UC undergoing surveillance endoscopy. MES ≥1 indicates the macroscopic inflammation seen at endoscopy. Geboes score >2.1 is the presence of acute inflammation (increased eosinophils and neutrophil count in lamina propria), whereas scores 0, 1 and 2.0 does not include acute inflammatory infiltrates (hence in our results we report Geboes >2.1 and not 2.0). Some of the studies in published literature assessed clinical outcomes using endoscopic scores such as MES and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) (126-129), and others used histological scores like Geboes score or Riley index (3, 4, 82, 125, 130) individually. To the best of our knowledge, ours is the only study in which both endoscopic and histologic disease activity indices are used to predict disease recurrence/relapse. In our study cohort, we found that the presence of either of these markers (MES>1 or Geboes ≥2.1) increases the risk of relapse up to 6 times in the subsequent twelve months period.

In a recent prospective study involving 187 patients with quiescent UC (MES 0=126 and MES 1= 61), Acosta et al(126) observed that in total 26% (n=49) of patients had relapsed in 12 months of follow up period. 41% of patients with MES 1 and 19.3% of MES 0 had relapsed at the end of 12 months. They noted a much stronger association of MES with relapse in the first 6 months period (9.6% of MES 0 and 36.6% of MES 1). Similar results were noted by Carvalho and colleagues(127) where patients with Mayo 1 relapsed significantly more than patients with Mayo 0 disease. In their subgroup analysis, they found that the findings were not dissimilar to patients with either extensive or left sided colitis (but not proctitis).

In another recent study, Arai et al(128) found that UCEIS correlated well with MES. The severity of disease activity noted at endoscopy with UCEIS had a linear correlation with the percentage of patients who had a relapse at the end of study period (5% of patients with UCEIS 0 relapsed compared to 75% of those with UCEIS 4-5).

Histology is the gold standard in the assessment of disease activity in UC. Various scoring systems are available in the literature (3, 72, 73) but none seems to have been rigorously validated externally. Presence of basal plasmacytosis in rectal specimens is reported to be an independent predictor of disease relapse(4, 82). However, opinion among the gastrointestinal histopathologists differs on this. Recently Feagins et al (16) in a retrospective review noted that presence of any of the histological markers of inflammation such as basal lymphoplasmacytosis, basal lymphoid aggregates, erosions/ulcers in the

epithelium, moderate/marked architectural distortion were independently associated with significant relapse risk in patients with clinical remission.

Bessissow and colleagues also reported that a Geboes score of >3.1 (Neutrophils in epithelium and crypt involvement of <5%) was significantly associated predicting a relapse within 12 months. Interestingly Jauregui-Amezaga et al in their prospective study involving 70 inactive UC patients found that only 7/38 patients who had basal plasmacytosis in biopsies relapsed whereas 10/24 patients with no basal plasmacytosis relapsed (p=0.78). In our study, we did not aim to look at the basal plasmacytosis as it was felt that the histological slides needed to be re-processed for accurate assessment.

In a recent systematic review and meta-analysis of 15 studies, Park et al(130) found that the absence of histological inflammation compared to its presence was associated with less risk of flare up [RR 0.48 (95%CI 0.39-0.60)]. They also reported similar outcomes for the specific histological subset with absence v/s presence of eosinophils and neutrophils in epithelium and lamina propria, Crypt abscesses, Basal plasmacytosis and basal lymphoid aggregates. Similar to our study, they noted that the presence of Eosinophils and neutrophils (graded as 2.1 on the Geboes score) is associated with increased risk of relapse. Interestingly they also found that absence of basal plasmacytosis was not associated with decreased risk of relapse.

The accuracy of CE and magnification endoscopy with or without CE in the prediction of disease flare is poor (37, 42, 44). Watanabe et al(44) studied the use of magnification chromoendoscopy in their prospective study. 57 patients with long standing UC were included, of whom 12 of 17 (70%) patients with ‘frank mucosal defects’ had a disease flare within 12 months of follow up. On the other hand only 10 of 22 (45%) patients with ‘some mucosal irregularity’ and 1 of 18 patients with ‘no mucosal abnormalities’ had a flare within the same period. Although there seems to be a linear relationship with the degree of inflammation to the risk of relapse, the diagnostic accuracy of this endoscopic modality was poor.

Jauregui-Amezaga et al(40) studied the use of high-resolution chromoendoscopy and NBI in predicting flare-up in patients with sustained clinical remission. In this study, neither advanced endoscopic techniques nor the histology was predictive of relapse within 12 months period. Unfortunately, uses of advanced imaging modalities have their own limitations. Only small areas can be assessed in detail, it is labour intensive, time-consuming and needs considerable expertise.

Our study had similar relapse rates to the published literature. Using both endoscopic and histological markers to predict disease relapse, we observed that presence of either will increase the probability of disease flare-up by six times [OR 6.19 (95% confidence interval (CI) 3.19-12.00)]. When used in combination or individually the parameters were significant in predicting disease flare (Table 3). However, the overall diagnostic accuracy of these parameters was low with low sensitivity, specificity, negative and positive predictive values. Overall diagnostic accuracy for MES>1 was 72%, and that of Geboes score >2.1 was 74%. When used both in combination (Mayo>1 and Geboes score >2.1) there was a minimal rise in overall diagnostic accuracy (76%) whereas when any combination (Mayo>1 or Geboes score >2.1) was used, the accuracy fell to 70%. One possible explanation for such low figures is the low pick up rate due to sampling error. It is possible that the biopsies were not adequately targeted.

Limitations of our study

One of the limitations of our study is the retrospective design. However, we have a robust electronic patient records system which is integrated with the General practitioners and Hospitals locally. All hospital episodes or GP reviews are pulled through to our system bound by the data-share agreement. Despite this, the data on smoking status and psychological stress 'at the time of procedure' was not complete and hence excluded. We also chose to exclude the serological markers of inflammation in our analysis as there was a considerable degree of variability in the timing of blood tests and the procedure in the initial 100 patients assessed.

The other limitation of our study is that we included only surveillance patients and patients in the early years of the diagnosis were excluded. However, the relapse rate in our study is similar to studies that were conducted in non-surveillance patients. Hence we believe that the data can be extrapolated to the non-surveillance population too.

The endoscopic scores for our study were provided by analysing the archived images in the reporting software. Although this is an accepted method of retrospective assessment in publications, we appreciate that it could potentially introduce bias.

Despite the above limitations, we believe that our data is robust in capturing all the relevant information surrounding a relapse, and we have taken appropriate steps to avoid potential bias.

Conclusion

Endoscopic and histological activity is an important driving force of inflammation in UC with the risk of subsequent relapse. However, only 60-70% of patients with risk of relapse are

identified using both. Further studies looking at combining endoscopic and histological markers are required.

3 Can Faecal Calprotectin predict a flare in IBD: A Meta-analysis

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the two major types of inflammatory bowel diseases (IBD) with characteristic relapsing and remitting disease course. Mucosal inflammation is associated with relapse. There are significant physical, mental and financial consequences associated with relapse. Deep remission with complete mucosal healing is the aim of treatment in IBD. Commonly used biomarkers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are used conventionally to monitor the disease activity; however, they have not been found useful to identify patients with high risk of relapse(4). Levels of CRP and ESR vary with the systemic illnesses making their interpretation difficult in IBD. The ability to predict relapse using biomarkers may be helpful to physicians in tailoring the treatment and preventing future relapses and its consequences.

Calprotectin is a calcium and zinc binding protein belonging to the SA100 group of proteins. It is found predominantly in the cytosol of neutrophils and to a lesser extent in monocytes and reactive macrophages. It is available in abundance in body fluids and is resistant to bacterial or enzymatic degradation. Calprotectin constitutes about 60% of the cytosolic protein in the granulocytes and about 5% of total body protein(54). Intestinal inflammation is marked by the influx of inflammatory cell infiltrates in the mucosal lining. Breakdown of cytosolic protein liberates calprotectin in the faeces, the concentration of which is found to be proportional to the intestinal inflammation(55) and on white cell scanning (56). A spot test of <5gm of faeces is shown to be as reliable as 24-hour stool collection and can remain stable for up to 7 days in room temperature (54). It can be readily quantified using enzyme-linked immunoassay testing (ELISA). Faecal calprotectin (FCP) is hence classed as a 'damage-associated molecular pattern' protein and fulfils desirable qualities of a non-invasive surrogate marker for intestinal inflammation. New generation FCP kits make it even easier with 'on table' testing. All these features make FCP an attractive alternative for the serum biomarkers.

Tibble et al (62) have demonstrated that FCP can be used to discriminate Crohn's disease from irritable bowel disease in adult patients with 100% sensitivity. FCP has been shown to correlate well with the endoscopic and histological inflammation (131-133). A growing body of evidence suggests that this can be used effectively to monitor disease activity and tailor treatment modalities to prevent relapses (134-136). More recently the role of FCP in predicting relapses in UC and CD has drawn interest from researchers. The results in the

published literature on this subject are variable. This meta-analysis aims at assessing the predictive capabilities of FCP in UC and CD population from prospective trials involving adult patients with no surgical history or biologic therapy.

Material and Methods

3.1.1.1 Literature search

Multiple electronic databases were searched including Pubmed, Embase and Ovid looking for studies providing data on relapse prediction in IBD using FCP up to July 2018. Search terms used included 'Calprotectin', 'Faecal calprotectin', Fecal calprotectin'. These terms were further intermixed with 'Crohn's disease', 'Ulcerative colitis', 'relapse prediction', and 'prediction of flare' respectively. Our search results were not restricted to English language literature alone. Only prospective studies satisfying the inclusion criteriae were included in the final analysis. Further details were requested from the authors, where felt appropriate, prior to inclusion/exclusion. Articles were first screened by all three investigators (NM, ET and VS) using the title and abstract. Full text of shortlisted articles was independently assessed and data extracted by NM and ET. Disagreements were resolved by discussions with the senior author (VS).

3.1.1.2 Study selection

A study was included if it met the following criteria as follows 1) the study prospectively evaluated the capability of FCP in predicting flare-up in adult patients with UC or CD, 2) the criteria for diagnosing a relapse was clearly mentioned and 3) the study provided deducible information or the authors provided further information to carry out the required statistical tests. Studies looking at the outcomes in paediatric/teenage population and post-operative patients were excluded, as were the studies looking at the predictive value of FCP in patients on anti-TNF therapy.

3.1.1.3 Data extraction

Data was collected independently (NM and ET) on author, year of publication, study design, type of Calprotectin kit, definition of relapse and cut off level used, along with the diagnostic accuracy tests including sensitivity, specificity, true positive (TP), true negative (TN), false positive (FP) and false negatives (FN) were calculated for each study. A 2X2 table was created to extract the information when it was not available from the papers. Where the published data was not sufficient the authors were contacted for further input. Differences were resolved by discussion with senior author (VS).

3.1.1.4 Quality assessment

Quality assessments of the included studies were conducted using QUADAS-2 (Quality Assessment of Studies of Diagnostic Accuracy included in Systematic reviews) which is a revised version of a previously used QUADAS tool(137). Answering terms 'yes', 'no' or 'unclear' were used in four different domains such as 'patient selection', 'Index test', 'reference standard' and 'flow and timing'. Assessment of bias and concerns regarding applicability was marked as 'low', 'high', or 'unclear'. Disagreements were resolved by discussions among the authors.

3.1.1.5 Statistical analysis

All standard methods for performing meta-analysis were used. Statistical analyses were performed using Meta-Disc version 1.4(138).

For each study, sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) of baseline FCP level. A 2X2 table was created for calculations. A random-effect model was used to create pooled estimates with 95% confidence intervals. Summary receiver operating characteristic curve (SROC) was created to assess the relationship between true and false positive rates. Study differences were calculated using the I^2 statistics (25% - low inconsistency, 50-moderate inconsistency and 75% - high inconsistency)(139).

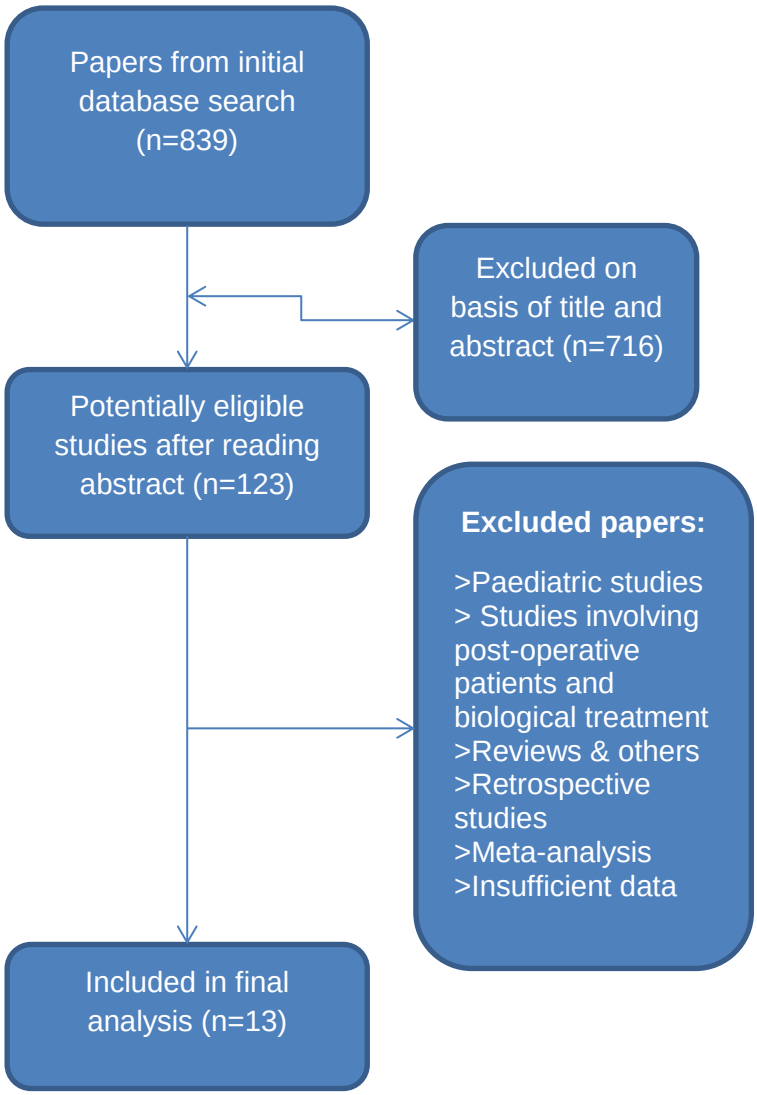

Figure 6 Flow chart of study selection (FCP Meta-analysis)

Table 12 Characteristics of included studies.

Study number	Author/year	Country	Calprotectin assay	Cut off level (UC/CD/IBD)	No. of Patients (UC/CD/IBD)	Age (years)	Definition of relapse	FU duration (months)
1	Tibble et al (2000)	UK	Roseth	50 mg/l (Adjusted value 250 µg/g)	37/43/80	16-77	CDAI>150 with >100 increase from baseline (CD), HBI >2 increase from baseline (UC)+change in treatment	12
2	Costa et al (2005)	Italy	Calprest	150 µg/g	41/38/79	24-54	CDAI>150 (CD), UCAI>4 (UC), plus change in treatment	12
3	D'Incà et al (2008)	Italy	Calprest	130 mg/kg Adjusted value 130 µg/g)	97/65/162	15-80	CDAI>150 or increase >50 or 75 from baseline (CD), or TW score≥4 (UC)	12
4	García-Sánchez et al (2009)	Spain	Calprest	150 µg/g	69/66/135	27-54	CDAI>150 (CD) or modified TW>11 (UC) plus change in treatment	12
5	Kallel et al (2009)	Tunisia	Calprest	340 µg/g	0/53/0	15-66	CDAI>150 or increase >100 from baseline that warranted treatment	12
6	Gisbert et al (2009)	Spain	Philcal	150 µg/g	74/89/163	--	CDAI>150 (CD) or modified TW>11 (UC)	12
7	Lasson et al (2013)	Sweden	Bühlmann	169 µg/g	69/0/0	18-74	Symptoms of UC requiring change in	3 months then yearly

							treatment	for 3 years
8	Yamamoto et al (2013)	Japan	Human Calprotectin ELISA Kit	170 µg/g	80/0/0	20-75	Worsening of stool frequency and/or rectal bleeding with an endoscopic score of 2 or 3.	12
9	Naismith et al (2013)	UK	Bühlmann	240 µg/g	0/92/0	18-83	Unplanned escalation of therapy, progression of disease (Montreal), or hospitalisation +/- emergency surgery.	12
10	Jauregui-Amezaga et al (2014)	Spain	Philcal	250 µg/g	64/0/0	--	Blood PR/Mayo≥ 1 with Histological activity	12
11	Hosseini et al (2015)	Iran	Bühlmann	341 µg/g	154/0/0	20-69	Elevated Seo index (>220), escalation of treatment	12
12	Scaioli et al (2015)	Italy	Calprest	193 µg/g	74/0/0	16-89	SCCAI>3	12
13	Theede et al (2016)	Denmark	Bühlmann	321 µg/g	70/0/0	--	Escalation of treatment	12

CD-Crohn's disease, UC-Ulcerative colitis, CDAI-Crohn's disease activity index, HBI-Harvey-Bradshaw Index, UCAI-Ulcerative Colitis Activity Index, TW-Truelove and Witts severity index, PR-Per rectum, SCCAI-Simple Clinical Colitis Activity Index, pMayo-Partial Mayo score.

Table 13 Quality Assessment of studies of Diagnostic Accuracy included in Systematic reviews-2 (QUADAS-2)

Domain 1 PATIENT SELECTION		Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Study 7	Study 8	Study 9	Study 10	Study 11	Study 12	Study 13
Risk of bias	Q1 Q2 Q3	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N	Y N N
Could the selection of patients have introduced bias?	**Risk**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Concerns regarding applicability														
Is there concern that the included patients do not match the review question?	**Concern**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Domain 2 INDEX TEST														
Risk of bias	Q4 Q5	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N	Y N
Could the conduct or interpretation of the index test have introduced bias?	**Risk**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Concerns regarding applicability														
Is there concern that the index test, its conduct, or interpretation differ from the review question?	**Concern**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Domain 3 REFERENCE STANDARD		CR	CR	CR	CR	CR	CR	CR	CR+ER	CR	CR+ER+ HR	CR	CR	CR

Risk of bias	Q6 Q7	Y U	Y U	Y U	Y U	Y U	Y N	N Y	Y U	Y N	Y Y	Y U	Y Y	Y Y
Could the reference standard, its conduct, or its interpretation have introduced bias?	**Risk**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**High**	**Low**	**Low**	**Low**	**Low**	**Low**	**High**
Concern regarding applicability														
Is there concern that the target condition as defined by the reference standard does not match the review question?	**Concern**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Domain 4 FLOW AND TIMING														
Risk of bias	Q8 Q9 Q10 Q11	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y	Y Y Y Y
Could the patient flow have introduced bias?	**Concern**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

Q1- Was a consecutive or random sample of patients enrolled?

Q2- Was a case-control design avoided?

Q3-Did the study avoid inappropriate exclusions?

Q4-Were the index test results interpreted without knowledge of the results of the reference standards?

Q5-If a threshold was used, was it pre-specified?

Q6- Is the reference standard likely to correctly classify the target condition?

Q7- Were the reference standard results interpreted without knowledge of the results of the index test?

Q8- Was there an appropriate interval between index test(s) and reference standard?

Q9- Did all patients receive a reference standard?

Q10- Did patients receive the same reference standard?

Q11- Were all patients included in the analysis?

Yes-Y, No-N, Unclear-U

CR- Clinical relapse

ER-Endoscopic relapse

HR-Histological relapse

Results

Electronic search for the terms mentioned earlier returned 3178 related publications, of which 839 articles were selected for detailed review. Further selection process is shown in the flowchart above. Studies assessing the ability of FCP in diagnosing IBD were not included. Those which assess the role of FCP in predicting relapses in postoperative Crohn's disease and in the paediatric/adolescent population were excluded. We also excluded studies including patients exclusively on biologic therapy.

Thirteen studies were included in the meta-analysis (40, 140-152). All the included studies were prospective studies with either a consecutive, systematic or a random selection of patients. Eleven studies provided (or extractable) data on Ulcerative colitis (40, 140-143, 145-147, 149, 150, 152) and 7 on Crohn's disease(140-145, 148). Data on combined UC and CD patients was extracted from 4 studies(140, 142, 145, 151), however, one study provided combined data alone without separate analysis of UC and CD patients (151). The characteristics of each of the included studies are mentioned in Table 12 and QUADAS-2 questionnaires in Table 13. Due to the small number of studies, it was felt that the analysis of combined data for UC and CD will not yield meaningful results.

Where appropriate the authors were contacted to provide additional data on their studies. Studies that were excluded from analysis include, a) those studies that did not provide a 12-month of data, b) those studies where the data was not extractable, and c) when the authors failed to respond to our email requests for providing us with the data (153, 154). A recent study assessing the role of consecutive sampling of FCP in predicting relapse was not included as extractable data was not available for analysis(155).

Diagnostic accuracy of FCP in predicting relapses in UC:

Eleven studies with a total of 932 patients with UC were included in the analysis. The pooled sensitivity and specificity are 0.70 (95% CI 0.65-0.76), 0.82 (95% CI0.79-0.86) respectively with a pooled DOR of 10.5 (95% CI 5.74-19.23). The pooled LR+ was 3.51 (95% CI 2.48-4.97) and LR- was 0.4 (95% CI 0.28-0.56). The AUC was 0.83 (SE 0.03) and the Q^* was 0.76 (SE 0.03) (Figures 7 and 8). The specificity of FCP for UC patients was improved compared to the overall IBD populations (82 versus 66%); however, the results demonstrated significant heterogeneity.

Diagnostic accuracy of FCP in predicting relapses in CD:

Seven studies involving 446 patients were included in this analysis. The pooled sensitivity and specificity was 0.74 (95% CI 0.65-0.82) and 0.75 (95% CI 0.71-0.80) respectively with a pooled DOR of 8.2 (95%CI 3.95-17.15). The pooled LR+ was 2.62 (95% CI 1.80-3.82) and

LR- was 0.35 (0.18-0.70). The AUC was 0.8 (SE 0.04) with a Q^* of 0.74 (SE 0.03) (Figures 9 and 10).

The above results suggest that FCP in UC has a slight advantage in predicting a relapse within 12 months compared to the CD patients.

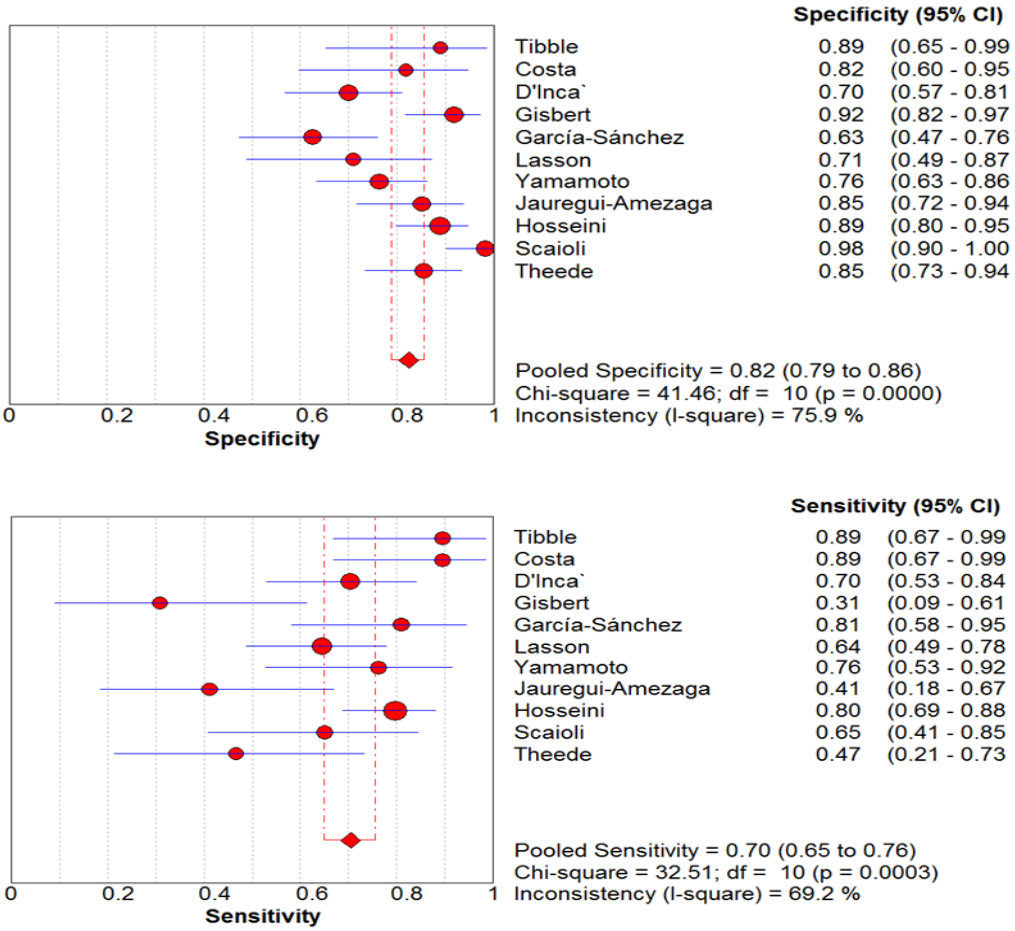

Figure 7 Pooled sensitivity and specificity for UC studies

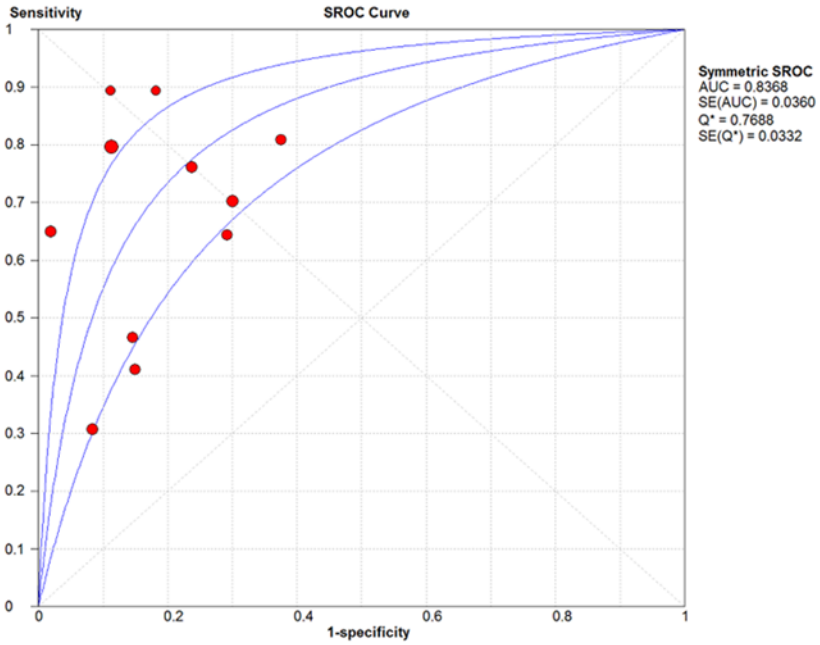

Figure 8Summary receiver operating characteristic curve (SROC) for UC studies

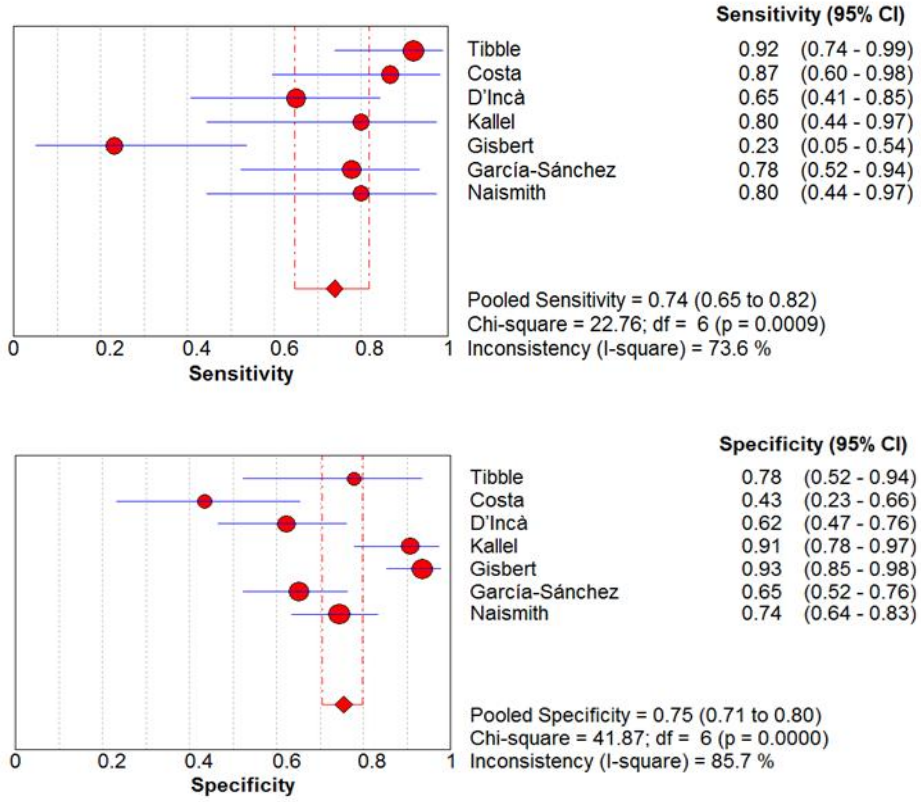

Figure 9 Pooled sensitivity and specificity for CD studies

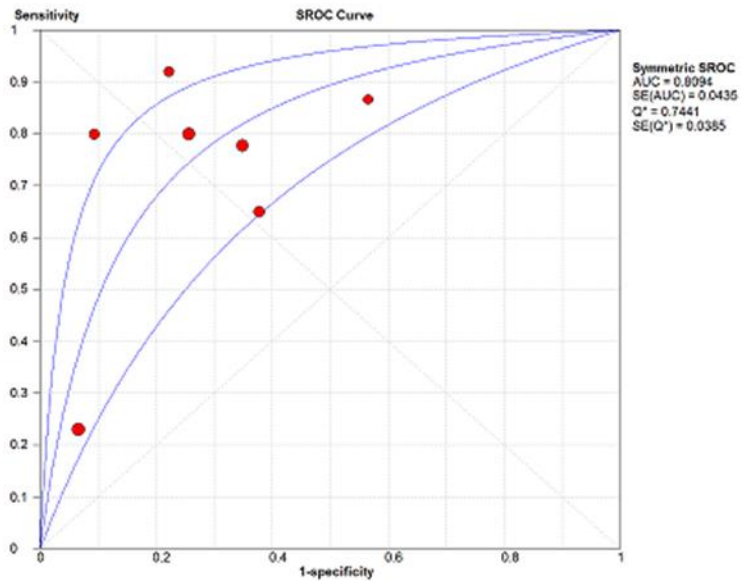

Figure 10 Summary receiver operating characteristic curve (SROC) for CD studies

Heterogeneity testing:

We identified three areas to test for heterogeneity among the studies; the FCP assays used, the cut-off levels of FCP used in studies to predict relapse, and the definition of relapse.

FCP cut off used in the studies ranged from 130 to 341 µg/gms for predicting relapse. We separated studies using 200µg/gm and tested for heterogeneity among the included studies. There were 7 studies in UC group with FCP cut off <200 and four studies with FCP cut off >200. However the data available to test heterogeneity among CD studies using cut off of 200 was not significant (only three studies with data on FCP cut off <200 and one study with cut off >200), and hence the analysis was not performed in this group.

Some studies have used clinical scoring systems to define a relapse (140-145, 149-151) while some used clinical symptoms (40, 146-148, 152) and others used a combination. The results are shown in Table 14.

Table 14 Heterogeneity testing of the studies

	Sensitivity [95% confidence interval 9CI)]	Specificity	LR+	LR-	DOR	SROC
FCP in UC >200	0.72 (0.63-0.79)	0.87 (0.81-0.91)	4.66 (2.67-8.11)	0.37 (0.17-0.78)	13.12 (3.69-46.63)	0.95 (SE=0.02, Q*=0.89)
FCP in UC <200	0.69 (0.62-0.76)	0.80 (0.75-0.84)	2.94 (2.05-4.21)	0.41 (0.28-0.60)	8.86 (4.60-17.04)	0.80 (SE=0.03, Q*=0.74)
Relapse defined with scores	0.70 (0.64-0.76)	0.85 (0.86-0.88)	4.74 (2.56-8.76)	0.36 (0.20-0.62)	14.80 (5.65-38.74)	0.87 (SE=0.87 Q*=0.80)
Relapse determined clinically	0.62 (0.52-0.71)	0.79 (0.73-0.83)	2.93 (2.23-3.87)	0.53 (0.39-0.72)	6.03 (3.46-10.51)	0.80 (SE=0.80, Q*=0.73)

LR+ likelihood ratio positive, LR- Likelihood ratio negative, FCP, Faecal calprotectin, DOR-Diagnostic odds ratio, SE-Standard error, SROC-Summary Receiver Operator curve

Discussion

FCP is a stable protein, resistant to degradation in room temperature for about 7 days, and the ability of spot testing (using stool sample of just around 5 grams) to detect the inflammatory burden, making it a simple, inexpensive and non-invasive biomarker. It is used to reliably distinguish IBD from functional bowel disease. A meta-analysis of 13 prospective studies including 1041 patients FCP was shown to be an effective screening tool to distinguish IBD from IBS with 93% sensitivity and 96% specificity (156). The study results also suggested that there would be a 67% reduction in endoscopic procedures if screened with FCP.

Another meta-analysis of 6 prospective studies involving 672 IBD patients assessed the value of FCP in predicting a relapse in IBD. In this study, the authors describe a pooled sensitivity of 78% and specificity of 73% of the baseline FCP values to predict a relapse in the 12 months follow up period(123). However in this meta-analysis authors have combined

studies which provided only CD(144) and UC data(143) along with the ones which provided combined data(140-142, 145). As we know that CD and UC have different disease pattern a combined analysis would not be suitable for diagnostic accuracy of FCP in predicting relapse. In our analysis, 8 additional prospective studies involving adult IBD patients were included with analysis of UC, CD and combined data separately. Our results suggest that FCP fares better in UC than CD population. The sensitivity of 70% and specificity of 82% with a pooled DOR of 10.51 and AUC was 0.83 in UC population. Although this is not as expected of a diagnostic test, it has good overall predictive value. The LR+ and LR- were not as expected to be of a predictive test. Ideally one would expect LR+ to be at least >5 and LR- to be <0.2 to be a strong diagnostic marker.

In three different studies, it has been noted that consecutive sampling of FCP helps predict relapse. The first study was conducted in patients with Crohn's disease treated with anti-TNF therapy(157), and second was in patients with mesalamine treated distal UC(158). The latter study only had a follow-up period of up to 40 weeks and hence did not qualify for our analysis. Both these studies suggested using FCP every 2-3 months in predicting relapse. Huge variation of cut off levels was observed in these studies. In the former study, a cut off of >300 mg/kg in two consecutive samples predicted a relapse with 61.5% sensitivity and 100% sensitivity. However, in the latter study, an FCP elevation of >55 µg/g was associated with relapse with 88% sensitivity and 80% specificity. In the third study by Zhulina et al (155), both UC and CD patients were included. A doubling of FCP value between two consecutive samples within 3 months was associated with 101% increased risk of relapse. This study could not be included in our study due to the lack of extractable data.

We acknowledge that our data has limitations. Heterogeneity across the studies existed due to the different assays used along with different cut-offs and the criteria used to define relapse in the studies. We could not statistically correct this. Studies not only varied in defining relapse but also in the methods used to confirm and assess the severity of relapse. Some studies used only clinical parameters with or without scoring systems to diagnose a relapse; others used endoscopic and histological markers of inflammation. Most of the scoring systems used in the studies are generally accepted in clinical practice; however, they are not externally validated. The parameters used in these indices to define relapse are different and could introduce bias.

Subjective interpretation of symptoms without using standard or validated scoring systems to define relapse could introduce inter-observer variations making the studies prone to bias(146, 152). In our analysis, we found that only a few studies used hard endpoints to define relapse like endoscopic and histological inflammation (40, 147, 151).

Data analysis based on the location of the disease (ileal, ileocolonic, colonic Crohn's disease and in UC proctitis, left sided colitis or pancolitis) was not possible owing to the relatively small number of studies providing such extensive data. Finally, publication bias with positive studies being published more often than negative studies is an established fact.

Conclusion

Our results suggest that FCP has a potential role in predicting disease relapses, more so for UC than CD patients. Repeated measurements may be useful than one-off baseline FCP value in predicting disease course. More research is needed in use of repeated FCP measurements at regular intervals with standardised cut off values.

4 Do newer generations of Narrow Band Imaging quantitatively improve contrast enhancement of endoscopic images?

Introduction

Narrow band imaging (NBI) digitally enhances the optical image and provides contrast enhancement resulting in better visualisation of the blood vessels and mucosal surface pattern. Hence it is also referred to as 'virtual chromoendoscopy' or 'Image-enhanced endoscopy' (IEE).

The Wavelengths of light visible to the human eye as white colour is between 400-700nm, in which 400nm is seen as blue, 550nm and 700 are seen as green and red colour respectively. Light undergoes degradation in three forms when illuminated on objects; absorption, reflection and scattering. Light with a wavelength of 400-550nm gets absorbed more due to less penetrance and scatter. Narrow band imaging is the technology which relies on the principle of depth of penetration of the light to visualise the tissue in wavelengths between 400-550 nm. NBI filters decrease the wavelengths of the light illuminated; the blue light is centred at 415nm and the green lights at 540 nm. Photons in light at these wavelengths are absorbed strongly by the haemoglobin and hence capillaries on the mucosa appear optically enhanced with NBI.

NBI allows superior assessment of vascular pattern and the intensity of capillary networking compared to white light endoscopy (159-163). These features help to differentiate polyps into adenomatous and non-adenomatous. Sano et al proposed a classification system using NBI with magnification (meshed capillary pattern I -non-adenomatous and capillary pattern II and IIIa, IIIb -neoplastic)(164). Diagnostic accuracy of NBI with magnification in differentiating adenomatous from non-adenomatous is reported at 95.3% compared with the histology with a sensitivity of 96.4% and specificity of 92.3%(162). Recently, NBI International Colorectal Endoscopic (NICE) classification is designed by an international group of experts with interest in NBI. The proposed classification helps to characterise polyps using NBI without magnification (165). Studies show that effective training in the use of NBI has resulted in high accuracy in diagnosing lesions in the colorectum (166-168).

There are three generations of endoscopes equipped with NBI capabilities currently available in the UK, the 240, 260, and the latest 290 series of Olympus endoscopes. Each upgrade has resulted in an improved resolution and enhanced image quality. The 240 series were standard definition whereas the 260 and 290s are higher definition endoscopes. Whether the improvement in resolutions and NBI among these successive generations of

endoscopes translates into the better visualisation of haemoglobin has not been studied. We aimed to quantitatively compare the effect of improvement in contrast enhancement among three generations of Olympus endoscopes using serial dilutions of haemoglobin in our *in vitro* study.

Methods

We compared the visibility of human blood on endoscopic still images captured in white light (WL) and NBI with three generations of endoscopes.

Images of human blood diluted with distilled water were taken with each generation of endoscopes, first with white light followed by NBI. A 24-well transparent plastic plate was used with one millilitre of distilled water pipetted in each of the flat wells. Human blood was added to the first well to set up a 1:1 concentration (1/2 dilution). Blood was diluted in subsequent wells so that each step resulted in doubling dilution. We used Olympus® Keymed gastroscopes for capturing images; GIF Q240 and GIF H260 endoscopes were used with a LUCERA spectrum CV260 processor and GIF H290 endoscope was used with an ELITE CV 290 processor. Still images of the overview of the plate and of each well were taken with WL and NBI as shown in images in Figure 11 and 12. It is worth noting that the effect of NBI does not vary between gastroscopes or colonoscopes of the same generation. We did not use digital enhancement techniques available on the endoscopes, as we felt that by doing so the image quality would be distorted.

Endoscopic images appear convex or curved and in order to eliminate the bias of curved effect on assessing the presence or absence of blood we imaged individual wells at an optimal distance to prevent the curving of the image. In total 150 images were taken (6 overview and 144 individual wells) which were then mixed in random order in a PowerPoint presentation. Participants who entered into the study were asked to identify the least noticeable blood in the overview image and to answer ‘yes’ or ‘no’ for the presence of blood in individual wells.

Out of 45 participants included for the study, 15 were novices with no prior endoscopic experience at all, 15 were endoscopist with accreditation to perform upper endoscopy independently but without NBI experience (NBI-Naïve) and 15 were NBI-experienced or experts in the field of NBI. The NBI-Naïve group had certification of upper gastrointestinal endoscopy skills by the Joint advisory group (JAG)-a quality improving and service accrediting body for endoscopy in the UK. The NBI experts included in the study are the endoscopists with either published record of expertise in NBI or those select consultant gastroenterologists who admitted to using NBI in “most of the cases” in the screening questionnaire.

Statistical analysis

The kappa values were calculated for an inter-rater agreement for the presence or absence of blood using IBM SPSS version 21. A kappa value of 0.01-0.20 means that the agreement is slight, a value of 0.21-0.40 - fair, 0.41-0.60 - moderate, 0.61-0.80 - substantial agreement and 0.81-0.99 is almost perfect agreement. (169)

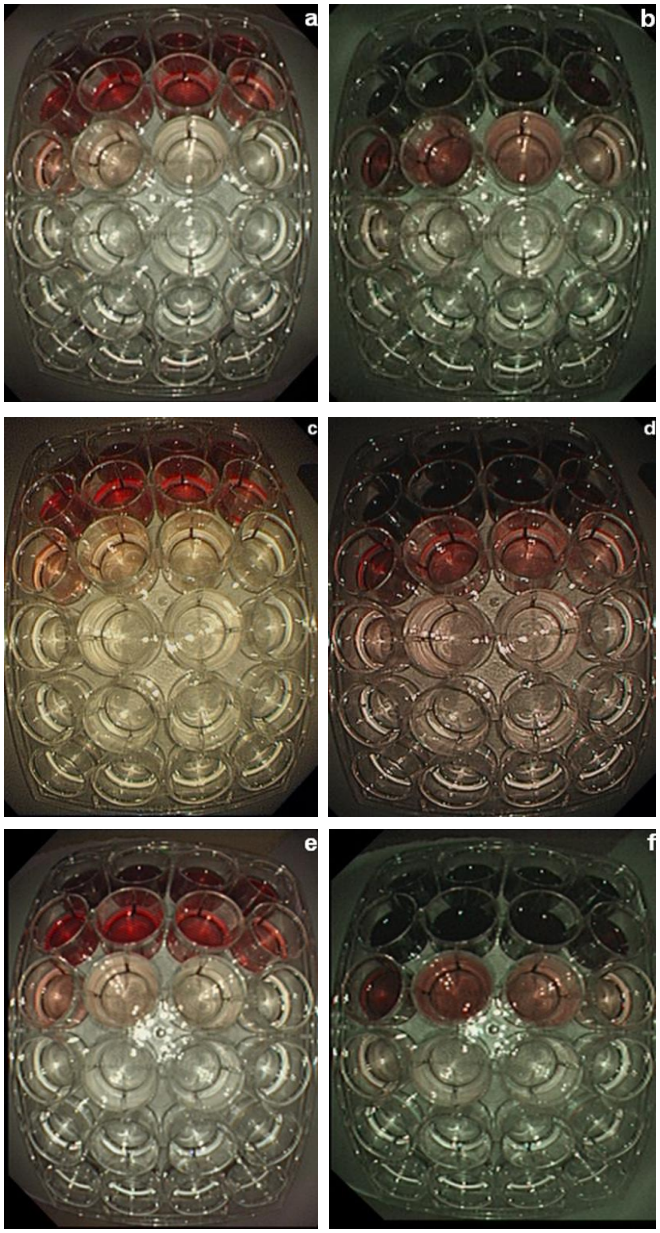

Figure 11 Overview images of 24-well plate using three series of endoscopes

White light and NBI images of the overview using 240 (a & b), 260 (c & d) and 290 series (e & f) respectively

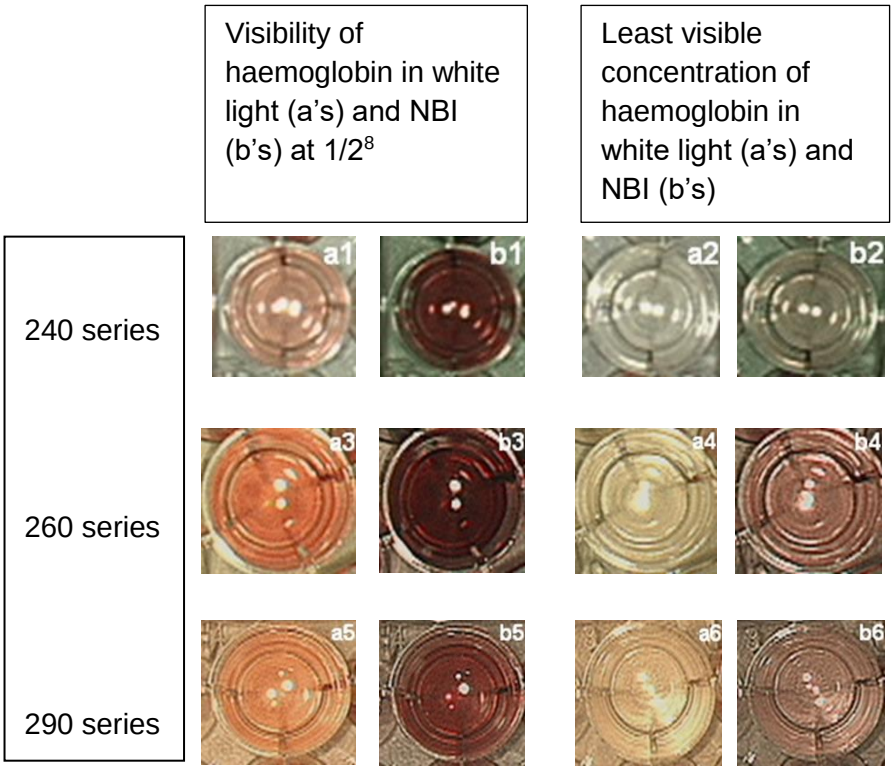

Figure 12 Comparative visibility of haemoglobin under white light and NBI with different dilutions of blood.

Results

4.1.1.1 Analysis of overview images:

Blood appears dark under NBI and hence more readily visible than under WLE. This pattern was observed in our analysis across all generations of the endoscopes. With conventional white light the median dilution at which all 3 groups noted the presence of haemoglobin was $1/2^{15}$ using all three generations of endoscopes. The average scores for WLE among the experts was 15.6, NBI naïve-15.2 and Novices-15.8. It was impossible to assess the presence of blood at further dilutions despite using higher resolution endoscopes under white light examination. The visualisation of blood improved using NBI across all three groups. The average score among experts was 18.9 whereas among NBI-naïve and novices it was 19.1 and 19.7 respectively. The kappa value for the inter-rater agreement was substantial for all 3 generations of endoscopes using conventional white light.

4.1.1.2 Analysis of individual images

The analysis of the presence of blood assessed using individual wells revealed similar results. Presence of blood identified with WLE was between $1/2^{12}$ - $1/2^{16}$ across the three groups with median visualisation at $1/2^{15}$. There was an improvement in visualising blood using NBI. It was noted that using NBI with GIF Q240 series, blood was seen at $1/2^{17}$. There was further improvement with GIF H260 series with blood seen at $1/2^{19}$ and to $1/2^{20}$ using GIF H290 series of endoscopes.

The kappa values for the inter-rater agreement was κ>0.7 for all 3 generations of endoscopes using conventional white light, while it was κ>0.5 for all 3 generations of NBI. The kappa value of >0.7 suggests there was substantial agreement whereas a kappa value of >0.5 means only moderate to fair agreement between the observers (169).

These results suggest that there was a significant improvement in assessing the presence of blood using NBI over WLE. There was a greater increment seen using NBI between Q240 series to H260 series of endoscopes than seen between H260 and H290 series of endoscopes.

Discussion

NBI is designed to allow maximum absorption of light by haemoglobin and provide contrast enhancement of the tissue vasculature. The vascular pattern is altered in the mucosa and submucosa when a tissue undergoes neoplastic change. Because of the increased uptake of NBI from within the vasculature, it allows characterisation and potentially assessing the deeper invasion of the neoplastic lesions. NBI, as discussed earlier is superior to WLE in assessment and characterisation of lesions in the colon and rectum.

Introduction of higher resolution and higher definition video endoscopes systems with digital and optical zoom capabilities have improved image quality. The Q 240 series of endoscopes were normal definition endoscopes equipped with the NBI technology. Later generation of endoscopes, the H260 and H290 series, are the higher definition endoscopes with further improvement in contrast enhancement. In our study, we compared the effect of improvement in contrast enhancement among the three generation of endoscopes. Not surprisingly the visualisation of blood was better with NBI compared to WLE. Similar findings were noted in one previous *in vitro* study which demonstrated the superiority of NBI in identifying blood compared to WLE and FICE(170). In our study, we compared WLE and NBI in three different generations of endoscopes in addition to quantitatively assessing the improvement between the three generations of endoscopes. To the best of our knowledge, this is the only study looking at such a comparison. This study is clinically relevant as all three generation of endoscopes are still in use in endoscopy units across the UK. And we believe that the results of the study can be translated into clinical practice.

We acknowledge the limitation of our study is that is an *in vitro* study. However since we aimed at visualising blood using three generation of endoscopes, an *in vitro* study design was felt appropriate. *In vivo* study for assessment of the presence of haemoglobin within a colorectal lesion with three different endoscopes would not have been feasible. It would require a tandem design with back to back examination of the lesion with three different endoscopes for such a study and recruitment would be a major problem. We also

considered confounding factors in an *in vivo* such as mucosal changes of the lesions impairing the true assessment of the haemoglobin such as oedema and collagen content within the lesion.

Conclusion

In conclusion, we demonstrated that NBI is superior to WLE in the assessment of the presence of blood. There was a significant improvement in NBI in the newer generation of endoscopes (H290 and H260) compared to the earlier endoscopes (Q240). The improvement in NBI was not as we expected between H260 to H290 in our study. Further studies comparing these two generations of endoscopes in humans to demonstrate the clinical utility must be considered.

5 Use of NBI in predicting disease outcomes in UC-a prospective observational study

Background

Ulcerative colitis (UC) is a chronic inflammatory condition of unknown aetiology, characterized by a diffuse confluent mucosal inflammation of the colon starting from the rectum with a relapsing and remitting course(134). Conventional endoscopy was thought to be a reliable parameter of disease activity(2), but microscopic inflammation can persist despite normal mucosal findings with conventional endoscopy(3). Histological detectable inflammation is thought to be associated with a greater risk of subsequent relapse(4, 5) A flare in UC activity is difficult to predict, but a simple, easily measured biological marker of relapse would be important in guiding the most appropriate and cost-effective therapy. High dose maintenance therapies could reduce the risk of relapse but carry their own risks.

Serum markers like erythrocyte sedimentation rate, C reactive protein as well as orosomucoid have been shown to have a relatively poor sensitivity and specificity for intestinal inflammation and correlate poorly with disease activity indices(55, 171). Although faecal calprotectin and lactoferrin have been shown to be sensitive but not very specific in predicting the risk of relapse(142, 145), they are still underutilized with no large prospective studies on a diverse patient population done to date. More recently small studies have shown that findings on magnifying colonoscopy modestly predict disease relapse, with 70% of patients having endoscopic mucosal defects relapsing in 12 months(44). A pilot study from Japan showed that the mucosal vascular pattern using narrow band imaging correlates well with histological grade of inflammation in ulcerative colitis(38).

Recent technological advances in fibre optics, light sources, detectors, and molecular biology have stimulated the unprecedented development of numerous optical methods that promise to significantly improve our ability to visualize and evaluate human epithelium in vivo. These methods collectively termed “optical biopsy,” are non-destructive in situ assays of mucosal histopathology using light that can provide instantaneous tissue assessment. NBI is a novel technique that enhances the diagnostic capability of endoscopes in characterising tissues by using narrow-band width filters in a red-green-blue (RGB) sequential illumination system. In NBI, the bandwidths of the standard red, green, and blue pass filters have been narrowed and the relative contribution of the blue filter has been increased resulting in improved mucosal contrast and detail(172)

UC always involves the rectum and activity is usually greatest distally. This makes an evaluation of the rectum alone an attractive marker in patients with UC. Unlike serum and

faecal markers, endoscopic assessment of the rectal mucosa is unlikely to be affected by systemic disease or inflammation in the small intestine/stomach and would be a relatively easy and acceptable test for patients and physicians. Utilizing magnifying colonoscopy using Indigo carmine dye spray (which improves surface mucosa resolution), Japanese researchers have shown that regular pit patterns are associated with a significantly reduced risk of relapse(44). Similarly, patients with the distorted mucosal vascular pattern are noted to have a higher grade of inflammation among UC patients in remission(38)

The primary aims of our pilot proof of concept study was to assess the correlation between NBI, white light endoscopy, and histological assessment of inflammation and clinical scoring systems in patients with ulcerative colitis.

Study design

This was a single centre study with a sequential trial design where all patients included in the trial had an endoscopic assessment of their rectum and sigmoid colon by either sigmoidoscopy or colonoscopy with white light and narrow band imaging. National research ethics committee and local 'research and development' department approval were obtained for the study (NREC reference number 13/YH/0115, and R&D approval number UR/13/10708).

The power calculation was for this pilot observational study was based on the estimated relapse rate of 20-30% among our study group. The risk of relapse in UC patients when followed up for 12 months was derived from the published literature (4, 40, 82). Similar findings were noted in our retrospective analysis mentioned in chapter 2. Hence, we estimated that recruitment of around 120 patients would yield the desired results in the follow up period of one year.

All adult patients undergoing sigmoidoscopy/colonoscopy at the Leeds Teaching Hospitals Trust (LTHT) for UC related assessment or surveillance on endoscopy waiting lists or those from gastroenterology outpatient clinics, were invited to take part in the study. All patients were under the care of Gastroenterology consultants (which may include the investigators) at LTHT. Patients were interviewed individually and details about the study were provided which included a written patient information sheet. Adequate time was given (at least 2 weeks) to consider participating in the study and to clarify any doubts before consent was obtained. Patients were clearly informed that they can withdraw their consent at any stage during the study without compromising their standard clinical care. Images or videos of all

endoscopic procedures were digitally recorded with no patient identifiable data included. The endoscopies were performed by the researchers with experience in advanced endoscopic imaging. Data were collected on three different activity indices for clinical, endoscopic and histologic findings. Walmsley, Lichtiger and Modified Mayo scores were used for clinical activity; Baron, Mayo endoscopic subscore and Ulcerative colitis endoscopic index of severity were used for endoscopic assessment. Histology slides were graded according to three scores, Geboes, Riley and Rubin scores.

Endoscopic assessment of UC plays a vital role in the assessment of disease activity. It also helps in assessing the response to treatment. Mucosal healing (MH) is increasingly adopted in research studies as a clinical endpoint. Although the definition of MH is not clear, it is complete absence of any inflammatory activity i.e. normal appearing mucosa. Endoscopic indices are developed to assess the inflammatory activity and may correlate with the histological grade of inflammation. However endoscopic examination has the disadvantage of being an invasive and hence not particularly desired by the patients. The problems with endoscopic assessment tools are two-fold; on one hand, there is lack of uniformity in definitions used to define severity and on the other hand there are numerous un-validated endoscopic indices of severity. Some of the indices use endoscopic findings alone while others use various combinations of clinical, biochemical, histological findings along with physicians own impression of the clinical situation(22, 27, 29, 30, 33, 34). On one hand, it may be argued that using these parameters separately allows for an objective assessment and perhaps make it easier, some researchers have questioned the need for endoscopy in the first place for knowing severity of disease activity, when similar findings can be derived by using clinical disease activity indices (26, 173). In one study researchers found that the absence of rectal bleeding and normal stool frequency can equate to complete mucosal healing(174). The clinical disease activity indices are non-invasive, rely on the clinical (and in some, biochemical) parameters to assess disease activity and may be preferred by patients compared to endoscopic examinations.

Histology is the gold standard in the assessment of inflammation in UC. 18 histological indices are available to assess disease activity in UC, however, none of them is validated externally nor a preferred scoring system that is used universally. We compared the disease activity indices to the histological scores. Three histological scores were selected for the purpose of the study. The process of selecting the scoring systems was purely on the basis of published literature and ease of use by the histopathologists. We also looked for the defined cut off for the active and inactive disease. We did not restrict the selection based on external validation of the scores as none of them would have been qualified. The three

selected scores are Geboes, Riley and Histological inflammatory activity (HIA) scores. For the ease of use, we have named the scores on the basis of the first author of the published article in which the scores were proposed. Hence HIA is referred as Rubin score in the thesis. For Geboes score the cut off for defining active disease was 3.2, and that for Riley was 12 and Rubin was 2. Any scores above the cut off levels were classed as an active disease (5, 72, 73, 82).

All three endoscopic indices used in the study are extensively used in the clinical research. Mucosal healing with a complete absence of inflammation is perceived as an important clinical outcome in the trials. Recent research suggests that patients with Mayo endoscopic score of 1 have a higher risk of relapse than those with a score of '0'(126, 127). Ikeya et al and Xie et al compared MES and UCEIS to the clinical outcomes in UC. In both these studies, patients with acute severe colitis were included. UCEIS score of ≥7 was a predictor for colectomy and was found to outperform MES (175). It was also found to accurately predict medium and long-term outcomes when tested against MES during the treatment phase of acute severe colitis(176).

In this prospective observational study, we aimed to assess the relationship of the clinical, endoscopic and histologic markers to the clinical outcomes of the disease. Three clinical disease activity indices were selected for assessment of inflammation for the purpose of our study. There are various scoring systems or activity indices available in the literature. The commonly used parameters for assessing the severity are stool frequency and rectal bleeding. The other clinically important factors such as urgency and incontinence were not commonly featured among the scoring systems.
For the purpose of the study, we wanted the scoring systems that used only clinical parameters and no biochemical markers were involved. We selected Walmsley index (also called as Simple Clinical Colitis Index), Lichtiger index (also called as Modified Truelove and Witts index) and Modified Mayo score (Mayo score without the endoscopic component).

Methods

5.1.1.1 Procedural details:

The endoscopies were performed by the researchers with experience in advanced endoscopic imaging. Rectum and Sigmoid colon were adequately washed and white light endoscopy performed followed by examination under NBI with Olympus endoscopes series numbers 260 and above.

The endoscopic findings and the biopsies (number, site, level) taken were recorded accurately in the patient case record form. Biopsies taken were according to the standard guidelines for UC surveillance.

5.1.1.2 Timescale

The patient's participation in the study is for twelve months after their sigmoidoscopy or colonoscopy performed for clinical indications as determined by the treating physician.

Patients with acute colitis who underwent sigmoidoscopy for assessment of disease severity and extent were followed up for the period of twelve months following the procedure.

Patients with Quiescent colitis who underwent colonoscopy for surveillance of their disease were included in the study for twelve months following the procedure. Flare up data was recorded during the follow-up period.

5.1.1.3 Primary endpoint

The primary endpoint is the assessment of inflammation in patients with ulcerative colitis using NBI and its correlation with standard endoscopy, histology and clinical scoring systems also called as Disease activity indices (DAIs).

5.1.1.4 Secondary endpoints

- To assess the accuracy of clinical DAIs and endoscopic scores in predicting inflammatory activity.
- To assess if DAIs, endoscopic and histological scores predict outcomes among the recruited patients.
- To assess the benefit of adding NBI to WLE endoscopy in predicting disease outcomes.

Statistical Analysis

Results are expressed as means ± standard deviations for continuous variables and as frequencies for categorical variables. P values from ANOVA or chi-square tests were considered statistically significant if ≤ 0.05. We used varying cut-offs of endoscopic scores (with white light and/or NBI imaging) and histologic scores to predict disease activity based on patient perspective of a flare, physician global assessment and clinical diseases actiivty indices. We also evaluated the ability of endosopic and histologic scores in prediting disease flare at 12 months of folow up. We calculated sensitivity, specificity, negative and positive predictive values, and diagnostic accuracy for each scoring system together with

95% confidence intervals (CI), according to standard definitions. All statistical tests were done using PASW version 20 (IBM Corp, NY).

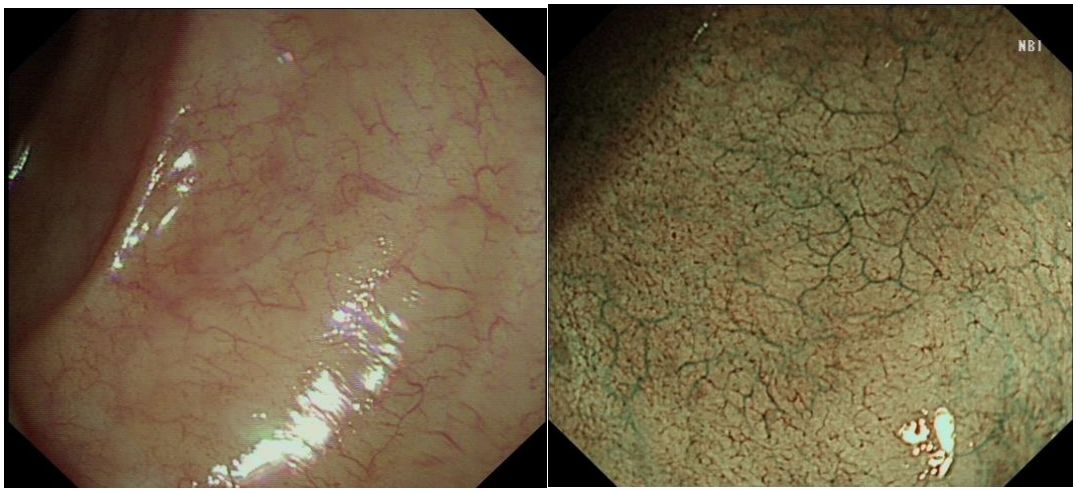

Figure 13 Inactive UC assessed with white light (left) and NBI (right)

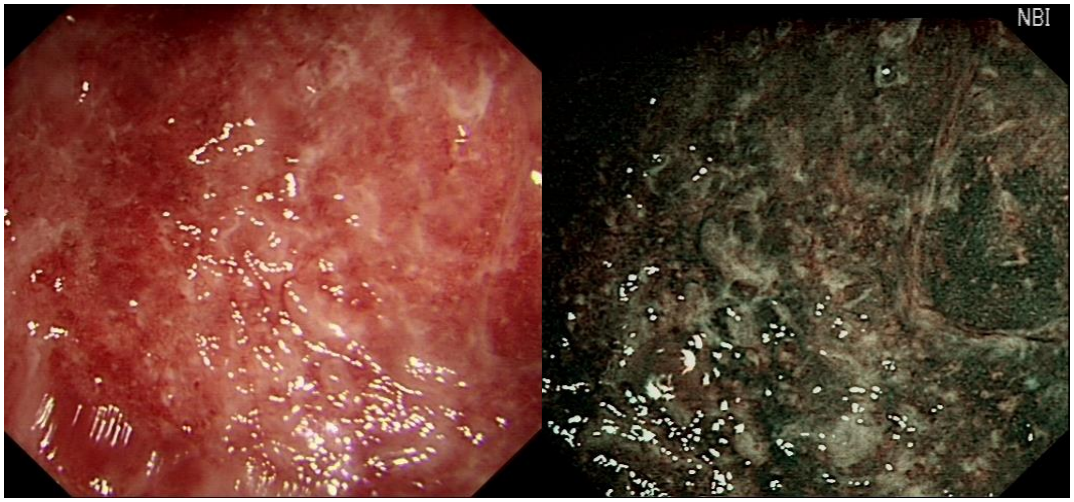

Figure 14 Active UC assessed with white light (left) and NBI (right)

Results

Patients were recruited for this trial from 20th September 2013 to 17th July 2014. All patients with acute severe colitis were contacted to participate in the trial by the research fellow (NM) and research nurses. Patients who were thought to have a flare up of their disease activity from the out-patient's clinic were contacted by the consulting physician. A sigmoidoscopy was arranged on the same day in most of the cases. Patients with quiescent disease were provided with the written information of the trial and were consented prior to procedure.

Baseline characteristics

A total of 116 patients were included in the study, 53 in acute and 61 in quiescent arm. Table 15 demonstrates the basic demographics of the patients involved. As expected from the disease pattern, the duration of disease is significantly different in the quiescent compared to

the acute arm. The flowchart describes the process of patient selection for the study. A total of 124 patients were contacted for the study; 4 did not consent for the trial, 2 withdrew consent during the procedure and 3 patients did not attend clinic appointments.

Table 16 describes the outcomes in the follow up period for patients in acute and quiescent arms. In the first year of follow up among the patients in acute arm, about 33% of patient flared. This is attributable to the fact that many of the patients included were newly diagnosed with Ulcerative colitis. 11 patients out of 53 (20.7%) were newly diagnosed colitis with recto-sigmoiditis diagnosed within the last 12 months. In the quiescent arm, however, the percentage of patients who experienced a flare up were 8.2 and 20% in first year of follow up respectively which is around the same in the published literature(4, 40)

The results of this study pertaining to our primary and secondary objectives are presented in different subheadings as follows.

Table 15 Characteristics of patients included in NBI prospective study

	Acute arm (n=53)	**Quiescent arm (n=61)**
Mean age in years (range)	37.6 (20-80)	55.8 (24-83)
Males	26	41
Females	27	20
Mean duration of disease in years (range)	8.07 (1-44)	20.2 (2-54)
Extent of disease (Montreal classification)		
E1	5	1
E2	34	23
E3	14	37
Previous flare up in months (range)	12.05 (1-60)	24.3 (2-60)

E1- Proctitis only, E2-Left sided colitis, E3-Extensive/Pan colitis

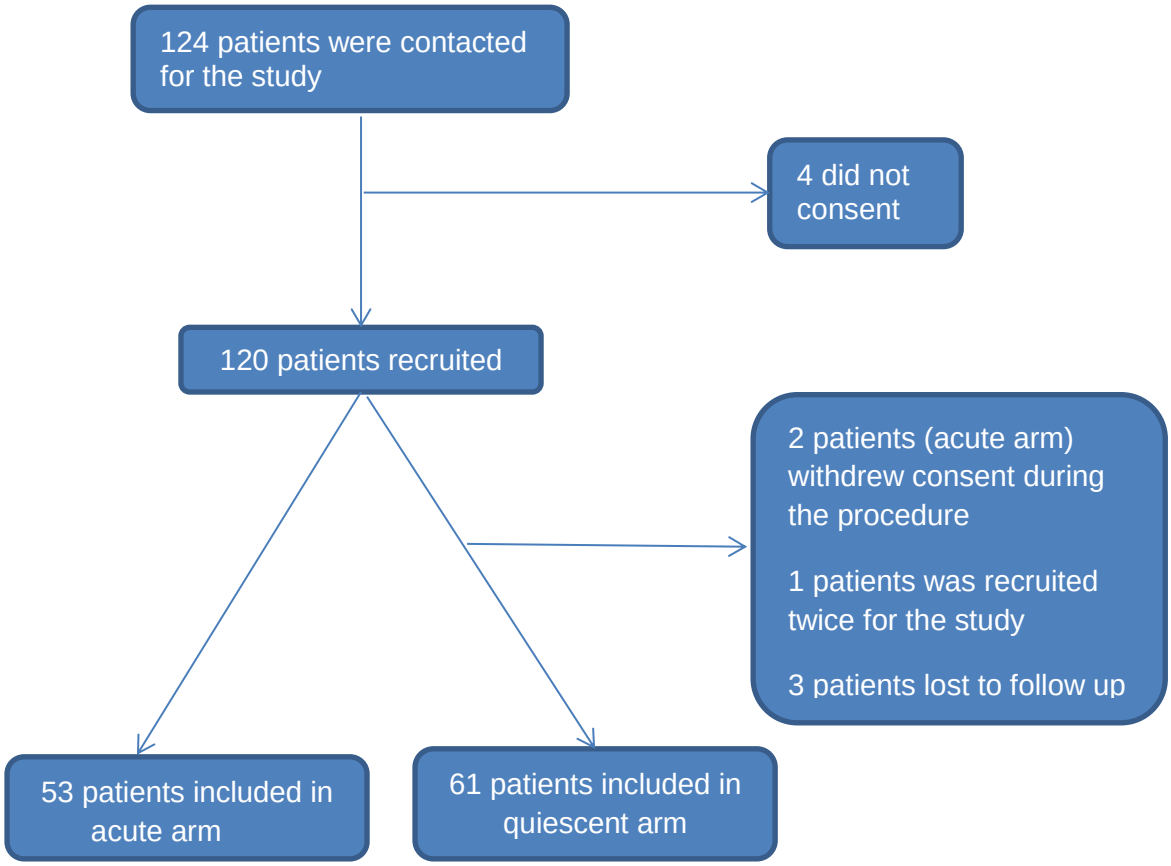

Figure 15 Flowchart of patient recruitment for NBI study

Table 16 Outcome data for patients with acute and quiescent colitis at twelve months

	Acute arm (n=53)		Quiescent arm (n=61)
	Outcome at 30 days (n=53)	**Outcome at 12 months (n=51)***	**Outcomes at 12 months (n=61)**
Remission attained (%)	47 (88.6)	34 (66.6)	56 (91.8)
Remission not attained (%)	5 (9.4)	17 (33.3)	5 (8.2)
Mean duration of relapse in months (range)	n/a	5.4 (2-11)	7.2 (6-11)
Lost to follow up	1	1	0
Escalation of treatment	5	19	3
Escalated treatment	INF alone-3 INF+AZA-1 Adacolum-1	INF alone- 11 INF+AZA- 5 Adacolum-1	Steroids+ AZA-1 AZA alone-1 Golizumab-1 No escalation -2
Surgery	None	2	None

INF-Infliximab, AZA-Azathioprine

Can disease activity indices predict endoscopic activity in UC?

All three DAIs were assessed against the three endoscopic indices separately to assess accuracy of DAIs in predicting endoscopic activity. We also checked other parameters such as 'patient's understanding of the symptoms' (flare or no-flare), and Physicians global assessment (PGA) against disease activity indices.

Statistical analysis was performed on the available cut off scores for each of the indices to assess quiescent and active disease. When there is more than one cut off scores available in the literature analyses was performed for on each of the scores. For example, a score of 2 and 3 are reported as cut-offs for Walmsley index in the literature and hence these scores were individually analysed to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), correlation and accuracy as shown in the tables below. Each of the three DAIs was compared to the three endoscopic indices and analysis was separately performed for WLE and NBI as shown in tables below.

The results in Tables 17-20, 21-24 and 25-28 show the comparative analysis of the DAIs to Mayo endoscopy sub-scores, Ulcerative colitis endoscopic index of severity and Modified Baron's scores with white light and Narrow band examinations respectively.

From the analysis, it is also evident that the use of DAIs with objective point-based scoring systems were better in predicting the endoscopic findings in both active (MES ≥1) and inactive/quiescent state (MES 0) of the disease. The PGA although scored better in assessing the active state, compared to the patient's interpretation of symptoms, the sensitivity still remained around 80%. All three DAIs faired more or less the same in predicting the activity, with sensitivity and accuracy around 80%. Addition of NBI to the three endoscopic scores does not seem to confer any added benefit in grading the severity further than white light examination.

5.1.1.5 *Comparison of disease activity indices in predicting endoscopic activity using Mayo endoscopic sub-score.*

Table 17 Comparison of three DAIs and Mayo endoscopic subscore '0' (with WLE)

	MES '0' WLE Sensitivity % (95% confidence interval)	MES '0' WLE Specificity % (95% confidence interval)	MES '0' WLE PPV % (95% confidence interval)	MES '0' WLE NPV % (95% confidence interval)	MES '0' WLE Accuracy % (95% confidence interval)
Patient's interpretation of symptoms	0.53 (0.46-0.57)	0.92 (0.81-0.97)	0.90 (0.78-0.97)	0.58 (0.51-0.61)	0.69 (0.60-0.73)
Physicians global assessment	0.72 (0.65-0.77)	0.87 (0.77-0.94)	0.89 (0.80-0.95)	0.69 (0.60-0.74)	0.78 (0.70-0.84)
Walmsley index ≥2	0.91 (0.81-0.97)	0.70 (0.63-0.74)	0.68 (0.61-0.72)	0.92 (0.83-0.97)	0.79 (0.71-0.84)
Walmsley index ≥3	0.93 (0.83-0.98)	0.67 (0.60-0.70)	0.67 (0.60-0.70)	0.93 (0.84-0.98)	0.78 (0.70-0.82)
Modified Mayo score '0'	0.89 (0.74-0.92)	0.77 (0.70-0.83)	0.73 (0.64-0.79)	0.91 (0.79-0.94)	0.81 (0.72-0.87)
Modified Mayo score ≥1	0.81 (0.79-0.96)	0.74 (0.67-0.78)	0.70 (0.62-0.75)	0.89 (0.82-0.96)	0.80 (0.72-0.85)
Lichtiger index ≥3	0.91 (0.81-0.97)	0.67 (0.60-0.71)	0.66 (0.59-0.70)	0.92 (0.82-0.97)	0.78 (0.69-0.82)

Disease activity indices – DAIs, White light examination (WLE). *'PPV=Positive predictive value, NPV=Negative predictive value.* Values expressed in % and 95% confidence intervals in the bracket.

Table 18 Comparison of the three DAIs and Mayo endoscopic subscore '≥1' (with WLE)

	MES '1' WLE Sensitivity % (95% confidence interval)	MES '1' WLE Specificity % (95% confidence interval)	MES '1' WLE PPV % (95% confidence interval)	MES '1' WLE NPV % (95% confidence interval)	MES '1' WLE Accuracy % (95% confidence interval)
Patient's interpretation of symptoms	0.63 (0.51-0.72)	0.84 (0.77-0.90)	0.72 (0.59-0.83)	0.78 (0.70-0.83)	0.76 (0.67-0.83)
Physicians global assessment	0.85 (0.74-0.92)	0.77 (0.70-0.82)	0.71 (0.62-0.77)	0.88 (0.80-0.94)	0.80 (0.71-0.86)
Walmsley index ≥2	0.78 (0.71-0.84)	0.80 (0.69-0.89)	0.86 (0.78-0.92)	0.71 (0.61-0.78)	0.79 (0.70-0.86)
Walmsley index ≥3	0.8 (0.72-0.86)	0.76 (0.64-0.85)	0.83 (0.75 0.89)	0.71 (0.60-0.79)	0.78 (0.69-0.86)
Modified Mayo score '0'	0.71 (0.64-0.76)	0.87 (0.75-0.94)	0.89 (0.80-0.95)	0.66 (0.58 0.72)	0.78 (0.69-0.83)
Modified Mayo score ≥1	0.75 (0.68-0.80)	0.84 (0.73-0.92)	0.88 (0.80-0.94)	0.70 (0.60-0.76)	0.79 (0.70-0.85)
Lichtiger index ≥3	0.80 (0.72-0.85)	0.78 (0.66-0.87)	0.85 (0.77-0.90)	0.72 (0.61-0.80)	0.79 (0.70-0.86)

Comparison of the three disease activity indices (DAIs) and Mayo endoscopic subscore '≥1' using white light examination (WLE). Values expressed in % and 95% confidence intervals in the bracket. *'PPV=Positive predictive value, NPV=Negative predictive value*

Table 19 Comparison of the three DAIs and Mayo endoscopic subscore '0' (with NBI)

	MES '0' NBI Sensitivity % (95% confidence interval)	MES '0' NBI Specificity % (95% confidence interval)	MES '0' NBI PPV % (95% confidence interval)	MES '0' NBI NPV % (95% confidence interval)	MES '0' NBI Accuracy % (95% confidence interval)
Patient's interpretation of symptoms	0.53 (0.46-0.57)	0.92 (0.81-0.72)	0.90 (0.78-0.97)	0.58 (0.51-0.61)	0.69 (0.60-0.73)
Physicians global assessment	0.70 (0.63-0.75)	0.87 (0.76-0.94)	0.89 (0.80-0.95)	0.66 (0.57-0.71)	0.77 (0.68-0.82)
Walmsley index ≥2	0.91 (0.80-0.97)	0.68 (0.61-0.72)	0.65 (0.58-0.69)	0.92 (0.82-0.97)	0.78 (0.69-0.82)
Walmsley index ≥3	0.93 (0.83-0.98)	0.65 (0.59-0.68)	0.64 (0.57-0.67)	0.93 (0.84-0.98)	0.77 (0.69-0.80)
Modified Mayo score '0'	0.84 (0.73-0.92)	0.75 (0.68-0.80)	0.69 (0.60-0.76)	0.69 (0.79-0.94)	0.79 (0.70-0.85)
Modified Mayo score ≥1	0.89 (0.78-0.95)	0.72 (0.65-0.77)	0.68 (0.60-0.73)	0.91 (0.82-0.96)	0.79 (0.71-0.84)
Lichtiger index ≥3	0.91 (0.80-0.97)	0.65 (0.58-0.69)	0.63 (0.56-0.67)	0.92 (0.82-0.97)	0.76 (0.67-0.80)

Comparison of the three disease activity indices (DAIs) and Mayo endoscopic subscore of '0' using Narrow Band Imaging (NBI). Values expressed in % and 95% confidence intervals in the bracket.
PPV=Positive predictive value, NPV=Negative predictive value

Table 20 Comparison of the three DAIs and Mayo endoscopic subscore '≥1' (with NBI)

	MES '≥1' NBI Sensitivity % (95% confidence interval)	MES '≥1' NBI Specificity % (95% confidence interval)	MES '≥1' NBI PPV % (95% confidence interval)	MES '≥1' NBI NPV % (95% confidence interval)	MES '≥1' NBI Accuracy % (95% confidence interval)
Patient's interpretation of symptoms	0.59 (0.51-0.64)	0.91 (0.82-0.96)	0.87 (0.75-0.95)	0.68 (0.62-0.72)	0.75 (0.66-0.80)
Physicians global assessment	0.80 (0.71-0.86)	0.86 (0.77-0.92)	0.85 (0.76-0.92)	0.80 (0.72-0.86)	0.83 (0.74-0.89)
Walmsley index ≥2	0.89 (0.80-0.95)	0.78 (0.69-0.83)	0.79 (0.71-0.84)	0.88 (0.78-0.94)	0.84 (0.75-0.89)
Walmsley index ≥3	0.91 (0.82-0.96)	0.74 (0.66-0.79)	0.77 (0.70-0.82)	0.89 (0.79-0.95)	0.83 (0.74-0.88)
Modified Mayo score '0'	0.82 (0.73-0.88)	0.84 (0.76-0.91)	0.83 (0.74-0.90)	0.83 (0.74-0.89)	0.84 (0.75-0.90)
Modified Mayo score ≥1	0.86 (0.77-0.92)	0.81 (0.72-0.87)	0.81 (0.73-0.87)	0.85 (0.76-0.92)	0.836 (0.75-0.90)
Lichtiger index ≥3	0.89 (0.80-0.95)	0.74 (0.66-0.80)	0.77 (0.69-0.82)	0.88 (0.78-0.94)	0.82 (0.73-0.88)

Table 4. Comparison of the three disease activity indices (DAIs) and Mayo endoscopic subscore of '≥1' using Narrow Band Imaging (NBI). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value.*

5.1.1.6 Comparison of disease activity indices in predicting endoscopic activity using Ulcerative colitis endoscopic index of severity (UCEIS).

Table 21 Comparison of the three DAIs and UCEIS '0' (with WLE)

	UCEIS WLE 0 Sensitivity	UCEIS WLE 0 Specificity	UCEIS WLE 0 PPV	UCEIS WLE 0 NPV	UCEIS WLE 0 Accuracy
Patient's interpretation of symptoms	0.55 (0.45-0.65)	0.91 (0.81-0.97)	0.90 (0.78-0.97)	0.57 (0.50-0.60)	0.68 (0.60-0.73)
Physicians global assessment	0.71 (0.64-0.76)	0.82 (0.76-0.94)	0.89 (0.80-0.95)	0.67 (0.59-0.73)	0.78 (0.69-0.83)
Walmsley index ≥2	0.91 (0.81-0.97)	0.69 (0.62-0.73)	0.67 (0.59-0.71)	0.92 (0.82-0.97)	0.78 (0.70-0.83)
Walmsley index ≥3	0.93 (0.83-0.98)	0.66 (0.59-0.69)	0.65 (0.58-0.68)	0.93 (0.84-0.98)	0.88 (0.67-0.97)
Modified Mayo score '0'	0.85 (0.74-0.92)	0.76 (0.69-0.82)	0.71 (0.62-0.77)	0.88 (0.79-0.94)	0.80 (0.71-0.86)
Modified Mayo score ≥1	0.89 (0.78-0.95)	0.73 (0.66-0.78)	0.70 (0.61-0.75)	0.91 (0.82-0.96)	0.80 (0.72-0.85)
Lichtiger index ≥3	0.91 (0.81-0.97)	0.66 (0.59-0.70)	0.65 (0.57-0.69)	0.92 (0.82-0.97)	0.77 (0.68-0.81)

Comparison of the three disease activity indices (DAIs) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) '0' using White light endoscopy (WLE). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Table 22 Comparison of the three DAIs and UCEIS '≥ 1' (with WLE).

	UCEIS WLE ≥ 1 Sensitivity	UCEIS WLE ≥ 1 Specificity	UCEIS WLE ≥ 1 PPV	UCEIS WLE ≥ 1 NPV	UCEIS WLE ≥ 1 Accuracy
Patient's interpretation of symptoms	0.59 (0.51-0.64)	0.91 (0.82-0.96)	0.87 (0.75-0.95)	0.68 (0.62-0.72)	0.75 (0.66-0.80)
Physicians global assessment	0.75 (0.65-0.82)	0.80 (0.71-0.88)	0.80 (0.70-0.88)	0.75 (0.67-0.82)	0.77 (0.68-0.85)
Walmsley index ≥2	0.82 (0.73-0.89)	0.71 (0.62-0.78)	0.73 (0.65-0.79)	0.80 (0.70-0.88)	0.76 (0.67-0.84)
Walmsley index ≥3	0.84 (0.74-0.91)	0.67 (0.58-0.74)	0.71 (0.63-0.77)	0.81 (0.70-0.89)	0.76 (0.67-0.83)
Modified Mayo score '0'	0.75 (0.65-0.83)	0.78 (0.68-0.85)	0.76 (0.67-0.84)	0.76 (0.67-0.83)	0.77 (0.67-0.84)
Modified Mayo score ≥1	0.78 (0.69-0.86)	0.74 (0.65-0.81)	0.75 (0.65-0.82)	0.78 (0.68-0.86)	0.77 (0.67-0.84)
Lichtiger index ≥3	0.82 (0.72-0.89)	0.67 (0.58-0.74)	0.71 (0.63-0.77)	0.80 (0.69-0.88)	0.75 (0.66-0.82)

Comparison of the three disease activity indices (DAIs) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) of '≥1' using White light endoscopy (WLE). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*.

Table 23 Comparison of the three DAIs and UCEIS '0' (with NBI)

	UCEIS NBI 0 Sensitivity	UCEIS NBI 0 Specificity	UCEIS NBI 0 PPV	UCEIS NBI 0 NPV	UCEIS NBI 0 Accuracy
Patient's interpretation of symptoms	0.52 (0.45-0.55)	0.93 (0.83-0.98)	0.92 (0.81-0.98)	0.55 (0.49-0.58)	0.68 (0.60-0.72)
Physicians global assessment	0.70 (0.63-0.75)	0.89 (0.78-0.96)	0.91 (0.82-0.96)	0.66 (0.57-0.70)	0.77 (0.69-0.83)
Walmsley index ≥2	0.91 (0.80-0.97)	0.67 (0.60-0.71)	0.64 (0.56-0.68)	0.92 (0.82-0.97)	0.77 (0.68-0.81)
Walmsley index ≥3	0.93 (0.82-0.98)	0.64 (95% CI 0.58-0.67)	0.62 (0.55-0.66)	0.93 (0.84-0.98)	0.76 (0.68-0.78)
Modified Mayo score '0'	0.84 (0.73-0.92)	0.74 (0.67-0.79)	0.67 (0.58-0.74)	0.88 (0.79-0.94)	0.78 (0.69-0.85)
Modified Mayo score ≥1	0.88 (0.77-0.95)	0.71 (0.64-0.76)	0.66 (0.58-0.71)	0.91 (0.82-0.96)	0.78 (0.70-0.84)
Lichtiger index ≥3	0.91 (0.80-0.97)	0.64 (0.57-0.68)	0.62 (0.54-0.66)	0.92 (0.82-0.97)	0.75 (0.66-0.80)

Comparison of the three disease activity indices (DAIs) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) 0f '0' using Narrow Band Imaging (NBI). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Table 24 Comparison of the three DAIs and UCEIS '≥ 1' (with NBI).

	UCEIS NBI ≥ 1 Sensitivity	UCEIS NBI ≥ 1 Specificity	UCEIS NBI ≥ 1 PPV	UCEIS NBI ≥ 1 NPV	UCEIS NBI ≥ 1 Accuracy
Patient's interpretation of symptoms	0.54 (0.47-0.59)	0.92 (0.82-0.97)	0.90 (0.78-0.97)	0.60 (0.54-0.64)	0.70 (0.62-0.75)
Physicians global assessment	0.71 (0.63-0.77)	0.84 (0.73-0.92)	0.91 (0.82-0.96)	0.66 (0.57-0.70)	0.78 (0.69-0.83)
Walmsley Index ≥2	0.88 (0.77-0.94)	0.69 (0.61-0.74)	0.68 (0.60-0.74)	0.88 (0.78-0.94)	0.78 (0.69-0.83)
Walmsley Index ≥3	0.84 (0.74-0.91)	0.67 (0.58-0.74)	0.71 (0.63-0.77)	0.81 (0.70-0.89)	0.77 (0.68-0.82)
Modified Mayo score '0'	0.82 (0.71-0.89)	0.77 (0.69-0.83)	0.73 (0.63-0.80)	0.85 (0.76-0.91)	0.79 (0.70-0.86)
Modified Mayo score ≥1	0.86 (0.75-0.93)	0.74 (0.66-0.79)	0.71 (0.63-0.77)	0.87 (0.78-0.93)	0.79 (0.70-0.85)
Lichtiger Index ≥3	0.88 (0.77-0.94)	0.66 (0.58-0.71)	0.66 (0.58-0.71)	0.88 (0.77-0.94)	0.76 (0.67-0.82)

Comparison of the three disease activity indices (DAIs) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) 0f '≥1' using Narrow Band Imaging (NBI). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

5.1.1.7 Comparison of disease activity indices in predicting endoscopic activity using Modified Baron's index of activity.

Table 25 Comparison of the three DAIs and Modified Baron index of '0' (with WLE).

	Baron WLE_0 Sensitivity	Baron WLE_0 Specificity	Baron WLE_0 PPV	Baron WLE_0 NPV	Baron WLE_0 Accuracy
Patient's interpretation of symptoms	0.53 (0.457-0.568)	0.92 (0.81-0.97)	0.90 (0.78-0.97)	0.58 (0.51-0.61)	0.69 (0.60-0.73)
Physicians global assessment	0.72 (0.65-0.77)	0.87 (0.77-0.94)	0.89 (0.80-0.95)	0.69 (0.60-0.74)	0.78 (0.70-0.84)
Walmsley index ≥2	0.91 (0.81-0.97)	0.70 (0.63-0.74)	0.68 (0.61-0.72)	0.92 (0.83-0.97)	0.79 (0.70-0.84)
Walmsley index ≥3	0.93 (0.83-0.98)	0.67 (0.60-0.70)	0.67 (0.60-0.70)	0.93 (0.84-0.98)	0.78 (0.70-0.82)
Modified Mayo score '0'	0.85 (0.74-0.92)	0.77 (0.70-0.83)	0.73 (0.64-0.79)	0.88 (0.79-0.94)	0.81 (0.72-0.87)
Modified Mayo score ≥1	0.89 (0.79-0.95)	0.75 (0.67-0.79)	0.71 (0.63-0.76)	0.91 (0.82-0.96)	0.81 (0.72-0.86)
Lichtiger index ≥3	0.91 (0.81-0.97)	0.67 (0.60-0.71)	0.66 (0.59-0.70)	0.92 (0.82-0.97)	0.77 (0.69-0.82)

Comparison of the three disease activity indices (DAIs) and Modified Baron index of '0' using White Light Endoscopy (WLE). Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value.*

Table 26 Comparison of the three DAIs and Modified Baron index of '≥1' (with WLE).

	Baron WLE ≥ 1 Sensitivity	Baron WLE ≥ 1 Specificity	Baron WLE ≥ 1 PPV	Baron WLE ≥ 1 NPV	Baron WLE ≥ 1 Accuracy
Patient's interpretation of symptoms	0.53 (0.46-0.57)	0.92 (0.81-0.97)	0.90 (0.78-0.97)	0.58 (0.51-0.61)	0.69 (0.60-0.73)
Physicians global assessment	0.72 (0.65-0.77)	0.87 (0.77-0.94)	0.89 (0.80-0.95)	0.69 (0.60-0.74)	0.78 (0.70-0.84)
Walmsley index ≥2	0.70 (0.63-0.74)	0.92 (0.81-0.97)	0.92 (0.83-0.97)	0.69 (0.61-0.73)	0.79 (0.71-0.84)
Walmsley index ≥3	0.68 (0.61-0.71)	0.94 (0.84-0.98)	0.94 (0.84-0.98)	0.67 (0.60-0.70)	0.78 (0.70-0.82)
Modified Mayo score '0'	0.78 (0.70-0.83)	0.85 (0.75-0.93)	0.88 (0.80-0.94)	0.73 (0.64-0.79)	0.81 (0.72-0.87)
Modified Mayo score ≥1	0.75 (0.68-0.79)	0.90 (0.79-0.96)	0.91 (0.82-0.96)	0.72 (0.63-0.77)	0.81 (0.72-0.86)
Lichtiger index ≥3	0.68 (0.60-0.71)	0.92 (0.81-0.97)	0.92 (0.82-0.97)	0.67 (0.59-0.71)	0.78 (0.69-0.82)

Comparison of the three disease activity indices (DAIs) and Modified Baron index of '≥1' using white light endoscopy (WLE). Values expressed in % and 95% confidence intervals in the bracket.
PPV=Positive predictive value. NPV= Negative predictive value

Table 27 Comparison of the three DAIs and Modified Baron index of '0' (with NBI)

	Baron NBI_0 Sensitivity	Baron NBI_0 Specificity	Baron NBI_0 PPV	Baron NBI_0 NPV	Baron NBI_0 Accuracy
Patient's interpretation of symptoms	0.51 (0.44-0.55)	0.91 (0.80-0.97)	0.90 (0.77-0.96)	0.55 (0.49-0.59)	0.67 (0.59-0.72)
Physicians global assessment	0.70 (0.63-0.75)	0.87 (0.76-0.94)	0.89 (0.80-0.95)	0.66 (0.57-0.71)	0.77 (0.68-0.82)
Walmsley index ≥2	0.91 (0.80-0.97)	0.68 (0.61-0.72)	0.65 (0.58-0.69)	0.92 (0.82-0.97)	0.78 (0.69-0.82)
Walmsley index ≥3	0.93 (0.83-0.98)	0.65 (0.59-0.68)	0.64 (0.57-0.67)	0.93 (0.84-0.98)	0.767 (0.686-0.805)
Modified Mayo score '0'	0.84 (0.73-0.92)	0.75 (0.68-0.80)	0.69 (0.60-0.76)	0.88 (0.79-0.94)	0.79 (0.70-0.85)
Modified Mayo score ≥1	0.89 (0.78-0.95)	0.72 (0.65-0.77)	0.68 (0.60-0.73)	0.91 (0.82-0.96)	0.79 (0.70-0.84)
Lichtiger index ≥3	0.91 (0.80-0.97)	0.65 (0.58-0.69)	0.63 (0.56-0.67)	0.92 (0.82-0.97)	0.76 (0.67-0.80)

Comparison of the three disease activity indices (DAIs) and Modified Baron index of '0' using Narrow Band Imaging. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Table 28 Comparison of the three DAIs and Modified Baron index of '≥1' (with NBI)

	Baron NBI ≥1 Sensitivity	Baron NBI ≥ 1 Specificity	Baron NBI ≥ 1 PPV	Baron NBI ≥1 NPV	Baron NBI ≥1 Accuracy
Patient's interpretation of symptoms	0.51 (0.44-0.55)	0.91 (0.81-0.97)	0.90 (0.78-0.97)	0.55 (0.49-0.59)	0.67 (0.59-0.72)
Physicians global assessment	0.70 (0.63-0.75)	0.87 (0.76-0.94)	0.89 (0.80-0.95)	0.66 (0.57-0.71)	0.77 (0.68-0.82)
Walmsley index ≥ 2	0.69 (0.62-0.72)	0.91 (0.81-0.97)	0.92 (0.83-0.97)	0.66 (0.58-0.70)	0.77 (0.69-0.82)
Walmsley index ≥3	0.66 (0.59-0.69)	0.93 (0.83-0.98)	0.94 (0.84-0.98)	0.64 (0.57-0.67)	0.77 (0.69-0.80)
Modified Mayo score '0'	0.76 (0.68-0.81)	0.85 (0.74-0.92)	0.883 (0.79-0.94)	0.70 (0.60-0.76)	0.79 (0.70-0.85)
Modified Mayo score ≥1	0.73 (0.66-0.77)	0.89 (0.78-0.96)	0.91 (0.82-0.96)	0.68 (0.60-0.73)	0.79 (0.71-0.84)
Lichtiger index ≥3	0.66 (0.59-0.69)	0.91 (0.81-0.97)	0.92 (0.82-0.97)	0.64 (0.56-0.68)	0.76 (0.67-0.80)

Comparison of the three disease activity indices (DAIs) and Modified Baron index of '≥1' using Narrow Band Imaging. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Can Disease Activity Indices predict histological activity?

In this section we compared DAIs with the three histological scores to check their accuracy in predicting the histology.

Statistical analysis was performed while comparing DAIs, patient's interpretation of a flare and PGA with the histologically active disease scored as Geboes score >3.2. Data was recorded on the 2x2 table to attain Sensitivity, Specificity, PPV, NPV, Correlation and accuracy of the scores.

The results in Tables 29-31 show the comparative analysis of the DAIs to Geboes score ≥3.2, Riley's score ≥12 and Rubin score ≥2 respectively.

Among the scores used, both Walmsley (cut off 2 and 3) and Lichtiger indices predicted the histological inflammation better than Modified Mayo score. Although the sensitivities were high, the accuracy remained low at around 70%. The patient's interpretation of the symptoms was poorly correlated with the histological findings ranging from 46-50% across the three scores.

Table 29 Comparison of the three DAIs and histology using Geboes score

	Geboes score ≥3.2 Sensitivity	**Geboes score ≥3.2 Specificity**	**Geboes score ≥3.2 PPV**	**Geboes score ≥3.2 NPV**	**Geboes score ≥3.2 Accuracy**
Patients perspective	0.49 (0.42-0.54)	0.87 (0.76-0.94)	0.85 (0.72-0.93)	0.54 (0.47-0.58)	0.65 (0.56-0.70)
Walmsley index ≥2	0.85 (0.74-0.93)	0.65 (0.58-0.70)	0.62 (0.54-0.68)	0.86 (0.76-0.93)	0.73 (0.64-0.79)
Walmsley index ≥3	0.87 (0.76-0.94)	0.62 (0.55-0.67)	0.61 (0.53-0.66)	0.88 (0.77-0.95)	0.72 (0.63-0.78)
Modified Mayo score 0	0.77 (0.65-0.86)	0.71 (0.63-0.77)	0.64 (0.54-0.72)	0.82 (0.73-0.89)	0.73 (0.64-0.81)
Modified Mayo score ≥ 1	0.81 (0.69-0.89)	0.68 (0.60-0.74)	0.63 (0.54-0.70)	0.84 (0.74-0.91)	0.73 (0.64-0.80)
Lichtiger index ≥ 3	0.85 (0.74-0.93)	0.62 (0.55-0.67)	0.60 (0.53-0.66)	0.86 (0.75-0.93)	0.72 (0.62-0.78)

Comparison of the three disease activity indices (DAIs) and histology using Geboes score with a cut off ≥3.2. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Table 30 Comparison of the three DAIs and histology using Riley score

	Riley score_12 Sensitivity	Riley score_12 Specificity	Riley score_12 PPV	Riley score_12 NPV	Riley score_12 Accuracy
Patients perspective	0.50 (0.42-0.55)	0.87 (0.77-0.94)	0.85 (0.72-0.93)	0.55 (0.48-0.60)	0.65 (0.56-0.71)
Walmsley index ≥2	0.85 (0.74-0.93)	0.66 (0.58-0.71)	0.64 (0.56-0.70)	0.86 (0.76-0.93)	0.74 (0.65-0.80)
Walmsley index ≥3	0.87 (0.77-0.94)	0.63 (0.56-0.68)	0.63 (0.55-0.68)	0.88 (0.77-0.95)	0.73 (0.64-0.79)
Modified Mayo score_0	0.77 (0.66-0.86)	0.72 (0.64-0.78)	0.66 (0.56-0.74)	0.82 (0.73-0.89)	0.74 (0.65-0.82)
Modified Mayo score ≥1	0.81 (0.70-0.90)	0.62 (0.61-0.75)	0.65 (0.56-0.72)	0.84 (0.74-0.91)	0.74 (0.65-0.81)
Lichtiger index≥3	0.85 (0.74-0.93)	0.63 (0.55-0.69)	0.62 (0.54-0.68)	0.86 (0.75-0.93)	0.72 (0.52-0.79)

Comparison of the three disease activity indices (DAIs) and histology using Riley score with a cut off ≥12. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Table 31 Comparison of the three DAIs and histology using Rubin score

	Rubin score_2 Sensitivity	Rubin score_2 Specificity	Rubin score_2 PPV	Rubin score_2 NPV	Rubin score_2 Accuracy
Patients perspective	0.46 (0.40-0.48)	0.91 (0.78-0.98)	0.92 (0.81-0.91)	0.42 (0.36-0.45)	0.59 (0.51-0.63)
Walmsley index ≥2	0.89 (0.75-0.96)	0.59 (0.53-0.62)	0.48 (0.41-0.53)	0.92 (0.83-0.97)	0.68 (0.60-0.73)
Walmsley index ≥3	0.91 (0.78-0.98)	0.57 (0.51-0.59)	0.48 (0.41-0.51)	0.94 (0.84-0.98)	0.67 (0.59-0.71)
Modified Mayo score_0	0.83 (0.68-0.92)	0.67 (0.60-0.71)	0.52 (0.43-0.58)	0.90 (0.81-0.92)	0.72 (0.63-0.77)
Modified Mayo score ≥1	0.89 (0.75-0.96)	0.65 (0.59-0.68)	0.52 (0.43-0.56)	0.93 (0.85-0.98)	0.72 (0.64-0.76)
Lichtiger index≥3	0.89 (0.75-0.96)	0.57 (0.51-0.60)	0.47 (0.40-0.51)	0.92 (0.82-0.97)	0.66 (0.58-0.71)

Comparison of the three disease activity indices (DAIs) and histology using Rubin score with a cut off ≥2. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*

Can the Endoscopic scores predict histological activity?

We performed a statistical analysis to compare the three endoscopic score to test their ability to predict histology. Both white light and Narrow band examinations were scored according to the three endoscopic indices. Tables 32-35 demonstrates the comparison between the three endoscopic and histological scores.

In our analysis all three endoscopic scores were able to predict histology with sensitivities around 80% when assessed against the three histology scoring systems. Among the individual scores, MES ≥1, UCEIS ≥1 and Modified Baron ≥1 predicted the histology with higher sensitivities (range 85-91%), however the overall accuracy remained around 83-84%. The addition of NBI to WLE marginally improved sensitivities when MES 0 and Modified Baron's ≥1 scores were used with Geboes and Rubin scores. When used against Riley index, addition of NBI was of little value. NBI seemed to play a role in upstaging the disease marginally with MES for inactive and with Modified Baron scores for active disease. However the overall correlation and accuracy remained under 90%.

Table 32 Comparison of the EIs and histological activity using Geboes score

	Geboes score _3.2 Sensitivity	Geboes score _3.2 Specificity	Geboes score _3.2 PPV	Geboes score _3.2 NPV	Geboes score _3.2 Accuracy
MES WLE_0	0.81 (0.70-0.89)	0.85 (0.78-0.91)	0.79 (0.69-0.87)	0.87 (0.79-0.92)	0.84 (0.75-0.90)
MES NBI_0	0.88 (0.81-0.93)	0.88 (0.81-0.93)	0.83 (0.72-0.90)	0.87 (0.80-0.92)	0.85 (0.77-0.91)
MES WLE ≥1	0.91 (0.81-0.97)	0.61 (0.54-0.65)	0.61 (0.54-0.65)	0.91 (0.81-0.97)	0.73 (0.65-0.78)
MES NBI ≥1	0.87 (0.76-0.82)	0.77 (0.69-0.82)	0.72 (0.63-0.78)	0.90 (0.81-0.95)	0.81 (0.72-0.87)
UCEIS WLE_0	0.81 (0.70-0.88)	0.87 (0.80-0.92)	0.81 (0.70-0.88)	0.87 (0.80-0.92)	0.84 (0.76-0.91)
UCEIS NBI_0	0.80 (0.70-0.88)	0.87 (0.80-0.92)	0.81 (0.70-0.89)	0.87 (0.80-0.92)	0.84 (0.76-0.91)
UCEIS WLE ≥1	0.89 (0.79-0.96)	0.78 (0.71-0.83)	0.74 (0.65-0.79)	0.91 (0.83-0.97)	0.83 (0.74-0.88)
UCEIS NBI ≥1	0.85 (0.75-0.92)	0.85 (0.78-0.94)	0.80 (0.70-0.87)	0.89 (0.82-0.94)	0.85 (0.77-0.91)
Baron score WLE_0	0.81 (0.70-0.87)	0.85 (0.78-0.91)	0.79 (0.69-0.87)	0.87 (0.79-0.92)	0.84 (0.75-0.90)
Baron score NBI_0	0.81 (0.70-0.88)	0.88 (0.81-0.93)	0.83 (0.72-0.90)	0.87 (0.80-0.92)	0.85 (0.77-0.91)
Baron score WLE ≥1	0.85 (0.78-0.91)	0.81 (0.70-0.89)	0.87 (0.79-0.92)	0.79 (0.69-0.87)	0.84 (0.75-0.90)
Baron score NBI ≥1	0.88 (0.81-0.93)	0.81 (0.70-0.88)	0.87 (0.80-0.92)	0.83 (0.72-0.90)	0.85 (0.77-0.91)

Comparison of the three endoscopic indices and histological inflammation (active disease) using Geboes score with a cut off ≥3.2. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*. *MES=Mayo Endoscopic Sub-score, UCEIS= Ulcerative Colitis Endoscopic Index of Severity, WLE=White Light Examination, NBI= Narrow Band Imaging, EI-Endoscopic indices*

Table 33 Comparison of the EIs and histological activity using Riley's score

	Riley score_ cut off 12 Sensitivity	Riley score_ cut off 12 Specificity	Riley score_ cut off 12 PPV	Riley score_ cut off 12 NPV	Riley score_ cut off 12 Accuracy
MES WLE_0	0.88 (0.81-0.93)	0.83 (0.73-0.90)	0.88 (0.81-0.93)	0.83 (0.73-0.90)	0.86 (0.78-0.92)
MES NBI_0	0.91 (0.84-0.96)	0.83 (0.73-0.90)	0.89 (0.82-0.93)	0.87 (0.77-0.94)	0.88 (0.80-0.93)
MES WLE ≥1	0.92 (0.81-0.97)	0.62 (0.54-0.66)	0.63 (0.56-0.67)	0.91 (0.81-0.97)	0.74 (0.66-0.79)
MES NBI ≥1	0.85 (0.75-0.93)	0.76 (0.69-0.82)	0.72 (0.63-0.78)	0.88 (0.79-0.94)	0.80 (0.71-0.86)
UCEIS WLE_0	0.83 (0.73-0.90)	0.90 (0.83-0.94)	0.85 (0.75-0.92)	0.88 (0.81-0.93)	0.87 (0.79-0.93)
UCEIS NBI_0	0.81 (0.71-0.88)	0.91 (0.84-0.96)	0.87 (0.76-0.94)	0.87 (0.81-0.92)	0.87 (0.79-0.92)
UCEIS WLE ≥1	0.90 (0.79-0.96)	0.79 (0.72-0.84)	0.75 (0.67-0.81)	0.91 (0.83-0.97)	0.84 (0.75-0.89)
UCEIS NBI ≥1	0.83 (0.73-0.91)	0.85 (0.78-0.90)	0.80 (0.70-0.87)	0.88 (0.80-0.93)	0.84 (0.76-0.91)
Baron score WLE_0	0.83 (0.73-0.90)	0.88 (0.73-0.90)	0.83 (0.73-0.90)	0.88 (0.81-0.93)	0.86 (0.78-0.92)
Baron score NBI_0	0.81 (0.71-0.88)	0.91 (0.84-0.96)	0.87 (0.76-0.94)	0.87 (0.80-0.92)	0.87 (0.79-0.92)
Baron score WLE ≥1	0.88 (0.81-0.93)	0.83 (0.73-0.90)	0.88 (0.81-0.93)	0.83 (0.73-0.90)	0.86 (0.78-0.92)
Baron score NBI ≥1	0.91 (0.84-0.96)	0.83 (0.73-0.90)	0.89 (0.82-0.93)	0.87 (0.77-0.94)	0.88 (0.80-0.93)

Comparison of the three endoscopic indices and histological inflammation (active disease) using Riley score with a cut off ≥12. Values expressed in % and 95% confidence intervals in the bracket.
PPV=Positive predictive value. NPV= Negative predictive value. *MES=Mayo Endoscopic Sub-score, UCEIS= Ulcerative Colitis Endoscopic Index of Severity, WLE=White Light Examination, NBI= Narrow Band Imaging, EI-Endoscopic indices.*

Table 34 Comparison of the EIs and histological activity using Rubin score

	Rubin Cut off_2 Sensitivity	Rubin Cut off_2 Specificity	Rubin Cut off_2 PPV	Rubin Cut off_2 NPV	Rubin Cut off_2 Accuracy
MES WLE_0	0.83 (0.78-0.84)	0.97 (0.85-0.99)	0.98 (0.92-0.99)	0.71 (0.62-0.73)	0.87 (0.80-0.89)
MES NBI_0	0.85 (0.80-0.86)	0.97 (0.86-0.99)	0.98 (0.93-0.99)	0.74 (0.65-0.76)	0.89 (0.82-0.90)
MES WLE ≥1	0.57 (0.52-0.57)	1.0 (0.89-1.0)	1.0 (91-1.0)	0.50 (0.44-0.50)	0.69 (0.63-0.70)
MES NBI ≥1	0.72 (0.66-0.73)	0.92 (0.85-0.99)	0.98 (0.91-0.99)	0.59 (0.52-0.61)	0.79 (0.72-0.81)
UCEIS WLE_0	0.84 (0.79-0.85)	0.97 (0.86-0.99)	0.98 (0.93-0.99)	0.72 (0.64-0.74)	0.88 (0.81-0.90)
UCEIS NBI_0	0.85 (0.80-0.87)	0.94 (0.82-0.99)	0.97 (0.91-0.99)	0.73 (0.64-0.77)	0.88 (0.81-0.91)
UCEIS WLE ≥1	0.73 (0.68-0.73)	1.0 (0.89-1.0)	1.0 (0.93-1.0)	0.61 (0.55-0.61)	0.81 (0.74-0.81)
UCEIS NBI ≥1	0.80 (0.75-0.81)	0.97 (0.85-0.99)	0.98 (0.92-0.99)	0.68 (0.56-0.70)	0.85 (0.78-0.87)
Baron score WLE_0	0.83 (0.78-0.84)	0.97 (0.85-0.99)	0.98 (0.92-0.99)	0.71 (0.62-0.73)	0.87 (0.80-0.89)
Baron score NBI_0	0.85 (0.80-0.86)	0.97 (0.86-0.99)	0.98 (0.93-0.99)	0.734 (0.65-0.76)	0.89 (0.82-0.90)
Baron score WLE ≥1	0.85 (0.78-0.91)	0.81 (0.70-0.89)	0.87 (0.79-0.92)	0.79 (0.69-0.87)	0.84 (0.75-0.90)
Baron score NBI ≥1	0.88 (0.81-0.93)	0.81 (0.70-0.88)	0.87 (0.80-0.92)	0.83 (0.72-0.90)	0.85 (0.77-0.91)

Comparison of the three endoscopic indices and histological inflammation (active disease) using Rubin score with a cut off ≥2. Values expressed in % and 95% confidence intervals in the bracket. *PPV=Positive predictive value. NPV= Negative predictive value*. *MES=Mayo Endoscopic Sub-score, UCEIS= Ulcerative Colitis Endoscopic Index of Severity, WLE=White Light Examination, NBI= Narrow Band Imaging, EI-Endoscopic indices.*

Can endoscopy and histology predict outcomes of the disease?

Here we compared the endoscopic and histological findings with the clinical outcomes to assess if they could predict disease relapse/remission or surgery.

Although overall accuracy did not reach ≥90% using all three of the endoscopic indices for inflammatory activity, the accuracy of UCEIS≥1 was 85% with a sensitivity of 89%. Among the histological scores Geboes≥3.2 predicted outcomes with accuracy of 88% and a sensitivity of 89%. The data was insufficient for analysis regarding surgical outcome.

Table 35 Comparison of the EIs and histological scores with the clinical outcomes

	Relapse Sensitivity	**Relapse Specificity**	**Relapse Correlation**	**Relapse Accuracy (95%CI)**
MES ≥1	0.73 (0.70-0.89)	0.79 (0.75-0.99)	0.71 (0.62-0.73)	0.72 (0.58-0.94)
MES NBI ≥1	0.71 (0.64-0.96)	0.81 (0.73-0.90)	0.77 (0.73-0.94)	0.78 (0.70-0.93)
UCEIS ≥1	0.89 (0.79-0.96)	0.78 (0.71-0.84)	0.90 (0.73-0.96)	0.85 (0.76-0.90)
UCEIS NBI ≥1	0.83 (0.73-0.91)	0.85 (0.78-0.90)	0.88 (0.72-0.96)	0.84 (0.76-0.91)
Baron≥1	0.81 (0.70-0.91)	0.67 (0.59-0.92)	0.77 (0.69-0.95)	0.79 (0.75-0.90)
Baron NBI ≥1	0.80 (0.70-0.88)	0.78 (0.61-0.90)	0.83 (0.72-0.90)	0.81 (0.71-0.91)
Geboes≥3.2	0.89 (0.80-0.92)	0.78 (0.70-0.82)	0.88 (0.72-0.93)	0.88 (0.74-0.93)
Riley≥12	0.71 (0.66-0.73)	0.89 (0.85-0.99)	0.85 (0.75-0.99)	0.79 (0.73-0.91)
Rubin≥2	0.81 (0.78-0.84)	0.85 (0.81-0.99)	0.75 (0.62-0.78)	0.83 (0.80-0.89)

Comparison of the three endoscopic indices and three histological inflammation (all representing active disease) to correlate with the outcomes at 12 months. Values expressed in % and 95% confidence intervals in the bracket. *MES=Mayo Endoscopic Sub-score, UCEIS= Ulcerative Colitis Endoscopic Index of Severity, WLE=White Light Examination, NBI= Narrow Band Imaging, EI-Endoscopic indices.*

Discussion

In this prospective observational study we found that the DAIs predict endoscopic and histological inflammation better than patient's own impression of disease activity and in most of the case they performed better than the PGA. Results from Walmsley index ≥3 were better with sensitivities consistently >90% when assessed against all three endoscopic and histological indices. It is simple to use in day to day clinical practice.

In a large retrospective study of 369 patients the 'clinical impression' of disease activity under-estimated inflammatory activity in one third of study population(177). This finding is similar to our study in which the accuracy of PGA was at best around 80%.

We also assessed the ability of WLE and NBI in assessment of inflammation. There was only marginal benefit of adding NBI to WLE. NBI assessment was of limited value in presence of severe inflammation as contact bleeding hampered detailed examination of the colon. The additional time spent on using NBI was thought to upstage the disease in selected population with very mild inflammation, however the results were not statistically significant. Although there are more descriptors in UCEIS to differentiate grades of inflammation, addition of NBI to the score did not contribute to upstaging of the endoscopic activity. Only marginal improvement was noted in MES (0) and Modified Baron (≥1) scores when used with NBI. As the results were unimpressive, we did not feel the need to investigate further to identify the predictive markers with NBI.

Among the endoscopic and histological scores UCEIS and Geboes scores predicted the outcomes of the disease activity in 12 months of follow up with reasonable accuracy, albeit falling short of 90%. However addition of NBI to the endoscopic scores was not helpful for predicting the outcomes. Similar outcome was found by Jauregui-Amezaga et al, when they examined the role of advanced endoscopy and histological markers to predict outcomes in patients with UC(40). In this study 17 out of 64 patients (27%) relapsed within 12 months follow up period. They found that the high resolution endoscopy with or without NBI did not confer any benefit in prediction of relapse. In addition to this histological findings assessed by Matt's grading and Riley's index did not predict relapse with accuracy. This study contradicted the previously observed histological criteria, the presence of basal plasmacytosis, as a predictor of relapse (4, 72, 82). Presence of plasma cells in excessive amounts in the lower third of the biopsy specimen is classed as 'Basal plasmacytosis'. Opinion among the gastrointestinal histopathologists differs on the significance of finding basal plasmacytosis. Recently Feagins et al (16) in a retrospective review noted that presence of any of the histological markers of inflammation such as basal lymphoplasmacytosis, basal lymphoid aggregates, erosions/ulcers in the epithelium,

moderate/marked architectural distortion were independently associated with significant relapse risk in patients with clinical remission. This study confirms that presence of any chronic inflammatory infiltrate predisposes to the risk of relapse, than only plasma cells.

In another prospective study of patients who were in clinical remission, UCEIS was found to be predictive of relapse in medium and long term with increasing severity of scores (5.0% for UCEIS=0, 22.4% for UCEIS=1, 27.0% for UCEIS=2, 35.7% for UCEIS=3 and 75.0% for UCEIS=4–5)(128). Despite aiming at including patients with clinical remission the study had significant proportion of patients with endoscopically active disease (67% of patients had MES score>0 and 75% had UCEIS score>0). This study found a suboptimal correlation of clinical severity with UCEIS; however these results cannot be generalised as there were no patients included with acute severe colitis. In our study we have included patients with both quiescent and active colitis.

Our findings support the recent studies in which UCEIS has outperformed MES in assessment and prediction of outcomes(126, 176). In another study UCEIS >7 is shown to predict colectomy rates(175). In our study we only had 2 patients who underwent surgery and hence we did not have enough of data to further analyse for predicting surgery.

In conclusion, DAIs are useful in clinical practice for objective assessment of the disease activity and predicting histology. We advocate using Walmsley or Simple clinical colitis activity index based on our findings. Addition of NBI to WLE is not found to be helpful in predicting outcomes and as such additional time spent may not be useful in assessing the endoscopic severity either.

6 Raman spectroscopy of endoscopic colonic biopsies from patients with ulcerative colitis to identify mucosal inflammation and healing

Abstract:

Raman spectroscopy was used to differentiate between mucosally healed (or quiescent) and inflamed colon tissue, as assessed endoscopically, in patients with ulcerative colitis. From the analysis of the Raman spectra of 60 biopsy tissue samples, clear differences were identified between the spectra of the quiescent and inflamed tissue. Three carotenoid peaks were found to be approximately twice as intense in the inflamed tissue. Two phospholipid peaks were found to be significantly lower in the inflamed tissue. Using multivariate statistical analysis, we show that these five peaks can be used to discriminate between endoscopically quiescent and inflamed tissue. We also correlated the Raman data with a histological assessment of the tissue. Four of the five peaks were found to be significantly different between the spectra of histologically healed (or quiescent) and histologically inflamed tissue. These findings indicate the ability of Raman spectroscopy to accurately classify colon tissue as either quiescent or inflamed, irrespective of whether an endoscopic or histological grading scheme is followed. We thus demonstrate that Raman spectroscopy could potentially be used as an early diagnostic tool for assessing the presence of mucosal healing or inflammation in patients with ulcerative colitis.

Introduction

Approximately 25% of patients with UC experience acute exacerbation of their disease activity during the course of their disease(7). The colectomy rate increases with more than one hospital admission with acute severe UC reaching up to 40% after two admissions(7). The treatment goals in UC focus on keeping the disease in remission and a colectomy-free survival. Endoscopic mucosal healing (MH) is characterised by the disappearance of endoscopic lesions such as erosions and ulcers. If MH is achieved then the short and long-term clinical outcomes for the patient tend to be favourable. However presence of histologically detectable inflammation despite normal endoscopic finding, has been shown to be associated with a greater risk of subsequent relapse(4, 5). That said, irrespective of whichever technique is used, a flare in UC activity remains difficult to predict. Therefore, a simple, easily measured biological marker that predicts relapse would be of great use in guiding the most appropriate and cost-effective therapy. Developing a complementary tool that can reliably and quickly identify the presence of inflammation or confirm that MH has occurred would help to guide patient management.

In this respect, the molecular vibrational spectroscopic analysis is a strong candidate for such a tool. The molecular vibrational spectroscopic analysis is used to characterise solids, liquids and gases and is especially relevant when the analyte is rare to procure and small in size for analysis, as is the case for endoscopic biopsy specimens of typical surface area ~5 mm^2. Further, vibrational spectroscopy has huge potential in medicinal applications as it is Non-destructive and has the ability to reveal the biochemistry of tissue. This allows, in principle, differentiation between healthy and anomalous tissue.

Raman spectroscopy was chosen as the vibrational spectroscopic technique for this study. In a Raman microscope, as used in this study, the incident monochromatic light of modest power (< 10 mW), controlled by focussing through an objective lens is directed at the sample. The light scattered by the sample is collected and detected. Raman scattering is an inelastic scattering process for which the probability of occurrence is 1000-100,000 times less than that of Rayleigh scattering. Only molecular vibrations which involve changes in the polarizability of the molecule are Raman active. In this respect, the vast majority of biomolecules provide rich Raman spectra as they have complex ring-like aromatic and/or long-chain aliphatic structures, which may be interconnected to enhance the probability of inelastic scattering, and, as a result, may be more Raman active. Such extended local order in the structures of biomolecules limits the dispersion of energy states in the resulting Raman spectra. Consequently, the peaks have well-defined shapes, unlike in amorphous materials where the lack of medium and long-range order yields dispersed phonon energy

states(178). In analytes consisting of multiple constituent molecules, a spectrum of Raman scattered light consists of a range of peaks corresponding to the Raman active-vibrational modes, stimulated by the incident laser. The peak intensities are proportional to the concentrations of the responsible molecules. The resultant spectrum may, therefore, be interpreted as a qualitative and quantitative measure of the analyte biochemistry(179). Importantly, the OH vibrational modes of water molecules produce weak Raman signals and thus do not contribute significantly to Raman spectra. This is in contrast to infrared (IR) spectroscopy where OH- ions and free water, often present in biomolecular and tissue media, are highly absorptive in the mid IR range from 2.7 to 4.5 μm. The absorption peaks of water thus tend to overlap with those due to representative aromatic ring C-C (wavenumber 1600-1585 cm−1, 1500-1400 cm−1), hydrocarbon C-H (2850-3100 cm−1) and C = O (1630-1780 cm−1) stretch vibrations of biomolecules. Fluorescence spectroscopy can also be used to characterise tissue. Once again in complex molecules, a range of electron-phonon coupled states might arise during excitation which during fluorescence decay might yield a broad spectrum of spontaneous emission. Fluorescence spectra from tissue thus tend to be relatively broad and featureless(180) with fewer specific differences in the spectra from healthy and anomalous tissue(181). In comparison, Raman spectroscopy has the advantage of delivering spectra with sharp, narrow peaks in different parts of the spectrum for lipids, proteins and nucleic acids(182). The main disadvantages of Raman spectroscopy are that the scattered intensity is inherently weak and that the Raman excitation laser can induce the aforementioned fluorescence in tissue, which can obscure the weak Raman signal(183). Despite these drawbacks, Raman spectroscopy has been shown to be successful in providing very sensitive biochemical information about the composition of biological tissue(184).

A number of vibrational spectroscopy studies have been performed on human colon tissue. Most of these studies have attempted to use vibrational spectroscopy to distinguish either between colon tissue containing polyps and healthy tissue or between cancerous and healthy tissue (185-189). However, very few studies have employed spectroscopy to study UC. Two such studies on UC and Crohn’s disease(190, 191) examined respectively, 21 and 38 samples.

In this study, therefore, we perform Raman spectroscopy on a large sample set (60 samples) of colonic biopsies, taken from patients who have been diagnosed with UC and are either in remission or still have the condition. We analyse the ability of Raman spectroscopy to differentiate between tissue that has, from an endoscopic point of view, mucosally healed and tissue for which endoscopic inflammation is still present. We provide insights into the pathogenesis of UC and put forward a biological explanation for the differences observed in

the levels of certain biomolecules between quiescent and inflamed tissue. In addition, we assessed the second set of colonic biopsies, taken from the same patients at the same sites at the same time as the first set, for histological activity. We assess whether Raman spectroscopy also provides a reliable means of discriminating between tissue that has, from a histological point of view, healed and tissue for which histological inflammation is still observable. We thus evaluate the ability of Raman spectroscopy to distinguish between quiescent and inflamed tissue, which has been graded by two different clinical techniques; endoscopy and histopathology. In this way, we aim to provide a more complete assessment of the utility of Raman spectroscopy as a potential, complementary tool for the assessment of UC.

Experiment

Patients, samples and tissue preparation

All patients in the study had initially been diagnosed with UC at the IBD clinic at St James University Hospital, Leeds, had followed a course of treatment and returned to the IBD clinic for further assessment during colonoscopy. Informed consent was obtained from all patients and ethical approval for the study including the collection of biopsies for spectroscopy and histological assessment was obtained from the Yorkshire and Humber–Yorkshire Bridge National Research Ethics Committee (13YH-0115). During a colonoscopy the colonic tissue was assessed endoscopically for endoscopic MH by expert (gastrointestinal GI) endoscopists, using the Mayo endoscopic score (See Table 3 in chapter 'Endoscopic assessment of disease activity) [34], and a score was assigned. A score of zero was taken to indicate that endoscopic MH had occurred and all scores greater than 0 indicated that endoscopic inflammation was still present (i.e. an absence of endoscopic MH).

Biopsies for the purposes of the Raman spectroscopy study and the histological assessment were then taken from the same area assessed by endoscopy. All biopsies were targeted, whether taken from active or inactive/quiescent area. The sample dimensions of biopsies were typically of length ~3 to 4 mm, width ~1.5 to 2 mm and thickness ~1 to 2 mm. For both the Raman study and the histological assessment, 60 biopsies were collected from 39 patients; 32 from the sigmoid and 28 from the rectum. Of the 60 samples for the Raman study, 24 were taken from areas with endoscopic MH and 36 from areas with signs of endoscopic inflammation. Immediately after taking the biopsies, they were snap-frozen in liquid nitrogen and stored at −80°C. The prompt snap-freezing of biopsies allowed the metabolic content of the tissue to be preserved as in the in-vivo state, as required to obtain Raman spectra which accurately reflect their biochemical composition at the time of collection of the biopsies. 60 biopsies were also taken at the same sites at the same time for

the histological assessment. These were fixed in formaldehyde and then later stained with haematoxylin and eosin. These were graded histopathologically by an expert GI histopathologist (O.R.), using the validated Geboes scoring system, which has been shown to have the best inter-observer agreement(192). A Geboes score of less than 3.1 was considered to indicate that the mucosa had histologically healed and a score of 3.1 or greater to denote histological activity (HA), when there is the presence of neutrophils in the epithelium(82, 125). Of these 60 samples, 27 were graded as histologically healed and 33 as histologically active (HA). In both the endoscopically assessed and in the histologically assessed groups of samples we can thus consider that there is a population of tissue samples with the status quiescent and another population with the status inflamed.

We compared the status given to each sample during endoscopic assessment with that given during histological assessment. Disagreement between the endoscopic and histological assessments of the tissue status was observed in 5 samples out of 60. The assessments of the tissue status via the two techniques, as being either quiescent or inflamed, thus matched for 92% of the samples, as might be expected for two techniques that are complementary.

Raman spectroscopy

We used an inVia Renishaw Raman microscope to obtain the Raman spectra for the tissue samples. Samples were removed from the −80°C freezer and placed on to low fluorescence glass microscope slides on the microscope stage. Samples were not rehydrated in saline. A continuous wave (cw) laser of wavelength 514.5 nm and 5 mW incident power was used as the excitation source. The laser beam was focused by a 50x microscope objective of numerical aperture 0.8 and working distance 1.1 mm to form a spot of diameter ~5 μm on the sample surface. Single scan exposures of 10 seconds were sufficient to obtain a good signal to noise ratio. The values used for the incident laser power, spot size and exposure time lead to an energy density which is similar or slightly lower than that employed in other Raman spectroscopy studies on tissue(187, 193, 194). The constituency, shape and composition of a biopsy sample can vary significantly from one region to another on its surface. Hence spectra were taken at four different points per sample. Spectra were collected for the Raman shift range of 400 to 3000 cm^{-1} with a spectral resolution of 4 cm^{-1}. This range and resolution are relatively favourable with respect to typical Raman instruments, which often provide a range of 700 to 1800 cm−1 and a resolution of 6 to 8 cm^{-1}, and are similar to those used in some recent Raman studies on colon tissue(195, 196).

Processing of Raman spectra

For each sample, the average Raman spectrum was calculated from the four measurements taken. As mentioned in Section 1, the tissue may produce fluorescence when excited with a short wavelength visible laser for Raman spectroscopy. The measured spectrum thus consists of Raman scattered light, fluorescent light emitted by the tissue (the fluorescent background) and noise(197). Three operations were performed; i) data smoothing, ii) background estimation and subtraction and iii) normalisation of the background-corrected spectrum. Data smoothing was achieved using a Savitzky-Golay filter with a smoothing width of 9 and a polynomial of degree 3 in order to increase the signal-to-noise ratio(187). An effective method for estimating the fluorescence background is modified polynomial fitting(198) and this technique was found to be optimal for the spectra in this study. The adapted polynomial form was subtracted from the averaged, smoothed spectrum to obtain the uncluttered Raman spectrum consisting of a set of peaks with a relatively flat baseline. In a Raman microscope, the cone of light backscattered from the sample, which enters the objective, forms the signal measured. For a particular illuminated sample area, the signal measured, therefore, depends not only on the concentrations of the Raman-active molecules in the area but also on the shape and the reflectivity of the surface and the accuracy of focussing on the surface. Since the biopsies were non-uniform and uneven, these factors lead to large variations in the signal measured from area to area on the sample and from sample to sample. To correct for the variations in absolute signal intensity and thus to be able to compare the peak intensities between samples, each background-corrected spectrum was thus normalised by dividing by the total area under the curve(194).

Statistical methods

For the evaluation of diagnostic sensitivity and tissue classification, two-tailed nonparametric Mann-Whitney U tests to identify statistically significant differences between the peak intensities in the Raman spectra for different sample groups were performed. One test was carried out on the Raman data for the group of samples assessed endoscopically in order to highlight the differences between the spectra of samples taken from areas of the colon which showed endoscopic MH and of those taken from areas which showed endoscopic inflammation. A second such test was performed on the group of samples assessed histologically in order to quantify the spectral differences between the samples which showed histological healing and those which showed HA. Non-parametric tests were used as the distribution of the Raman spectral peak intensities did not follow a normal distribution. A p-Value of ≤ 0.05 was used as a cut-off of significance. Results are expressed as the mean ± standard deviation for continuous variables.

Multivariate analysis was also performed by logistic regression analysis to calculate odds ratios (OR) and their 95% confidence intervals. All variables with a p-value of < 0.1 in the Mann-Whitney U analysis were included (as planned a-priori) in the final multivariate model. Correlation matrices were used to identify collinearity. If collinearity was detected we minimised this by inputting the variable separately in the multivariate analysis. Hosmer-Lemeshow's test was used to verify the null hypothesis that there is a linear relationship between the predictor variable and the log odds of the outcome variable. All statistical tests were done using PASW version 21 (IBM Corp, NY).

Results and discussion

Analysis of Raman spectra

Figure 16 displays the average Raman spectra of the endoscopically assessed biopsy samples taken from areas of the colon which showed endoscopic MH and of those taken from areas which showed endoscopic inflammation (i.e. where endoscopic MH was absent).

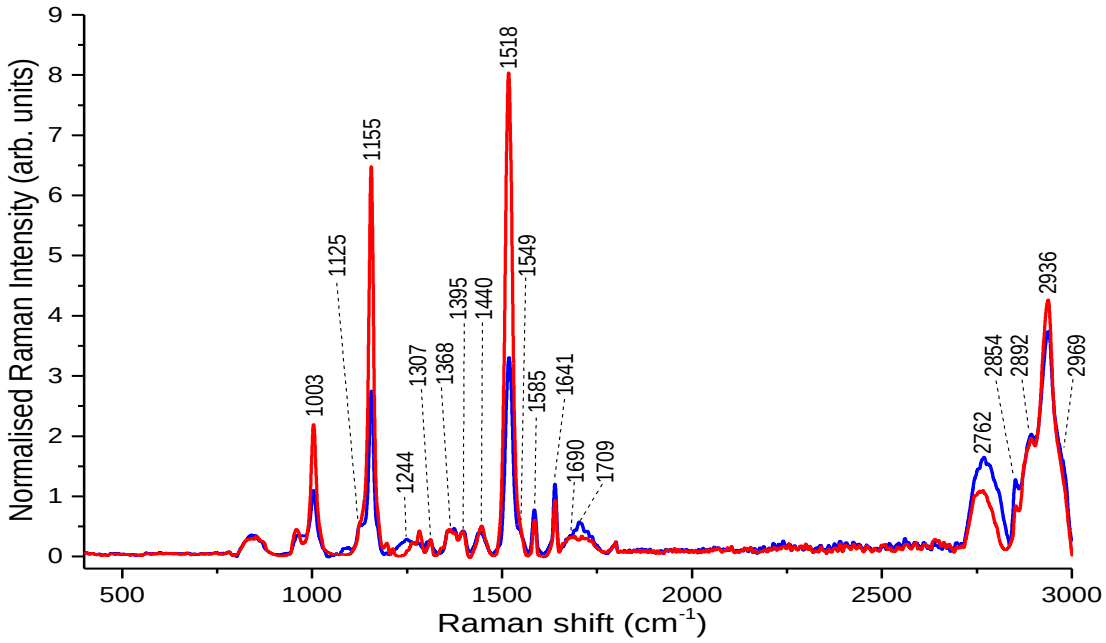

Figure 16 Raman shift

The above figure shows the average background-subtracted normalised Raman spectra for endoscopically assessed colonic mucosa which showed either a) endoscopic MH (black) or b) endoscopic inflammation (red).

The form of the spectra for both the colonic mucosa which showed endoscopic MH and those which exhibited endoscopic inflammation is similar with primary peaks observable at Raman shifts of 1003, 1155, 1244, 1307, 1368, 1395, 1440, 1518, 1585, 1641, 1690, 1709,

2762 and 2936 cm^{-1} and shoulder peaks at 1125, 1549, 2854, 2892 and 2969 cm^{-1}. Strong peaks are found in both tissue types at 1003, 1155, 1518 (the largest peak in the endoscopically inflamed tissue), 2762, 2892 and 2936 cm^{-1} (the largest peak in the endoscopic MH tissue). The most striking difference between the two tissue types is in the intensity of the peaks at 1003, 1155 and 1518 cm^{-1}. These are considerably higher in the endoscopically inflamed than in the endoscopic MH tissue.

In terms of constituent biomolecules, both spectra contain contributions from vibrational modes of proteins, amino acids, lipids and nucleic acids as well as other compounds such as carotenoids and myoglobin. A detailed list of the peaks observed and their possible assignments are described in Table 36. Regarding the peaks at 1125, 1307, 1368 and 1395 cm^{-1} several different assignments for each one are possible. The peak at 1125 cm^{-1} could be attributed to vibrations of phospholipids or proteins(199); the peak at 1307 cm^{-1} could be assigned to phospholipids(200) or lipids or the nucleotide, adenine(201); the peak at 1368 cm^{-1} to the nucleotides, guanine or thymine or to the amino acid, tryptophan (199)and that at 1395 cm^{-1} to the nucleotide, uracil(201). However, the fact that these four peaks as well as peaks at 1549 cm−1 (assigned to deoxy-myoglobin in colon tissue) and at 1585 cm^{-1} and 1641 cm^{-1} (both assigned to oxy-myoglobin in colon tissue(183) are present in the spectra plus the experimental observation that the inflamed colonic mucosa often showed signs of bleeding mean that we assign the peaks at 1125, 1307, 1368 and 1395 cm^{-1} to haeme groups, in particular to the haeme core of myoglobin. In terms of the other peaks, the peak at 1440 cm^{-1} is characteristic of scissoring vibrations of CH2 in phospholipids(197) and lipids(200). Signals characteristic of the amide bands of proteins is found at 1244 cm−1 (amide III, β-sheet conformation) and 1690 cm^{-1} (amide I, β-sheet conformation(194)). The band at 1709 cm^{-1} is consistent with C = O vibrations in phospholipids and triglycerides. The signal at 2762 cm^{-1} corresponds to a CH stretch, possibly in phospholipids. Vibrations that are characteristic of the CH groups in lipids (fatty acids, triglycerides) are observed at: 2854 cm^{-1} (symmetric stretching of the CH2 group), 2892 cm^{-1} (antisymmetric stretching of the CH2 group [43]), 2936 cm^{-1} (symmetric stretching of the CH3 group) and 2969 cm^{-1} (antisymmetric stretching of the CH3 group).

Considering the three sharp peaks at 1003, 1155 and 1518 cm^{-1}, the peak at 1003 cm^{-1} has previously been considered to be due to phenylalanine. However, when peaks at 1155 cm^{-1} and 1518 cm^{-1} are also present, forming the triplet combination seen in Fig. 1, it has become common to assign these three peaks to vibrations of carotenoids (193, 195, 202). In an attempt to improve the characterisation of the carotenoid groups, we have compared our data with the Raman data for a wide range of carotenoids. The most dominant phonon vibration is the approximate in-phase stretching vibration of C = C bonds (v1, 1490-1540 cm^{-1}), followed by the C-C stretching mode (v2, 1140-1160 cm^{-1}), which may be mixed with

C-H in-plane vibrations, and, finally, the in-plane rocking mode of CH3 (v3, ~1005 cm^{-1}). This vibration is part of the carotenoid “fingerprint region” from 1100 to 1400 cm^{-1}, which contains weak peaks sensitive to the terminal groups and chain conformation in the carotenoid. In our data, the peaks at 1003 cm^{-1} (in-plane rocking of CH3), 1155 cm^{-1} (stretching of C-C) and 1518 cm^{-1} (stretching of C = C) are thus consistent both in terms of position and relative intensities with the data reported for carotenoids(203).

We take the analysis of our data further by examining the relationship between π-electron conjugation and the wavenumber of the C-C stretching mode (v2) for different carotenoids. In polyenes, such as carotenoids, as the number of C = C double bonds and, in that way, length of the conjugated chain increases, the space for the Π electrons to delocalise increases, leading to a decrease in the order of the C = C bond. This causes a reduction in bond strength and thus a decrease in the frequency of vibration of the C = C stretching mode (v1). This can be seen in Fig. 17, where, for example, v1 for decapreno-beta-carotene, which has 10 more C atoms than beta-carotene, is 25 cm−1 lower than for beta-carotene. A decrease in the position of the v1 mode is usually accompanied by an increase in the position of the v2 mode(203). However, interestingly, carotenoids show the opposite trend in Fig. 17.

Table 36 Tentative assignments of peaks in the Raman spectra of colon tissue

Peak No	Centre (cm^{-1})	Vibrational mode	Major Assignments
1	1003	Ring breathing	Phenylalanine
		ρ(C-CH_3)	Carotenoids
2	1125	ν_{22}-ν(Pyr ½ ring)$_{as}$	Myoglobin (haeme core)
		ν(C–C)	Phospholipids
		ν(C–N)	Proteins
3	1155	ν(C–C)	Carotenoids
4	1244		Amide III, ß sheet
5	1307	τ(CH_2)	Phospholipids, lipids
			Adenine
		ν_{21}-δ_{as}(C_mH)	Myoglobin (haeme core)
6	1368	ω(CH_2),	δ(CH) Tryptophan
			Guanine
			Thymine
		ν_4-ν(Pyr ¼ ring)$_s$	Myoglobin (haeme core)
7	1395	ν_{20}-ν(Pyr ¼ ring)	Myoglobin (haeme core)
			Uracil
8	1440	δ_{sc}(CH_2)	Phospholipids, lipids
		δ(CH_2), δ(CH_3)	Collagen
9	1518	ν(C=C)	Carotenoids
10	1549	ν_{11}-ν($C_\alpha C_ß$)$_{as}$	Deoxy-Myoglobin (haeme core)
11	1585	δ(C=C)	Phenylalanine
			Hydroxyproline
		ν_{37}-ν($C_\alpha C_m$)$_{as}$	Oxy-Myoglobin (haeme core)
12	1641	ν_{10}-ν($C_\alpha C_m$)$_{as}$	Oxy-Myoglobin (haeme core)
13	1690		Amide I, ß sheet
14	1709	ν(C=O)	Phospholipids, triglycerides
15	2762	ν(C-H)	Phospholipids
16	2854	ν_s(CH_2)	Lipids
17	2892	ν_{as}(CH_2)	Lipids, proteins
18	2936	ν_s(CH_3)	Lipids, proteins
19	2969	ν_{as}(CH_3)	Lipids, proteins

ν – stretching vibration; ν_s – symmetric stretch; ν_{as} – antisymmetric stretch; δ – bending vibration; δ_{sc} – in-plane bending (scissoring); ρ – in-plane bending (rocking); τ – out-of-plane bending (twisting); ω - out-of-plane bending (wagging).

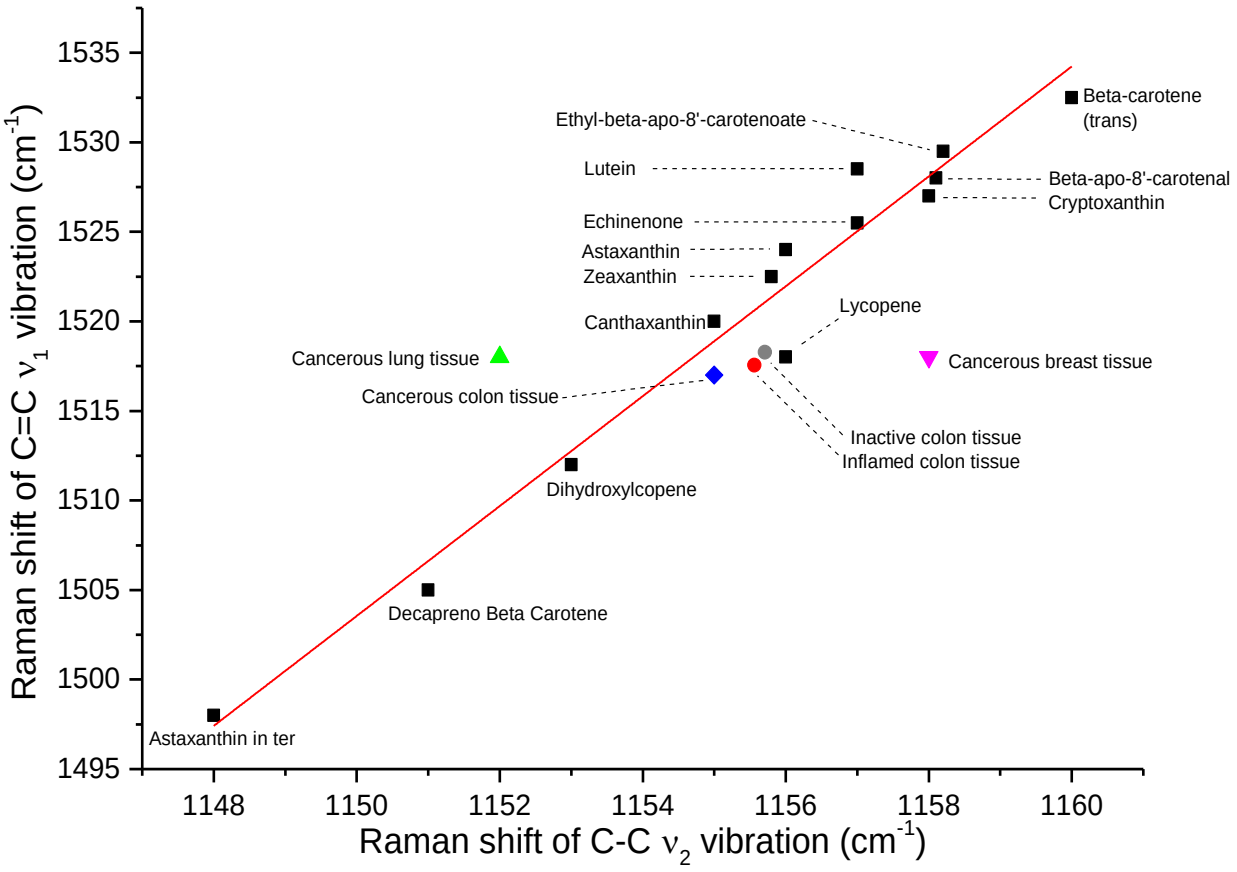

Figure 17 Characteristics of beta-carotene

This figure shows the plot of frequency of C = C (v1) versus C-C stretching vibration (v2) for beta-carotene isomers (black squares), colonic mucosa showing endoscopic MH (grey circle, our data), colonic mucosa showing endoscopic inflammation (red circle, our data), cancerous colon tissue (blue diamond), cancerous breast tissue (pink triangle), cancerous lung tissue (green triangle [37],). Figure adapted from(203).

This may be due to the presence of CH3 groups which disturb the C-C stretching modes [53]. From Fig. 17 we suggest, that in both our colonic mucosa which showed endoscopic MH and in those with endoscopic inflammation, the carotenoid found may be of canthaxanthin and lycopene types. The carotenoid present in cancerous colon tissue also appears to be of these types.

Statistical analysis of data

In Fig. 18 we compare the average Raman peak intensities of the endoscopically assessed biopsy samples which showed endoscopic MH with those of the samples which showed endoscopic inflammation. The p values, obtained in the non-parametric Mann Whitney U test for this group of endoscopically assessed samples, are also shown in Fig. 18. It can be seen from Fig. 18 that in the endoscopically inflamed tissue the average intensity of the carotenoid

peak at 1003 cm−1 is ~75% greater than in the endoscopic MH tissue, whilst the carotenoid peaks at 1155 cm−1 and 1518 cm−1 are almost double those in the endoscopic MH tissue. The standard deviation of the peak intensities is substantial, which reflects the inhomogeneity of the tissue samples. Differences in the peak intensity between endoscopic MH and endoscopically inflamed tissue with a significance level of $p \leq 0.05$ are found for peaks 1 (1003 cm−1), 3 (1155 cm−1), 9 (1518 cm−1), 14 (1709 cm−1) and 15 (2762 cm−1). Differences with a significance level of $0.10 \geq p > 0.05$ are obtained for peaks 6 (1368 cm−1) and 8 (1440 cm−1). These statistics clearly indicate that the Raman spectral differences between endoscopic MH and endoscopically inflamed colon tissue are significant and are consistent with previous reports(190, 191).

Figure 4 presents the same information as Fig. 3 but for the group of histologically assessed samples. The p values come from the non-parametric Mann Whitney U test for this group.

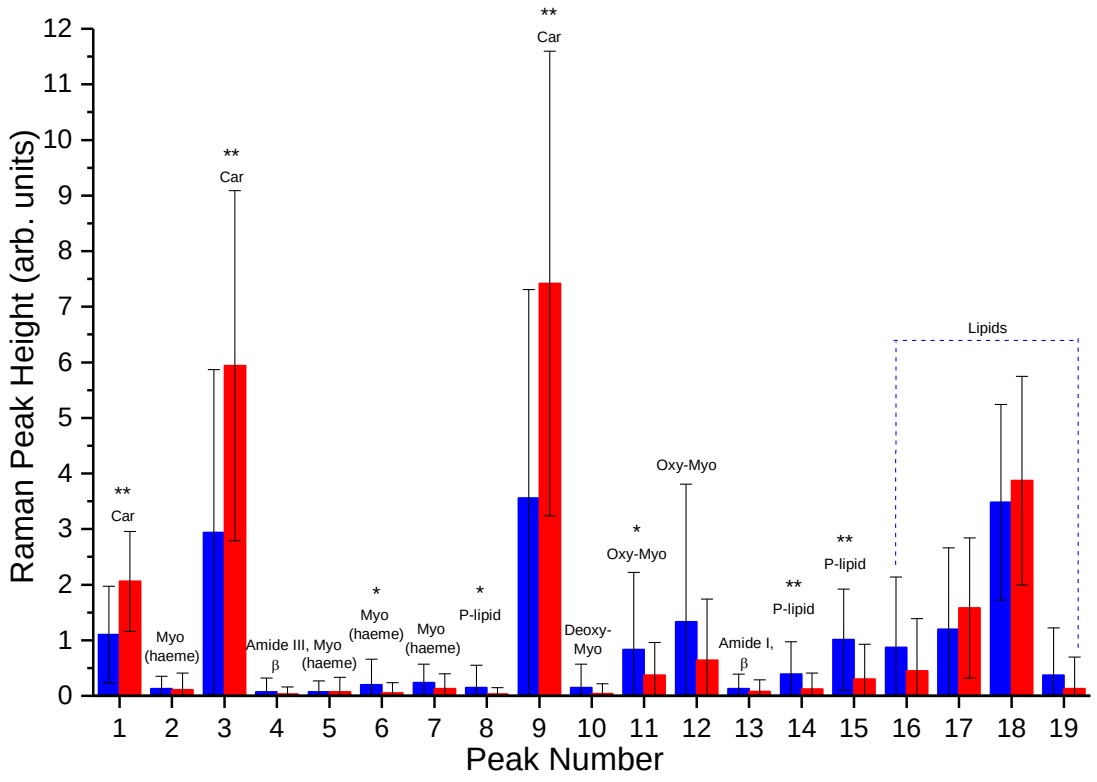

Figure 18 Histogram of Raman peaks in endoscopic assessment of UC activity

This figure is a Histogram displaying average Raman peak intensities, standard deviations and p values for endoscopically assessed colonic mucosa which showed either a) endoscopic MH (N = 24) or b) endoscopic inflammation (N = 36). No asterisk represents $p > 0.10$, a single asterisk * represents $0.10 \geq p > 0.05$ and two asterisks ** represent $p \leq 0.05$. Abbreviations: Car = carotenoids; Myo = myoglobin, Oxy-Myo = oxy-myoglobin, Deoxy- Myo = deoxy-myoglobin, P-lipid =phospholipids.

The peak intensities are very similar to those in Fig. 18, with the carotenoid peaks much greater in the samples which showed HA than in the samples which exhibited histological

healing. Differences in the peak intensity between histologically healed samples and samples which showed HA with a significance level of $p \leq 0.05$ are again observed for peaks 1, 3, 9, 14, 15 and also 19 (2968 cm−1). Just as for the endoscopically assessed group, Fig. 19 indicates that there are significant differences in the Raman spectra of histologically healed and histologically active samples.

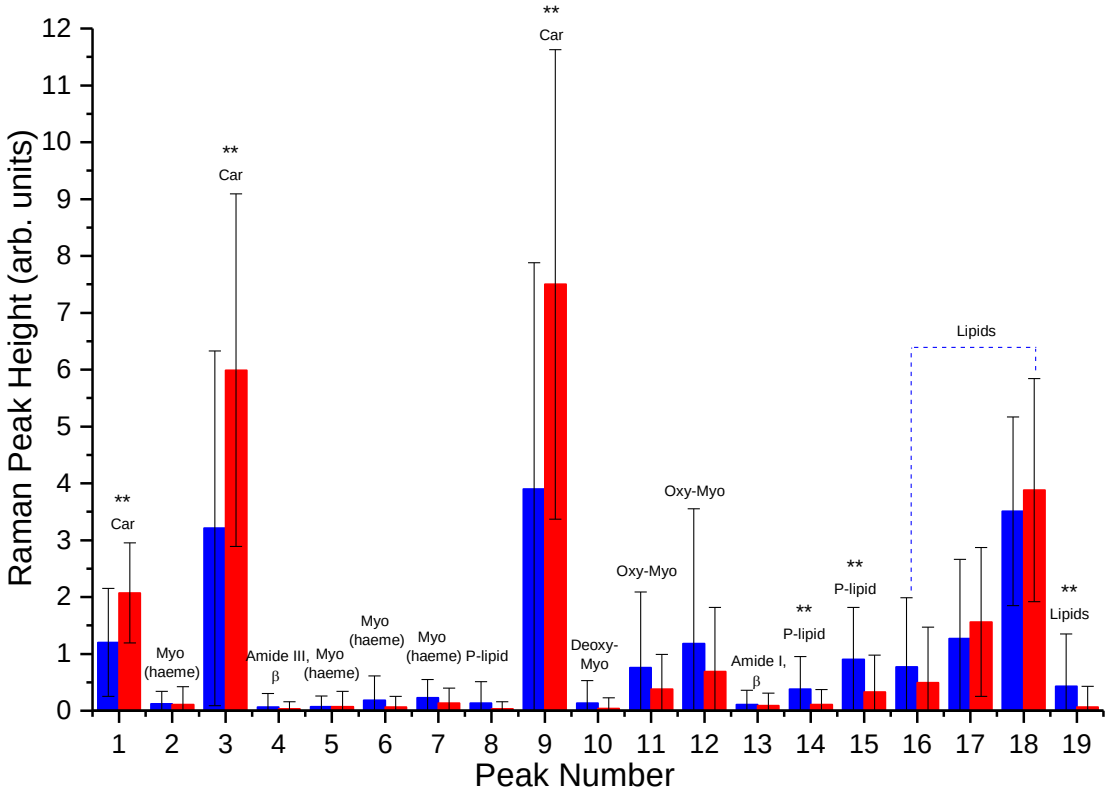

Figure 19 Histogram of Raman peaks in histological assessment of UC activity

This figure shows the Histogram displaying average Raman peak intensities, standard deviations and p values for histologically assessed colonic mucosa which showed either a) histological healing (N = 27) or b) HA (N = 33). The same key and abbreviations as for Fig. 18 apply.

The non-parametric analysis is extended to a multivariate analysis, which has been shown to be more accurate and reliable when analysing multiple peaks over a large Raman spectral range, as is the case for our colon tissue spectra. The results of the multivariate analysis are presented in Table 37.

In the multivariate model peaks 1, 3, 8, 9 and 15 turn out to be significantly different between endoscopic MH and endoscopically inflamed samples. All of these except peak 8 are also significantly different between histologically healed samples and samples which showed HA.

A visual comparison is presented in the 3D scatter plots in Figs. 20(a) and 5(b) which show the distribution of the intensity of the important peaks 1, 3 and 15 in the groups assessed endoscopically and histologically. In both figures, the separation between the two

populations (the quiescent population and the inflamed population) is clearly visible. Both figures suggest that in a Raman spectrum where peaks 1 and 3 are high, whilst in comparison peak 15 is medium to low, the sample is more likely to be inflamed than quiescent. The strong likeness between the distributions in both figures is to be expected in view of the close match between the endoscopy and histology grades for the vast majority of samples.

Table 37 Multivariate analysis of the patients included in Raman spectroscopy study

Peak no.	MH: absent versus present OR (95% CI)	HA: histologically active versus inactive (quiescent) OR (95% CI)
1#	**3.71 (1.79-7.67)**	**1.92 (1.28-2.87)**
3#	**1.50 (1.19-1.89)**	**1.22 (1.07-1.39)**
6	0.68 (0.05-9.48)	n/a
8	**0.05 (0.004-0.55)**	n/a
9#	**1.36 (1.14-1.61)**	**1.17 (1.09-1.30)**
11	0.82 (0.32-2.07)	n/a
14	0.68 (0.15-3.04)	0.48 (0.08-2.80)
15	**0.24 (0.09-0.64)**	**0.40 (0.19-0.84)**
19	n/a	0.65 (0.21-2.04)

Multivariate analysis for the a) endoscopically assessed group and b) histologically assessed a group of colonic mucosa

inputted separately into the model as they were highly correlated (rho > 0.6). Significant differences are in **bold**. N/A implies the p-value of the peak in the Mann Whitney U test was > 0.10 and therefore was not included in the multivariate analysis.

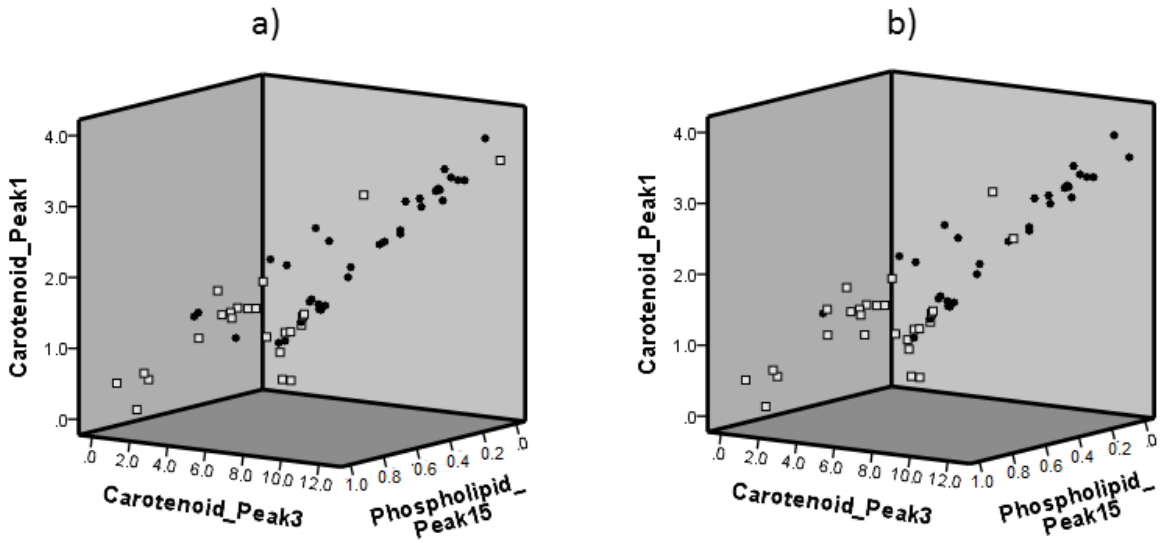

Figure 20 Scatter plot

This figure shows Scatter plot of intensities of peaks 1, 3 and 15 for a) endoscopically assessed colonic mucosa which showed either endoscopic MH (open squares) or endoscopic inflammation (filled circles) and b) histologically assessed colonic mucosa which showed either histological healing (open squares) or HA (filled circles).

Biomolecular explanation for differences between quiescent and inflamed Raman spectra

We attempt to explain the similarities and differences between the Raman spectra of colonic mucosa showing endoscopic MH and of colonic mucosa showing endoscopic inflammation in terms of the biomolecular composition of the mucosa, based on the peak assignments. The fact that the same set of peaks occurs in both the endoscopic MH and endoscopically inflamed tissue indicates that their biochemical composition is very similar. This is consistent with the common understanding of inflammation. Inflammation does not introduce new metabolites to the system but rather leads to overproduction or overuse of the existing metabolites. One would thus expect the same peaks to be found in the two tissue types. Variations in peak intensities between the two tissue types are due to differences in the concentrations of biomolecules in the two. The endoscopically inflamed tissue contains very high amounts of carotenoids. Carotenoids are known to act as anti-oxidants(184) in the defence mechanism of tissue against inflammation. Beta-carotene, for instance, has been shown to suppress the activation of nuclear factor kappa-beta and thereby inhibit pro-inflammatory gene expression(204). Carotenoid compounds could thus be expected to be strongly present in the endoscopically inflamed tissue, as observed. Additionally, we found that the phospholipid components (peak 8 at 1440 cm−1 and peak 15 at 2762 cm−1) were

markedly higher in samples where endoscopic MH had occurred. This observation would reflect the fact that when, endoscopically, the tissue is visibly inflamed, there is a marked loss of tissue integrity, characterised by ulceration or erosion of the mucosa with loss of the cell membrane. Phospholipids are well known to be a major component of the colonic cell membrane and if the tissue is disrupted, as is the case when it is inflamed, their levels would be expected to decrease.

Conclusion

An emerging goal of gastroenterology is to establish whether mucosal healing (MH) has occurred in patients treated for UC, as MH appears to lead to favourable outcomes for the patient. To this end, a spectroscopic tool which could assist current techniques such as endoscopy and histopathology in examining colonic mucosa for evidence of MH would be of great benefit. In this study, we thus employed Raman spectroscopy to evaluate its potential for such a tool.

60 biopsy samples were taken from areas of the colon which showed endoscopic MH and from areas which showed endoscopic inflammation, snap frozen at −80°C in order to preserve their metabolic content and their Raman spectra were obtained. Simultaneously, we collected a second group of 60 samples from these areas of the colon and assessed them histologically.

We analysed the Raman spectra of the colonic mucosa, assigned the peaks to vibrations of biomolecules and performed Mann Whitney U analyses and multivariate statistical analyses on the spectral peak intensities. The essential findings can be summarised as follows:

1. A similar set of Raman peaks corresponding to vibrations of proteins, amino acids, lipids, nucleic acids, myoglobin and carotenoids was observed in the endoscopic MH and the endoscopically inflamed tissue, indicating that similar biomolecules are present in each. This suggests that inflammation can be thought of as a state of activity where greater or lower quantities of existing biomolecules are produced by the body's response.
2. The major visual difference between the Raman spectra of the biopsy samples which showed endoscopic MH and those which showed endoscopic inflammation was found to be in three carotenoid peaks. Carotenoid levels were found to be very high (almost double) in the inflamed compared to in the quiescent tissue. This finding is consistent with the role they play as anti-oxidants in fighting inflammation. Significant differences were also observed in two phospholipid peaks. Phospholipid levels were found to be lower in the inflamed tissue. This is also consistent with

studies which indicate that phospholipids are a key component of the colonic cell membrane. Their levels may thus be expected to decrease when tissue is inflamed and thus damaged.

3. Using multivariate analysis, the intensities of these five peaks (the three carotenoid and the two phospholipids) were found to be statistically significantly different between the Raman spectra of the endoscopic MH and the endoscopically inflamed tissue. A similar result was obtained for the histologically assessed samples with four of the same five peaks (three carotenoid and one phospholipid) also significantly different between the spectra of the histologically healed and the histologically active tissue.

This study shows that Raman spectroscopy can be used to discriminate between quiescent and inflamed colon tissue, as assessed either endoscopically or histologically, and thus illustrates its potential as a diagnostic tool for the evaluation of MH in patients with UC.
Possible applications of Raman spectroscopy could thus be as an in-vivo adjunct during endoscopy or for rapid assessment of tissue samples taken in endoscopy units. Larger studies to look at whether using these spectral biomarkers can help predict patients at risk for adverse outcomes like a relapse of disease activity or lack of response to medical therapy are required.

7 The utility of routine chromoendoscopy for the detection of dysplastic lesions during surveillance colonoscopy in patients with Ulcerative Colitis. Does research translate into clinical practice?

Introduction

There is an increased risk of cancer in patients with ulcerative colitis (UC). The risk increases with the duration of the disease and reaches approximately 18% after 30 years of disease(111). The dysplasia in UC is usually flat and difficult to detect. Random colonic biopsies have been the mainstay of detecting the dysplasia in UC. However, random sampling method can easily miss the dysplastic areas. It is estimated that approximately 33 biopsies are needed to achieve 90% confidence interval for detecting dysplasia(205). The other main feature that renders this method somewhat ineffective is that the dysplasia in UC is multifocal and it is difficult to map them to a particular colonic segment(206). And besides being ineffective, it is time-consuming, laborious and expensive. In a retrospective analysis of 167 patients undergoing 466 colonoscopies over a period of 10 years (1998-2008), dysplasia was detected on random colonic samplings on only 5 colonoscopies in 4 patients. Only one of these patients had advanced neoplasia confirmed on the proctocolectomy specimen(207). The quality of surveillance colonoscopies is variable among colonoscopists and could also be dependent on factors influencing day to day running of endoscopy lists like the timing of the list and involvement of fellows.

New generation High definition (HD) endoscopes have shown to be superior in the identification of dysplastic areas in IBD surveillance compared to the standard definition (SD) endoscopes(208). Contrast enhancement is used to improve the detection of abnormalities in the gastrointestinal tract. Non-dye based contrast enhancement methods, also called virtual chromoendoscopy (VCE) involves optical filters or software-based technologies to provide contrast enhancement without the use of dyes. Narrow band imaging (NBI), Fujinon intelligent colour enhancement (FICE) and iSCAN (are examples of VCE in clinical use. There is, however, no convincing evidence to support their routine use in surveillance colonoscopies in colonic IBD and this view has been endorsed by the international SCENIC consensus guidelines(209) Dye based contrast enhancement, also called as chromoendoscopy (CE), refers to application of diluted indigo carmine or methylene blue to the colonic mucosa to highlight subtle mucosal abnormalities. By providing a rim of contrast around the lesions, CE helps in detection, delineation, and characterisation of dysplastic tissue. There is growing evidence to suggest CE is superior in detecting dysplasia in UC

surveillance compared with the standard white light examination (WLE). A Meta-analysis including 665 patients from 6 studies in 2013 confirmed that CE detects more dysplastic lesions compared to random biopsies obtained from standard WLE(118). CE is recommended by British Society of Gastroenterology(119) and European Crohn's and Colitis Organisation(120) as a preferred method of surveillance in colonic IBD. However, the uptake of CE among the colonoscopists has been variable. It may be due to the steep learning curve involved, time constraints on endoscopy lists and the common perception that this is a 'messy' time-consuming procedure.

We aimed to look at the practice among the endoscopists in our hospital who offer surveillance colonoscopies for patients with UC to see if CE improves the detection of dysplasia in routine clinical practice and audit the uptake of this technique in routine clinical practice.

Methods

All patient undergoing surveillance colonoscopy for long-standing UC (>7 years) between January 2012 to December 2013 in Leeds teaching hospitals were included in this retrospective cohort study. This study was conducted as an audit on local practice of surveillance procedures. This study was approved by the local audit governance committee and used for quality assurance and improving local practice. The study protocol conforms to the ethical guidelines of the World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects" (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004). All procedures were done as a part of routine patient care. There has been no extra tests or procedures done as a part of the study.

Data was collected using electronic records for endoscopy reports and the subsequent clinical care. Demographic details of the patients were recorded along with the duration and extent of the disease, medications (current and past), and colonoscopy outcomes.

Inclusion criteria: All adult patients (Age ≥18 years) who underwent colonoscopy for surveillance of UC with a disease duration of ≥7 years were included in the study.

Exclusion criteria: Patients were excluded from the study if they were having severe colitis or undergoing surveillance of only rectal stump or pouch.

Equipment: High definition and High-resolution endoscopes (CF HQ260DL, CF Q260SL, PCF Q260AL colonoscopes from Olympus Keymed®) with Lucera series processors (CV 260) were used for all the procedures. Biopsies were obtained using single-use Radial

Jaw™ biopsy forceps from Boston Scientific®. Standard equipment available in the department were used for resecting polyps when identified.

Biopsy materials were processed according to standard procedures and read by an expert gastrointestinal pathologist. When there was dysplasia or cancer the consensus opinion of two pathologists was reported. Histology was classified according to the Vienna criteria of gastrointestinal epithelial neoplasia ranging from no intraepithelial neoplasia to invasive neoplasia(210)

Statistics

Numeric variables are summarized with the sample mean and standard deviation and categorical variables with the number and percentage. The primary endpoints of interest were the number of patients with any dysplastic lesion detected and the number of patients with endoscopically visible dysplastic lesions. The secondary endpoints were the number of high-grade dysplasia's/cancers detected and the number of patients with endoscopically visible flat non-polypoid dysplastic lesions. The risk of association between the outcome variables of interest and type of endoscopy (HD or SD) was estimated using Poisson regression with robust standard errors rather than a more commonly used logistic regression(211, 212)The choice of the Poisson model, which provides a prevalence ratio (PR), was based on the fact that the outcomes were relatively common. The Poisson model provides a more conservative estimate of the relative risk that is closer to its sample value than when logistic regression is used in cross-sectional studies. Potential confounders that were included in multiple regression models included age, gender, duration of disease, 5 amino-salicylate use, the extent of disease, exposure to immunomodulators or biologics, the experience of colonoscopist, adequacy of bowel preparation and a total number of biopsies taken. A two-sided significance level was set at $p < 0.05$. The statistical software's SPSS version 20 (SPSS Inc, Chicago, Illinois) and Stata version 16 (Stata Corp, College Station Texas) were used in all analyses.

Results

Total of 10831 colonoscopies was performed in Leeds teaching hospitals during the study period. We selected the procedures undertaken for the surveillance of UC for this study. A total of 336 patients were included in the study after applying our exclusion criteria (5 pouchoscopies) and removing wrongly coded procedures (n=3). 120 patients underwent surveillance colonoscopy with CE and 216 patients underwent WLE alone.

Table 38 provides the summary of the patient demographics, disease characteristics, equipment used and the grade of endoscopist for the colonoscopies included (120 CE and

216 WLE). The groups were well matched for age, gender, diagnosis, extent of disease, disease duration and drug therapy (5-Aminosalicylates and Immunomodulators). There were significantly more patients who had their procedures performed by consultants among CE (89%) than WLE group (55%). Significantly more colonoscopies in the WLE group (21%) were done by trainees compared to CE group (2%). Significantly more CE procedures involved HD scopes (34%) compared to the WLE group (19%).

9 dysplastic lesions (all low-grade dysplasia) were detected in 7/216 colonoscopies in the WLE group. Of these 2 lesions were detected outside the extent of colitis leaving 7 dysplastic lesions detected in 5/216 colonoscopies in the WLE group. 27 dysplastic lesions (all low-grade dysplasia) were detected in 20/120 colonoscopies in the CE group. Of these 3 lesions were detected outside the extent of colitis leaving 24 dysplastic lesions noted in 17/120 colonoscopies in the CE group. There were 2 patients in the WLE group with endoscopically visible non-polypoid flat dysplasia and 11 in the CE group. Table 39 provides details of the number and characteristics of lesions detected by the two modalities.

Table 40 provides the adjusted and unadjusted prevalence ratio of picking up any dysplastic lesion, and endoscopically visible non-polypoid flat dysplastic lesions with CE compared to WLE. Significantly more dysplastic lesions and non-polypoid endoscopically visible flat dysplastic lesions were detected by CE compared to WLE even after adjusting for age, gender, diagnosis, duration of disease, extent of disease, 5 amino-salicylate use, immunomodulator or biologic drug exposure, number of biopsies taken experience of colonoscopist, previous dysplasia, high definition scope use and bowel preparation score.

Table 38 Details of patients include in the study

Variable	WLE (n=216)	Chromoendoscopy (n=120)
Age (mean ± SD) years	48.8 (15.2)	49.7 (14.8)
Gender: Male (%)	122 (56.5%)	72 (60%)
Female (%)	94 (47.3)	48 (40%)
Disease extent: Pancolitis (%)	104 (48.1%)	66 (55%)
Left sided UC (%)	112 (51.9%)	54 (45%)
5-aminosalicylate use (%)	167 (77.3%)	92 (76.6%)
Immunomodulator or biological drug exposure (%)	48 (22.2%)	28 (23.3%)
Disease duration (mean ± SD)	19.9 (9.8)	20.3 (12.1)
Adequacy of bowel preparation#		
Poor (%)	42 (19.4%)	13 (10.8%)
Adequate (%)	43 (19.9%)	49 (40.9%)
Good (%)	131 (60.7%)	58 (48.3%)
Completion rate*	200 (92.6%)	119 (99.2%)
Grade of colonoscopist**		
Consultant (%)	118 (54.6%)	107 (89.2%)
Nurse Endoscopist (%)	53 (24.5%)	11 (9.2%)
Trainee (%)	45 (20.9%)	2 (1.6%)
High Definition endoscope use**	41/216 (19%)	42/120 (34%)
Mean number of biopsies (SD)**	22 (10)	13 (9)

p=0.004,* p=0.008 and ** p<0.001

Table 39 provides comparative details of the total number of dysplastic lesions in each group along with the patients with dysplasias, site of the lesions and the number of flat dysplastic lesions.

Table 39 Characteristics of dysplastic lesions detected

	WLE (n=216)	CE (n=120)
Total number of dysplastic lesions#	7	24
Number of patients with dysplasia#	5	17
Anatomic location of lesions: **Right Colon** **Sigmoid/Descending** **Rectum**	 3 3 1	 8 11 5
Number of patients with non-polypoid dysplastic lesions# (endoscopically visible)	2	7

Location and number of dysplastic lesions within the colitic area detected according to modality.

WLE: White light endoscopy, CE: Chromoendoscopy5 adenomatous polyps in 5 patients outside the segment of colitis (3 in chromoendoscopy arm and 2 in WLE arm).

all lesions were low-grade dysplasia noted on targeted biopsies. No dysplasia was noted on random biopsies.

Table 40. Multiple regression analysis

	Prevalence ratio (95% CI)	P value	Adjusted prevalence ratio* (95% CI)	P value
Any Dysplasia	6.12(2.25-16.58)	<0.001	5.43 (1.3-17.55)	0.004
Flat dysplastic lesions detected by targeted biopsies	7.19 (1.52-33.9)	0.01	4.30 (1.02-18.16)	0.04

Multiple regression analysis of the association between type of colonoscopy (WLE or CE) and detection of any dysplastic lesions and endoscopically visible non-polypoid dysplastic lesions on a per patient basis.

* Adjusted for age, gender, diagnosis, duration of disease, extent of disease, 5 amino-salicylate use, immunomodulator or biologic drug exposure, number of biopsies taken the experience of colonoscopist, previous dysplasia, high definition scope use and bowel preparation score.

Discussion

Our study demonstrates that CE improves detection of dysplastic lesions during surveillance colonoscopy of patients with ulcerative colitis even in routine clinical practice. CE was found to be superior to WLE in detecting all dysplastic lesions and the detection of endoscopically

visible flat non-polypoid lesions. This is a significant finding as dysplastic lesions in long-standing UC are usually non-polypoid and flat.

This study was conducted after the guidelines by British Society of Gastroenterology recommending the use of CE for surveillance colonoscopies in long-standing UC(119) and we aimed to understand the uptake among the colonoscopists in our centre. Despite being an active academic centre with robust IBD service, CE was performed only in 1/3rd of the study population, and the remaining patients underwent standard WLE with random colonic biopsies. Among those who underwent CE, 89% of procedures were performed by the consultants and 54% of the WLE were also performed by the consultants who opted not to perform CE for reasons we have been able to capture from our study. This highlights the lack of a uniform approach for endoscopic surveillance even in academic units. Our results are very similar to a recent physician survey from Canada looking at the practice of surveillance colonoscopy in patients with UC among the academic gastroenterologists (121). This study showed results similar to ours with only 26.5 % of Canadian Gastroenterologists routinely using CE, despite the fact that the majority (71%) of the participants were physicians with IBD as their subspecialty.

Although there is ample evidence in favour of CE and targeted biopsy over WLE and random biopsies, results from a retrospective study from the Netherlands found contrasting findings(213). In their retrospective study, the authors collected data from 2000 to 2013 and included 401 and 772 patients in CE and WLE arm respectively. The rate of dysplasia in the CE group was 11% and that in WLE group was 10%. These findings must be interpreted with caution as the study spanned a 13 year period with historical controls as the WLE arm. All the surveillance procedures in the initial part of the study were done with WLE and CE was introduced only in the later years of the study period. Factors that could have influenced these results could be the decreasing incidence of dysplasia over time and the relative in-experience of colonoscopists with the technique of CE when it was first introduced.

A much larger retrospective study from St Mark's Hospital(214) reported outcomes of colonoscopic surveillance in UC over a period of forty years and included 1375 patients followed up for 15,234 patient-years. They reported a 2.5 fold increase in dysplasia detection with CE compared to WLE. In their time-trend analysis, they also report a significantly reduced incidence rate of colorectal cancer among patients who had at least one or more CE procedures (2.2 per one thousand patient-years) compared to those who never had CE (4.6 per one thousand patient-years). In this study mainly, standard definition endoscopes were used and the study was spread over a long period of time with different technological advancements like video endoscopy and ever-improving image resolution. We have

previously shown that new generation High definition (HD) endoscopes with over 1 million pixels image density improve the detection of dysplastic lesions during routine surveillance colonoscopies in colonic IBD(208). Results of a recent randomised controlled trial by our group comparing the outcomes of HD-WLE and HD-CE has shown a significant increase in detection of dysplastic lesions by HDCE(215). This present study confirms that CE during routine clinical care with modern endoscopes does increase the detection rate of all dysplastic lesions. This increase appears to be driven by the increased detection on non-polypoid flat endoscopically visible lesions which may be missed by WLE alone.

We understand that the retrospective nature of our study is an important limitation with the potential to introduce bias. Standardisation of data collection between the two groups was not possible as there was lack of information on factors such as reasons for not performing CE, withdrawal time and the procedures were performed by endoscopists of varying grade and expertise. We used appropriate statistical tests to exclude confounding factors in our study. The retrospective design could also be considered a strength as we aimed at capturing data in routine clinical practice outside of randomized trials. It has been argued that randomized controlled studies in endoscopy could also be considered biased as endoscopists perform the procedure more diligently than in routine clinical practice. The Institute of Medicine has issued recommendations and priorities on “comparative effectiveness research” to include colorectal cancer screening strategies to be done on routine clinical practice(216). A prospective study design would have potentially introduced researcher bias by influencing the endoscopist’s decision to perform CE. This would not have represented real-world practice. We did not have data on smoking status and family history of colorectal cancer as these could not be reliably confirmed by the patient records. The mean number of biopsies taken in this study was 22 in the WLE group and 13 in the CE group. This is less than the recommended 33 biopsies that are needed to achieve 90% confidence to detect dysplasia(205). A survey of UK gastroenterologist revealed that 57% take fewer than 10 biopsies per patient(217). The figures we observed in this audit are similar to the UK clinical practice. This could have led to the lower identification of dysplasia but many studies have shown an extremely low yield of dysplasia from random biopsies suggesting that the incremental benefit from multiple random biopsies is small(209). However, we felt that major limitation of our study was the inability to perform a more qualitative work to identify the reasons of not performing CE when it was clinically indicated. Further research in this area will enhance our understanding in a challenging aspect, which is the behaviour of endoscopists affecting their performance and outcomes.

In conclusion, CE picks up significantly more dysplastic lesions especially non-polypoid flat lesions even when performed in routine clinical settings. However, the uptake for CE among endoscopists is poor with only 1/3 of the procedures in our tertiary centre being done using CE. Increased time taken, lack of training, the perception that CE is a messy procedure could be speculated to be among the reasons. Further work is needed to understand the reasons for the poor utilization of CE in routine clinical practice and make innovations to the procedure that can increase the uptake of this technique to improve surveillance outcomes in this high-risk population.

8 High definition white light endoscopy versus high definition chromoendoscopy in detecting dysplasia in Ulcerative colitis

Background

Patients with ulcerative colitis (UC) have an increased risk for colorectal cancer (CRC) compared to the general population(111). Cancer in UC occurs at a younger age and increases with time, approaching 18% after 30 years of disease(111). This increased risk has prompted both the North American and UK gastroenterology societies to recommend cancer prevention strategies(218, 219). Random sampling throughout the colon has been the mainstay of conventional surveillance practice. Surveillance colonoscopy requires multiple biopsies, to be taken and processed which is tedious, expensive and time-consuming. It has been estimated that at least 33 biopsies are needed to achieve 90% confidence to detect dysplasia if it is present(205). Surveillance colonoscopy practices are not uniform and less than 10 biopsies were noted to be taken based on gastroenterologists self-reported practices for colonoscopic surveillance for UC(217). The focus of dysplasia in UC is flat and multifocal and can be easily overlooked with conventional white light endoscopy(206). There is growing evidence that the yield of surveillance can be improved by the addition of newer endoscopic methods that enhance the detection of subtle mucosal abnormalities like chromoendoscopy (CE) and autofluorescence with narrow band imaging(106). CE refers to the topical application of dyes or pigments to improve detection and delineation of surface abnormalities and is an inexpensive adjunct to conventional endoscopy. It has been shown to be useful in the detection of flat adenomas in the sporadic setting as well as in patients with familial polyposis syndromes(220, 221). There is increasing evidence that most dysplasia in UC is associated with visible mucosal abnormalities(222, 223). This has, in turn, led to increasing use of endoscopic resection techniques to treat areas of raised, visible dysplasia in patients with UC, without the need for colectomy. There is, therefore, a need to improve detection techniques during surveillance endoscopy and CE has been advocated as a promising method. A recent meta-analysis has shown that CE is better than the standard white light endoscopy in detecting dysplasia in UC. However, despite several studies showing the utility of CE in detecting subtle mucosal abnormalities in CE, this technique has not been widely accepted in routine clinical practice. CE is time consuming increasing the extubation time by 9-26 minutes, and even when performed with specially designed dye-spray catheters, complete and even mucosal dye coverage is not guaranteed(112). CE has only been compared in clinical trials with standard definition endoscopy rather than the currently available high definition endoscopes with

better resolution and picture. High definition (HD) endoscopy uses a high definition (1080 lines of vertical resolution) monitor and a high resolution CCD (charge coupled device) with up to a million pixels which provides much better images than standard video endoscopy. A recent meta-analysis showed that HD colonoscopy was better than standard endoscopy in polyp detection but not in the detection of high risk adenomas(113). HD colonoscopy promises therefore to provide an alternative to chromoendoscopy in UC surveillance without the need for the extra time and experience required for dye spraying for both endoscopists and nursing staff.

At the time we started this study there were no trials comparing these 2 modalities. The recent SCENIC guidelines endorsed the view that HDCE was the preferred modality for surveillance in these patients based on one study from the Mayo Clinic on 75 patients which showed a more than 2 fold increase in dysplasia detection (9.3% vs 21.3%) with HDCE using a 0.2% indigo carmine solution. A subsequent randomized trial from Canada showed no differences in detection rates between HDWL and HDCE using a 0.03% methylene blue solution

The aim of study was to a randomized trial to compared HDWL colonoscopy alone compared to HDWL with chromoendoscopy (HDCE) for dysplasia detection during surveillance for extensive ulcerative colitis.

Methods

This was a parallel group randomized controlled trial (clinicaltrials.gov number NCT02138318) in a single tertiary centre in the UK. Ethical approval for the study was obtained from the UK National Research Ethics Committee (Reference number 12/YH/0228).

Patients with extensive colitis on a surveillance program at the Leeds Teaching Hospital NHS trust (150 patients per year) and those referred for a surveillance colonoscopy from the outpatient clinics at the Leeds Teaching Hospital NHS trust were screened by a research nurse/ fellow and were invited to join the study by letter along with their colonoscopy appointment if they meet the inclusion and exclusion criteria. A patient information leaflet (PIL) explaining the study was sent to the patient. On arrival in the endoscopy reception area, they were given the opportunity to ask questions to the endoscopists/member of the research team and be invited to participate and sign the written consent form. Preparation prior to colonoscopy including bowel preparation and dietary restriction were according to local unit protocol.

Consecutive eligible patients undergoing surveillance colonoscopy for long standing ulcerative colitis were approached for inclusion in this trial. Patients were reassured that participation in the trial would ensure they have a thorough procedure with a possible chance of increased detection rates. The increased procedure time of around 10 minutes with the use of chromoendoscopy was explained beforehand.

Inclusion criteria

1. Patients with longstanding (more than 8 years of disease), extensive (extending proximal to splenic flexure) colitis attending for surveillance colonoscopy
2. Patients aged over 18 years of age.

Exclusion criteria

Pre-intubation

1. Pregnancy
2. Unwilling or unable to give informed consent
3. Severe active colitis (as assessed by endoscopists)

Pre-randomization

1. Poor bowel preparation (solid stool or <90% of mucosal area cannot be visualized even after jet washing using the Aronchik scale score of > 3)

8.1.1.1 Standardisation for colonoscopy

All efforts were made to standardise every step of the patient journey. A standard split dose polyethylene glycol based bowel preparation was used as per unit protocol. Colonoscopies were performed in the standard manner till caecum or terminal ileum as appropriate. Hand pressure, position changes, sedation and antispasmodics were used as per unit protocol. The Olympus Lucera spectrum processor, with an Olympus CFH260 DL scope and high definition monitor, were used for all procedures. After informed consent for the trial and procedure patients had an intravenous cannula sited. Sedation and antispasmodics were used as per unit protocol at the discretion of the endoscopists. As much fluid residue was washed out at insertion as possible, using a jet wash system (Olympus or Mediavators). Caecal intubation time was recorded and caecum was identified by IC valve, appendicular orifice and tri-radiate fold. Bowel preparation quality was noted as per the Aronchick scale(224) which is score as 1. Excellent > 95% of mucosa seen, 2. Good: clear liquid covering <25% of mucosa but > 90% mucosa seen, 3. Fair semisolid stool could be suctioned off but > 90% of mucosa seen, 4. Poor: semi solid stool could not be suctioned off and < 90% of mucosa seen, 5. Inadequate, repeat preparation required.

Randomization

Patients were randomized to withdrawal with either high definition white light endoscopy or chromoendoscopy when caecum is reached. A closed envelop randomisation with block sequence was used and minimization techniques were utilized to ensure a balance between groups on known risk factors like family history, primary sclerosing cholangitis and previous dysplasia.

8.1.1.2 Withdrawal

Withdrawal was also standardised for every patient. The colon was examined in segments with at least 2 targeted biopsies per suspected lesion noted or snare resection of lesion detected if deemed appropriate by the endoscopists. Suspicious areas were defined as mucosal irregularities not consistent with chronic or active ulcerative colitis. Quadrantic biopsies were taken from around each suspicious lesion to look for field change. Random biopsies from each segment were taken as per the guidelines.

In patients randomized to the CE arm, dye spraying was performed using 0.2% Indigocarmine solution via a spray catheter inserted via the biopsy channel of the colonoscopy and sprayed from a 50cc Luer lock syringe prefilled with dye solution. Extubation time (excluding time for resection and biopsies) was calculated and a minimum of 8 minutes were used to examine mucosal surface. If there was marked psuedopolyposis then representative biopsies of any atypical looking psuedopolyps were taken. Sizes of lesions were measured against an open biopsy forceps. Segment of lesions, type (according to Japanese Research Society classification) and endoscopic diagnosis (hyperplastic, adenoma, carcinoma, DALM) were recorded. Figure 21 shows assessment of polyps with HD-WLE and HD-CE. Where possible all lesions had still and video images recorded.

Endoscopists made an assessment of mucosal inflammation of each segment as per the Mayo score

0= normal or inactive disease

1= mild (erythema, decreased vascular pattern, mild friability)

2= moderate (marked erythema, absent vascular pattern, friability, erosions)

3= severe (spontaneous bleeding, ulceration)

Endoscopist also commented on the presence of tubular colon, featureless colon, strictures, scarring, psuedopolyps and backwash ileitis. Figure 22 shows the assessment of active disease with HD-WLE and HD-CE.

Histology

All specimens were fixed in 10% formalin. Slides were read by an experienced gastrointestinal histopathologist with confirmation of any equivocal cases with a second experienced gastrointestinal histopathologist. Lesions were graded as 1-Normal, 2- indefinite for dysplasia 3-Low grade 4-High grade 5-Invasive cancer(210).

Non-targeted biopsies were additionally assessed for inflammation: 0= normal, 1=mild chronic inflammation only 2= mildly active (cryptitis but not crypt abscess) 3= moderately active (few crypt abscesses) and 4= severely active (numerous crypt abscesses)

Statistical analysis

At the time of study initiation there were no data available on the potential incremental yield with HDCE. Hence a sample size calculation prior to starting the study was not possible; instead we agreed to perform an interim analysis to guide us estimate the number needed for completion of study. Based on our preliminary results after the first 103 patients were recruited we showed a 9.4% yield with HD and 22.4% yield with HDCE. With this yield, for a once sided (HDCE unlikely to be worse than HD alone) test of significance, with a power of 80% (beta error 0.2, alpha error 0.05) we estimated 79 patients in each group had to be enrolled.

Primary Outcomes

1. Number of lesions with at least low grade dysplasia detected by targeted biopsy
2. Total number of patients with dysplastic lesions in each group

Both primary outcomes were analysed on a superiority basis. For the detection of dysplasia on a per patient 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 negative binomial regression was used to compare the means in this group.

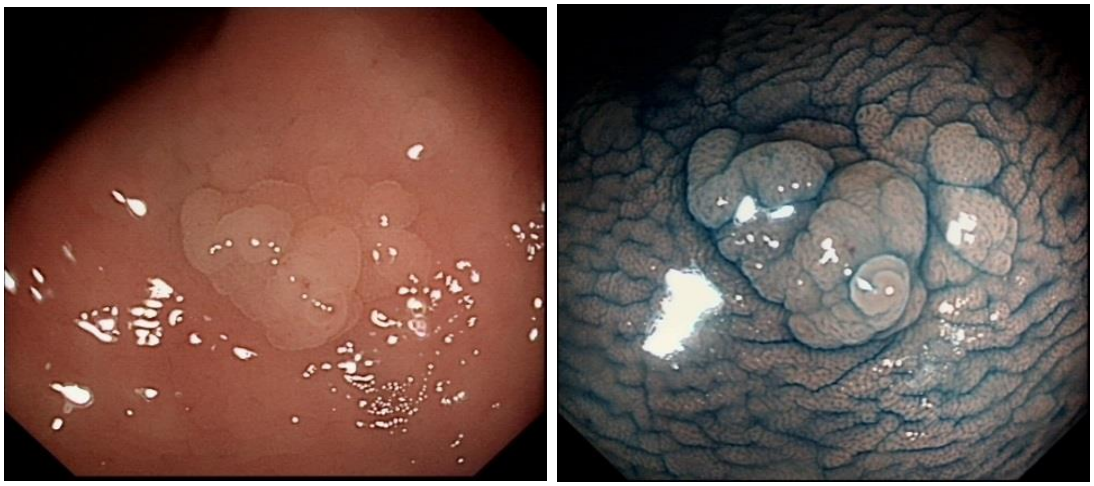

Figure 21 Assessment of polyp assessed using HD-WLE (left) and HD-CE (right)

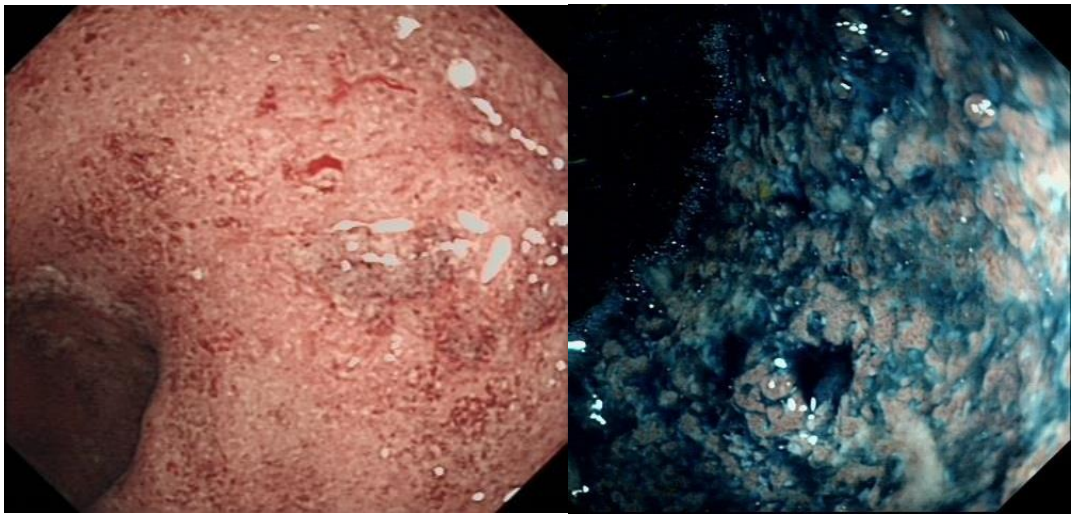

Figure 22 Active UC assessed using HD-WLE (left) and HD-CE (right)

Results

From 23/08/2013 to 31/08/2015, 297 patients with ulcerative colitis underwent surveillance colonoscopy at Leeds Teaching Hospital NHS trust. Of these 137 were not included (110 did not have extensive colitis, 4 did not consent to trial, 13 had active disease in the endoscopists opinion). In all 160 patients consented to the trial of which 2 were excluded (1 was noted to have a subtotal colectomy and the other had Mayo Grade 3 colitis). The remaining 158 were randomly assigned by block randomization with 79 in each arm and were analyzed on an intention to treat bases. No patient was excluded due to poor bowel preparation as the endoscopists made every attempt using a jet wash system to achieve adequate mucosal visualization. Figure 23 provides an overview of the study enrollment.

Flowchart

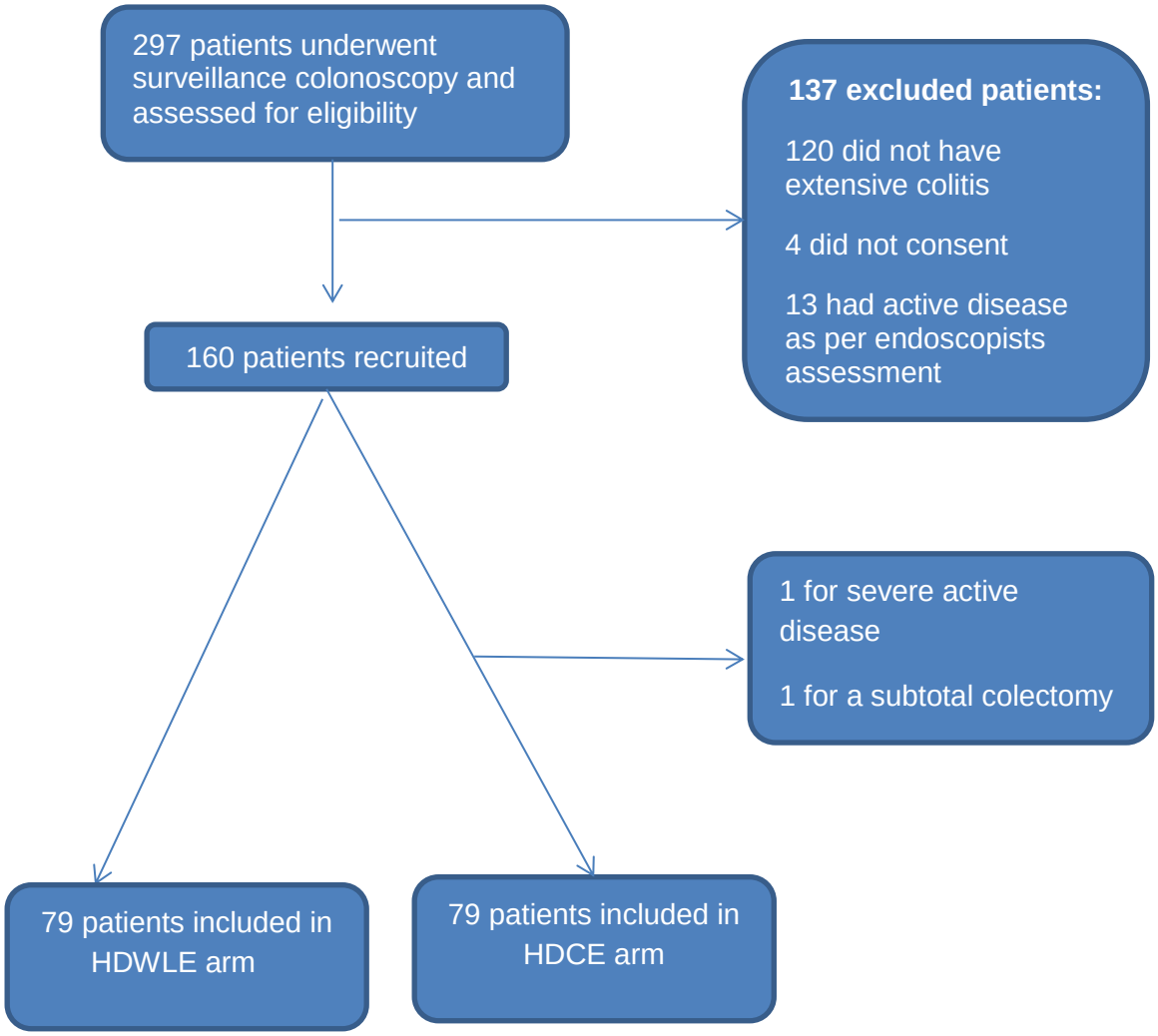

Figure 23 Flowchart of patient selection-RCT

HDWLE- High definition white light endoscopy, HDCE-High definition chromoendoscopy

Baseline characteristics

Table 41 provides information on the baseline characteristics of the population enrolled. There were 79 patients in each arm and no significant differences were noted between the two groups. The mean age was around 55 years in each group with predominantly male patients 62%). There were slightly more patients with PSC in the HD group compared to the HDCE group but this was not statistically significant, but overall 17% of our cohort had PSC.

Characteristic of the study procedures is shown in Table 42. The caecum was reached in 99% of patients in each group. A completion CT-Colonography done in both patients in whom the caecum was not intubated did not show any additional polyps. Both have been included in the analysis on an intention to treat basis. The median time taken for the procedure was significantly higher in the HDCE group which was mainly due to a difference in about 9 minutes in withdrawal time which was a combination of the additional time taken to spray the dye and the time taken for additional targeted biopsies or polyp resections. The median number of biopsies taken was similar (32) but more targeted biopsies were taken in the HDCE group.

Table 41 Patient characteristics

	High Definition alone (n=79)	**High Definition with chromoendoscopy (n=79)**
Mean age in years	55.5 (14.2)	55.0 (14.0)
Male	49 (62)	49 (62)
Female	30 (38)	30 (38)
Current or previous smoking history**	34 (43)	31 (39)
Median disease duration (years)	17 (9-25)	20 (12-28)
Previous dysplasia	5 (6.3)	6 (7.6)
Primary sclerosing cholangitis	16 (20.2)	11 (11.5)
Family history of colorectal cancer	5 (6.3)	4 (5.1)
Previous or current immunomodulator use*	27 (34.2)	34(43.0)
Previous or current 5 aminosalicylate use	75 (94.9)	68 (86.1)

Data are mean (SD), n (%) and median (IQR). *immunomodulator therapy is thiorpurines, methotrexate or biologics. **only 2 patients in the HD group and 1 patient in the HDCE group were current smokers

Primary end points

47 dysplastic lesions were identified in 30 patients. The overall dysplasia detection rate was 19%. 10 patients (12.6%) in the HD arm had dysplasia compared to 20 patients (25.3%) in the HDCE arm. This gave HDCE an incremental yield of 12.7% (p=0.04). The mean number of dysplastic lesions detected in the HDCE arm (0.37) was also significantly higher than the HD arm (0.16). Table 43 provides an overview of these results.

Histological and morphological characteristics of detected lesions

Table 44 provides details of the detected lesions and their characteristics. Of the 47 lesions detected using HD colonoscopy, 13 had dysplasia (27.6%) and of the 103 lesions detected using HDCE, 29 had dysplasia (28.1%). There were 64 targeted biopsies taken in the HD group (mean 0.81 ±1.5) and 176 targeted biopsies taken in the HDCE group (mean 2.2 ± 2.7). This difference was statistically significant (p <0.01). A total of 2305 random biopsies were taken in the HD group of which one showed low grade dysplasia and a total of 1937 random biopsies were taken in the HDCE group of which none had any dysplasia noted. No dysplasia was noted on random biopsies taken from the mucosa surrounding each suspicious lesion. Agreement between the blinded histopathologists (OR and PP) for the presence of dysplasia was excellent with disagreement in only 1 polyp in a patient who had an additional dysplastic lesion and therefore was included in the per patient detection analysis.

Table 42 Colonoscopy characteristics

	High Definition alone (n=79)	High Definition with chromoendoscopy (n=79)	P value (two sided)
Post inflammatory polyps	16	19	0.28
Mayo 1 colitis	12	9	0.41
Tubular shortened colon	6	4	0.36
Bowel preparation **			
1. Excellent	47	53	0.38
2. Good	24	21	
3. Fair	8	4	
Caecal intubation	78$	78$	1.00
Total time of colonoscopy	26 (21-31)	32 (24-36)	<0.01
Withdrawal time (minutes)	15 (10-20)	24 (20-28)	<0.01
Number of biopsies taken	32 (29-35)	30 (24-36)	0.36
Number of targeted biopsies taken	64	176	<0.01

Data are n (%) or median (IQR)

** The Aronchick bowel preparation scale was used to assess the quality of bowel preparation $ In both cases the ascending colon was reached but caecum was visualized but not intubated, completion CTC done showed no additional polypoid lesions beyond the point of insertion.

Table 43 Outcome measures of the trial

	High Definition alone (n=79)	High Definition with chromoendoscopy (n=79)	P value (2 sided)
Proportion of patients with ≥ 1 dysplastic lesions	10 (12.6%)	20 (25.3%)	0.04
Mean number of dysplastic lesions per patient	0.16 (0.5)	0.37(0.7)	0.04

Table 44. Characteristics of detected lesions and details of histology

	High Definition alone (n=79)	High Definition with chromoendoscopy (n=79)
Total number of lesions detected	47	103
Total number of dysplastic polyps/lesions	13	29
Number of patients with >1 dysplastic polyp/lesion	2	7
Location of dysplastic polyps 1. Caecum 2. Ascending 3. Transverse 4. Descending 5. Sigmoid 6. Rectum	 1 4 3 2 2 1	 4 6 10 6 1 2
Median size of polyp in mm (IQR)	3.5 (3-4.5)	4 (3-8)
Morphology of dysplastic lesions by Paris classification 1. Subpedunculated (Isp) 2. Sessile (Is) 3. Flat or flat elevated (IIa or IIb) 4. Flat and Depressed component (IIa + IIc)	 1 4 8 0	 3 8 16 2
Histology of dysplastic lesions detected a. Adenocarcinoma b. HGD/adenocarcinoma c. LGD	 0 0 13*	 0 1 28
Mean number of targeted biopsies	0.81 ±1.5	2.2 ± 2.7
Total number of random biopsies	2305	1937

* One patient with a dysplastic adenoma in ascending colon also had low grade dysplasia noted on a random biopsy from the descending colon with no visible endoscopic lesion.

Discussion

In this randomized controlled trial we detected a significant difference between HD colonoscopy and HDCE for the detection of colitis associated neoplasia in patients with extensive long standing ulcerative colitis in remission. We used a standard 0.2% indigocarmine spray introduced via a spray catheter for all patients in the HDCE arm. This is the first parallel group randomized controlled study to compare HD and HDCE. The only other published study on this was a three arm study comparing HD, HDCE and ISCAN

technologies(225). This study however used a 0.03% indigo carmine dye or 0.04% methylene blue sprayed using the jet wash system rather than a spray catheter. All the randomized studies comparing standard definition colonoscopy and CE in the literature have used concentration of methylene blue and indigocarmine of 0.1% and higher(112, 209). The validity of the use of the lower concentration of dyes used in the Canadian study have never been shown previously to improve neoplasia detection in IBD surveillance in any published study to date.

We found that HDCE has an incremental yield of about 12.7% with a NNT of about 8, suggesting that HDCE would detect one additional patient with a dysplastic lesion for every 8 patients on whom this procedure is done. Studies on the use of CE with standard definition technology showed an incremental yield of 7% with a NNT of about 14(118). Our overall detected rate for dysplasia in this study was 1 in 5 which is higher than that found with standard definition technology where around 1 in 12 patients had dysplasia(209). This increase in yield may partly be due to the higher risk patients enrolled in this trial (extensive long standing colitis with 17% having concomitant PSC). The majority of lesions detected were however small (median size 4 mm) and it is unclear if these small lesions have the same malignant potential and if removal of these lesions would improve long term outcomes. Our detection rates are however similar to two recent studies comparing HDCE with NBI(226) and AFI(107) respectively. HDCE had a detection rate of 19% in the Vleugels et. al. AFI study and 21.2% in the Bisschops et.al. NBI study. The median size of dysplastic lesions detected was 3 mm in both studies.

The withdrawal time with HDCE was on average 9 minutes longer. This is similar to studies comparing standard definition colonoscopy with CE(112, 209). The withdrawal time with HDCE in our study (24 minutes) is in between those in the 2 recently published studies comparing AFI with HDCE(107) where the withdrawal time with HDCE was 16 minutes and in that comparing NBI with HDCE(226) where the withdrawal time with HDCE was 27 minutes. One of the reasons for the shorter withdrawal time in the AFI study may be because they separated the time for inspection (16 minutes on average) and dye application and lesion removal (7 minutes on average). Like in the Bisschops et.al study we did not attempt to separate these out as it would be impractical and difficult to measure accurately in a busy standard clinical endoscopy list and the total time taken would be a more accurate reflection of the costs involved with the procedure.

As expected CE detected more lesions overall (103) compared to HD alone (47). However the proportion of lesions that were dysplastic was similar with 28.1% of lesions being dysplastic with HDCE and 27.6% with HD. This was slightly higher than the 17% of lesions

detected that were dysplastic in the Bisschops et.al. study(226). This difference may be due to the relative experience of the endoscopists involved in this single tertiary academic centre study who performed the study procedures (NM, VS and JH) compared to the 3 centre, 5 endoscopist (4 inexperienced) study by Bisschops et. al(226). However, a Spanish study on the role of routine CE in dysplasia detection did not find a learning effect for CE with less experienced endoscopists nor a difference in detection rates between experts and non-experts(227).

In addition to targeted biopsies we took random biopsies from each segment with a median number of 30 and 32 biopsies per patient in the HDCE and HD arm. NICE guidelines in the UK do not recommend taking random biopsies when CE is used and the recent SCENIC guidelines(209) have been equivocal on their use with no consensus reached. Interestingly more random biopsies were taken in the HD arm in our RCT than the HDCE arm perhaps reflecting an unconscious bias on the part of the endoscopists in this trial feeling that HD was not detecting enough suspicious lesions. The lack of random biopsies have been a criticism of several recent trials including the Canadian study comparing HD,HDCE and ISCAN(225) and Bisschops et al(226) comparing HDCE and NBI. We had included the addition of random biopsies in both arms apriori to deflect this criticism that lesions may have been missed in either arm that might have been noted on random biopsy and also because random biopsies were part of routine clinical practice when this study was designed and an application for ethical approval made. Reassuringly only 1 of the 2305 random biopsies taken in the HD arm had a focus of low grade dysplasia and the patient was offered colectomy as he had an additional dysplastic polyp in the ascending colon, but declined this. On follow up colonoscopy 6 months later no lesion was found and no dysplasia noted on random biopsies from the same or other segments. Additionally no dysplasia was noted on biopsies taken from around each suspicious lesion in this study. These findings support the view that when using HD colonoscopy, taking routine 10 cm, 4 quadrant biopsies is not likely to increase yield.

There are several limitations to our study. Firstly we used a randomized parallel group study design and cannot make assumptions on the neoplasia miss rate of either modality. However no cancers have developed on follow up on any of these patients till date. We chose a parallel group study as back to back study designs where HDCE would have followed withdrawal in HD is also inherently biased as it assumes all lesions noted on white light would be visible with chromoendoscopy. Due to our selection criteria these results are only valid for patients with extensive ulcerative colitis with long standing (> 8 years) disease and in endoscopic remission. As this was done in a tertiary academic centre the proportion of high risk patients was more than average with 17% of the cohort having PSC and 7%

having previous dysplasia. Finally the power calculation of our study was done on a superiority design based on our interim results. As the majority of studies using CE with standard definition colonoscopies had shown superiority of CE over white light endoscopy we felt this was a reasonable assumption to make. A total sample size of 158 patients with extensive long standing ulcerative colitis makes this one of the largest RCT's to date to include such a high risk cohort where surveillance is presumed likely to have the greatest benefit. Nevertheless, the results of this study need to be confirmed in larger multi-centre trials with a more inclusive group of patients and endoscopists of varying experience.

In conclusion this RCT shows that chromoendoscopy should remain the preferred surveillance technique especially in high risk groups (extensive colitis, PSC and previous dysplasia) and validates the recommendations of the SCENIC guidelines(209). Further studies comparing HDCE with HD in multiple centres including a wider cohort of patents (both low and high risk) are needed before this approach can be advocated all for patients. Clinical societies (like the AGA, ASGE, BGS and ECCO) should facilitate the adoption of chromoendoscopy as the preferred surveillance method in daily practice while we await the results of this large multi-centre study.

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10 Appendices:

Appendix A: Approval Letter: NBI observational study

The Leeds Teaching Hospitals NHS
NHS Trust

Michael Wood

15/07/2013

Research & Development

Leeds Teaching Hospitals NHS Trust
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r&d@leedsth.nhs.uk
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Dr Venkataraman Subramanian

Leeds Teaching Hospitals NHS Trust
Gastroenterology department
Bexley Wing
St James" University Hospital
LS9 7TF

Dear Dr Venkataraman Subramanian

Re: NHS Permission at LTHT for: Utility of narrow band endoscopy in predicting short and long term risk of relapse in patients with quiescent ulcerative colitis.
LTHT R&D Number: UR13/10708
REC: 13/YH/0115

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&D Department.

Permission is granted on the understanding that the study is conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable) and NHS Trust policies and procedures available at http://www.leedsth.nhs.uk/academic/research-development/

This permission is granted only on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2978.

Indemnity Arrangements

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers

Chairman Mike Collier CBE Chief Executive Maggie Boyle

The Leeds Teaching Hospitals incorporating:
Chapel Allerton Hospital Leeds Dental Institute Seacroft Hospital
St James's University Hospital The General Infirmary at Leeds Wharfedale Hospital

use the NHS Number

with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as investigator and the researchers listed on the Site Specific Information form. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an appropriate contract, or letter of access, with the Trust if required.

Yours sincerely

Dr D R Norfolk
Associate Director of R&D

Approved documents
The documents reviewed and approved are listed as follows

Document	*Version*	*Date of document*
NHS R&D Form	3.5	08/04/2013
SSI Form	3.5	21/05/2013
Directorate Approval		12/07/2013
REC Letter confirming favourable opinion		20/06/2013
Protocol	1.0	01/02/2013
Patient information sheet	1.3	06/06/2013
Consent form	1.1	06/06/2013
GP/Consultant information sheets	1.1	06/06/2013
Topic guides		

Appendix B: Substantial amendment_Spectrometry study

NHS
Health Research Authority
National Research Ethics Service

NRES Committee Yorkshire & The Humber - Humber Bridge
HRA NRES Centre North West
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7816
Fax: 0161 625 7299

15 August 2013

Dr Venkataraman Subramanian
Leeds Teaching Hospitals NHS Trust
Gastroenterology department
Bexley Wing
St James's university Hospital
Leeds
LS9 7TF

Dear Dr Subramanian

Study title: **Utility of narrow band endoscopy in predicting short and long term risk of relapse in patients with quiescent ulcerative colitis.**
REC reference: **13/YH/0115**
Amendment number: **1**
Amendment date: **10 July 2013**
IRAS project ID: **120491**

- The amendment proposed to take two biopsied for research purposes
- Add Professor Animesh Jha as co-investigator

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

- The Sub-Committee suggest you now delete the last sentence from clause 6 of the consent form, as you are now taking extra samples for research purposes.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Information Sheet	1.4	10 July 2013
Notice of Substantial Amendment (non-CTIMPs)	1	10 July 2013
Investigator CV	Professor Animesh Jha	

A Research Ethics Committee established by the Health Research Authority

GP/Consultant Information Sheets	1.2	10 July 2013
Participant Consent Form	1.2	10 July 2013
Protocol	1.1	10 July 2013
Part B Section S		12 August 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

13/YH/0116:	**Please quote this number on all correspondence**

Yours sincerely

Dr Lynn Cawkwell
Chair

E-mail: nrescommittee.yorkandhumber-humberbridge@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Dr Derek Norfolk, Leeds teaching Hospitals NHS Trust
Miss Anne Gowing

Appendix C: Approval letter_RCT HDCE v/s HDWLE

NHS
Health Research Authority
NRES Committee Yorkshire & The Humber - Bradford
Yorkshire & Humber REC Office
Millside
Mill Pond Lane
Meanwood
Leeds
LS6 4RA

Telephone: 0113 3050128

13 November 2012

Dr Venkataraman Subramanian
Consultant Gastroenterologist
Leeds Teaching Hospitals NHS Trust
St James University Hospital
Beckett Street
Leeds
LS9 7TF

Dear Dr Subramanian

Study title: **Randomized controlled trial of High Definition white light endoscopy versus Chromoendoscopy for dysplasia detection in ulcerative colitis surveillance**
REC reference: **12/YH/0228**

Thank you for your letter of 12 November 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter	1	18 April 2012
Investigator CV		07 February 2012
Letter of invitation to participant	2	29 September 2012
Other: letter from Dr D R Norfolk		29 March 2012
Other: R&D Peer Review Form		18 April 2012
Other: GP letter	1	18 April 2012
Participant Consent Form	3	12 November 2012
Participant Information Sheet	3	12 November 2012
Protocol	1	18 April 2012
REC application		01 November 2012
Referees or other scientific critique report		18 April 2012
Response to Request for Further Information		29 September 2012
Response to Request for Further Information		12 November 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/YH/0228	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Pp Dr Ian Woollands
Chair

Email: nrescommittee.yorkandhumber-bradford@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Derek Norfolk, Leeds Teaching Hospitals NHS Trust*

Appendix D: Patient information leaflet for NBI prospective trial

The Leeds Teaching Hospitals NHS
NHS Trust

PATIENT INFORMATION SHEET

Project title: Utility of narrow band imaging endoscopy in assessment of colonic inflammation and prediction of relapse in patients with Ulcerative colitis: a pilot study

Invitation

You are being invited to take part in a research study. Before you decide we would like to explain why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

It is known that flare up of disease activity in people with ulcerative colitis is unpredictable. It is also known that inflammation can persist in the deeper lining of bowel wall despite the normal endoscopic appearance. This resistant inflammation contributes for further flare up of the disease. Flare ups cause health and economic burden to both the patients and carers. If the intestinal inflammation can be accurately assessed endoscopically and if we are able to predict future flare ups of the disease, this would improve health related quality of life of patients.

The usual way to look at the inflammation in the colon is by Flexible sigmoidoscopy (examination of left side of the bowel) or colonoscopy (examination of whole colon) using a flexible endoscope. This uses standard white light to look at the inner lining of the bowel wall. Technological advances in endoscopy help explore options to improve assessment of intestinal inflammation using different modalities. Narrow band imaging technology is one such advancement. We wish to see if this correlates well with intestinal inflammation and if this could predict future flares in ulcerative colitis. This could potentially help us treat colitis better using the predictive capabilities of NBI endoscopy.

2. Why have I been chosen?

We are currently inviting all patients with ulcerative colitis who are scheduled to have routine surveillance colonoscopy or flexible sigmoidoscopy for assessment of disease activity.

3. Do I have to take part?

NO. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign an extra consent form in addition to the one just before your procedure. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What does the study involve?

You will undergo examination of the bowel with a flexible endoscope (camera). The endoscope has an ability to look at the inner lining of the bowel with different colours. Normally we perform the test with a white light, but in this study we would examine with white light first followed by blue light (called as Narrow band imaging or NBI). The blue light examination is only limited to lower left side of the bowel called sigmoid and rectum. If you agree, we would take digital images and videos of the lining of the gut during the NBI endoscopy before biopsy samples are taken. The NBI endoscope is a standard endoscope with all the functions of regular endoscopy, but can additionally visualize the lining under NBI light. This additional examination will only take 3-4 minutes more than standard examination.

5. What do I have to do?

There are no restrictions, other than the usual advice for flexible sigmoidoscopy or colonoscopy. This study involves no extra visits to hospital before or after the procedure.

6. What are the possible disadvantages and risks of taking part?

Taking NBI endoscopic images or videos is painless but will prolong your endoscopy by about 3-4 minutes.

7. What are the possible benefits of taking part?

This research is being done to try to make scientific progress. This study could potentially help improve our understanding about the flares in ulcerative colitis and help treating the disease better. The NBI endoscopic images and videos obtained may help improve our knowledge to treat your condition (colitis) better.

8. What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions [contact Dr V Subramanian, Tel 01132067575]. If you remain unhappy and wish to complain formally, you can do this 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: 01132067188.

9. Will my taking part in this 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.

When your samples are analysed in the laboratory, they will be identified only by a code so that your name will not be known to laboratory staff. Samples will also be stored in a secure laboratory for future studies. Only the research team and your usual doctors will be able to link your tissue samples to your personal and medical details.

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

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

11. What will happen to the results of the research study?

The results of the research may be published in a scientific journal when the study is complete. This means that results may not be published for a couple of years from now. If you would like a copy of the published results, you should contact us on following address; Department of Gastroenterology, 4th Floor, Bexley Wing, St James's University Hospital NHS Trust, Leeds LS9 7TF.

Please note that you will not be personally identified in any report or publication.

12. What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

13. Who is organising and funding the research?

This study is organised and funded by Department of Gastroenterology, Leeds Teaching Hospitals NHS Trust.

14. Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by National Research Ethics Committee.

Contact for further information

If you would like further information about this study at any stage, please contact:

Dr. N Mohammed

Endoscopy Research Fellow,

St James's university Hospital,

4th Floor, Bexley wing,

Leeds LS9 7TF.

☏07886577132

Dr Venkat Subramanian

Consultant Gastroenterologist

St James's university Hospital,

4th Floor, Bexley wing

Leeds LS9 7TF.

☏ 0113 2062288 (secretary)

15. Your consent

In order to obtain images and videos for research we need to ask your permission:

- To take them.
- For your medical records to be looked at by responsible people involved in this research.

You are free to give or withhold permission for all, or some of these activities, without prejudice to your medical care. People not involved in your care or in the research will not gain access to your notes as a result of your giving this consent.

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 Noor Mohammed
Dr Venkat Subramanian
Dr John Hamlin
Professor Mark Hull

Appendix E: Consent form_NBI observational study

The Leeds Teaching Hospitals NHS
NHS Trust

Title of Study: Utility of narrow band imaging endoscopy in assessment of colonic inflammation and prediction of relapse in patients with Ulcerative colitis: a pilot study

Name of Participant:

Please initial box to confirm

DOB

1. I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to ask questions about the study, and to discuss with family and friends. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the Leeds teaching Hospitals, University of Leeds, the research group and regulatory authorities. I give permission for these individuals to have access to these records. I understand that my personal details will be kept confidential. ☐

4. I agree that digital images and videos of the lining of the bowel will be taken during the endoscopy and stored for research analysis. ☐

5. I understand that tissue samples are used to asses inflammation under microscope. These will be stored anonymously, identifiable only to the research team and my usual clinical team using coded sequence. No extra samples will be obtained for research purpose. ☐

6. I agree to my GP being informed of my participation in this study. ☐

7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent (If different from Principal Investigator)	Date	Signature
Name of Principal Investigator	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Appendix F: Patient information leaflet_HDWLE v/s HDCE

The Leeds Teaching Hospitals NHS
NHS Trust

High definition colonoscopy versus chromoendoscopy for dysplasia detection in Ulcerative colitis.

You are being invited to take part in a **RESEARCH** study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

It is known that people with ulcerative colitis have a higher risk of developing bowel cancer than healthy people. For this reason it is recommended that you undergo regular checks on the health of your bowel by having a colonoscopy. Often bowel cancer develops from small patches of bowel lining which become abnormal and unstable. The aim of having a colonoscopy is to try and find these patches and to remove them before they can turn into a cancer. The higher the chance of finding these abnormal patches, the better the chance of picking up early bowel cancer and removing it.

The usual way that we look for these patches is by colonoscopy. To help highlight early abnormalities, it is recommended that applying a special blue dye to the bowel lining can improve their chance of detection. This has the disadvantage of being more time consuming and occasionally messy. There are now newer more advanced instruments available which, using high-definition technology gives much clearer images of the bowel lining. We wish to investigate whether these new instruments are as good at picking up abnormal patches without the need for the additional blue dye. If this is the case, then these procedures can be performed quicker, be more comfortable for the patient, yet still pick up early abnormalities.

Why have I been chosen?

We are currently inviting all patients with ulcerative colitis who are scheduled to have routine surveillance colonoscopy.

Do I have to take part?

No, participation is entirely voluntary and is completely separate from your usual care. If you do decide to take part you will be given this information sheet to keep and be asked to sign an extra consent form in addition to the one just before your colonoscopy. If you decide to take part, you are still free to withdraw at any time and without giving a reason. **This will not affect your usual medical care.** If you decide **not** to take part, this will also not affect you in any way.

What will happen to me if I take part?

The study doctor may have already explained the study at your clinic appointment. If not, you will be contacted soon by the research team to ask you if you are interested in taking part and will arrange to see you on the day of your colonoscopy, before the test. On the day, you will have an opportunity to ask any questions you may have about the study to the study doctor who will also be performing your colonoscopy. At this time, if you are willing to participate, you will be asked to sign a consent form.

You will then 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 all the way to the caecum (the end of your large bowel). We then aim to look for abnormalities as we slowly bring the tube out. Just before we do this, we will decide whether we do this using the blue dye or not. The decision will be made using a special computer code.

If you are to receive blue dye we will slowly pull the tube back 10 cm at a time, spraying the blue dye down the colonoscope, onto the lining and closely inspecting the lining for any abnormalities. If any abnormalities are found, we will take samples from them for further analysis. If any polyps are found, these may be removed. We will also take a series of samples from the bowel lining which looks normal. The tube will be gradually removed and this procedure will be repeated every 10 cm.

If we decide not to use the blue dye, we will gradually pull back the tube 10 cm at a time and closely inspect the bowel lining. We will again be looking for abnormal patches, from which we will take samples and also a round of samples will be taken from the normal looking lining.

At the end of the procedure you will receive the same care you would usually receive after a colonoscopy and be allowed to go home shortly after the procedure if you are well enough.

Is there anything else I have to do?

No, this study involves no extra visits to hospital either before or after the 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, determining whether the new high-definition colonoscopies are as good on their own, compared with using the additional blue dye to detect abnormal patches, will help improve our understanding of the best way of looking for these abnormalities.

Are there any risks in taking part?

Having any form of colonoscopy test for any reason carries a very small risk of making a hole in the bowel. This risk is less than 1 in 1,000. The new colonoscopes carry no additional risk and are in widespread use for many endoscopy procedure of both the stomach and the large bowel. The use of the blue dye is entirely safe and is recommended by all the international gastroenterology societies. A very small amount is absorbed into the bloodstream and may make your urine or stool and light shade of green just for a day or so. This is entirely harmless and will return to normal.

What if 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 someone's negligence, then you may have grounds for a legal action but you may have to pay for it.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions [contact Dr Prashant Kant (07753659893). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure or PALS. Details can be obtained from either your doctor or the Leeds Teaching Hospital 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 on them. 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.

When your samples are analysed in the laboratory, they will be identified only by a code so that your name will not be known to laboratory staff. Samples will also be stored in a secure laboratory for future studies. Only the research team 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 Leeds Teaching Hospitals and University of Leeds, known as the research tissue bank. The samples may be used for further projects in the future after successful application for permission has been made from 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 that this study is being carried out was reviewed and given a favourable ethical opinion by the Bradford Research (Ethics) Committee. All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by The Leeds Research Ethics Committee.

Who to contact for further information

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

Dr Noor Mohammed, Specialist Registrar in Gastroenterology, Leeds Teaching Hospitals on 0113 3925811 or 07886577132

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 Noor Mohammed
Dr Prashant Kant
Dr Venkat Subramanian

Appendix G: Consent form_HDWLE v/s HDCE

CONSENT FORM

Comparison of high definition colonoscopy versus high-definition chromoendoscopy in the detection of dysplasia in UC

Name: **DOB:**

Patient Identification No:

	Please initial to confirm
• I have read the information sheet for the above study.	___
• 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.	___
• I have had the opportunity to ask questions about the study, and to discuss it with family and friends.	___
• I understand the purpose of the study, and how I will be involved.	___
• I understand, and accept, that if I take part in the study I will not gain any direct, personal benefit from it	___
• 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. ___

- 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. ___
- 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. ___
- 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.
- I give permission for my GP to be informed about my inclusion in this study ___

___

I agree to take part in the above study.

_____	_____
Signed	_Date
_____	_____
Person taking consent	_ Date
_____	_____
Researcher (if different from above	_

The Leeds Teaching Hospitals NHS
NHS Trust