Prevalence and detection of serrated colorectal neoplasia in a screened population

---

A thesis submitted for the degree of

Doctor of Medicine (MD)

Cardiff University

Dr Rajeswari Ramaraj MBBS FRCP

C1338965

May 2023

Statements:

1. This thesis is being submitted in partial fulfilment of the requirements for the degree of Doctor of Medicine.

2. This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is it being submitted concurrently for any other degree or award (outside of any formal collaboration agreement between the University and a partner organisation).

3. I hereby give consent for my thesis, if accepted, to be available in the University's Open Access repository (or, where approved, to be available in the University's library and for inter-library loan), and for the title and summary to be made available to outside organisations, subject to the expiry of a University-approved bar on access if applicable.

Declaration:

This thesis is the result of my own independent work, except where otherwise stated, and the views expressed are my own. Other sources are acknowledged by explicit references. The thesis has not been edited by a third party beyond what is permitted by Cardiff University's Use of Third-Party Editors by Research Degree Students Procedure.

WORD COUNT: 59045

(excluding summary, acknowledgements, contents pages, appendices, tables, diagrams and figures, references, bibliography, footnotes and endnotes).

SIGNED: **DATE: 20/05/2023**

Contents

List of Figures..... vii

Acknowledgements..... 5

Abstract..... 6

Chapter 1: Introduction..... 9

1.1 Introduction..... 10

1.2 Prevalence and anatomical distribution of colorectal cancer..... 10

1.3 The rationale and limitation of the current CRC screening Strategies..... 12

1.3.1 Rationale of screening in the UK..... 14

1.3.2 Limitations of strategies used in screening programmes:..... 17

1.4 Biology and its relation to the anatomical distribution of colorectal cancer..... 18

1.4.1 Molecular heterogeneity of CRC:..... 19

1.4.2 Molecular mechanisms of colorectal cancer development:..... 20

1.4.2.1 Chromosomal instability (CIN):..... 21

1.4.2.2 Signal transduction pathways:..... 22

1.4.2.2.1 WnT Pathway:..... 23

1.4.2.2.2 MAPK/ERK pathway:..... 25

1.4.2.2.3 PI3K-AKT pathway:..... 26

1.4.2.2.4 TGF-β pathway:..... 26

1.4.2.2.5 P53 mediated pathways:..... 27

1.4.2.3 Microsatellite instability (MSI):..... 28

1.4.2.4 CpG-island methylator phenotype (the serrated pathway):..... 30

1.4.3 Transcriptional subtypes and the relation to conventional subtypes:..... 31

1.4.4 Anatomical distribution of CRC: (Right sided versus Left sided CRC)..... 33

1.4.4.1 Right-sided CRC..... 35

1.4.4.2 Left-sided CRC..... 36

1.4.5 Hereditary colorectal cancer..... 39

1.4.6 Family history of CRC:..... 41

1.4.7 Probable Non-Genetic Risk factors for CRC..... 41

1.5 The pathogenesis of serrated neoplasia..... 42

1.5.1 Histopathological classification of serrated polyps:..... 43

1.5.2 Molecular Pathways-the serrated neoplasia pathway:..... 44

1.5.3 Molecular features of serrated precursor Lesions:..... 47

1.5.3.1 Hyperplastic polyps (HP):..... 47

1.5.3.2 Sessile serrated lesions (SSLs):..... 47

1.5.3.3 Sessile Serrated Lesion with Dysplasia (SSLD):..... 47

1.5.3.4 Traditional Serrated Adenomas (TSA):..... 48

1.5.4 Molecular Subtypes of CRC and the role of precursor serrated lesions..... 50

1.5.5 Risk factors for serrated neoplasia..... 53

1.5.5.1 Serrated polyposis syndrome (SPS)..... 54

1.5.5.2 Lifestyle risk factors for serrated neoplasia..... 56

1.5.5.2.1 Tobacco Smoking..... 56

1.5.5.2.2 Alcohol..... 57

1.5.5.2.3 Obesity..... 58

1.5.5.2.4 Dietary factors..... 58

1.5.5.2.5 Medications..... 59

1.5.5.3 Factors that contribute to epigenetic changes in colonocytes..... 59
1.5.5.4 Role of inflammatory bowel disease (IBD) in serrated neoplasia..... 62
1.5.6 Summary..... 62

1.6 Endoscopic characteristics and appearance of colorectal serrated neoplasia..... 64
1.6.1 Endoscopic appearance..... 66
1.6.1.1 Hyperplastic polyps..... 66
1.6.1.2 Sessile Serrated lesions (SSL)..... 66
1.6.1.3 SSL with dysplasia (SSLD)..... 66
1.6.1.4 Traditional Serrated Adenoma (TSA)..... 67
1.6.2 Endoscopic management and surveillance of serrated neoplasia..... 69
1.6.3 Artificial intelligence and serrated neoplasia..... 71

1.7 Current methods of estimating prevalence of serrated neoplasia..... 72
1.7.1 Methods of Estimating Prevalence:..... 73
1.7.1.1 Diagnostic modalities:..... 73
1.7.1.2 Diagnostic accuracy i.e. the histological interpretation..... 79
1.7.1.3 Epidemiology..... 80
1.7.2 Limitations:..... 80
1.7.3 Summary:..... 81

1.8 Post colonoscopy colorectal cancer..... 83
1.8.1 Definitions and standardisation of terminology..... 83
1.8.2 Factors that may contribute to aetiology and potential mechanisms..... 85
1.8.3 Proximal CRC versus distal CRC..... 86
1.8.4 PCCRC and serrated neoplasia:..... 88

1.9 The influence of colonoscopy quality on the detection of Proximal colon neoplasia and Serrated neoplasia..... 89

1.10 Research gaps and questions:..... 93
1.10.1 Research gaps:..... 93
1.10.1.1 Epidemiology:..... 93
1.10.2 Malignant potential:..... 98
1.10.3 Research Questions..... 102

1.11 The Aims of the Research:..... 104

Chapter 2: Feasibility and acceptability of chromocolonoscopy for the detection of proximal serrated neoplasia in a population-based screening programme (CONSCOP study)..... 105

2.1 Introduction..... 106
2.1.1 The challenges in detecting proximal colon neoplasia and serrated neoplasia..... 107
2.1.1.1 Stool Based tests..... 108
2.1.1.2 Colonoscopy..... 110
2.1.1.3 CT Colonography (CTC) and Colon Capsule Endoscopy (CCE)..... 111
2.1.2 The influence of colonoscopy quality on the detection of proximal serrated neoplasia..... 112
2.1.2.1 Operator factors..... 113
2.1.2.2 Quality of Bowel preparation in colonoscopy..... 114
2.1.2.3 Other factors to help improve mucosal visualisation..... 115
2.1.3 The known and potential impact of chromocolonoscopy in the detection of colorectal neoplasia 117
2.1.3.1 Limitations or Barriers to dye based chromocolonoscopy..... 121
2.1.4 The current understanding of methods in improving colonoscopy quality..... 124

2.1.4.1 Technical factors..... 124
2.1.4.1.1 Bowel preparation..... 124
2.1.4.1.2 Withdrawal Technique..... 125
2.1.4.1.3 Withdrawal time..... 125
2.1.4.1.4 Antispasmodic agent..... 125
2.1.4.1.5 Water infusion techniques..... 126
2.1.4.2 Device factors..... 126
2.1.4.2.1 Technology used to enhance optical Images..... 126
2.1.4.2.2 Ancillary technology and techniques to improve mucosal visualisation and polyp detection 128
2.1.4.3 Training..... 130
2.1.5 The Rationale and Aims of the CONSCOP Study..... 131
2.1.5.1 Rationale..... 131
2.1.5.2 Aims of the study..... 134

2.2 Methodology..... 135
2.2.1 Funding and ethical approval..... 135
2.2.2 Study Design and Participants..... 135
2.2.2.1 The Inclusion criteria for participants..... 136
2.2.2.2 Exclusion criteria..... 136
2.2.3 Recruitment Process..... 137
2.2.3.1 Colonoscopists and local assessment bowel screening centres in Wales..... 137
2.2.3.2 Recruitment process of participants..... 138
2.2.3.2.1 Before the procedure..... 138
2.2.3.2.2 During the procedure..... 139
2.2.4 Randomisation and masking..... 140
2.2.5 Data Collection and Process..... 142
2.2.6 Statistical Analysis..... 145

2.3 Results..... 146
2.3.1 Recruitment and participation..... 147
2.3.2 Baseline demographics and pre-procedure characteristics..... 148
2.3.3 Intra-procedural characteristics for index colonoscopy..... 151
2.3.3.1 Quality Indicators..... 151
2.3.3.2 Procedure time and withdrawal time:..... 154
2.3.4 Post- Procedure findings..... 156
2.3.4.1 Repeat Procedures..... 156
2.3.4.2 Polyp and Cancer Detection..... 158
2.3.4.3 Adenoma detection rate..... 158
2.3.4.4 Serrated neoplasia detection rate..... 159
2.3.4.4.1 Serrated Lesion (SL)..... 159
2.3.4.4.2 Significant SL..... 160
2.3.4.4.3 Sessile Serrated Lesion (SSL)..... 160
2.3.4.4.4 Serrated lesion and synchronous advanced adenoma..... 164
2.3.4.4.5 Advanced neoplasia..... 164
2.3.5 Serious Adverse Reactions (SARs)..... 171
2.3.6 Health Economic Evaluation (HEE)..... 171

2.4 Discussion..... 174

Chapter 3: Histopathological aspect of serrated neoplasia (CONSCOP study)..... 181

3.1 Introduction..... 183
3.1.1 Terminology and histological features of serrated neoplasia..... 184
3.1.2 Aim of the study..... 189

3.2 Methodology....190
3.2.1 Data collection method:....192
3.2.2 Data collection and statistical analysis....196
3.3 Results....199
3.3.1 Demographics....199
3.3.2 Central Pathology review of proximal polyps....200
3.3.3 Reclassification of Hyperplastic Polyps and SSLs:....205
3.3.4 Inter-observer variability (IOV) of serrated neoplasia....208
3.3.4.1 IOV by polyp type....208
3.3.4.1.1 Between expert pathologists....208
3.3.4.1.2 Between Experts and Local Pathologists....209
3.3.4.2 IOV of grade of dysplasia:....211
3.3.4.2.1 Between the experts....211
3.3.4.2.2 Experts and LAC....211
3.3.4.3 IOV between the subtypes of serrated lesions:....212
3.3.5 Prevalence of serrated neoplasia....215
3.3.6 Novel findings:....217
3.4 Discussion....221
Chapter 4- Mutational Signatures in Serrated Neoplasia....233
4.1 Introduction:....234
4.1.1 Mutational signatures:....234
4.1.2 Methods of detecting mutational signatures:....236
4.1.3 Whole genome sequencing (WGS):....237
4.1.4 Whole Exome Sequencing (WES):....237
4.1.5 FFPE samples:....239
4.1.6 Aim of the study:....241
4.2 Materials and Methods:....242
4.2.1 Reagents and Equipment:....242
4.2.2 Case Cohort....244
4.2.3 Workflow of FFPE sample DNA extraction and sequencing:....246
4.2.4 Preparation of slides from FFPE Blocks....248
4.2.4.1 FFPE Block sectioning:....248
4.2.4.2 Identification of abnormal areas on the FFPE sectioned slide:....248
4.2.4.3 Macro dissection of tissue from FFPE sections:....248
4.2.5 DNA extraction, purification, and quantification from FFPE samples:....248
4.2.5.1 DNA extraction and purification:....249
4.2.5.2 DNA quantification:....251
4.2.6 DNA Library preparation for next-generation sequencing:....253
4.2.6.1 Target enrichment:....255
4.2.6.1.1 PCR set up:....255
4.2.6.1.2 Sample pooling and purification using AMPure XP beads:....257
4.2.6.2 Library construction:....258
4.2.6.2.1 End repair of DNA:....258
4.2.6.2.2 A-addition:....258
4.2.6.2.3 Adapter ligation:....259
4.2.6.2.4 Clean-up of adapter ligated DNA with AM Pure XP beads:....259
4.2.6.2.5 Amplification of purified library:....259
4.2.6.2.6 Clean-up of amplified library with AM Pure XP beads:....260
4.2.6.2.7 Library quality control (QC):....260

4.2.6.3 NGS Run:.... 262
4.2.6.3.1 Targeted and Comprehensive gene panel:.... 262
4.2.6.3.2 Data analysis.... 263
4.3 Results:.... 263
4.4 Whole Exome Sequencing:.... 273
4.4.1 Methodology.... 274
4.4.1.1 Quality control.... 274
4.4.1.2 Pre-processing.... 275
4.4.1.3 Mapping.... 275
4.4.1.4 Post-processing.... 275
4.4.1.5 Variant discovery — identification of somatic variants.... 276
4.4.1.6 Inputs.... 276
4.4.1.7 Mutational signatures inferred.... 279
4.4.2 Results and discussion.... 279
4.4.2.1 A. Inferring mutational signatures by group.... 279
4.4.2.1.1 Stage 1. Determine mutational spectrum.... 279
4.4.2.1.2 Stage 2: Identifying mutational signatures.... 282
4.4.2.2 B. Inferring mutational signatures by sample.... 287
4.4.2.2.1 Stage 1: Determine mutational spectrum for individual samples.... 287
4.4.2.2.2 Stage 2: Identifying mutational signatures for individual samples.... 290
4.4.3 Conclusion from WES results:.... 294
4.5 Discussion:.... 294
5. Summary of Research and Future directions.... 299
5.1 Overview of chapter structure:.... 300
5.2 Research methodology:.... 301
5.2.1 Chapter 2.... 301
5.2.2 Chapter 3:.... 303
5.2.3 Chapter 4:.... 304
5.3 Summary of research findings:.... 305
5.4 Limitations of the Studies:.... 307
5.5 Future directions and research:.... 310
Bibliography.... 315
Appendices.... 339

List of Figures

Figure 1: Previous BSG Surveillance Guidelines following adenoma removal 2009 [54] 16
Figure 2: Conventional adenoma to carcinoma sequence [63]. 21
Figure 3: Canonical WnT signalling [80] 24
Figure 4: MAPK Pathway [82] 25
Figure 5: Molecular Pathways to CRC development [98] 29
Figure 6: Consensus molecular subtypes (CMS) classification [101] 32
Figure 7: Summary of the differences between right and left-sided CRC [100, 104, 111] 38
Figure 8: Factors contributing to the serrated pathway in CRC [62] 42
Figure 9: The serrated pathway to carcinogenesis [131] 45
Figure 10: Molecular subtypes of CRC and the role of precursor serrated lesions [145] 52
Figure 11: Environmental, Physiological, and other risk factors that contribute to CRC through DNA Methylation [64]. 61
Figure 12: The WASP classification [182]. 65
Figure 13: Sessile serrated lesion 1a: sessile serrated lesion on white light imaging; 1b: SSL with Indigo carmine dye; 1c: SSL following submucosal lift where margins appear well defined; 1d: Type II-0 pit pattern 68
Figure 14: TSA and hyperplastic polyp.2a: traditional serrated adenoma NBI; 2b: TSA on white light imaging- "pinecone like" appearance; 2c: NBI image of hyperplastic polyp; 2d: white light image of hyperplastic polyp 68
Figure 15: TSA and hyperplastic polyp.2a: traditional serrated adenoma NBI; 2b: TSA on white light imaging- "pinecone like" appearance; 2c: NBI image of hyperplastic polyp; 2d: white light image of hyperplastic polyp 68
Figure 16: Cumulative hazard rates for PCCRC according to the endoscopist ADR [1]. 91
Figure 17: Chromocolonoscopy can highlight flat lesions like serrated lesions 123
Figure 18: Trial Schema 141
Figure 19: Participating bowel cancer screening sites with distribution of eligible participants into different arms 147
Figure 20: CONSORT diagram-Trial Profile 148
Figure 21: Repeat procedures following index colonoscopy-white light endoscopy (WLE) versus Chromocolonoscopy 156
Figure 22: Key Polyp detection rates 161
Figure 23: Histopathological images of serrated lesions adapted from [122] 187
Figure 24: Flow diagram to demonstrate the methodology used to collect histopathology data 197
Figure 25: CONSCOP-Polyp Processing Flowchart 198
Figure 26: Distribution of polyps by location in the proximal colon 199
Figure 27: Flowchart demonstrating slides discussed among 3 GI Experts (LAC-local assessment centre) 201
Figure 28: Serrated neoplasia reported by Local assessment centre (LAC) vs. Experts 207
Figure 29: Interobserver variability of serrated neoplasia subtypes between the 3 experts 212
Figure 30: Comparison of Kappa agreement of serrated neoplasia between expert pathologists (EP) and EP vs local assessment centre (LAC) pathologists 214
Figure 31: Distribution of serrated lesions in the proximal colon 215
Figure 32: Marking of areas of interest on H&E slides 245
Figure 33: Workflow for FFPE DNA extraction and sequencing 247

Figure 34: Workflow of purification of DNA from FFPE samples using gene read and quantification of DNA using Qubit assay....252
Figure 35: Workflow for Generead DNAseq Targeted Panel V2 Procedure....254
Figure 36: Preparation of PCR Master mix for each primer mix pool....255
Figure 37: 4- pool panel....256
Figure 38: Sample Agilent bioanalyzer image of a MiSeq sequencer library for illumina....261
Figure 39: Adequate fragment size noted for polyp 449 measured by bioanalyser....265
Figure 40: Observed motif frequency for each of the three patient groups, collectively referred to as the mutational spectrum of the data....280
Figure 41: Hierarchical clustering of the mutational spectrum, according to motif....281
Figure 42: Mutational signatures inferred with the NMF method, represented both as a bar chart (Panel A) and a heat map (Panel B)....283
Figure 43: Contribution of the three inferred mutational signatures to observed mutational spectrum, visualised as a heat map (Panel A) and a bar chart (Panel B)....284
Figure 44: Mutational signatures inferred with PCA, represented as a bar chart (Panel A) and a heat map (Panel B)....285
Figure 45: Contribution of the three inferred mutational signatures to observed mutational spectrum, visualised as a heat map (Panel A) and a bar chart (Panel B)....286
Figure 46: Observed motif frequency for each of the eighteen samples, collectively referred to as the mutational spectrum of the data....288
Figure 47: Hierarchical clustering of the mutational spectrum per sample, according to motif....289
Figure 48: Summary statistics for selecting the number of signatures, determined for NMF (panel A) and PCA (panel B)....291
Figure 49: Mutational signatures inferred with NMF assuming 8 signatures....292
Figure 50: Mutational signatures inferred with NMF assuming 3 signatures....292
Figure 51: Mutational signatures inferred with PCA assuming 8 signatures....293
Figure 52: *Mutational signatures inferred with PCA assuming 3 signatures*....293

List of Tables

Table 1: Molecular features of preneoplastic lesions and CRCs by anatomical site -adapted from[99] 37
Table 2: Molecular features of precursor serrated lesions modified from (62)..... 49
Table 3: Recommendations for surveillance sessile serrated polyps adapted from [203]..... 70
Table 4: Baseline Demographics of the participants..... 149
Table 5: Quality indicators including Bowel Preparation quality, technical factors, sedation, procedural difficulty, and patient comfort score..... 152
Table 6: Procedure and withdrawal time by mean, median and inter quartile range (IQR)..... 155
Table 7: Repeat Procedures for the different trial arms.....157
Table 8: Polyps (WHO classification) retrieved over first and repeat procedures.....163
Table 9: Univariable logistic regression for advanced adenoma detection rates by categories of serrated lesions..... 166
Table 10: Univariable and multivariable logistic regression for Serrated Lesions.....167
Table 11: Univariable and multivariable logistic regression for Significant Serrated Lesions..... 168
Table 12: Univariable and multivariable logistic regression for Sessile Serrated Lesions..... 169
Table 13: Univariable and multivariable logistic regression for advanced neoplasm detection rates 170
Table 14: Cost analysis of all index and repeat procedures (£ per procedure)..... 172
Table 15: Cost analysis of all repeat procedures only (£ per procedure)..... 173
Table 16: Histological types and features of serrated lesions (WHO 2019)..... 186
Table 17: Estimated 10-year risk of CRC for polyp subtypes [237].....189
Table 18: Consensus in polyp diagnosis among 3 GI Expert Pathologists..... 202
Table 19: SSLs that needed discussion at the consensus meeting..... 202
Table 20: Proximal Polyps that did not reach a consensus after review by the Experts..... 203
Table 21: Summary of Polyp characteristics including demographics, location and histology..... 205
Table 22: Comparison of individual polyp type reports between the experts and LAC..... 206
Table 23: IOV of Polyp type between Expert Pathologists (EP) and IOV between EP and Local Pathologists (LP)..... 210
Table 24: Based on Dysplasia grade, IOV between EP and IOV between EP and LP..... 211
Table 25: Interobserver variability of serrated neoplasia between the expert and local pathologists 213
Table 26: Distribution of serrated neoplasia in participants based on expert and local pathology review..... 216
Table 27: PCR program..... 256
Table 28: Samples processed, categorised by smoking status, with raw DNA yield from sequencing specified..... 277
Table 29: Outcome of Mutect2 analysis.....278

Abbreviations

ACG	American College of gastroenterology
ACPGBI	The Association of Coloproctology of Great Britain and Ireland
ADR	Adenoma detection rate
AFI	Autofluorescence imaging
AGA	American gastroenterology Association
AI	Artificial Intelligence
APC	Adenomatous polyposis coli
BLI	Blue light imaging
BMI	Body mass index
BRAF	B-Raf proto--oncogene
BSG	British Society of gastroenterology
BSIMS	Bowel screening information management system
BSW	Bowel screening Wales
CAD	Computer aided Detection
CC	Chromocolonoscopy
CCD	charge-coupled device
CCE	Colon Capsule endoscopy
CE	Chromoendoscopy
CI	Confidence interval
CIMP	CpG Island methylator phenotype
CIN	Chromosomal instability
CMS	consensus molecular subtypes
CONSCOP	Feasibility of reduction of right sided bowel cancer through **CON**trast Enhanced colono**SCOP**y
COSMIC	Catalogue of Somatic Mutations and Cancer
CRC	Colorectal cancer
CRF	Case Report Form
CRP	C-reactive protein
CRUK	Cancer Research UK
CT	Computed tomography
CTC	CT colonography
DBS	Doublet Base Substitutions
Distal Colon or Left Colon	Descending colon to rectum
dMMR	Mismatch repair deficiency
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
ECF	Ectopic Crypt Foci
EGFR	Epidermal growth factor receptor

EP	Expert Pathologist
ESGE	European Society of gastroenterology and endoscopy
EU	European Union
FAP	Familial adenomatous polyposis
FFPE	Formalin Fixed Paraffin Embedded
FICE	flexible spectral imaging colour enhancement
GCHP	Goblet cell-rich type hyperplastic polyp
GFOBT	Guaiac Faecal occult blood
GI	Gastrointestinal
H&E Stain	Haematoxylin and Eosin stain
Hb	Haemoglobin
HD	High Definition
HD WLE	High-Definition white light endoscopy
HDC	High-Definition Colonoscopy
HGD	High-grade dysplasia
HP	Hyperplastic polyp
HR	Hazard ratio
HRT	Hormone Replacement Therapy
IARC	International Agency for Research on Cancer
IBD	Inflammatory Bowel Disease
ICER	incremental cost-effectiveness ratio
ICV	Ileocecal Valve
IHC	Immunohistochemistry
IOV	Inter Observer Variability
IRAS	Integrated research application service
IRR	Incident Rate Ratio
JNET	Japan NBI Expert Team
KPI	Key Performance Indicators
KRAS	Kirsten rat sarcoma virus
LAC	Local assessment centre
LCI	Linked colour imaging
LGD	Low-grade dysplasia
LOH	Loss of heterozygosity
LP	Local Pathologist
MAPK	Mitogen- Activated Protein Kinase pathway
MLH	mutL homologue
MMR	Mismatch repair
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
MSS	Microsatellite stable
MV	Microvesicular
MVHP	Microvascular type hyperplastic polyp
NBI	Narrowband imaging
NGS	Next Generation Sequencing

NHS	National Health Service
NICE	National Institute for health and care excellence
NISCHR	National Institute for social care and health research
NMF	Nonnegative matrix Factorisation
NOS	Dysplasia not otherwise specified
NRAS	neuroblastoma RES viral oncogene homolog
NSAID	Non-Steroid Anti-inflammatory Drug
OR	Odds ratio
PC	Pathology coordinator
PCA	Principal Component Analysis
PCCRC	Post colonoscopy colorectal cancer
PCR	Polymerase Chain Reactions
PDR	Polyp detection rate
PIK3CA	phosphatidylinositide 3-kinase
Proximal Colon or Right Colon	Extending from caecum to splenic flexure
QC	Quality Control
qFIT	Quantitative faecal immunohistochemical test
RCT	Randomised controlled trial
RTK	receptor tyrosine kinase
SAR	Serious Adverse Reactions
SBS	Single Base Substitutions
SD	Standard Deviation
SEER	Surveillance, epidemiology, and end results
SNV	Single Nucleotide Variation
SP	Serrated Polyp
SPDR	Serrated Polyp Detection Rate
SPS	Serrated polyposis syndrome
SSL	Sessile serrated lesion
SSLD	Sessile serrated lesion with dysplasia
SSP	Specialist screening practitioner
TA	Tubular adenoma
TCGA	The Cancer Genome Atlas
TNM	Tumour Node Metastasis (Classification of malignant tumours)
TP53	Tumour Protein 53
TSA	Traditional serrated adenoma
TVA	Tubulovillous adenoma
UC	Unclassified
UHB	University Health Board
UK	United Kingdom
UNG	uracil N-glycosylase
USA	United States of America

VA	Villous Adenoma
VCF	Variant Call Format
WASP	Workgroup Serrated Polyps and Polyposis
WCTU	Wales Cancer Trials Unit
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
WHO	World Health Organisation
WLE	White light endoscopy

Acknowledgements

I would like to express my heartfelt gratitude to many people who have supported me throughout my research journey.

I am profoundly grateful to my supervisor, Professor Sunil Dolwani, who has been a fantastic mentor and supervisor throughout this journey. His patient guidance, wisdom and continued support and encouragement has been invaluable, and I am very thankful for the opportunity given to me to complete this research.

I would also like to thank all the professional teams that have been involved in this research. These include the Wales cancer trials unit team- Chris Hurt, Georgina Gardiner, and Catherine Porter. The expert pathology team, Professor Geraint Williams, Professor Namor Williams, Dr Meleri Morgan, and the pathology coordinator Andrew Coombes. The genetics team- Shelley Rundle, Gareth Marlow, Kevin Ashelford, Karen Read, and Rachel Butler. The health economics team in Swansea- Professor Ceri Phillips and Angela Farr. The bowel cancer screening team, Dr Hayley Heard who was the head of the Bowel Screening Programme at the time of my research, Richard Wakeley and Helen Davies from the IT team and all the Specialist Screening Practitioners in Cardiff and throughout Wales.

It has been an absolute pleasure to have collaborated and worked with all of these enthusiastic teams that has enabled the success of this research.

I would like to thank my family especially my parents who have always been an inspiration in resilience, strength and perseverance. And also, to my wonderful family of sisters, nieces, nephews, and all my friends who have been like family, thank you all for the moral support and encouragement. Last but not the least, to my better half. Thank you for everything and reminding me every day of what truly matters.

Abstract

Word count: 300 words

Prevalence and detection of serrated colorectal neoplasia in a screened population

Introduction: Serrated lesions (SL), precursors of colorectal cancer (CRC), account for 15 to 30% of all CRC cases. Post-colonoscopy CRC (PCCRC) may occur due to missed proximal serrated neoplasia. Existing heterogeneity in SL reporting and detection makes the actual prevalence uncertain. This study aims to assess the feasibility of introducing enhanced colonoscopy and estimating proximal SL detection and prevalence.

Method: A multicentre randomised controlled trial was conducted in bowel cancer screening centres in Wales. Participants aged 60-74, testing positive for faecal occult blood, were randomised to undergo standard white light colonoscopy or chromocolonoscopy. All removed proximal colon polyps were centrally reviewed by expert pathologists. Data analysis included serrated neoplasia detection rate, procedural time, resource utilisation, and interobserver variability among pathologists. A subsequent genetics exploratory study was conducted using Next Generation sequencing.

Results: The study recruited 741 patients, achieving 82% patient participation and 87% colonoscopists. The chromocolonoscopy procedure was marginally longer (6.3 minutes) and showed enhanced detection rates for proximal sessile serrated lesions (11.8% vs 6.4%) and significant serrated lesions (4.2% vs 1.9%). Central histopathology review revealed a higher prevalence of significant serrated neoplasia in the proximal colon (7.6% vs 3.3%).

There was good concordance for non-dysplastic SLs however this was fair for dysplastic SLs. Lastly, an exploratory study revealed the feasibility of extracting DNA from FFPE blocks with a weak mutational signature in ex-smokers, albeit with significant background noise.

Conclusions: This study demonstrated that chromocolonoscopy could be implemented in a UK-based population bowel screening programme for improved detection of proximal serrated neoplasia with acceptable time and resource usage, and bias minimisation. Further trials and longitudinal studies to assess clinical effectiveness, economic evaluation, translational studies, and the impact on surveillance and reducing PCCRC is necessary.

Research Dissemination

Publication:

Feasibility and economic assessment of chromocolonoscopy for detection of proximal serrated neoplasia within a population-based colorectal cancer screening programme (CONSCOP): an open-label, randomised controlled non-inferiority trial. Lancet Gastroenterol Hepatol 2019; 4: 364-75

R Ramaraj*, C Hurt*, A Farr, M Morgan, N Williams, CJ Philips, GT Williams, G Gardner, C Porter, J Sampson, S Hillier, H Heard, S Dolwani, on behalf of the CONSCOP Clinical Research Consortium

*Joint first authors

Conference abstracts (**Poster Presentation**):

OTU-026 Feasibility & economic evaluation – chromoendoscopy for detecting proximal serrated neoplasia: randomised controlled trial, conscop. (British Society of Gastroenterology 2018). 10.1136/gutjnl-2018-BSGAbstracts.366

S Dolwani*, R Ramaraj, C Hurt, A Farr, M Morgan, N Williams, C Phillips, G Williams, G Gardner, C Porter, J Sampson, S Hillier, H Heard, CONSCOP Investigators

Chapter 1: Introduction

1.1 Introduction

According to global cancer statistics 2018 (2), Colorectal cancer (CRC) ranks third in terms of cancer incidence (10.2 %) after lung and breast cancer in both sexes but is the second most frequent cause of death in both sexes (9.2%) after lung cancer in the world. In the United Kingdom, CRC is the second most common cause of cancer death accounting for 10% of all cancer deaths. It is the third most common cancer in the UK in both males and females (2-4).

1.2 Prevalence and anatomical distribution of CRC

There remains a wide geographical variation in the incidence of CRC and related mortality with similar patterns noted in both genders (5). Age standardised incidence rates (ASRi) vary significantly when comparing world regions classified by the United Nations. For example, the highest rates occur in Australia and New Zealand (ASRi 44.8 and 32.2 per 100,000 in men and women respectively) whereas it is the lowest in Western Africa (ASRi 4.5 and 3.8 per 100,000 in men and women respectively) (5, 6). However, CRC estimates in Africa are heterogenous and may be underestimated as there is a possibility of underreporting due to the known lack of consistent and systematic ascertainment and cancer registries. (5, 7).

In the United Kingdom where there has been an established organised CRC screening programme using stool-based screening tests since 2006 (England 2006, Scotland 2007, Wales 2008, and Northern Ireland 2010) the ASRi is 30.2 per 100,000 in both genders with the age standardised mortality rates (ASRm) being 10.7 per 100,000.

According to the bowel cancer incidence statistics from cancer research UK 2020 (CRUK) (4), age specific incidence rates rise steeply from (4) ages 50 to 54 with the highest rates in the 85 to 89 age group for males and females. The rate of bowel cancer incidence has increased by 48% in the younger age group (25 to 49 age) and in the 60 to 74 age group has decreased by 6% (4, 8). The incidence of CRC in this older age group is possibly attributable at least partly to the population-based screening programme that have been ongoing for over a decade and a half.

The majority of CRCs in the UK are located in the distal or left colon with a slight preponderance in males (65% in males and 56% in females) of which the most common location is the rectum (22% in females and 31% in males). However the incidence in the right or proximal colon (caecum including appendix to splenic flexure) has a preponderance in females (44% females and 35% males). (4, 8). A study using the Data from the National Cancer Institute's surveillance, epidemiology and end results (SEER) registry (9, 10) showed that female sex, age over 60, and black ethnicity were associated with an increased risk of proximal CRC.

Though population-based bowel screening programmes have been shown to reduce the incidence and mortality of bowel cancer, this seems to have had a substantial impact on distal CRC but only a modest impact on proximal CRC (11, 12). Post colonoscopy colorectal cancers (PCCRC) also seem to occur more in the proximal location (13, 14).

The factors that may contribute to less accurate detection of proximal CRC at colonoscopy include quality related issues such as colonoscopists key performance indicators, missed polyps and cancers due to poor bowel preparation (15), failure to detect polyps due to morphology i.e. flat polyps (16), incompletely resected polyps (17) and a small proportion of accelerated biology related cancer (e.g. those progressing via the serrated neoplasia pathway) that could result in either a PCCRC or new CRC (18-21).

The other potential factor that impacts on the detection of proximal colon cancer during screening is the poor sensitivity of stool-based screening tests (gFOBT and FIT) to detect flat non-bleeding polyps such as significant serrated lesions which are predominantly located in the proximal colon and more common in women. (22-24)

There is no doubt that CRC is a huge burden on healthcare and the society and early detection strategies such as screening seem cost-effective (25) and may enable the prevention of the development or progression of cancer and thereby reduce morbidity and mortality.

1.3 The rationale and limitation of the current CRC screening Strategies

CRC screening is cost-effective and may decrease the incidence and mortality of CRC by detection and removal of cancer precursors and early-stage detection associated with lower mortality and morbidity.

Stage of CRC is one of the most important predictors of survival and the office for National statistics (ONS) in the United Kingdom (4, 8) shows that the five-year survival rate for stage I and II is 91 to 93 % (both genders), around 60% in stage III and 10 % in case of stage IV (distant metastases).

Hence early detection is key to reduce mortality. However despite this it is not widely accessible throughout the world as only a small proportion of the target population is offered this (5). Globally there remains a widespread difference in the implementation status and CRC screening strategy. This is thought to be due to a geographical variation in CRC incidence, availability of economic resources, infrastructure, and healthcare systems to support screening such as having the ability to identify a population at risk and being able to maintain a cancer registry (5).

The current CRC screening strategies in the world include the use of different screening methods such as organised or opportunistic screening (5) and the use of different screening modalities such as invasive and non-invasive tests (26). Organised screening (27, 28) is distinguished from opportunistic screening as invitations for screening are offered for a defined population and there exists a centralised process for quality assurance, eligibility requirements including target population, follow-up, and further evaluation.

The target population for an average risk population group includes an asymptomatic population that is at an average risk of CRC and the age range for this population has been influenced by national and international guidelines (5, 26, 29, 30) and though most screening programmes commence mainly over the age of 50 years, it remains constantly over the age of 40 years.

Globally, there appears to be a variation in the type of screening modality used for an average risk population. These modalities (5) can include either a non-invasive test or an invasive test. Non-invasive testing includes stool-based testing (faecal immunochemical tests-FIT, guaiac faecal occult blood test-gFOBT and newer tests such as stool DNA, RNA and protein biomarkers) and blood tests (the detection of methylated DNA in blood) (31). Invasive tests include colonoscopy which is considered to be the gold standard for the detection of CRC, virtual colonoscopy, or CT colonography (CTC).

However, in most of the organised screening programs, a stool-based test is used initially and if the test is positive (FOBT) or if the quantification satisfies the threshold criteria for screening (FIT), then this usually triggers a further invasive test such as a colonoscopy examination. Opportunistic screening which is what is predominantly practised in the USA and some European countries depends on members of the public or healthcare providers initiating screening and hence involves fewer formal decisions on appropriateness and follow-up (27). This often involves the primary screening test being either a stool test or a primary invasive test such as a colonoscopic examination. In 2015, 24/28 European Union countries had established or were in the process of establishing a nationwide organised or opportunistic CRC screening programme (5).

1.3.1 Rationale of screening in the UK

The United Kingdom began implementation of the National Health Service bowel cancer screening programmes by using gFOBT in 2006 for England and the programme achieved full roll-out in England and Wales in 2010 (32).

This was supported by evidence from multiple large randomised controlled trials that showed that FOBT-based CRC screening showed a reduction in CRC mortality of 15 to 33% (33-39).

The programme identified eligible people within the target population (age range of both genders 60 to 74 years) who were invited to complete a home FOBT biennially. Following a positive test, the participants were subsequently contacted and directed to a specialist screening practitioner (SSP) who then provided more information on the implications of a positive test, including clinical evaluation of the participant assessing fitness to undergo a colonoscopic examination and discussing the risks and benefits of having a colonoscopy examination. In more recent years, the FOBT has been replaced by the more sensitive quantitative test called the faecal immunochemical test (FIT) which has the advantage of not being as influenced by factors such as diet and medications and being a quantitative test allows the sensitivity and specificity to be varied by adjusting the cut off of a positive test (40).

There is considerable variability in the procedural quality of a colonoscopy examination (41-43) amongst operators hence to ascertain quality based on evidence there are key performance indicators (KPI) that help to achieve the necessary standard needed for colonoscopy. Screening colonoscopy in England and Wales are only undertaken by accredited colonoscopists who satisfy certain quality KPIs which are regularly reviewed by screening programmes to ensure consistent performance at a very high standard.

CRC screening programmes aim to reduce mortality and longer-term incidence and allow the early-stage detection (3, 44-47). Populations that have implemented screening strategies and programmes have shown an overall reduction in mortality from CRC varying between 22 to 68% (46-52). Though the effect of FOBT-based screening does not influence all-cause mortality, the sustained reduction in CRC mortality is likely to be due to the colonoscopic detection of adenomas/polyps and polypectomy (33).

The British Society of Gastroenterology (BSG) had recommended surveillance guidelines following the removal of adenomas which was published in 2009 (53) (Figure 1) following an update from 2002. This has recently been updated in 2020 (54) and have included recommendation for surveillance for serrated polyps alongside adenomas.

My research study was completed prior to the recent updated guidelines and hence the 2009 guidance was followed (Figure 1).

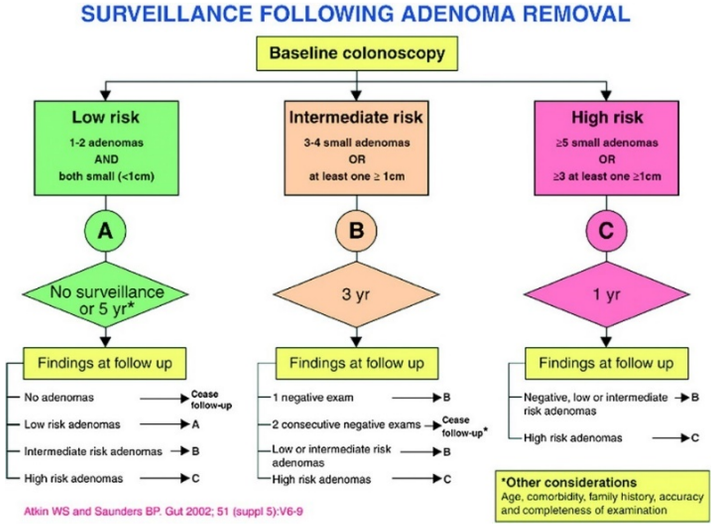


Figure 1: Previous BSG Surveillance Guidelines following adenoma removal 2009 (54)

"Reproduced from [Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002), Stuart R Cairns et al, developed on behalf of The British Society of Gastroenterology, and the Association of Coloproctology for Great Britain and Ireland, Volume 59, Issue 5, 666-689. Gut 2010 with permission from BMJ Publishing Group Ltd."

1.3.2 Limitations of strategies used in screening programmes:

Though national screening programmes that use faecal occult blood and subsequent colonoscopy have reduced the incidence and mortality of CRC, participation in the programme could be improved and there remains a relatively high false positive rate in faecal tests.

There also is high variability in the FIT test thresholds set for screening and possible laboratory variation in reporting unless standardised and external quality assured (48). Though FIT is a sensitive test to detect CRC at lower thresholds, the sensitivity is low to detect adenomas and even poorer to detect serrated polyps.

FIT sensitivity for CRC varies between 71-91% depending on the Hb cut off used with a specificity of 90-95% (48) however the sensitivity for advanced adenomas reduces to 25-40% (55, 56) and for serrated lesions reduces further to 4 to 16.3% (22, 57).

Hence in stool-based screening programmes, the prevalence of serrated lesions may not be accurate and variable in comparison to countries such as USA that adopt primary colonoscopy as a screening strategy.

However primary colonoscopy is resource dependent, expensive, invasive and the quality of the procedure is dependent on patient and operator factors. Standard colonoscopy can miss polyps particularly flat polyps due to variation in technique, equipment, standards, and detection rates amongst colonoscopy operators. This could explain the variation in the detection of proximal colon cancers to distal CRCs particularly missing flat precursor polyps that follow the serrated pathway to carcinogenesis.

1.4 Biology and its relation to the anatomical distribution of CRC

Overview: this section aims to briefly describe the biology and molecular pathways that lead to CRC. It also aims to discuss the anatomical distribution of CRC i.e., proximal colon versus distal colon and the differences between them along with briefly describing hereditary CRC and family risk as relevant to the topic of this dissertation.

CRC occurs due to an accumulation of genetic abnormalities in the nuclei of clones of colonocytes that allow these cells to escape the normal regulatory mechanisms that control cell growth, death, and differentiation (58). The causes of these genetic abnormalities include chance events that happens during cell division, pattern of inheritance and other factors including lifestyle and personal characteristics.

Sporadic CRC i.e., CRC with no apparent genetic predisposition accounts for nearly 75% of the cases (6) and only 3 to 5% of new CRC diagnosis are related to hereditary conditions such as Lynch syndrome or familial adenomatous polyposis (FAP).

Around 20% of the cases report a family history of CRC with no known germline mutation (59). Inherited syndromes of CRC and the presence of a readily identifiable precursor lesion such as an adenoma has facilitated research into genetic mechanisms responsible for tumorigenesis (60).

1.4.1 Molecular heterogeneity of CRC:

The pathogenesis of CRC is due to the progressive accumulation of genetic and epigenetic alterations some of which are implicated for activating oncogenes or inactivating oncosuppressor genes that has the potential to change normal epithelium to early neoplastic lesions finally leading to the development of CRC (61-63).

The ongoing process of classifying CRC based on these molecular changes is crucial in understanding the essential events in CRC evolution and the clinical significance (64).

With advancements in molecular and genomic technologies CRC has been classified into different subtypes over the years. Earlier molecular classification was mainly based on genetic and epigenetic parameters with the recognition of two distinct morphological pathways of carcinogenesis that results in the neoplastic transformation in the colonic epithelium. These are the conventional adenoma-carcinoma sequence pathway and the alternative pathway i.e., the serrated neoplastic pathway (62, 65).

More recently, The Cancer Genome Atlas (TCGA) project has shown crucial relationships among genetic, epigenetic, and transcriptional changes (66). Recent classifications (67) have incorporated transcriptional signatures that resulted in a new classification system comprising of four consensus molecular subtypes (CMS 1-4) to provide more robust clinical implications (66, 68, 69).

Despite the genetic heterogeneity of CRC, the conventional classification of CRC is based on molecular pathways that cause genomic instability and epigenetic molecular features such as methylation.

Each of these mechanisms result in different types of CRC biology and clinical phenotypes (70, 71).

1.4.2 Molecular mechanisms of CRC development:

Conventional pathway: The precursor lesion for CRC can either be an adenoma or serrated polyp and the molecular events that lead to carcinogenesis are through one of the three molecular pathways (Figure 2) i.e., 1. Chromosomal instability (CIN), 2. Microsatellite instability (MSI) or the 3. CpG island methylator phenotype (CIMP) or Serrated pathway.

In addition to this there is also inflammatory bowel disease (IBD) associated CRC that in contrast develops through the “inflammation-dysplasia-carcinoma” sequence and the dysplastic lesions in IBD have increased chromosomal instability compared to sporadic adenomas (72).

The conventional pathways lead to the development of both hereditary and sporadic cases of CRC and each pathway is underpinned by mutations in key tumour suppressor genes and oncogenes. Based on the current body of evidence and understanding most CRCs develop from a precancerous lesion such as an adenoma or serrated polyp.

At a molecular level, Fearon and Vogelstein in 1990 (73) described this conventional model called the adenoma-carcinoma sequence of colorectal carcinogenesis which describes a multi-step process that leads to transformation of normal colon epithelium into the earliest dysplastic lesion called the aberrant crypt focus that then develops into a premalignant adenomatous polyp that transforms into low-grade, high-grade dysplasia and subsequently into a carcinoma based on a series of cumulative genetic mutations over a period of time (Figure 2).

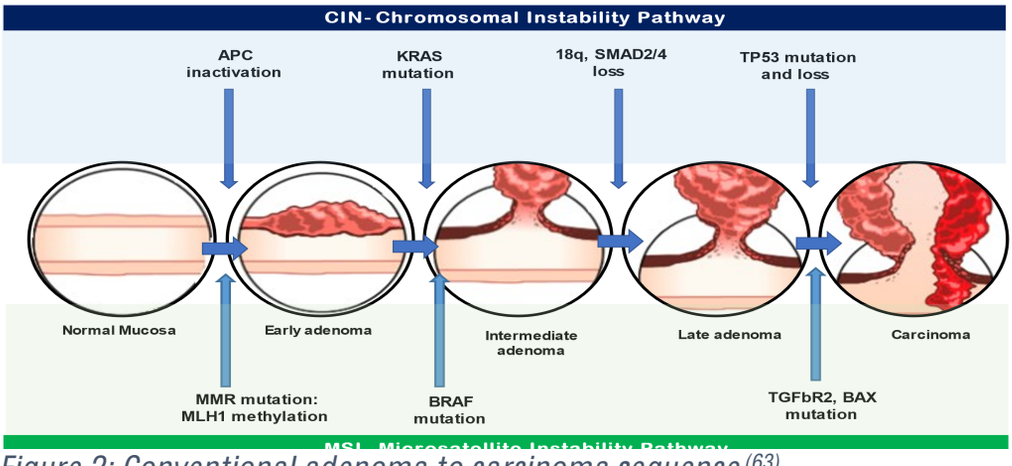


Figure 2: Conventional adenoma to carcinoma sequence [63].

Abbreviations: APC-Adenomatous Polyposis Coli; MMR-Mismatch Repair; MLH1-MutL Homolog 1; KRAS- Kirsten rat sarcoma virus; BRAF- B-Raf proto—oncogene; SMAD-Suppressor mothers against decapentaplegic; TP53- Tumour Protein 53; TGFbR2-Transforming Growth Factor Beta Receptor 2; BAX-BCL2 associated X. Copyright © 2019 by the authors. De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The Molecular Hallmarks of the Serrated Pathway in Colorectal Cancer. Cancers. 2019;11(7):1017. Licensee MDPI, Basel, Switzerland.

1.4.2.1 Chromosomal instability (CIN):

CRC with CIN is frequently located in the distal colon (74). Errors that occur during cell division can lead to loss of parts of a chromosome called loss of heterozygosity (LOH) or an abnormal number of chromosomes called aneuploidy. Aneuploidy and loss of heterozygosity are the hallmarks of tumours displaying CIN in CRC (58).

CIN can be observed in around 70% of CRC cases (75, 76). The first event that happens in this pathway is bi-allelic mutations of the tumour suppressor gene APC within the normal colonic mucosa. This is followed by mutation of the KRAS gene (75).

The key genes involved in CIN include APC, TP53, KRAS, PI3KCA (76) with consequent dysregulation of the WnT/β-catenin, MAPK, PI3K and TGF-β signalling pathways (62).

LOH in chromosome 18q containing tumour suppressor genes SMAD2, SMAD4 and DCC is also common as these genes are transcriptional mediators of the transforming growth factor (TGF) beta signalling pathway that regulates cell growth, differentiation and apoptosis and promotes MYC activation (64).

An APC mutation can occur either in sporadic CRC or if a germline APC mutation occurs then this leads to hereditary familial adenomatous polyposis (FAP).

1.4.2.2 Signal transduction pathways:

In CRC, although multiple gene mutations occur, only a few are responsible for driving the cancer process and are known as driver genes. The mutated genes that do not contribute to the progression of cancer are called passenger genes. According to Vogelstein (77) , only three driver mutations are required to produce CRC.

The first driver gene is a gateway gene that initiates the breakthrough phase, the second leads to neoplasia or the expansion phase and the third enables invasion and metastasis. These driver genes are part of a limited number of key signal transduction pathways that must be inactivated for neoplasia to progress.

The WnT pathway (driver genes APC, CTNNB1/ β catenin), MAPK pathway (driver genes KRAS and BRAF), of PI3K-AKT pathway (PIK3CA and PTEN), TGF-β pathway (SMAD) and p53 mediated pathways (TP53 driver gene) are signal transduction pathways that contribute to the development of the CIN phenotype in CRC. The TP53 pathway is responsible for cell cycle arrest, apoptosis and DNA repair (78).

1.4.2.2.1 WnT Pathway:

This pathway is frequently activated in CRC due to mutations in the APC or β-catenin genes. In normal cells, the WnT/β-catenin pathway is tightly regulated, but in CRC cells, this pathway is constitutively activated leading to the stabilization of β-catenin, which translocate to the nucleus and activates the transcription of various target genes involved in cell proliferation, survival, and differentiation. Dysregulation of this pathway has been implicated in the initiation and progression of CRC (73).

The WnT signalling pathway is altered in the majority (up to 92%) of all CRCs (66). Multiple genes are affected in this pathway however the main driver mutations occurs either through the inactivation of the tumour suppressor gene (APC) or through the activation of the proto-oncogene (CTNNB1) (66). This pathway consists of three subtypes, 1. Canonical pathway 2. Noncanonical planar cell polarity pathway and 3. Noncanonical WnT/calcium pathway. The canonical pathway is the most understood pathway(Figure 3).

When there is absence of WnT signalling during normal cell function the following processes occur. Beta catenin forms a destruction complex that contains proteins such as AXIN and APC along with GSK3beta and CK1alpha. This is then subjected to phosphorylation and degradation which allows the regulation of gene expression in the cell nucleus and subsequent suppression of WnT signalling.

However, if there are mutations in APC or CTNNB1 then this subsequently leads to disruption and inactivation of the destruction complex that leads to the accumulation of beta catenin in the nucleus that initiates the transcription of genes linked to tumourigenesis (79, 80).

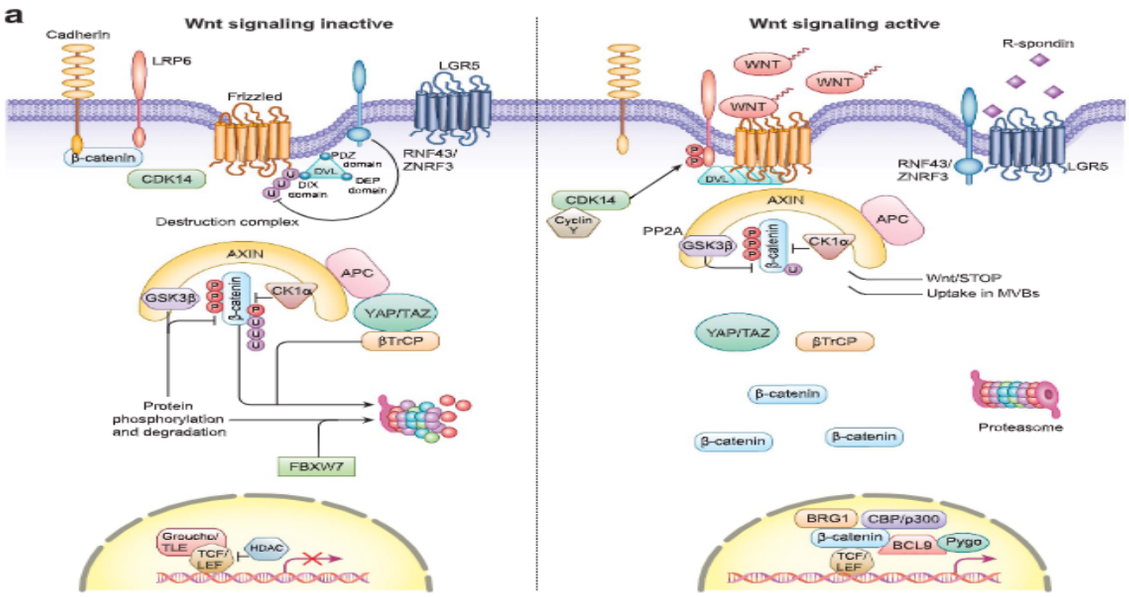


Figure 3: Canonical WnT signalling [80]

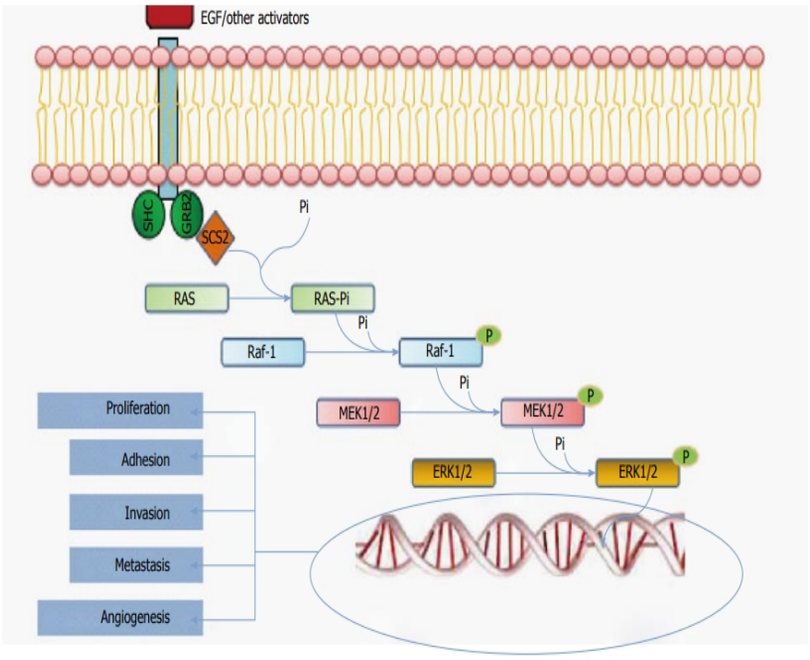
Abbreviations: FZD: Frizzled receptor; LRP5/6: Low-density lipoprotein receptor-related protein 5/6; DVL: Dishevelled protein; GSK36: Glycogen synthase kinase 3 beta; APC: Adenomatous polyposis coli protein; CK1α: Casein kinase 1 alpha; 6-catenin: Beta-catenin; TCF/LEF: : T-cell factor/lymphoid enhancer factor; Axin: Axis inhibition protein; TCF7L2: Transcription factor 7-like 2; R-Spondin: Roof plate-specific spondin; R-Spondin: Porcupine protein; LGR: Leucine-rich repeat-containing G-protein coupled receptor; RNF43: Ring finger protein 43; ZNRF3: Zinc and ring finger 3; YAP: Yes-associated protein; LEF (lymphoid enhancer factor) ; TLE/Groucho: transducing-like enhancer protein

"Material from: Zhan T, Rindtorff N, Boutros M, Wnt signalling in cancer, Oncogene, published [2017], [Publisher: Springer Nature, CC BY license]"

1.4.2.2.2 MAPK/ERK pathway:

The Mitogen- Activated Protein Kinase pathway (Figure 4) is key in the control of the cell cycle and is a critical mechanism for cell signal conduction and for transmitting signals from the extracellular environment into the cell nucleus where specific genes are activated for cell growth, cell proliferation, migration and apoptosis or programmed cell death (81). The activation of this pathway occurs when a signalling molecule binds to a protein receptor tyrosine kinase (RTK) such as epidermal growth factor receptor (EGFR) on the surface of the cell. EGFR is most frequently altered in carcinoma. This subsequently results in the phosphorylation of a cascade of signalling proteins such as RAS, RAF, MEK and ERK. The family of RAS proto-oncogenes consists of three oncogenes in human beings namely KRAS, NRAS (neuroblastoma RES viral oncogene homolog) and HRAS (Harvey rat sarcoma viral oncogene homolog).

Figure 4: MAPK Pathway [82]



This simplified diagram of the MAPK pathway shows the RAS/Raf-1/MEK/ERK pathway. RAS mutations are found in 36% of serrated polyps, and Raf-1 mutations are found in 9%-11%. These mutations promote gene transcription and cellular growth that results in cellular adhesion, invasion, metastasis, and angiogenesis.

Abbreviations: MAPK: Mitogen-Activated Protein Kinase; RAF: Rapidly Accelerated Fibrosarcoma; MEK: Mitogen-Activated Protein Kinase Kinase; ERK: Extracellular Signal-Regulated Kinase; RAS: Rat Sarcoma; GRB2: Growth Factor Receptor-Bound Protein 2; RTK: Receptor Tyrosine Kinase; EGFR: Epidermal Growth Factor Receptor

BRAF (B-raf proto-oncogene) mutations occur in the RAF oncogene most commonly resulting from a V600E substitution. Uncontrolled cell proliferation occurs due to mutations in RAF and RAF oncogenes that result in constitutive activation of downstream signalling components (82) .

The activation of the MAPK/ERK pathway is frequently observed in CRC due to mutations in the KRAS, BRAF, or PIK3CA genes. Dysregulation of this pathway has been implicated in the development of CRC and resistance to targeted therapies (83).

1.4.2.2.3 PI3K-AKT pathway:

The PI3K-AKT pathway is involved in regulating cell growth, survival, and metabolism. The PI3K-AKT pathway can be triggered by signals that bind to cell surface receptors, such as the EGFR receptor. This initiates a series of events that activate kinases within the phosphatidylinositide 3-kinase (PI3K) family. Consequently, AKT is phosphorylated by PI3K proteins and mTOR complexes. AKT influences several downstream targets that regulate cell growth, differentiation, tumorigenesis, and apoptosis prevention. Alterations in the AKT or PIK3CA proto-oncogenes, or the PTEN tumour suppressor gene, can cause AKT signalling activation and subsequent cancer cell proliferation (84-86).

1.4.2.2.4 TGF-β pathway:

This pathway is involved in the control of several biological processes that include cell proliferation, differentiation, migration, apoptosis, and adhesion.

The TGF-β family of proteins is activated when various ligands bind to transmembrane receptors on the cell surface, leading to the activation of SMAD proteins. SMAD4 protein then moves to the nucleus, where it plays a role in regulating transcription during DNA replication.

TGF-β signalling encourages apoptosis, and mutations in the SMAD genes can lead to a loss of tumour suppression properties, with SMAD4 being the most commonly implicated gene(87).

Loss of heterozygosity (LOH) involving chromosome 18q is connected to mutations in the SMAD2 and SMAD4 genes, which are located on this chromosome (88).

1.4.2.2.5 P53 mediated pathways:

The TP53 tumour suppressor gene produces the p53 protein, which manages gene targets involved in DNA repair, cell cycle arrest, and apoptosis through various pathways (89).

Under normal cell conditions, p53 levels are regulated by negative regulators MDM2 and MDM4. However, when cells experience stress (such as DNA damage), the interaction between p53 and MDM2/MDM4 is disrupted, enabling p53 activation and the corresponding tumour suppression activity (90) .

TP53 mutations lead to a loss of this tumour suppression function. TP53 mutations are more frequently found in carcinomas than adenomas, suggesting they are a later event in the development of CRC via the adenoma-carcinoma sequence, as well as being a crucial driver gene for CRC development (91, 92).

1.4.2.3 Microsatellite instability (MSI):

Microsatellites are short repeating nucleotide base sequences that occur both in noncoding and coding parts of the genome. During DNA replication these microsatellites are prone to slippage and results in loop mismatches (58). MSI refers to the shortening or lengthening of such repetitive sequences that happened during DNA replication. This primarily occurs as a result of DNA polymerase slippage that causes insertion or deletion (Indel) of one or more repeating unit which causes frameshift of the sequence and can also happen as a result of DNA damage due to insults such as methylation, alkylation and base deamination (93).

Mismatch repair (MMR) proteins play a crucial role in the maintenance of normal cell function, recognition and correction of base mismatches and insertion and deletion loops that are randomly generated during the DNA replication process (94). Loss of function of one or more of the mismatch repair proteins leads to MMR deficiency (dMMR) and this leads to accumulation of spontaneous gene mutations that leads to either activation of oncogenes or impairment of tumour suppressor genes (95). If either of these occurs, then this leads to hypermutability and the molecular hallmark of this being an MSI-H tumour.

MSI develops due to the inactivation of DNA mismatch repair genes and accounts for 15 to 20% of sporadic CRC (64). Based on the number of microsatellites associated, MSI tumours have been subclassified into three groups: 1. High MSI (MSI-H) 2. Low MSI (MSI-L) and microsatellite stable (MSS) (64).

Sporadic MSI-H occurs due to somatic hyper methylation of both alleles of MLH1 with the initiating event that could be BRAF mutation leading to tumours that could share characteristics from MSI and serrated neoplasia pathway. CRC with MSI-H status is more frequently reported in the proximal colon and in contrast to MSI-H tumours CIN tumours are not hypermethylated and MSS (74).

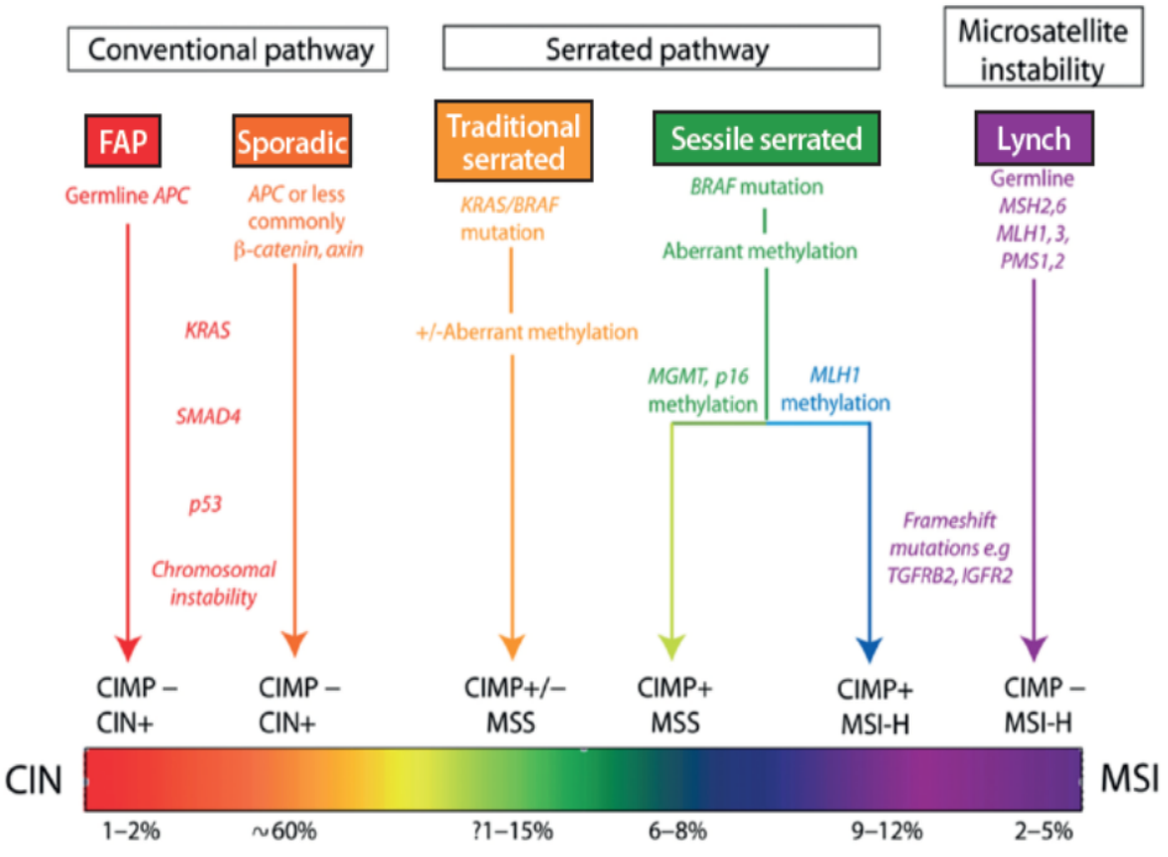


Figure 5: Molecular Pathways to CRC development [98]

Abbreviations: CIN: Chrmosomal instability; MSI: microsatelite instability; FAP: Familial adenomatous polyposis; CIMP: CpG island methylator phenotype; MSS: microsatelite stable; IGF2R: insulin-like growth factor 2 receptor; MGMT: 06-methylguanine-DNA methyltransferase; TGFR62: transforming growth factor receptor beta 2

"Reproduced from [British Society of Gastroenterology position statement on serrated polyps in the colon and rectum, James E East, Wendy S Atkin, Adrian C Bateman, Susan K Clark, Sunil Dolwani, Shara N Ket, Simon J Leedham, Perminder S Phull, Matt D Rutter, Neil A Shepherd, Ian Tomlinson, Colin J Rees, 66 (7): 1181–1196, Gut. 2017 Jul;] with permission from BMJ Publishing Group Ltd."

Inherited germline mutations can also occur in mismatch repair genes (MSH1, MSH2, MSH6 and PMS2) which predispose to Lynch syndrome which accounts for about 20 % of MSI CRC (64).

Hereditary CRC-Lynch syndrome results from the germline mutation of one of the two alleles of any MMR gene followed by somatic inactivation of the other wild-type allele (96). Hence Lynch- CRC can develop from precursor lesions by the adenoma carcinoma pathway with mutations in APC, CTNNB1 or TP53 as well as being MSI-H and not from a serrated precursor.

1.4.2.4 CpG-island methylator phenotype (the serrated pathway):

DNA methylation is a physiological process that has a wide-ranging functions that include genomic imprinting, the timing of DNA replication and in the regulation of chromatin and structure and gene transcription (81). In some circumstances DNA methylation can lead to silencing of a gene. CpG sites are regions in the DNA where a cytosine nucleotide occurs next to a guanine nucleotide separated by a phosphate group in the linear sequence of bases along its length (C-Phosphate-G).

The regions of the genome that have a high concentration of CpG sites are called CpG Islands (97). These islands are found in the promoter regions of DNA that usually initiates transcription of a particular gene and DNA replication. In the normal physiological state CpG Islands are not methylated however methylation of CpG sites (addition of methyl group) also known as hypermethylation within promoters of genes can lead to the inactivation of promoter sequence and corresponding gene that can cause loss of gene function or inactivation of the gene silencing (95).

As many tumour suppressor genes such as P16 and MLH1 harbour CpG Islands in the promoter regions, CpG island methylation is a potential mechanism for carcinogenesis (81, 98). Methylation is one of the hallmarks of serrated pathways to colorectal carcinogenesis. The serrated molecular pathway will be discussed in detail in the following section 1.4.

1.4.3 Transcriptional subtypes and the relation to conventional subtypes:

CRC is a heterogenous disease with distinctive gene expression patterns. The standard classification of CRC described above is a crucial starting point for comprehending the molecular mechanisms behind CRC and directing therapeutic approaches and response patterns. Nonetheless this classification does not cover the range of CRC phenotypes or the impact of the transcriptional landscape on these molecular genetic phenotypes (64). Based on transcriptome classification (67) CRC can be classified into the consensus molecular subtypes (CMS) that has widened understanding of the biological and molecular properties of the various types of CRC. Based on this around 80- 90% of CRC falls into one of the four major transcriptional subgroups termed as CMS 1-4 (Figure 6) with the remaining cases of CRC being heterogenous with indeterminate gene expression patterns (64). The CMS classification not only assists in classifying CRC into molecular pathways but also helps to understand the site of the colon at which the cancer originates (99).

1. CMS1 or MSI immune, is characterised by hypermutated state, CIMP-H, high MSI, BRAF mutation and increased expression of genes associated with diffuse immune infiltrate and clinically has good prognosis however worse survival after relapse. This is also predominantly located in the right colon.

2. CMS2, this is the canonical subtype and characterised by CIMP-H/MSI-L or MSS, CMS 2 has a strong epithelial signature with marked activation of WnT and MYC pathways.

3. CMS3, Metabolic Subtype: this is characterised by deregulation of metabolic signature pathways.

4. CMS4, Mesenchymal Subtype: this is the worst overall survival among all stages and worst relapse-free survival amongst those who initially stage I-III. This is enriched for signatures of epithelial-mesenchymal transition (EMT), pro-EMT transforming growth factor beta and angiogenic pathways.

Figure 6: Consensus molecular subtypes (CMS) classification [101]

	CMS1	**CMS2**	**CMS3**	**CMS4**
Alternate name	MSI immune subtype	Canonical subtype	Metabolic subtype	Mesenchymal subtype
Incidence	14%	37%	13%	23%
Molecular features	MSI, CIMP-high, hyper mutation	SCNA high	Mixed MSI status,SCNA low,CIMP low	SCNA High
Mutations	BRAF		KRAS	
Signatures	Immune infiltration and activation	WNT and MYC activation	Metabolic dysregulation	Stromal infiltration,TGF-βactivation, angiogenesis
Clinical features	Worse survival after relapse			Was relapse free and overall survival
Potential precursor lesion	Serrated	Tubular adenoma	Uncertain (tubulovillous adenoma with serrated features)	Serrated
Location	Proximal colon 	Distal Colon rectum 	No specific localisation 	Distal Colon rectum 

Abbreviations: CMS: Consensus Molecular Subtypes; MSI: Microsatellite Instability; CIMP: CpG Island Methylator Phenotype; BRAF: B-Raf Proto-Oncogene, Serine/Threonine Kinase; SCNA: Somatic Copy Number Alteration; KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog; TGF-ß: Transforming Growth Factor Beta.

1.4.4 Anatomical distribution of CRC: (Right sided versus Left sided CRC)

CRC is a heterogeneous disease that arises from different regions of the colon and rectum, which have distinct embryological origins and different molecular characteristics. There is no uniform definition of the dividing point between right and left CRCs and the most common distinction defines cancers proximal to the splenic flexure as being right-sided or proximal CRC and that distal to the splenic flexure as left-sided or distal CRC (66, 99).

In 1990, Bufill (100) proposed the existence of two distinct categories of CRC according to the location of the tumour in the proximal and distal segments of the large bowel and his review article was the first to comprehensively review the evidence in favour of this concept (101). Subsequent research has shown distinct differences in epidemiology, pathogenesis, molecular pathways, alterations in genetic and epigenetic factors and clinical outcomes depending on the location of the CRC and hence understanding the differences between the two is important in the management of CRC (102).

Though it may be convenient to categorise CRC into proximal or distal location relative to splenic flexure any definition related to the anatomical region of the colorectum cancer is an oversimplification (101) and the molecular features are responsible for determining the phenotype of the tumour whereby there is likely to be overlap between right and left-sided cancers (101).

The potential factors related to differences between the normal right and left colon include:

1. Differences in embryological origin,

2. Apparently distinct environmental milieus such as the

a). Difference in mucosal microbiota such as bacterial biofilms (defined as mucin layers with associated bacteria on the luminal surface of the colonic epithelium) where majority of invasive bacterial biofilms were present in CRCs proximal to the hepatic flexure (99) and

b). Differential bile acid levels where epidemiological studies have shown that the luminal level of bile acids in the metabolites can be carcinogenic and epidemiological studies have called related elevated faecal bile acid levels with increased CRC incidence. There is a tenfold increase in concentration of primary bile acid conjugated colon cancer in the right colon compared to the left colon and therefore the differential concentrations of bile acid in the right and left colon may contribute to the differential mechanisms in tumour formation.

3. Differential gene expression and methylation in the normal right and left colon. The right colon has a higher expression of cytochrome P-450 family genes than the left colon and there are significant differences in the pattern of gene methylation between the right and left colon (99). The prevalence of promoter methylation of hMLH1 mismatch repair genes and MGMT (O-6-methylguanine DNA methyltransferase) is significantly greater than normal right colon mucosa in older women (103) which indicates that the epigenetic aberrations increased neoplastic right colon mucosa that may be reflected in the subsequent biology of right-sided CRC (99).

A summary of the differences in right and left sided CRC is shown in Figure 7.

1.4.4.1 Right-sided CRC

Epidemiology: Sporadic right-sided CRC occurs predominantly in females and older people(101, 104).

Morphology and histology: right-sided CRCs are more likely to have flat morphology that may be difficult to detect (105, 106). These cancers tend to exhibit different histology such as origin from sessile serrated lesions or mucinous adenocarcinoma. They also tend to have advanced tumour pathology and are often poorly differentiated mucinous tumours. Mucinous adenocarcinomas have a faster progression in comparison to adenomatous polyps and are commonly seen in inflammatory bowel disease (74).

Genetics: Right CRCs have more microsatellite instability high (MSI-high) tumours which are characterised by mutations or inactivation of DNA mismatch repair system. Sessile serrated lesions and mucinous adenocarcinoma are frequently observed in the right side and are MSI high and have mismatch repair deficiency (17). Hereditary cancer syndromes with the exception of FAP tend to occur in the right side of the colon particularly in Lynch syndrome (102).

Presentation: This tends to present at a higher TNM stage and larger tumours are more of the mucinous type.

Immunology: MSI high tumours also have more T cell infiltrates and hence carry more immunogenic mutations in histologically many of these tumours have shown clones like lymphoid reaction in the invasive front of the tumour. Presence of T cells in these tumours has a better prognosis and a low chance of metastases.

Metastases tend to occur in the peritoneum (107). Targeted therapies-MSI high tumours which are located mainly in the right side are highly activated lymphocytic microenvironment and have a high degree of neoantigens (74).

Tumours with high antigenic load seem to benefit from immunotherapy. Hence immunotherapy is a promising therapeutic option for MSI high tumours resistant to systemic chemotherapy (108).

Clinical outcomes: Higher incidence of poorly differentiated tumours, mucinous histology, and a higher frequency of BRAF mutations. It is also associated with a higher risk of developing synchronous and metachronous tumours in other regions of the colon, and it has a worse prognosis compared to left-sided CRC. Right-sided CRC is more commonly observed in older patients and is more often diagnosed at an advanced stage (107). This may be attributed to delay in symptomatic of the earliest stage for example bleeding is less frequently associated with right-sided colon cancers and more so with advanced staging abdominal mass or obstructive symptoms are common in this group (104).

1.4.4.2 Left-sided CRC

Epidemiology: The incidence is higher than right sided CRC though has been decreasing in incidence over the years and more common in males. Left-sided CRC is more commonly observed in younger patients and is more often diagnosed at an early stage. Patients with left-sided CRC have a better prognosis compared to those with right-sided CRC (102).

Morphology and histology: Left-sided CRC is characterized by a higher incidence of well-differentiated tumours, signet-ring cell histology, and a higher frequency of KRAS mutations. They are more likely to be polypoid in morphology and hence easier to detect in the early stages (104) in comparison to the flat morphology that is difficult to detect in the right side.

Presentation: They usually present at a lower TNM stage and are smaller tumours.

		CIMP-H	MSI-H	MLH1 methylation	BRAF mutation	CIN
Pre-neoplastic lesions	Sessile serrated lesions (right sided)	+	+/-	+/-	+	-
	Conventional adenoma (right and left sided)	-	-	-	-	+
CRCs	Right-sided CRC	High prevalence	High prevalence	High prevalence	High prevalence	Low prevalence
	Left-sided CRC	Low prevalence	Low prevalence	Low prevalence	Low prevalence	High prevalence

Table 1: Molecular features of preneoplastic lesions and CRCs by anatomical site - adapted from[99]

Abbreviations: CRC-colorectal cancer; CIMP-CpG island methylator phenotype; MSI-microsatellite instability; CIN-chromosomal instability; MLH: MutL homolog.

Genetics: common site for CRC and FAP. They predominantly follow the CIN molecular pathway are CIN high tumours (17).

Clinical outcomes: They have an overall better survival outcome. There is a stark difference in genomic make-up of right and left CRC (Table 1) Right sided CRC tends to be microsatellite instability high tumours and left tend to have CIN high tumours (17).

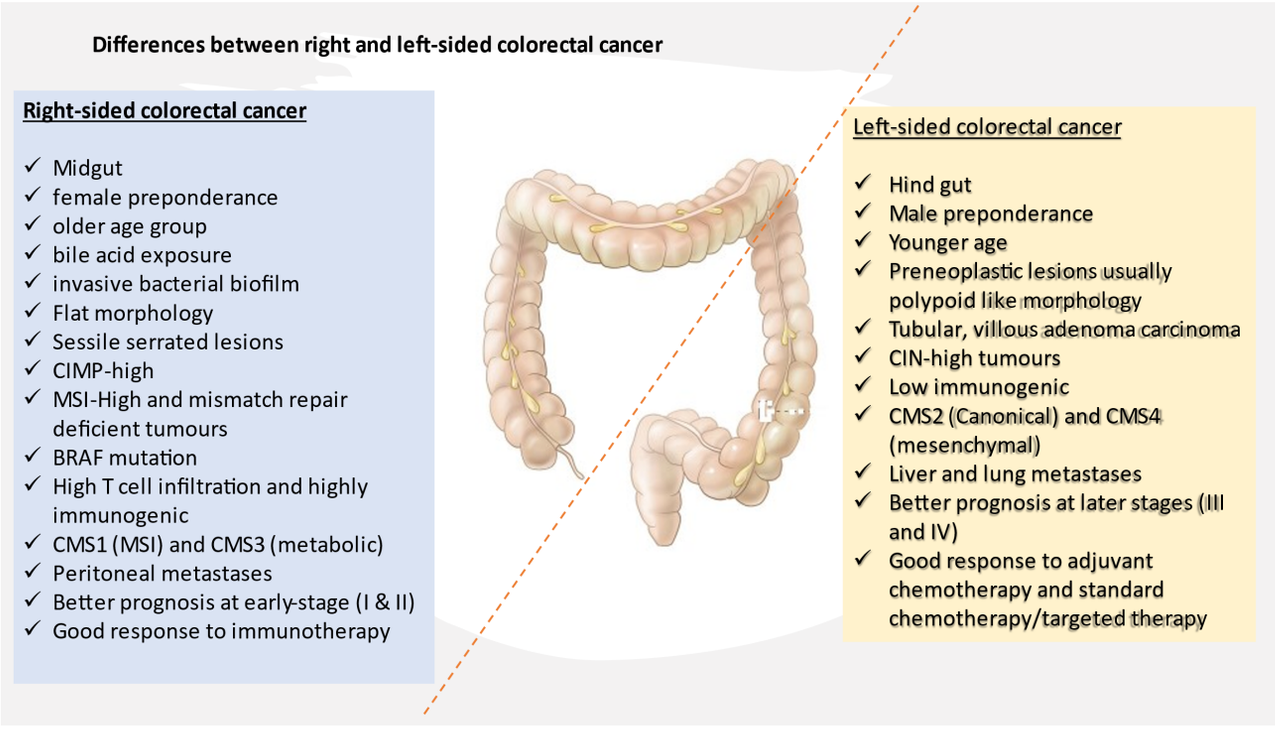


Figure 7: Summary of the differences between right and left-sided CRC (100, 104, 111)

In summary, right and left sided CRC have markedly different molecular characteristics (Figure 7) with right-sided CRCs being CIMP high, MSI high and BRAF mutated cancers which is different from left-sided (distal) and rectal cancers (Table 1).

The characteristics are also included in the CMS1 genomic subtype which is predominantly present in the right sided CRC and also in the CMS3 subtype which again is predominantly present in right sided CRCs (99). In addition to the differences in molecular characteristics, anatomical, embryological and biological differences exist between right-sided and left-sided CRC.

Understanding the location of CRC has a key role in metastatic cancer as this is increasingly being recognised as a predictor marker of response to anti-eGFR drugs (109). Right-sided and left-sided CRC tumours exhibit different histological and molecular characteristics. The location of the tumour in the colon and rectum plays an important role in the clinical and molecular characteristics of CRC. Understanding these differences is important for tailoring treatment strategies and improving patient outcomes.

1.4.5 Hereditary CRC

Hereditary CRC accounts for 3 to 5% of all CRCs and develops in patients that have germline mutations associated with well-defined cancer predisposing syndromes. These syndromes include familial adenomatous polyposis (FAP) and Lynch syndrome.

FAP occurs in less than 1% of cases of CRCs and is caused by mutations in the adenomatous polyposis coli (APC) gene which controls the activity of the WnT signalling pathway (110).

This disease is characterised by a very large number of colorectal adenomas that develop in the colon that predisposes the individual to CRC at a very young age.

Lynch syndrome is the most common form of hereditary CRC constituting 1 to 3% of cases and is characterised by microsatellite instability (MSI) as a consequence of a germline mutation in a DNA mismatch repair (MMR) gene (111, 112). It is an autosomal dominant condition affecting males and females in the same family equally. The cancers associated with Lynch syndrome tend to affect the caecum or the right colon and constitute 70% of the 40% of the sporadic cases. They appear in polyps or adenomas which are large and flat with a high degree of dysplasia and possess hereditary mutations in MMR, MSH 2, MLH 1, MSH 6, PMS 2 and PMS 1 repair genes.

Patients with Lynch associated CRC develop a finite number of adenomas which could become malignant in a short period of time compared to those with FAP where the polyps are more diffuse. The tumours most often show a mucinous histology with lymphocytic infiltration and are usually poorly differentiated having similar characteristics to sporadic tumours that have high microsatellite instability. Lynch syndrome occurs due to mutation in one of the DNA mismatch repair genes (MMR) which are MLH1, MSH1, MSH2, MSH6, PMS2 or EPCAM deletion mediated MSH2 hyper methylation (6, 110, 113).

This impaired mismatch repair that occurs during replication leads to an accumulation of DNA mutations particularly in microsatellite DNA fragments with repetitive nucleotide sequence.

This MSI can be identified by polymerase chain reaction (PCR) testing that compares normal and tumour DNA of the same patient. Patients with Lynch syndrome historically used to be identified by clinicopathological criteria such as Amsterdam and Bethesda criteria however more recently clinical practice has shifted to using MSI PCR and immunohistochemistry for lack of expression of MMR proteins (6, 113). The other rare forms of hereditary CRC include hamartomatous polyposis syndrome (i.e., Peutz-Jeghers syndrome, juvenile polyposis and Cowdens disease) and MYH-associated polyposis (MAP) which constitutes less than 1% of all CRCs globally (59).

1.4.6 Family history of CRC:

In the Western world, the average risk of developing CRC in the general population is estimated to be between 3 and 5%. The average lifetime risk of CRC has been estimated to have a 4.7% for men and 4.4% for women (114). However, this risk increases significantly for individuals with a first-degree family member who has been diagnosed with CRC, particularly if the diagnosis occurred between the ages of 50 and 70 years of age. In these cases, the risk of developing the disease nearly doubles, and it triples if the relative was diagnosed before age 50. Furthermore, individuals with two or more affected family members have an even higher risk of developing CRC. Low penetrance genetic factors contribute to this increased risk in cases of sporadic CRC with affected family members (6). As a result, a positive family history is a factor in approximately 15 to 20% of CRC cases.

1.4.7 Probable Non-Genetic Risk factors for CRC

Consumption of red and processed meat, obesity, alcohol and smoking are all linked to an increased risk of CRC (4).

There is evidence that physical activity, consuming wholegrains, fibre, and calcium supplements reduce the risk of CRC. While the review reported a nonsignificant reduction in CRC mortality, low-dose aspirin significantly reduced mortality from proximal CRC by 66% (115). In summary, CRC is a heterogenous disease that has established molecular pathways to carcinogenesis. There are differences between proximal and distal CRCs. The CMS classification aids in defining the molecular pathways are clinical prognostic factors associated with this.

1.5 The pathogenesis of serrated neoplasia

It has been estimated that approximately 15 to 30% of all CRCs arise from a range of precursor serrated lesions (71, 97, 116). These lesions histologically are characterised by a serrated or saw-toothed appearance of epithelial glandular crypts within the precursor lesions which have previously been thought to lack malignant potential (81, 97, 117). Current literature shows that serrated lesions could be responsible for post colonoscopy CRC (PCCRC) or interval cancers and are associated with synchronous and metachronous advanced colorectal neoplasia (62, 118).

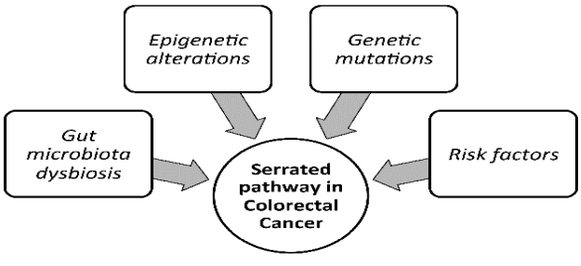


Figure 8: Factors contributing to the serrated pathway in CRC [62]

The serrated pathway to carcinogenesis involves a combination of molecular genetic factors that include epigenetic alterations and genetic mutations along with risk factors that can trigger the above mechanisms such as environmental, lifestyle, presence of inflammation and alteration in gut microbiota (Figure 8).

This section outlines the pathogenesis of serrated neoplasia and includes an outline of histopathology (a further detailed review will be described in chapter 3). It goes on to summarise the molecular genetic pathways that lead to carcinogenesis including the molecular subtypes of CRC in relation to precursor serrated lesions and finally describes the risk factors associated with serrated neoplasia.

1.5.1 Histopathological classification of serrated polyps:

Serrated neoplasia is histologically classified by the recent updated WHO criteria 2019 (119) into 3 morphological categories. This includes, 1. Hyperplastic polyp (HP) which further includes microvascular type (MVHP) and goblet cell-rich type (GCHP).

The 2010 WHO criteria also included another subtype which was mucin poor type (MPHP), but this is now deleted from the terminology. 2. sessile serrated lesion (SSL) and SSL with dysplasia (SSLD) 3. traditional serrated adenoma (TSA) 4. serrated adenoma unclassified (this is a new entity).

These subtypes have distinct endoscopic appearance (described in Chapter 1.5), share some histological features, and have unique molecular pathology and biology. The various histological types and characteristics will be described in detail in chapter 3. The molecular features of individual serrated precursor lesions along with pathways will be discussed in this chapter.

1.5.2 Molecular Pathways-the serrated neoplasia pathway:

In the previous Section 1.3, the molecular pathways of conventional adenomas including a detailed description on signal induction pathways and brief introduction into CIMP was described. This chapter will mainly focus on the molecular pathways involved in the serrated neoplasia pathway.

The serrated neoplasia pathway leads to CRC through a range of precursor serrated lesions (Figure 9). At a molecular level, there are multiple interrelated serrated pathways to carcinogenesis. The serrated neoplasia pathway combines three major molecular mechanisms that include the CpG island methylator phenotype (CIMP) , the major mechanism that drives the serrated neoplasia pathway (120) and microsatellite instability pathway (MSI) which are both consequences of epigenetic DNA changes and the alteration of the MAPK pathway (121). As precursor serrated lesions progress through the pathways from the early-stage to the malignant stage, (Figure 9) they are characterised by molecular signatures (81) at each stage. The molecular mechanisms that underpin the serrated neoplasia pathway have been described below:

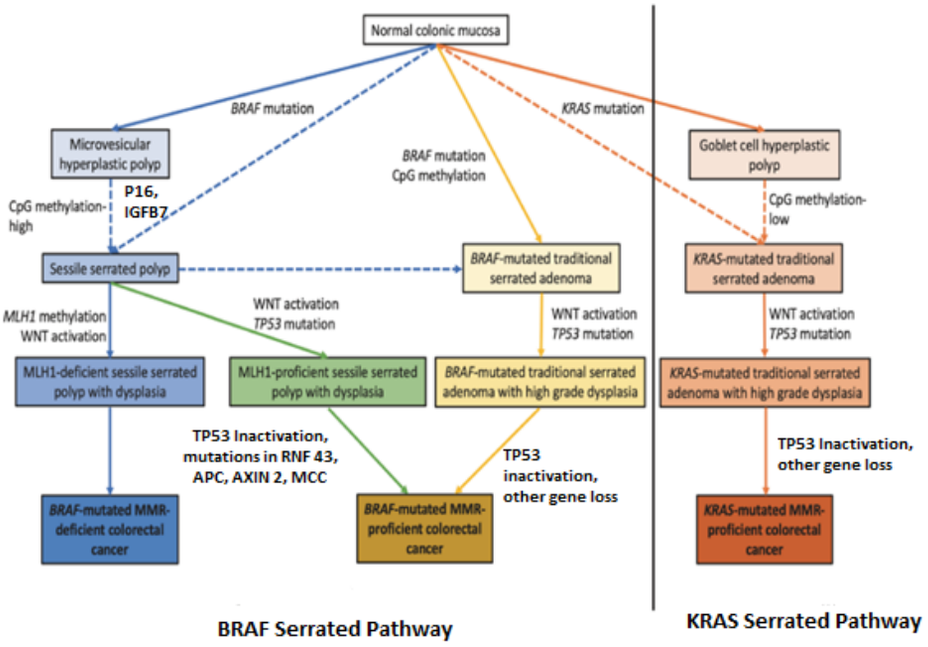
1. MAPK pathway activation (Figure 4): MAPK pathways moderate the extracellular signals from the cell surface to the nucleus to control growth, proliferation, differentiation, migration, and apoptosis. Activation of the MAPK-ERK pathway induces apoptosis arrest, proliferation of colonocytes and overexpression of P16 and IGFBP7 (122). This initiating event results in BRAF/KRAS mutation that leads to the constitutive activation of this pathway causing the normal colonic mucosa to develop into either MVHP, SSL, TSA or GCHP (73, 123-125).

Moreover BRAF and KRAS activity mutations have been found to be mutually exclusive in both the precursor lesions and CRCs (126).

2. Following BRAF mutation, aberrant promoter hyper methylation of CpG Islands occurs and is the major oncogenetic mechanisms that drives the serrated pathway to colorectal neoplasia. The MLH1 gene and its promoter methylation is the most relevant and is found in 75% of cases (127).CpG island methylator phenotype (CIMP) can either be at high level (CIMP-H) or low level (CIMP-L).

3. Sporadic MSI Pathway: This pathway is complex and involves multiple interrelated pathways that overlap with the MSI pathway which is not fully understood however eventually lead to CRC (Figure 9). Sporadic MSI-H occurs due to somatic hyper methylation of both alleles of MLH1 that leads to CRC (64).

Figure 9: The serrated pathway to carcinogenesis[131]



Abbreviations: MLH1: MutL Homolog 1; APC: Adenomatous Polyposis Coli; KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog; BRAF: B-Raf Proto-Oncogene; CIMP: CpG Island Methylator Phenotype; MMR: mismatch repair

"Reprinted from Modern Pathology, Vol 32/ edition number 10, Author (s) Rish K. Pai, Mark Bettington, Amitabh Srivastava, Christophe Rosty, An update on the morphology and molecular pathology of serrated colorectal polyps and associated carcinomas, Pages No. 1390-1415., Copyright (2019), with permission from Elsevier."

BRAF Serrated Pathway: Microvesicular hyperplastic polyp and sessile serrated lesions share molecular features with the initiating event being BRAF mutation that occurs by activation of the MAPK pathway. CRC that arises from these polyps typically have high levels of methylation that results in CIMP. Following BRAF mutation, there are two routes in which carcinogenesis can occur: One route is through the MSI pathway that leads to methylation of the mismatch repair genes MLH1 which is a key epigenetic change in this pathway that then leads to sporadic CRC (BRAF mutated, MMR deficient or MSI high and hyper methylated (CIMP-high tumours). These CIMP high tumours tend to occur in the proximal colon with a preponderance in the older age group and women (128). In contrast to CIN tumours that are usually MSS and are not hypermethylated (74).

The other route to colorectal carcinogenesis in the BRAF serrated pathway is the mutations that results in inactivation of TP53 gene and methylation of other genes such as MCC, AXIN2 and SFRP (129) involved in the WnT pathway. Unlike in conventional adenomas where APC inactivation occurs, in the serrated neoplasia pathway APC mutation is less common (64, 73). Following this, BRAF mutated, MSS, CIMP-H CRC that generally has a poorer prognosis. SSLs or TSAs are the precursor serrated lesion in this type of CRC.

TSAs can arise as a result of BRAF mutation or KRAS mutation and progression through dysplasia leads to CRC that is microsatellite stable (MSS). BRAF mutated TSA can either arise denovo from normal colonic mucosa or from SSL and the KRAS counterpart TSA can arise de novo or from a goblet cell hyperplastic polyp.

The following describes the molecular features of precursor serrated lesions.

1.5.3 Molecular features of serrated precursor Lesions:

The molecular features of precursor serrated lesions are summarised in Table 2.

1.5.3.1 Hyperplastic polyps (HP):

These are histologically subclassified into goblet cell hyperplastic polyp (GCHP) and Microvesicular hyperplastic polyp (MVHP) as per the updated WHO classification (119). MVHP is molecularly characterised by BRAF V600E which induces the constitutive activation of the MAPK pathway that leads to proliferative activity and inhibition of apoptosis (81).

They also have CIMP-H without promoter methylation of MLH1 gene and hence do not have microsatellite instability (MSS). They are therefore considered to be a precursor of sessile serrated lesions (130, 131). GCHP is linked to KRAS mutation and is CIMP-Low and is MSS (131).

1.5.3.2 Sessile serrated lesions (SSLs):

At a molecular level where the precursor lesion is Microvesicular hyperplastic polyp (MVHP) this has the characteristic of having BRAF mutation, CIMP-H, MSS and unmethylated MLH1 and is the molecular hallmark of the sessile serrated pathway that subsequently leads to carcinogenesis (Figure 9) (Table 2). However the mechanism that underlies spontaneous hypermethylation from normal colonic mucosa to SSL that results from BRAF mutation and CIMP-H that synergistic facilitate carcinogenesis, is poorly understood (122).

1.5.3.3 Sessile Serrated Lesion with Dysplasia (SSLD):

SSLDs exhibit BRAF V600 mutation along with CIMP-H which is regarded as the molecular hallmark of the serrated neoplasia pathway.

Seen in Figure 9, SSLD develop from 2 pathways, one by MLH1 gene silencing leading to MSI and secondly by activation of the WnT signalling pathway which usually occurs with the CIN pathway leading to MSS and the progression of an SSL to a dysplastic SSL (81).

CIMP-H causes genetic silencing of tumour suppressor genes by hypermethylation of their promoter regions (132). MLH1 gene silencing (133) causes an SSL to acquire dysplasia and leads to MSI-H status. The acquisition of MLH1 silencing and MSI status in SSLs is the molecular hallmark of progression of dysplastic SSLs into CRC. MLH1 hypermethylation and CIMP-H pattern also occurs in the elderly who have large SSLs in the proximal colon (122, 134, 135).

As a result of MSI-H status these lesions also harbour other different genetic mutations (132) this includes increased mutational rate of FBXW7 and alterations in the WnT signalling pathway associated genes such as the protein truncating mutations of RNF43, APC, ZNRF3 and the hypermethylation of AXIN2, MCC (122, 134, 136-138). The SSLD that is MLH1 proficient or MSS display the TP53 mutations without FBXW7 mutations (62). The serrated pathway has two end results i.e., the serrated adenocarcinoma or the sporadic colorectal carcinoma showing molecular features of MSI-H. Both of these differ in their molecular profile, prognostic and clinical characteristics (122).

1.5.3.4 Traditional Serrated Adenomas (TSA):

There are two different pathways recognised in the molecular pathogenesis of TSA. The first pathway is the KRAS mutation pathway and the second pathway BRAF mutation pathway (Figure 9) (139, 140).

The majority of the TSAs are located in the distal colon and follow the KRAS mutation pathway. These TSAs are usually CIMP-L or CIMP-H and are MSS (139, 140). Conversely, the TSAs that follow the BRAF mutation pathway are most likely located in the proximal colon and either undergo transformation from normal colonic mucosa or from an SSL.

They show features of CIMP-H and MLH1 expression is preserved and hence MSS. Both BRAF/KRAS mutated TSAs can progress to carcinoma by the TP53 inactivating mutations (71). In both the pathways, there is nuclear β-catenin accumulation and impairment of the WnT signalling pathway that leads to the transition to a dysplastic TSA (122, 139, 140).

Table 2: Molecular features of precursor serrated lesions modified from (62)

Molecular features of precursor serrated lesions

	BRAF Mutation	KRAS Mutation	CpG island methylation	Gene Methylation	MSI rate
Microvascular hyperplastic polyp	70 - 80%	0%	CIMP-H	MLH1 not methylated	MSS
Goblet cell hyperplastic polyp	0%	50%	CIMP-L	MLH1 not methylated	MSS
Sessile serrated lesion	>90%	0 - 5%	CIMP-H	MLH1 not methylated	MSS
Sessile serrated lesion with dysplasia	>90%	0%	CIMP-H	MLH1 Hypermethylated	MSI
Traditional serrated adenoma	20 - 40%	50 - 70%	CIMP-H / CIMP-L	MLH1 not methylated	MSS
Sessile serrated adenoma unclassified	Uncertain	Uncertain	Uncertain	Uncertain	Uncertain

However, WnT pathway activation that occurs in TSA differs from conventional adenomas in that it does not occur due to APC inactivation but probably due to PTPRK-RSPO3 fusions or RNF43 mutations (141). PTPRK-RSPO3 fusions-positive TSAs are usually KRAS mutated and located in the distal colon whereas RNF43 mutations are more likely to be found in the BRAF mutated TSA (142, 143).

There are no specific biomarkers for TSA however a recent study (144) (122) demonstrated that there was an overexpression of LEFTY1 which is a protein that down regulates the TGF-β pathway in TSAs.

1.5.4 Molecular Subtypes of CRC and the role of precursor serrated lesions

The precursor lesions of SSLs and TSAs show unique molecular features that can be matched to the consequential molecular subtypes of CRC (145). In 2015, Phipps et al (146, 147) proposed a CRC classification of five molecular subtypes based on CIMP, MSI status and KRAS/BRAF mutation status with successful validation of prognostic significance in large scale cohort studies (Figure 10). The molecular subtypes were defined into five types as follows (Figure 10):

Type 1: MSI +, CIMP+, BRAF-mutated, KRAS-wild-type,

Type 2: MSI -, CIMP+, BRAF-mutated, KRAS-wild-type,

Type 3: MSI -, CIMP-, BRAF-wild-type, KRAS-mutated,

Type 4: MSI -, CIMP-, BRAF wild-type, KRAS-wild-type,

Type 5: MSI +, CIMP-, BRAF- wild-type, KRAS-wild-type,

The prognosis of the above-described subtypes was ranked from best to worst as the following order: types 1,5,4,3,2 (145-147). The types that originate from serrated precursor lesions are 1, 2 and 3.

Type 1 CRC indicates sporadic MSI-H caused by promoter methylation associated MLH1 silencing. The majority of type 5 CRC arises as Lynch syndrome whereby there is hereditary/germline MSI-H that occur due to mutations in one of the MMR genes. Since type 1 and 5 have MSI-H, they have a favourable prognosis and survival outcome (132).

Type 2 CRCs have molecular features similar to SSLs that are MSS (MLH1 proficient) and BRAF mutated TSAs that are located in the proximal colon and have the worst prognosis compared to the other types (146, 147). Type 3 CRC also has a poor prognosis though survival is slightly better than type 2 CRC. The precursor lesions include distal Colon TSA that are mostly KRAS mutated along with other types that potentially harbour KRAS mutation i.e. unclassified serrated adenomas, serrated Tubulovillous adenoma, superficial serrated adenoma and can also include some subsets of conventional adenomas (145) (Figure 10). Type 4 CRC has the common subtype that develops through the classical adenoma carcinoma sequence molecularly characterised by a chromosomal instability and hence the precursor lesions are subtypes of conventional adenomas (145).

Types 1, 2 and 3 whereby the main precursor is an SSL can either have the best or the worst prognosis. Though type 1 and 2 CRC share CIMP and BRAF mutation, the difference in prognosis is related to the presence of MSI (145). (MLH 1 methylation). If an SSL has undergone MLH1 methylation, then this lesion is at high risk of progressing to an advanced lesion (SSLD) which can then transform into a type 1 CRC that has a favourable prognosis. However, if the SSL progresses to a high-risk dysplastic lesion without MLH1 methylation (148) then the eventual progression to a type 2 CRC has a poor prognosis.

Hence the use of molecular and immunohistochemical tests to determine the CIMP status and MLH1 methylation status in those patients who have proximal SSLs could be used as part of risk stratification and inform precision surveillance strategies to reduce the risk of interval cancers (145)

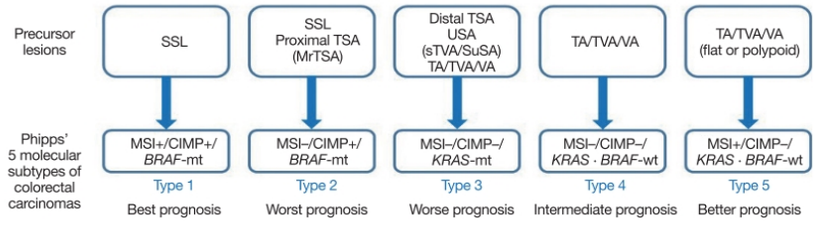


Figure 10: Molecular subtypes of CRC and the role of precursor serrated lesions [145]

Abbreviations: SSL-sessile serrated lesion, TSA-traditional serrated adenoma, TA-Tubular adenoma, TVA-Tubulovillous adenoma, VA-villous adenoma; MrTSA-mucin rich traditional serrated adenoma, USA-unclassified serrated adenoma; sTVA-serrated Tubulovillous adenoma; SuSA-superficially serrated adenoma, MSI-, microsatellite instability negative, MSI +, MSI positive, CIMP-CpG island methylator phenotype, CIMP +, CIMP positive; CIMP -, CIMP negative; mt-mutant type, wt- wild-type

1.5.5 Risk factors for serrated neoplasia

The approaches to reduce CRC incidence include primary prevention strategies such as dietary changes or increasing physical activity and secondary prevention strategies which include screening (149).

The role of various modifiable and lifestyle risk factors has been described for colorectal pathologies particularly colorectal adenomas (150). These include modifiable factors such as alcohol intake, smoking, consumption of processed and red meat and obesity. Non-modifiable factors showing a positive association with CRC include inflammatory bowel disease (IBD), a family history of CRC and advancing age (149).

This section outlines the risk factors for serrated neoplasia in particular and this includes the following:

1. Inherited risk – serrated polyposis syndrome.
2. Lifestyle -related risk factors such as obesity, sedentary lifestyle, Dietary factors, alcohol intake and tobacco smoking, medications (NSAIDs, aspirin, hormone replacement therapy (HRT), folate, calcium).
3. Factors that contribute to epigenetic changes in colonocytes such as ageing, inflammation, microbial dysbiosis that can lead to DNA methylation and eventually CRC.
4. Underlying bowel conditions such as IBD.

1.5.5.1 Serrated polyposis syndrome (SPS)

SPS was first described in 1980 as hyperplastic polyps which were the only recognised serrated polyps at the time and these were considered benign and non-neoplastic lesions (151). Although there were only reports of an association between CRC and SPS they received little attention (81). In 1996 (152) it was demonstrated that there were histological differences between polyps in SPS and sporadic hyperplastic polyps.

SPS is characterised by the presence of multiple serrated lesions distributed throughout the colon that has an increased risk of developing into CRC (122). This is diagnosed by colonoscopy appearance and histological confirmation of the polyps removed. The criteria used for diagnosis has been described by the WHO.

The 2010 World Health Organization (WHO) criteria (153) for SPS encompassed three criteria: (I) presence of at least 5 Serrated Polyps (SP) located proximal to the sigmoid colon, with at least two measuring ≥10mm in size; (II) presence of at least 1 SP located proximal to the sigmoid colon in a patient with a first-degree relative diagnosed with SPS; or (III) presence of >20 SPs distributed throughout the colon.

The updated 2019 (119) WHO SPS criteria now include patients who meet either of the following conditions: (I) presence of at least 5 SPs located proximal to the rectum, all measuring ≥5mm in size, with at least two being ≥10mm; or (II) presence of >20 SPs of any size distributed throughout the colon, with at least 5 being located proximal to the rectum.

The updated criteria now encompass SPs located in the sigmoid colon as well as more proximal regions, as opposed to the previous criteria which only considered SPs above the sigmoid colon and more proximal areas. This revision was informed by several cohort studies conducted in the past decade, which revealed that nearly 50% of CRCs in patients with SPS arise in the rectosigmoid region. It is crucial to note that CRC in SPS can develop from either SPs or adenomas, and this awareness is essential in clinical practice (154).

The reported prevalence of SPS in a screening population is estimated to be 0.5% at baseline colonoscopy increasing to 0.9% on follow up examination as per international studies from CRC screening programs (154-156).

However SPS largely remains underdiagnosed due to lack of awareness of the condition, difficult detection due to the subtle appearance of SSLs, absence of an efficient process to obtain previous colonoscopy/pathology results and poor tracking systems to calculate the individuals cumulative polyp count, size, location of SSLs (154).

In one study (157), the use of high definition endoscopes with chromocolonoscopy (the use of indigo carmine dye spray) showed an increased detection of serrated lesions compared to standard colonoscopy (1.19 vs 0.49 per patient , $p<0.001$).

The genetic background of SPS remains largely unknown however a small proportion of patients less than 3% who have SPS have a germline mutation in RNF43 which is involved in the WnT pathway.

The other genes that are thought to potentially have a role in the pathogenesis of SPS include EPHB2 (158), ATM, PIF1,TELO2 and XAF1 (122). However, the exact role in carcinogenesis remains unconfirmed. SPS appears to be familial and very rarely have a strong autosomal dominant pattern with frequency of germline pathogenic variants in RNF43 reported to be 1.76% (3/170) (154, 159).

Management of SPS is mainly endoscopic clearance by repeated colonoscopies however in those with unmanageable polyp burden, surgical options could be considered. The current recommendations for surveillance as per the BSG (97, 160) is 1-3 yearly after polyp clearance along with recommendations for first degree relatives to have a screening colonoscopy from the age of 35-40 every 5 years till the age of 75.

1.5.5.2 Lifestyle risk factors for serrated neoplasia

Bailie et al (150) showed in their large systematic review and meta-analysis that collectively investigated modifiable lifestyle factors in the influence of the risk of serrated colorectal polyps. The meta-analysis reported statistically significant increased risk of having a serrated polyp associated with smoking, alcohol consumption, obesity, dietary factors and meat intake, with a statistically significant inverse relationship with NSAIDs, aspirin and dietary folate.

1.5.5.2.1 Tobacco Smoking

Studies have consistently identified tobacco smoking as a risk factor for MSI high or CIMP- high CRCs (161) which are part of the serrated pathway. It is also associated with the risk of sporadic serrated neoplasia (162-164) and the risk of SPS (165).

Smoking cessation of 10 years is associated with a decreased risk of all polyps compared with current smokers and the risk was similar to people who had never smoked. This was particularly strongly noted for serrated lesions versus adenomas (166-168). Cigarette smoking status, duration and intensity were associated with increased polyp risk for all types of polyps with a strong association associated with serrated neoplasia (OR 1.74, 95% CI 1.16-2.624 current smokers versus never smoked (166).

In a meta-analysis that compared the highest versus lowest exposure of smoking, there was a 2.5-fold increased risk of serrated polyps (RR, 2.47; 95% CI, 2.12-2.87) (150). And when the risk of SSL was analysed, this risk increased to 3.4 fold (RR, 3.40; 95% CI, 1.90 - 6.07) compared to hyperplastic polyp risk (RR, 2. 34; 95% CI, 2.00-2.73) (150). There was high heterogeneity present in all analysis. The causes for this could be explained at a molecular level. A population-based cohort study(169) that investigated smoking and CRC overall risk by mutation status showed a strong correlation between cigarette smoking and MSI high, CIMP-H and BRAF mutations. Smoking increases the risk of DNA mutations within the cells of the colon that may eventually undergo malignant transformation through the serrated pathway.

1.5.5.2.2 Alcohol

Alcohol is a known risk factor for a number of cancers. Increased alcohol intake revealed a statistically significant 33% increase in the risk of serrated polyps (RR, 1.33 ; 95% CI, 1.17-1.52) for highest versus lowest intakes however this increased to 85% (RR, 1.85 ; 95% CI, 1.03 -1.32) for SSL risk specifically (150) (167, 168).

1.5.5.2.3 Obesity

There are a wide range of inflammatory cytokines produced from adipose tissue, of which some can be procarcinogenic. Those individuals who have high BMI also have a high level of C-reactive protein (CRP). A systematic review in 2008 showed a direct association between CRP and CRC risk (170). And meta-analysis also showed that there was a 42% increased risk of serrated polyps (RR, 1.42; 95% CI, 1.24 -1.63) in those with high BMI versus those with low BMI (150). The meta-analysis also showed that there was almost a twofold increased risk of hyperplastic polyps in individuals with the highest waste to hip ratio and visceral adipose volumes (150).

1.5.5.2.4 Dietary factors

A meta-analysis of observational studies showed a significant increased risk of serrated neoplasia for individuals consuming highest compared to lowest intakes of fat (RR, 1.25; 95% CI, 1.10-1.41), red meat and processed meat (RR, 1.23; 95% CI, 1.07-1.41) with associated low heterogeneity (150). In another study red meat intake was strongly associated with serrated neoplasia risk (OR 2.59, 95% CI 1.41-4.74) highest versus lowest intake with the association being stronger with serrated neoplasia compared to conventional adenoma (166).

Other dietary factors such as calcium, fibre and folate showed reduced serrated neoplasia risk (150) for individuals consuming highest compared with lowest intakes of the above with the latter (folate) showing significance (R, 0.65; 95% CI, 0.49-0.85). Vitamin D intake was not associated with serrated polyp risk (150).

1.5.5.2.5 Medications

The use of non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin associated with a significant 19 to 23% decreased risk of serrated neoplasia corroborating results from a randomised controlled trial of aspirin (150, 171). One RCT demonstrated a significant protective association for proximal and not distal colon for serrated neoplasia when 81 mg or 325 mg of aspirin was taken compared to placebo (150, 171). One study of pooled data from three trials found that aspirin, family history of polyps and folate treatment were all associated with the incidence of serrated polyps in the proximal colon at follow-up (171).

Other factors that associated with serrated neoplasia include female sex, smokers with more than 20 pack year history, diabetes, and obesity (171, 172)

1.5.5.3 Factors that contribute to epigenetic changes in colonocytes

As has been described above certain subsets of colorectal serrated neoplasia have a malignant potential that can progress to CRC. The hallmark of the serrated neoplasia pathway is methylation. There are multiple factors such as environmental and physiological risk factors that contribute to DNA methylation alterations and linked to the risk for CRC development.

These factors (Figure 11) contribute to the early acquisition of epigenetic alterations that occur over a period of time and lead to the dysregulation of signal transduction pathways that cause genetic driver mutations to eventually lead to CRC (64). This includes physiological factors and environmental factors such as ageing, obesity and diet, inflammation and the microbiome (64).

The mechanisms that cause accumulation of age-related hypermethylation or hypomethylation are not entirely clear. However, the constant cell division occurs in the context of environmental exposures (tobacco smoke, infection) and physiological exposures (obesity, hormonal changes) are an important component of ageing. Multiple studies have shown that constant cell divisions lead to methylation changes (64).

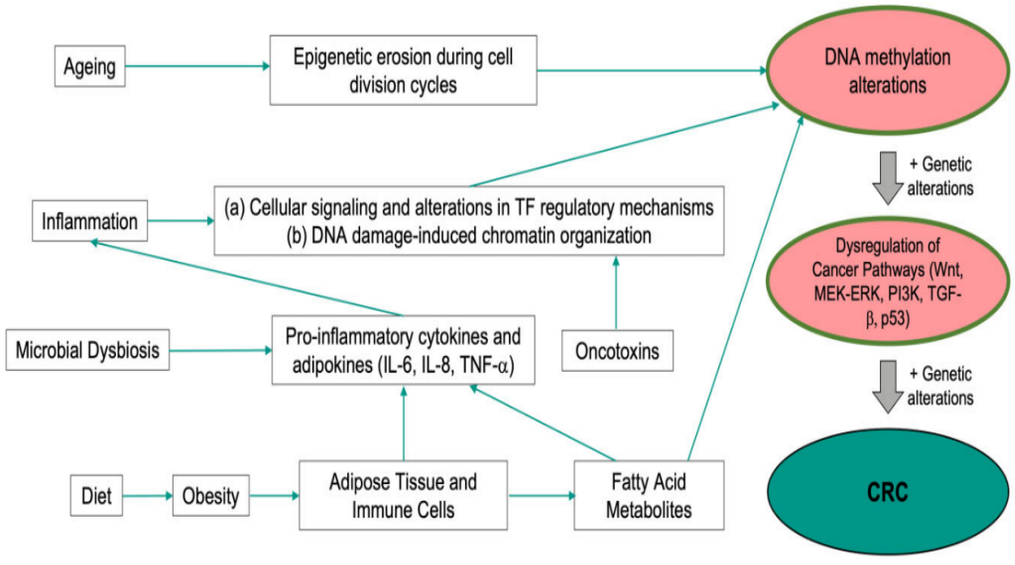
Microbial Dysbiosis: The gut microbiome represents an important micro-environmental exposure component essential for the normal functioning of the colon (64). Dysbiosis is defined as pathogenic changes that occur in the micro-biome profile and functions. Intestinal Dysbiosis is recognised in patients with CRC (173).

Alterations in healthy intestinal microbes can promote chronic inflammatory conditions in the gut that lead to the production of carcinogenic metabolites that subsequently lead to neoplasia (173). Dysbiosis in the gut can occur due to antibiotic use. Dietary factors such as red meat are associated with hostile gut microbiome compared to high-fibre fruits and vegetables (64).

Epidemiological studies involving patients with premalignant lesions and CRC have shown association with Fusobacterium nucleatum with specific clinical and molecular features that include right-sided anatomical location, hypermutation with microsatellite instability and mutations in BRAF (173) and given that these features characterise serrated neoplasia, the overgrowth of Fusobacterium nucleatum might be implicated in the progression of serrated lesions to CRC (174, 175).

Metabolic influences such as obesity is one of the major risk factors for colon cancers. Adipose tissue is enriched with immune cells such as macrophages that secrete pro inflammatory adipokines such as human process factor that can result in chronic low-grade systemic inflammation (64).This can subsequently lead to gene expression changes that occur as a result of an increase in prooncogenic signals due to alterations in histone modification. Histones are proteins that package DNA into compact structures called chromatin and modification to these proteins can affect gene expression which can contribute to colorectal tumourigenesis.

Figure 11: Environmental, Physiological, and other risk factors that contribute to CRC through DNA Methylation[64].



Abbreviations: CRC, colorectal cancer; TF, transcription factor

Inflammation can occur in the colon either due to conditions such as IBD, changes in the gut microbiome or as a result of diet and obesity. Chronic inflammation increases the risk of cancer initiation and progression and leads to an inflamed microenvironment.

Future larger studies are required to determine the potential interaction between risk factors with genetic or molecular risk factors to determine whether preventative strategies can be adopted in serrated carcinogenesis (150, 166).

1.5.5.4 Role of inflammatory bowel disease in serrated neoplasia

Serrated lesions are not uncommon in patients with IBD (176) however the incidence of serrated lesions in colitis is unknown as robust data is still lacking. Molecular studies have shown that all serrated lesions in IBD demonstrate either KRAS or BRAF mutation. KRAS mutation was frequently found in the part of the colon that was associated with inflammation particularly on the left side (177, 178).

1.5.6 Summary

This section briefly describes the morphological features of serrated lesions with more discussion of this in chapter 3. It also describes the molecular pathways involved in the serrated neoplasia pathway and explores the neoplastic potential of the different precursor serrated lesions. Although the preneoplastic potential of hyperplastic polyps have not been established completely, it is apparent that the precursor lesions that have true neoplastic potential are the SSL and TSA which are the important precursors in the serrated pathway.

The morphology of the serrated polyp pathway partially matches with specific driver molecular alterations with the most frequent mutation being in the BRAF proto-oncogene and additional epigenetic changes such as the hyper methylation of the MLH1 gene that leads to microsatellite instability and subsequent development of CRC.

The classification of CRC based on molecular subtypes is helpful to understand the prognosis of CRC and clinical outcome of the individual patient. It can be used to complement the traditional histopathological classification of CRC providing a more accurate and comprehensive prognostic assessment for individual patients.

This section also briefly explores the apparently non-genetic risk factors such as smoking, alcohol, increased BMI and consumption of dietary fat or meat which were shown to be associated with an increased risk of serrated polyps. Although epigenetic changes seem to play important role in the classical pathway, early epigenetic changes seem to be important in the serrated pathway. Mutational Signatures will be discussed in chapter 4 which describes this in context of the molecular genetic study performed in this research study (CONSCOP).

In summary, right-sided CRCs probably originate from serrated neoplasia. They are flat polyps that are often difficult to detect due to their morphology. There seems distinct molecular pathway, histopathology, and other risk factors that along with epigenetics can contribute to the development of serrated neoplasia.

The following section will discuss the endoscopic features of serrated neoplasia and the current surveillance strategies followed globally.

1.6 Endoscopic characteristics and appearance of colorectal serrated neoplasia

The flexible fibre-optic endoscope was introduced in the 1960s, since then there has been a quest to develop new techniques to enable better visualisation of the colonic mucosa. This has evolved over the years from fibreoptics to the charged coupled device (CCD) to acquire images and thereafter the focus has been on high resolution image acquisition(179).The use of dye spray to stain the colonic mucosa was first introduced and described in Japan in the 1970s. This technique is now popularly known as chromoendoscopy/ chromocolonoscopy (180). In the last two decades, advanced imaging endoscopy techniques such as narrow spectrum endoscopy and auto fluorescence imaging are used as standard to assess images and all modern endoscopes have this inbuilt by merely pushing a button on the endoscope. Narrowband spectrum imaging and chromoendoscopy helps to improve visualisation of the superficial details of polyps that include highlighting the micro-vessel network on the surface of the polyp and defining the surface mucosal pit pattern that helps to distinguish between neoplastic and non-neoplastic lesions in comparison to standard white light colonoscopy (181).

The paris classification system has been classically used to describe the morphology of a colonic polyp which broadly includes sessile, pedunculated, and flat or non-polypoidal lesions. There are several classification systems used to describe pit pattern, microvascular and surface patterns such as Kudo, Sano, Japan NBI expert team (JNET) and narrow banding imaging international colorectal endoscopic (NICE) NBI classification (179).

The NICE NBI classification was a consensus derived validated classification system that used colour change, vessel thickness and surface pattern of the polyp. Based on these criteria, polyps are classified into type I (hyperplastic), type II (adenoma) and type III (deep submucosal invasion or cancer).

The endoscopic differentiation of serrated lesions from hyperplastic polyps and adenomas has been difficult even with the presence of advanced and enhanced imaging techniques/NBI (182) and there were no available validated classification systems to distinguish between the above using NBI till the work group serrated polyps and polyposis (WASP) classification was introduced in 2016 (183).

The WASP classification (Figure 12) combines the NICE classification and for sessile serrated lesion like features i.e., cloud -like surface, indistinct border, irregular shape, and dark spots inside the crypts (179, 182). To diagnose a serrated lesion at least two of the above features is sufficient.

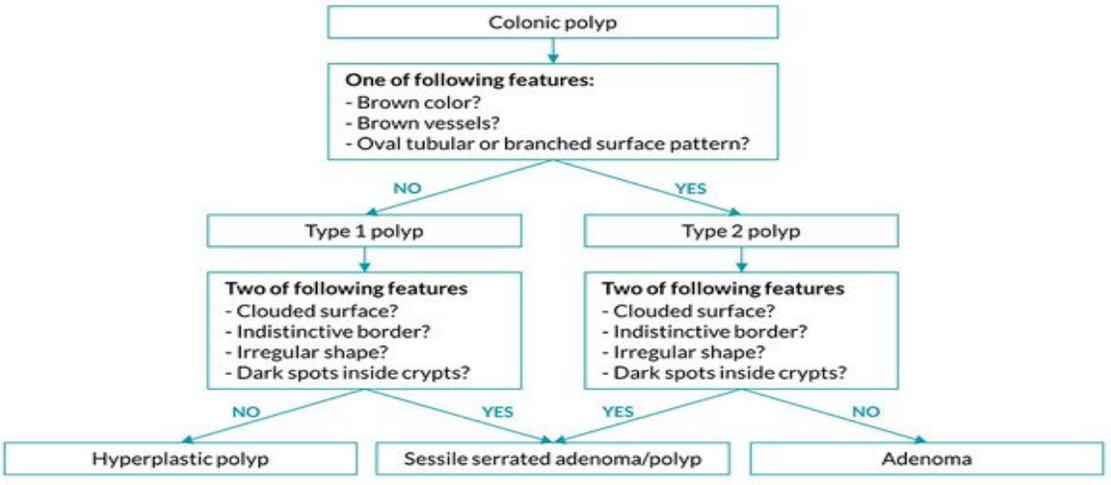


Figure 12: The WASP classification [182].

"Reproduced from [Development and validation of the WASP classification system for optical diagnosis of adenomas, hyperplastic polyps and sessile serrated adenomas/polyps, Joep E G IJspeert et al, Dutch Workgroup serrAted polypS & Polyposis (WASP), Gut. 65(6):963-70,2016] with permission from BMJ Publishing Group Ltd."

1.6.1 Endoscopic appearance

1.6.1.1 Hyperplastic polyps

Hyperplastic polyps are usually diminutive (<= 5 mm) and the majority of them are located in the sigmoid colon and rectum. Morphologically on white light endoscopy they appeared to be flat elevated lesions and are pale in colour. On narrowband imaging (NBI) they appear whitish lesions (NICE type I) without expanded, brown meshed capillary vessels (MC vessels) which are also seen in conventional adenomas(184). The surface pit pattern on chromoendoscopy is described as a Kudo Type II asteroid pit pattern.

1.6.1.2 Sessile Serrated lesions (SSL)

The endoscopic appearance of an SSL includes a size often though not always >=10 mm, proximal location, irregular shape, indistinct border, cloud like surface, the presence of a mucus cap and rim of debris on white light endoscopy (185). On NBI magnification endoscopy, a red cap sign (186), dilated and branching vessels (187) along with expanded crypt openings (188). The dilated crypts that are seen with NBI and magnification chromoendoscopy are described as type II-O (189). They are usually interspersed with star like pits (type II). Most of the crypts are filled with mucus and are dilated giving the appearance of the mucus cap. Distinguishing between hyperplastic polyps and SSLs endoscopically remains challenging in clinical practice as they often resemble each other.

1.6.1.3 SSL with dysplasia (SSLD)

SSLD are high risk lesions, and they can rapidly progress to CRC hence it is important to recognise these lesions endoscopically.

SSLs (Figure 13) are often incompletely resected (17) and incomplete resection of an SSLD due to failure to recognise the full extent and margins of the lesion may be responsible for a proportion of interval CRCs(190). Endoscopically, some indicators of dysplasia described in a study with 93.3% accuracy (191) were small or large nodules on the surface and partial protrusion an SSL. Other studies (192) have reported 71% accuracy for dysplasia when there is presence of a nodular or sessile component (0-1s) within an SSL over 20 mm in size. In the same study (192), on examination with chromoendoscopy, SSLD had the appearance of an adenoma with Kudo type III, IV and V. The adenomatous pit pattern along with type II or type II-O has also been described in other studies (193, 194). On NBI endoscopy, a demarcated area which is often hyper vascular or dark compared to the surrounding serrated tissue is seen. There is a transition area from Type II pit pattern to type III or type IV typical of a tubular or tubulovillous adenoma (190).

1.6.1.4 Traditional Serrated Adenoma (TSA)

TSAs (Figure 14) are mostly seen in the left colorectum with a similar distribution to hyperplastic polyps. Endoscopically on white light imaging they appear to be reddish, pedunculated lesions and have a “branch coral like” or a “pinecone like” appearance (195). On NBI, expanded brown capillary vessels that differ from MC vessels in adenomas are seen around the crypts. They are described as “leaf vein like” and in fact resemble conventional adenomas (NICE type II). On chromoendoscopy they have Kudo type IIIL, or Kudo type IV pits accompanied with serration in the crypt margin (195, 196).

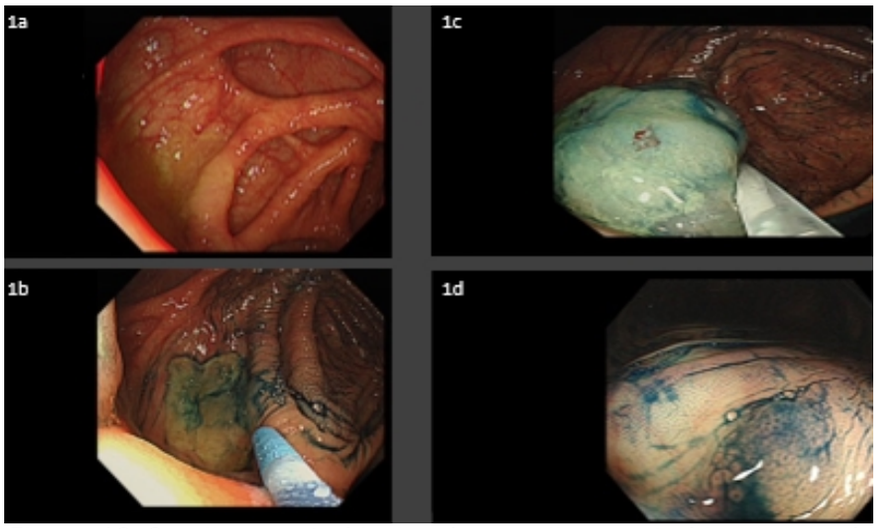


Figure 13: Sessile serrated lesion 1a: sessile serrated lesion on white light imaging; 1b: SSL with Indigo carmine dye; 1c: SSL following submucosal lift where margins appear well defined; 1d: Type II-O pit pattern.

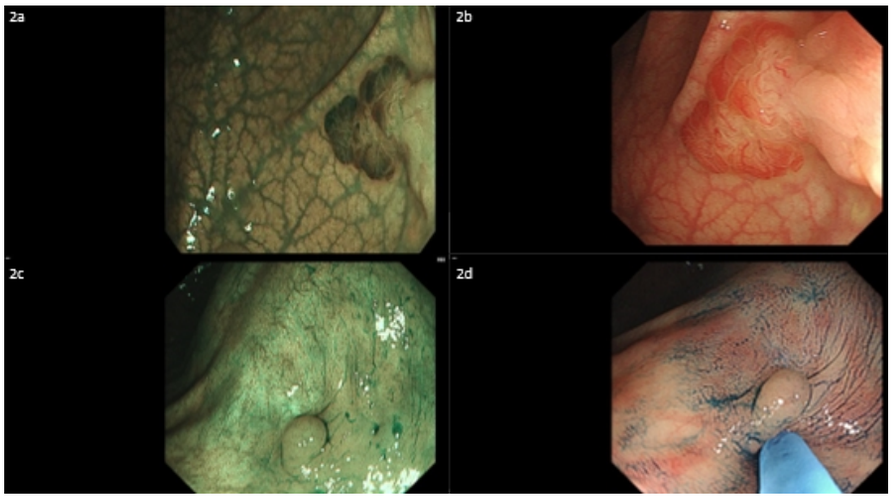


Figure 14: TSA and hyperplastic polyp.2a: traditional serrated adenoma NBI; 2b: TSA on white light imaging- "pinecone like" appearance; 2c: NBI image of hyperplastic polyp; 2d: white light image of hyperplastic polyp.

**All photo Images taken from own practice with patients consent*

1.6.2 Endoscopic management and surveillance of serrated neoplasia

Although there is no conclusive evidence-based guideline on the management of serrated polyps, new guidelines recommend that all serrated polyps should be endoscopically removed the exceptions being diminutive hyperplastic polyps in the rectosigmoid area due to their negligible malignant potential.

Sessile serrated lesions are reported to have a higher incomplete resection rate due to the flat shape and as the borders are not well demarcated (182). As discussed above, chromoendoscopy contrast dye helps to define the margins and border of serrated lesions (197, 198) and submucosal lifting of the lesion can also help to accurately identify the margin of the lesion (Figure 13; 1c).

Endoscopic mucosal resection is often used to remove these polyps >10 mm (197, 199). In larger lesions between 10 to 20 mm, piecemeal endoscopic mucosal resection can be performed to ensure that the margin of the serrated lesion is completely resected. The CARE study (17) revealed that the residual (incomplete resection) rate for serrated lesions is much higher than for adenomas. Hence serrated lesions as with other similar adenomas that are removed by piecemeal resection should have a resection site check performed between 2 to 6 months after removal of the polyp (97).

Surveillance guidelines following removal of serrated polyps are based on expert opinion and observational data due to the lack of prospective control data and surveillance intervals differ in various countries (200).

A summary of the recommended guidance for the surveillance of serrated polyps by the British Society of gastroenterology (BSG) position statement (97) versus the European Society of gastrointestinal endoscopy (ESGE) (201) versus the US multi-Society task force (US MSTF) (202) is represented in Table 3.

Table 3: Recommendations for surveillance sessile serrated polyps adapted from [203].

Baseline colonoscopy finding	BSG	ESGE	US MSTF
Non-Dysplastic SSL <10mm	No surveillance	10 years	5 years
Non-Dysplastic SSL >10mm or SSLD	One off colonoscopy at 3 years	3 years	3 years
TSA	One off colonoscopy at 3 years	3 years	3 years
Serrated Polyposis Syndrome	1- 2 years following polyp clearance	3 years	1 year

1.6.3 Artificial intelligence and serrated neoplasia

One of the causes of post-colonoscopy interval CRC or missed lesions is the failure of the endoscopist to recognise a lesion or polyp on the screen. This can occur due to incomplete exposure of the colonic folds and mucosa combined with a suboptimal withdrawal technique of the scope (203, 204).

Artificial intelligence (AI) assistance may help to increase the detection of polyps and thereby reduce the risk of miss rates and consequently interval cancers by alerting the endoscopist to the presence of a polyp on the screen. This is done by the adoption of convoluted neural networks (CNN) or deep learning that has led to the feasibility of real-time detection by computers (CADe-computer-aided detection) that are capable of highlighting polyps on the screen to the endoscopist by a visual or acoustic alarm (205).

A recent meta-analysis (206) looked at five randomised control studies that evaluated the role of CADe systems in lesion detection or mucosal exposure and the main features of the detected lesions. This meta-analysis showed that there was a 44% increase in adenoma detection rate and 70% relative increase in detection of adenoma per colonoscopy when adding CADe to colonoscopy.

CADe led to a statistically significant increase in the detection rate of diminutive, small, and large adenomas both in the proximal and distal colon. Additionally, there was an increase in detection of sessile serrated lesions per colonoscopy in the CADe group compared to the control group with low level of heterogeneity in all four RCTs (207-211) and a twofold increase in detection of advanced neoplasia.

Hence incorporating the use of AI in the future may help to improve optical diagnosis and detection of polyps and further help to improve the quality of a colonoscopy examination.

1.7 Current methods of estimating prevalence of serrated neoplasia

Serrated polyps are precursors of CRC and are probably responsible for 15-30 percent of all colorectal malignancies. They also have a similar molecular genetic pattern to some PCCRCs giving rise to the theory that missed proximal serrated polyps could be responsible for a significant proportion of PCCRC (12, 18, 212). Though it is increasingly recognised that certain types of serrated polyps can be precursors to CRC through the serrated neoplasia pathway, there remains a heterogeneity in the reporting of prevalence of serrated lesions and hence the true or actual prevalence of serrated neoplasia is uncertain (155). This is due to among other reasons the variation in the way in which the prevalence rates for serrated neoplasia have been described in the literature due to inconsistent diagnostic criteria, lack of consensus among histopathologists and inappropriate histological classification of different subtypes, variation in polyp detection rates amongst colonoscopists, use of different and endoscopic enhancing modalities and population selection criteria (97).

The prevalence of serrated neoplasia may be influenced by the detection rate of these lesions. Improved detection methods that include screening modalities, improved techniques and increased awareness affects the observed prevalence rates. Prevalence can also be influenced by other factors such as the natural progression of the disease, risk factors, the effectiveness of treatment and population demographics.

This section explores some of the current methods that are used to estimate the prevalence of serrated neoplasia and describes the limitations of each method that can have an impact on how prevalence has been reported.

1.7.1 Methods of Estimating Prevalence:

The factors that influence the estimation of prevalence of serrated neoplasia will be discussed under the following: 1. Diagnostic modalities used for detection 2. Diagnostic accuracy i.e. The histological interpretation and 3. Epidemiology

1.7.1.1 Diagnostic modalities:

The diagnostic modalities that are currently used in screening and early detection of colorectal polyps and cancer are faecal blood tests, colonoscopy, flexible sigmoidoscopy, colon capsule and CTC.

a) Stool markers:

The use of guaiac Faecal Occult Blood Test (gFOBT) in large, randomised population screening studies has shown that CRC mortality is reduced by 30% (34). gFOBT used in the UK until recently has been replaced by a more sensitive quantitative faecal immunochemical test (FIT) that directly assays the presence of haemoglobin in the stools. FIT is superior to gFOBT and has a much higher sensitivity (73.3% in comparison to 33.3% in gFOBT). Faecal blood testing i.e. either by gFOBT (55, 213) or FIT (214, 215) demonstrates low detection rates for adenomas especially those located in the proximal colon. Studies have shown that gFOBT. has a negligible or minimal effect on CRC incidence (20, 21) and data is limited as to the sensitivity of detecting proximal serrated neoplasia.

Though FIT is quite sensitive to detect CRC, it has modest sensitivity in the detection of advanced adenomas at around 23.8% (216) and this is even lower for serrated lesions. The sensitivity of FIT for SSLs using a threshold of 20ug Hgb/g faeces was reported in one study to be 6.2% (in comparison to 20.9% for advanced adenomas) (24).

Similarly, another study (217) that evaluated the quality and performance indicators of endoscopists showed a high ADR at 45% in a FIT positive sample and a low serrated lesion detection rate at 1.8%. Even if the FIT cut off is lowered from 20 to 10 ug Hgb/g faeces this only modestly increases the sensitivity to increase SL detection from 6.2 to 12.3% (24). In UK-based screening programmes that use a much higher quantitative threshold these values could be even lower. The low sensitivity of FIT for serrated lesions could be related to the fact that they bleed less and are flat and not protruding with a predisposition to be located in the proximal colon. Hence FIT appears to have very limited value in serrated neoplasia detection as a primary diagnostic tool.

Stool DNA testing could potentially aid the detection of serrated neoplasia in screening methods that use a stool test with a sensitivity higher than just using FIT (213). In a large average risk cohort study (216) using FIT versus FIT plus multitarget stool DNA, the latter significantly improved the detection of serrated polyps over 1 cm in size (42.4% versus 5.1% if using FIT alone, $P < 0.001$).

b) Colonoscopy:

Colonoscopy is the best current test for serrated lesion detection with other diagnostic modalities performing less well.

In the average risk population, there has been variability in reporting prevalence rates. The prevalence rates of serrated neoplasia differ between centres and colonoscopists reflecting a variation in practice and KPI.

The prevalence of sessile serrated adenoma/polyp has been described in previous studies to range from 1% to 14% (124, 218-220). Most of the studies have a retrospective design that include symptomatic patients or high-risk patients.

The prevalence rates of serrated lesions in the proximal colon, including hyperplastic polyps (HPs) and sessile serrated polyps (SSPs), differ between medical centres (221) and even among operators within the same centre (222, 223) (224).

In a study reported in the USA, the colonoscopic prevalence rate of serrated polyps among 15 endoscopists performing screening colonoscopy ranged from 1-18% (223). A dutch study involving five endoscopists showed a range of 6-22% (225). When a highly skilled colonoscopist and an experienced gastrointestinal pathologist collaborated, an SSP prevalence rate of 8.1% was found in a series of 1,910 screening colonoscopies, with 0.6% displaying cytological dysplasia (226).

Many researchers opt to analyse all right-sided serrated lesions (HPs and SSPs) collectively as the outcome measure, due to the unreliable distinction between HPs and SSPs by pathologists (224). Prevalence rates of proximal serrated lesions are higher in FOBT based colonoscopy programs to the link with advanced synchronous conventional adenomas detected by FOBT (227, 228).

These prevalence rates vary globally, from 2.8% to 13% (in countries such as the Netherlands, Spain, USA, Hong Kong, and Korea) (221, 227-232). Even within these estimates, there is variation between centres, with one study of 32 US and German centres reporting a range of 0-9.8% (221). Additionally, differences in serrated lesion rates may be associated with ethnicity, with higher rates reported in caucasian populations.

One study (233) described the prevalence and distribution of different serrated polyp subtypes in individuals undergoing primary screening colonoscopy. The report included hyperplastic polyps, serrated lesions and TSA were detected in 23.8%, 4.8% and 0.1% of individuals respectively.

Sessile serrated adenomas comprised 7.3% of all histopathological classified polyps. The prevalence and distribution of each subtype was evaluated within an invitational population-based colonoscopy screening programme. This finding is comparable to a study which demonstrated a prevalence of 2.3% in a symptomatic average risk patients (234)

In a meta-analysis (235), high-definition colonoscopy (HDC) in comparison to standard definition colonoscopy only provides a marginal incremental increase in serrated polyp detection rates of 3.8% (95% CI, 1-6.7%). Hence HDC is unlikely to be of major benefit. Narrow spectrum endoscopy regardless of type has also not shown any promising results (224). Chromocolonoscopy improves adenoma detection rate and in studies prior to the recognised definition of serrated lesions as shown an improved detection of 9 to 16% of hyperplastic polyps in the proximal colon (236).

A small proportion of sessile serrated lesions may progress into dysplastic lesions (SSLD) that can progress to CRC in a relatively short interval (127). The exact proportion of those lesions that become dysplastic is unknown, however the prevalence of these lesions in the screening population has been reported in the literature to be approximately 3.8-5.8% with the majority located in the proximal colon (27, 32). Patients with a dysplastic SSL have a significantly increased risk of CRC (237) (OR 4.76, 95% CI 2.59-8.73). Those with TSA also had an increased malignancy risk (OR 4.8, 95% CI 2.4-9.9). Traditional serrated adenomas (TSA) have a reported low prevalence in most series as 1-2% (238).

Larger serrated polyps have been found to be strongly associated with synchronous CRC (239) and sessile serrated lesions to be associated with an increased risk of metachronous CRC (240, 241). A meta-analysis(242) showed that there was a strong risk relationship between the presence of proximal serrated polyps and synchronous advanced neoplasia (OR = 2.77, 95% CI 1.71-4.46). The pooled prevalence of serrated polyps in this meta-analysis was 15.6%. Patients with proximal serrated polyps and larger serrated polyps were found to be associated with a threefold increase in the detection of advanced neoplasia (OR = 3.35, 95% CI, 2.51-4.46).

The meta-analysis did not find a significant association between serrated polyps and synchronous CRC however there was high heterogeneity and limited studies that were analysed (242). Based on this the authors concluded that serrated polyps and synchronous advanced colorectal neoplasia may define a high-risk phenotype leaving patients at highest risk for synchronous lesions and studies are needed to determine whether these individuals would require more intensive surveillance (242).

Subsequent to this, (237) a population-based study showed that the estimated 10 year risk of CRC risk was high with serrated lesions with dysplasia, women with serrated lesions, those with proximal serrated lesions and a history of TSA with a low risk of CRC associated with hyperplastic polyps. These data generally support the recommendation for closer follow-up for these individuals.

Inflammatory bowel disease (IBD): Serrated neoplasia occurs in colitis however there are limited studies for any surveillance recommendations to be made in this regard. Prevalence rates are variable ranging from 1-4% A retrospective study in Canada (243) in 83 patients showed that the prevalence of SSL was 1.39% and TSA 0.31% with the former located proximally and the latter evenly distributed.

Serrated polyposis syndrome (SPS): The prevalence estimates of SPS in a screening population is higher than what it was previously thought to be, with it being previously reported as exceeding 1:300 (244) (245) in FOBT-based screening and approximately 1:2000 (223) and colonoscopy-based programmes. Currently, In FIT/FOBT screening cohorts, the prevalence has been noted to be 1: 111 to 1: 127 colonoscopies (156) and in screening colonoscopy cohorts found to be 1 : 238 colonoscopies (155).

c) Flexible sigmoidoscopy:

Sigmoidoscopy is limited to visualisation of the left colon and hence has a limited value and impact on detecting proximal CRC and proximal polyps (224, 246, 247). When sigmoidoscopy is used for screening, this generally leads to a colonoscopic examination based on if there are any adenomas or advanced lesions seen in the left colon (246, 247).

One study found that the finding of conventional adenomas in the distal colon did not predict the occurrence of advanced proximal serrated neoplasia (SSL >1 cm) (248).

d) CT Colonography (CTC) and Colon Capsule (CCE):

The sensitivity for CTC to pick up flat lesions was thought to be low in earlier studies (249, 250) which may partly be due to lack of recognition of their existence and clinical importance. One study (251), showed that CTC based screening can detect sessile serrated lesions in the proximal colon with a non-diminutive (6 mm) prevalence of 3.1%. However in a dutch RCT study (252) that compared CTC with colonoscopy showed that CTC was outperformed by colonoscopy and it underperformed for flat proximal and dysplastic SSL.

A prospective single centre RCT (253) that compared CCE with CTC for FIT positive patients in a screening programme showed that the sensitivity CCE versus CTC for the detection of serrated lesions was 73.6 versus 32.9 (P <0.001).

1.7.1.2 Diagnostic accuracy i.e. the histological interpretation

The prevalence rates of serrated neoplasia in the published literature are extremely variable and one of the main reasons for an effect on the prevalence rates apart from detection modalities are the inconsistent diagnostic criteria that has been used for serrated neoplasia. The recent WHO 2019 has helped to standardise terminologies and the quality and comparability of emerging future studies should improve as a result of this.

In addition to this there has been inappropriate histological classification or misclassification where a number of hyperplastic polyps have been reclassified as serrated lesions. Furthermore, there is significant interobserver variation amongst GI pathologists in diagnosing SSL.

1.7.1.3 Epidemiology

The prevalence rates of serrated lesions based on autopsy studies have been reported between 13 to 35% (97) with the common subtype being hyperplastic polyps (24 to 42% of all respected colorectal polyps), SSL represented 2 to 4% of all polyps and TSA less than 1%. The prevalence of serrated neoplasia has also been shown to vary by geography based on a systematic review (254) reporting prevalence rate of 2.6% in Asia, 3.9% in Europe, 5.1% in USA and 10.5% in Australia. Long-term follow-up studies are needed to obtain further robust data.

1.7.2 Limitations:

Most data for colonoscopy detection of serrated lesions have been drawn from studies where serrated neoplasia was detected incidentally with adenoma detection being the primary endpoint (97). In addition to this many studies predate the accurate pathological distinction of serrated lesions. Hence the data for serrated lesion detection rate is not as robust as the ADR data (97).

The main limitation in detecting serrated neoplasia is a sensitivity of screening methods. Stool based screening methods have a very low sensitivity which may result in false-negative results. Similarly, image-based screening methods such as CTC and CCE have limited evidence.

Colonoscopy though is the gold standard test for identifying these lesions and is dependent on high quality intra procedural factors such as bowel preparation, operator factors such as polyp detection rate and visibility.

Most studies that report prevalence are based on a retrospective study design and the majority of them predate initiation of standardisation of terminology of serrated neoplasia. Hence inconsistent terminology, misclassification of serrated polyps and high interobserver variability between pathologists all contribute to variable detection rate impacting on prevalence estimation.

The detection rate of serrated lesions has improved in recent years and the factors associated with this include increased awareness amongst endoscopists, histopathological awareness of classification and description and newer endoscopy techniques to aid detection. There is significant variation in the reported detection rate of serrated polyps based on endoscopic factors that include studies using different imaging modalities, the quality of the procedure (adequate bowel preparation, change in position to allow examination behind the folds, sufficient withdrawal time) and based on the endoscopists key performance indicators. (222, 226, 229, 255).

1.7.3 Summary:

In order to ensure adequate detection of serrated neoplasia, diagnosis, and removal there is a need to have standardised diagnostic criteria that are adopted universally, increased awareness and quality verification for endoscopists and pathologists by way of training.

In addition to this using existing diagnostic modalities such as colonoscopy-ensuring that high quality colonoscopy is performed along with the use of additional newer emerging enhancement and assistive technologies. There is a need for long-term follow-up studies (longitudinal studies) to determine population prevalence in relation to demographics such as age, gender, ethnicity, and geographical variation.

In addition to this there are other factors that can influence detection such as a lack of awareness and recognition of SPS, inadequate detection methodology due to variation in screening modalities in different countries (FOB/FIT/primary colonoscopy) and variation in surveillance strategies. Environmental and demographic factors such as diet (red meat), smoking, high BMI, gender could potentially increase the risk of serrated neoplasia. The relationship of these factors to geographical variation within different countries is not fully understood.

Genetic factors such as the recognition of SPS (256, 257) is important as this increases the risk of CRC and hence important to recognise to be able to advise on surveillance intervals and screening of first-degree family members.

Adenomas were previously thought to be lone precursors of CRC and hence the adenoma detection rate (ADR) is an established key performance indicator (KPI) in a colonoscopy (258). ADR is inversely associated with the risk of developing post colonoscopy CRCs and colonoscopists who have a high ADR can reduce the risk of PCCRC by up to 50 to 90%. (259, 260).

Now that serrated lesions are also recognised to be precursors of CRC, the focus should be on reporting of serrated polyp detection rate as a performance indicator with the goal to help to improve the overall quality of a colonoscopic examination in order to reduce serrated lesion related PCCRC (261). However, it is only recently (262) in 2021 that the American Gastroenterology Association (AGA), is the first society to recommend serrated polyp detection rate (SDPR) as a parameter to measure KPIs.

1.8 Post colonoscopy colorectal cancer (PCCRC)

PCCRC can occur as a result of poor-quality colonoscopy or from missed polyps due to their morphology or de novo development after colonoscopy which may be attributed to the biology of polyps. They are most likely to be located in the proximal (right colon) colon having a flat macroscopic appearance and share genetic characteristics such as microsatellite instability (MSI) and have CpG island methylator phenotype (CIMP) similar to serrated neoplasia (18, 19). Hence one of the causes of proximal PCCRC could be related to missed serrated neoplasia. This chapter explores the possible aetiology and factors associated with PCCRC, however prior to this, understanding the current definitions and standardisation of terminology is necessary.

1.8.1 Definitions and standardisation of terminology

Interval CRCs are cancers that occur in patients after they have had an initial (index) examination test that has not identified CRC.

Hence this can occur in individuals screened by any modality used for colorectal screening which can include faecal occult blood testing, radiological imaging, and colonoscopy. The terminology PCCRC which was coined in 2010, is specifically used for those interval cancers that occur after patients have undergone a colonoscopy where no cancer was initially diagnosed (20, 21).

The PCCRC nomenclature is designed for colonoscopy detected colorectal adenocarcinoma and does not include those cancers for which colonoscopy is not considered gold standard for their diagnosis. For example, neuroendocrine tumours, squamous cell carcinomas of the anorectum or adenocarcinoma of the appendix which may not be apparent on colonoscopy examination (21).

According to the world endoscopy organisation consensus statement group (21), PCCRCs can be sub- categorised into the following:

1. True interval cancers, which is a cancer identified before the next recommended surveillance screening procedure.

2. Non-interval cancers, which is further subdivided into-

i. Type A-CRC detected at the time of screening colonoscopy.

ii. Type B-CRC detected after recommended surveillance of screening interval (this could be due to poor adherence to surveillance intervals, sub optimal bowel preparation, incomplete colonoscopy).

iii. Type C-CRC detected where there was no screening or surveillance interval recommended for up to 10 years after a colonoscopy.

The interval PCCRC is a measure of colonoscopy quality as it presumes that the recommended surveillance interval would pre-empt the occurrence of a CRC prior to the subsequent planned procedure (21). Non-interval PCCRC on the other hand, would not just measure the quality of the colonoscopy but would also reflect the adherence, appropriateness, or accuracy of the recommended surveillance interval.

1.8.2 Factors that may contribute to aetiology and potential mechanisms

It is very challenging to determine the precise aetiology of PCCRC due to the uncertainties related to cancer biology and the multiple pathways that could lead to the initiation and progression of cancer. However, there are other plausible factors that could contribute to the development of PCCRC which include those that can arise from missed cancers, missed premalignant lesions (polyps) and incompletely resected benign polyps (21, 212, 263-265). In addition to this rarely there could be a small proportion of accelerated biology -related cancer i.e., those progressing by the serrated neoplasia pathway that could contribute to PCCRC or a new CRC (18, 21).

The reasons for missed lesions include patient dependent factors i.e., poor bowel preparation and operator dependent factors such as incomplete colonoscopy, rapid withdrawal time and inadequate inspection technique (265-268). It is well-recognised that adenoma miss rates and incomplete polypectomy rates can vary between operators and a low adenoma detection rate has been associated with higher interval cancer rates (258, 266).

The PCCRC rate is an important performance measure of the ability of colonoscopist to detect and prevent CRC (21). The quality of a colonoscopy can be determined by surrogate measures such as the caecal intubation rate, adenoma or polyp detection rate and withdrawal times and associated patient factors such as age, the presence of diverticular disease and significant comorbidities (269) as these measures are easier to capture than the PCCRC rate (21, 270, 271).

1.8.3 Proximal CRC versus distal CRC

Various population-based case-control and observational studies have demonstrated that PCCRCs seem to occur more frequently in the proximal or right colon than the distal colon. The factors include quality of the procedure and biology.

The study by le Clercq 2013 (272) included a total of 5107 patients with CRC over a 10 year period and defined PCCRC as cancers diagnosed within five years after an index colonoscopy. They found that 2.9% (147 patients) had PCCRC's diagnosed on an average of 26 months after the index colonoscopy. The centre in the Netherlands showed in their cohort that 86.4% of all PCCRC's could be explained by procedural factors especially missed lesions and incompletely resected polyps. The majority (60%) were in the proximal colon and were noted to be small and macroscopically flat in appearance in comparison to the prevalent CRC's leading to the suggestion that the cancers could have originated from overlooked precursors at the initial colonoscopy.

Previous case-control or observational studies have shown that proximal CRC was more common in patients with interval CRC over a 10 year follow-up (14) and a subsequent study from Ontario (273) showed over the 14 year follow-up period, a negative complete colonoscopy was associated with subsequent reduced incidence of distal colon CRC however there was no reduction in the incidence of proximal colon CRC until year 8 of follow-up.

In a study from Ontario evaluating the association between colonoscopy and CRC deaths, a population-based case-control study by Baxter et al. 2009 (15) showed that colonoscopy was associated with fewer deaths from CRC and this association was primarily limited to deaths from cancer developing in the left side of the colon. The study identified that the right colon may not be evaluated completely due to poor bowel preparation and quality -related issues. In addition to this it suggested that the biology of right and left colonic neoplasia may be different. For example right sided colonic polyps are less pedunculated and more flat (274) and hence difficult to detect in comparison to left colon polyps. In addition to this the histology and molecular features of right sided cancers may be different due to different genetic pathways.

In another population-based case-control study from Germany, Brenner et al. 2011 (275) aimed to assess the characteristics and predictors of interval cancers occurring within 10 years after a negative colonoscopy. They demonstrated that interval cancers were more common amongst women and the location being in the proximal colon (caecum or ascending colon OR 1.98).

They also occurred often after incomplete negative colonoscopy where the caecum was not reached particularly in women and after a negative colonoscopy following a positive faecal occult blood tests particularly amongst men. The conclusion of the paper suggested that a substantial proportion of interval cancers were due to neoplasms missed at colonoscopy which could potentially be prevented by enhancing the quality of the colonoscopy.

Hence the associations with previous FOBT positivity and accompanying incompleteness of a previous negative colonoscopy support that missed detection could be one of the causes of interval cancers following a negative colonoscopy (16).

Most gFOBT deductible CRCs seem to be located in the left colon and rectum and the sensitivity of gFOBT due to adenomas seem to be higher in men, particularly those using low-dose aspirin than in women (275).

1.8.4 PCCRC and serrated neoplasia:

It appears that missed polyps particularly serrated neoplasia contributes to PCCRC. Biological factors such as the endoscopic appearance of serrated neoplasia and screening methodologies such as low sensitivity of faecal occult blood tests and quality assurance issues during the procedure (poor bowel preparation, incomplete resection etc.) poses challenges in detection and resection.

Molecular and genetic factors of proximal CRC share characteristics similar to some sessile serrated lesions i.e., microsatellite instability (MSI) and have CpG island methylator phenotype (CIMP +) supporting the theory that some SSLs could be precursor missed lesions (18, 19). In addition to this advanced stage of PCCRC found in cancers less than 3 years indicates biology or an accelerated (serrated) pathway could also be responsible for this.(275)

In conclusion, PCCRC seem more common in the proximal colon and the reasons include quality -related factors such as inadequate bowel preparation, the endoscopists key performance indicators, missed lesions and incompletely resected lesions along with poor sensitivity of gFOBT in detecting certain lesions. Hence improving colonoscopy quality could help in detection of proximal neoplasia and serrated neoplasia which could help to reduce the occurrence of post colonoscopy CRCs.

The following section briefly describes the influence of colonoscopy quality in the detection of proximal and serrated neoplasia.

1.9 The influence of colonoscopy quality on the detection of Proximal colon neoplasia and Serrated neoplasia

Colonoscopy along with the recognition and removal of precancerous polyps has been shown to decrease the incidence, mortality and morbidity associated with CRC. However, despite advancement in technology, it is recognised that it is not a perfect tool in completely preventing CRC as there are various factors that influence the quality of a colonoscopic examination.

This includes intra procedural factors such as the quality of the bowel preparation, operator factors such as the colonoscopists knowledge and technique, and other technical factors such as the use of adjunctive devices that can help to improve mucosal visualisation. In the bowel screening programmes there are a number of quality assurance measures to assess the quality of a colonoscopy that include the quality of the bowel preparation, caecal intubation rate, complications, detection of cancer, adenoma detection rate (ADR) and withdrawal time.

One of the most important quality indicators is the ADR of a colonoscopist. This is defined as the proportion of colonoscopies that pick up a histologically confirmed adenoma in an endoscopist's practice. A high-quality colonoscopy minimises the risk of missed lesions and hence reduces the risk of PCCRC.

Studies have shown that colonoscopists with a high ADR had a reduced risk of developing PCCRC (258, 266, 276) compared to those who have an ADR below 20% where there was an increased risk of PCCRC noted (Figure 16). The quality of bowel preparation is also an important factor as a higher quality bowel preparation also significantly improves ADR (277). Additionally, morphologically flat, and depressed lesions are subtle and are often difficult to detect endoscopically and are more likely to harbour dysplasia or malignancy regardless of size (278, 279). These polyps are also often located in the proximal colon which could explain why colonoscopy has not helped to reduce CRC mortality in the proximal colon in comparison to the benefit it has had in the distal colon (15, 275).

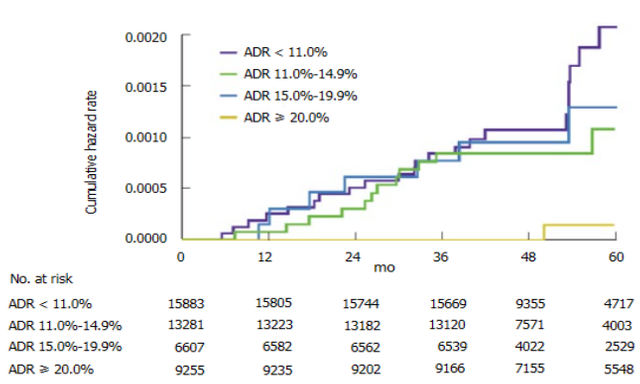


Figure 16: Cumulative hazard rates for PCCRC according to the endoscopist ADR (1).

The graph shows the cumulative hazard rates for PCCRC among participants undergoing a screening colonoscopy performed by an endoscopist with an ADR in the following categories-<11%, 11-14.9%, 15-19.9%, >= 20%. ADR = adenoma detection rate; PCCRC = post colonoscopy colorectal cancer

In order to maximise the detection and visualisation of these flat polyps the following factors needs to be taken into consideration which inadvertently helps to improve the quality of colonoscopy and thereby improve the detection of proximal neoplasia and serrated neoplasia.

This includes improving the cognitive knowledge of the endoscopist by means of training. Studies have shown that there is a learning curve (280-282) associated with recognising flat polyps that include serrated neoplasia and adenoma detection rate. Along with improving knowledge other operator dependent factors to enable adequate visualisation of the mucosa around colonic folds and to minimise missed polyps that could be in “blind spots” include changing the position of the patient, adequate insufflation, suctioning liquid, and good colonoscopy technique enabling adequate withdrawal time. A longer withdrawal time of around eight minutes has been shown to be associated with a high ADR (283) and studies have shown a withdrawal time of nine minutes is associated with a higher detection of serrated neoplasia (284).

There are other factors including the use of techniques to help to improve mucosal visualisation such as dye-based chromoendoscopy. This involves spraying the surface of the colon with dyes such as methylene blue or cresyl violet which can get absorbed through the mucosa and indigo carmine which is a food colouring agent and is not absorbed by the mucosa. Indigo carmine is usually diluted and acts by pooling in the mucosal crevices and thereby highlighting various patterns over the surface of the polyp.

Studies have consistently shown that chromoendoscopy improves ADR particularly flat adenomas compared to standard definition or high-definition white light colonoscopy (157, 285, 286).

There are other techniques that could help to improve the visual contrast of the mucosa, including the use of high definition colonoscopes that help in narrowband imaging, flexible spectral imaging colour enhancement and I-Scan.

There are other newer technologies that can help to improve the field of view and thereby improving visualisation of the blind mucosal areas such as Endocuff device, cap assisted colonoscopy, balloon colonoscopy, third eye retroscope and the use of artificial intelligence that could also help to improve proximal colon ADR and consequentially serrated polyp detection rate. However more data is required for the evaluation of the above before any definitive conclusions can be made.

This section provides a brief overview of the importance of ensuring colonoscopy quality to help to improve the detection of proximal colon neoplasia. The exploration of the individual factors including the pros and cons of each of the newer techniques will be discussed in detail in section 2.1 of Chapter 2.

1.10 Research gaps and questions:

While serrated polyps are known to play a significant role in the development of CRC particularly PCCRC as discussed in detail in the previous sections, there are still many gaps in the understanding of their prevalence, risk factors, and natural history. The following section briefly considers the existing comprehension of the literature and describes the research gaps in understanding the epidemiology, prevalence, and malignant potential of serrated neoplasia. The section will thereafter discuss the research questions pertinent to my thesis.

1.10.1 Research gaps:

1.10.1.1 Epidemiology:

Understanding the precise prevalence estimates of serrated neoplasia:

The current challenges include detection strategies in screening programmes, variation in detection during colonoscopic examination and the variation in terminology that has been used for reporting serrated neoplasia. As a result of this there have been variable prevalence rates reported and hence understanding the precise prevalence estimates of serrated neoplasia has been challenging.

As elaborated in chapter 1.6, the prevalence of serrated neoplasia particularly sessile serrated lesions is difficult to determine accurately as the results of the published literature are variable (97). This is because of various factors that cause a limitation in the detection rate.

This includes inconsistent terminology and nomenclature, changing taxonomy, variations in polyp detection by colonoscopy endoscopists (222, 287), resection practices and the use of different types of endoscopic enhancing modalities and the different criteria used for population selection for screening (97).

Hence it is quite difficult to draw conclusions from epidemiological studies that group all serrated class lesions together (288).

The published literature on colonoscopy detection of serrated lesions in the average risk screening population (97, 221, 222, 229, 289) are mostly retrospective studies which have reported serrated polyp detection rate between .6% to 20%. Some retrospective studies (230, 289) that have reported proximal serrated polyp prevalence rates at 11% and 2.7%.

Diagnostic modalities such as stool-based faecal blood tests (23) (290) (23), CT imaging (249, 250), colon capsule (253) have not shown promising results in the accuracy of detecting proximal colon serrated neoplasia.

High quality colonoscopy remains the best method to detect these lesions. However, colonoscopy is not a perfect test (224) as this is subject to operator factors such as technique and adenoma detection rate, patient factors such as bowel preparation, anatomical factors and procedural factors including difficult access and visualisation, all of which can lead to missed polyps that include serrated neoplasia and hence contribute to the occurrence of post colonoscopy CRCs in the proximal colon (17, 258, 291).

There is a considerable variation in the histopathological interpretation of serrated polyp subtypes that can influence the precise categorisation of the potential precursors to the serrated pathway (226, 292).

Significant interobserver variability has been recognised with the pathological reporting of serrated lesions arising from variation in terminology and diagnostic criteria between hyperplastic polyps and SSLs (97, 221, 293, 294). This can result in a variation or inconsistencies in the surveillance interval recommendation given to participants.(295-299).

Discrepancies in classification of those polyps that have a higher risk of cancer particularly those with high grade dysplasia or dysplastic serrated lesions or dysplastic TSA could lead to a recommendation of a longer surveillance interval that could contribute to the development of interval cancers due to the underlying biology of these polyps. Hence developing an understanding that would help to limit the variation in practice along with developing further knowledge that can reduce interobserver variability would impact on prevalence rates and clinical outcome.

Therefore, more research is needed to develop technologies and methods that help to improve the detection of serrated neoplasia in the proximal colon and understand the precise prevalence of proximal serrated neoplasia.

In addition to this, there is a need for long-term longitudinal studies to answer the question as to whether proximal colon cancers and PCCRC can be reduced by improving the detection and management of proximal serrated neoplasia.

Risk Factors:

The complete understanding of the absolute risk associated with inherited, environmental or lifestyle factors for CRC that results from the serrated pathway remains incomplete.

Studies (171) including meta-analysis and systematic reviews (150) have identified that there are some risk factors associated with serrated neoplasia such as an increased risk with smoking (162-164, 166), alcohol consumption, body fatness, dietary factors and meat (150). The meta-analysis also showed a statistically significant inverse relationship with NSAIDs, aspirin and dietary folate. However, a greater understanding is needed to understand the interplay between these factors including gut microbial dysbiosis that can contribute to epigenetic changes in the colonocytes. Uncertainty remains as to how these factors exactly initiate and enable the development and progression of serrated neoplasia and CRC along with understanding any potential benefit of prophylactic interventions such as aspirin and lifestyle modification.

There also remain gaps in knowledge regarding the genetic background of serrated polyposis syndrome with less than 3% having a germline mutation in RNF43 with identification of a few other genes (122, 158) that may play a role in the pathogenesis of SPS.

However, the exact role in carcinogenesis is not confirmed and though there is observation that it can be familial there is rarely a strong autosomal dominant pattern with the frequency of germline pathogenic variants in RNF43 being less than 2% (154, 159). The current recommendation of colonoscopy surveillance (97, 160) for these individuals and their 1st degree relatives is based on expert opinion only.

Age and gender: The proportion of proximal CRC and PCCRC increases with age and has a preponderance in women which could be related to proximal serrated neoplasia (237).

Racial and ethnic disparities:

There is limited information on the epidemiology of serrated neoplasia among different racial and ethnic groups and further research is needed to explore potential disparities in prevalence and risk factors in outcomes which could inform targeted prevention and screening efforts. Trying to identify the groups of population that could potentially be at risk of developing serrated neoplasia is also key.

A single centre, single endoscopist study from Australia showed that detection rate of sessile serrated lesions was significantly higher in caucasians when compared to the Chinese population (97, 300).

A cross-sectional study (301) that looked at the occurrence of colorectal neoplasia in different ethnic and social economic groups in the United States, interestingly showed that with the exception of Japanese patients serrated neoplasia was less prevalent among East Asians and contrary to adenomas and adenocarcinoma was positively associated with markers of high social economic status.

Hence future larger studies including large sample longitudinal studies with long follow-up periods are required to determine the prevalence of serrated neoplasia in different population groups and age groups.

In addition to this to understand the potential interaction between risk factors with genetic or molecular risk factors to determine whether preventative strategies can be adopted in serrated carcinogenesis (150, 166). This may help to inform appropriate surveillance and screening methods that can help in early diagnosis and improve survival.

1.10.2 Malignant potential:

There is a lack of consensus on the natural history and progression to CRC of serrated polyps. While adenomatous polyps are well-known to progress from benign to malignant over time, the natural history of serrated polyps is less clear. Some studies have suggested that serrated polyps may have a more aggressive natural history, with a higher risk of progression to CRC compared to adenomatous polyps (81, 123, 139). However, other studies have found conflicting results, with some serrated polyps remaining stable or regressing over time (302).

Studies from the last two decades have suggested that serrated neoplasia are precursors of CRC and can be responsible for up to 15 to 30% of all CRC (71, 98, 116, 303). The serrated neoplasia precursor lesions are hyperplastic polyps, SSLs, and TSAs.

Hyperplastic polyps are the commonest subtype accounting for 70 to 80% of serrated neoplasia and per se are thought to have little risk of malignant potential. However, molecular events such as BRAF and KRAS mutation can initiate transformation of these subtypes of polyps into more significant lesions such as the microvesicular hyperplastic polyp that follows the BRAF serrated pathway and the goblet cell hyperplastic polyp that can progress via the KRAS serrated pathway (73, 123-125).

SSLs which constitutes about 10 to 20% of serrated polyps along with TSAs which represents around 1% of SPs and have a risk of malignant transformation occurring through the serrated neoplasia pathway (122).

However, only certain types of SSLs undergo malignant transformation through this pathway which follow an accelerated pathway to carcinogenesis. The time taken for this transformation is uncertain and more data is required to risk stratify high risk serrated lesions.

TSA and SSLs with dysplasia have significant malignant potential (129, 139). There are no current specific surveillance guidelines when superimposed dysplasia is diagnosed in either particularly if they do not satisfy the number and size of polyps that triggers surveillance guidelines (129).

A Danish population-based case-control study (237) showed that there was an increased risk of CRC associated with dysplastic serrated lesions and TSA with the 10-year CRC risk being 4.4% and 4.5% respectively in comparison to conventional adenomas that had a risk of 2.3%.

There is limited data on the optimal management and surveillance strategies for serrated polyps. Current guidelines recommend surveillance colonoscopy for individuals with serrated neoplasia, but the optimal timing and frequency of surveillance is based on expert consensus view due to lack of high-quality evidence. There is also a lack of consensus on the management of advanced serrated polyps that have higher malignant potential.

One of the major differential diagnoses of sessile serrated lesions is the microvascular hyperplastic polyp (MVHP) and distinguishing between the two can be difficult (221, 304, 305). This is not just in the case of non-specialised pathologists but also in expert centres where GI pathologists have an interest in serrated polyps (129).

Though it is exceedingly rare to find MVHP that are >=10 mm in size which is the size that is used to help to decide on surveillance strategies, the problem arises with polyps that are less than 10 mm in size that are in the proximal colon. If all hyperplastic polyps in the proximal colon were assumed to be sessile serrated polyps or sessile serrated lesions then this may lead to increased frequency of colonoscopic surveillance and burden on surveillance programs (129).

Equally precursor lesions that have a high malignant potential such as dysplastic sessile serrated lesions and traditional serrated adenomas can be of varying sizes and can be less than 10 mm in size and diminutive and may not satisfy the criteria that triggers surveillance that includes the number of polyps detected and the size of the polyp. Individuals who have the above may not have optimal surveillance as a result.

Further research is needed to determine the specific genetic and epigenetic changes that drive malignant transformation and to identify which serrated lesions have the highest risk of progression to cancer. The current guidelines for the detection and management of serrated neoplasia primarily based on expert opinion and limited evidence. Additional research is needed to optimise screening and surveillance strategies including the identification of high-risk populations, the most effective methods for lesion detection and removal and the optimal surveillance intervals. More research is required to elucidate the specific molecular mechanisms and biomarkers associated with serrated neoplasia development and progression.

The CRC risk associated with proximal hyperplastic polyps remains poorly understood (302). More robust data is needed in different population subgroups in order to understand and inform screening and prevention strategies.

In summary, further research is needed to understand:

1. The natural history of serrated polyps, including their prevalence, progression rates, factors that influence progression, and potential differences in outcomes based on polyp size, location, histological and molecular features.
2. The key molecular and genetic alterations associated with serrated neoplasia, including specific gene mutations, interplay of lifestyle and environmental risk factors with epigenetic modifications, and other molecular changes that may contribute to the development of serrated adenocarcinoma.
3. The malignant potential of various subtypes of serrated neoplasia. This includes a deeper understanding of the difference between the different types of serrated polyps and to better predict which lesions are at higher risk to progression. This includes the validation of potential biomarkers and identification of new biomarkers that can reliably risk stratify the malignant potential of the subtypes of serrated neoplasia.
4. The most effective and cost-effective clinical management and surveillance strategies for serrated neoplasia. This includes the best approaches and modalities for detection, removal, and surveillance intervals. To determine evidence-based surveillance strategy minimises the risk of malignant transformation whilst balancing potential harm such as overtreatment or complications from invasive procedures.

There is a lack of high-quality evidence to inform the risk benefit ratio of surveillance procedures due to largely retrospective study designs, a lack of long follow-up data and hence large prospective studies are needed to identify high-risk patients who may benefit from shorter surveillance intervals versus low-risk patients.

5. Understanding the long-term outcomes and prognosis of serrated neoplasia which includes the rate of malignant transformation and the recurrence rates after removal. Long-term longitudinal studies with longer follow-up are needed to understand the prognosis of serrated neoplasia and whether strategies to help reduce the risk of serrated neoplasia has an impact on reducing proximal CRC and PCCRC.

The following section discusses research questions pertinent to the thesis.

1.10.3 Research Questions

1. How can we improve the detection and diagnosis of sessile serrated neoplasia in a screening population?

    a. What cost-effective technologies and techniques will help to increase the detection of serrated lesions at colonoscopy?

    b. Does chromocolonoscopy improve the detection of serrated lesions and if so, is it cost-effective and possible to implement in a population-based screening programme with acceptability from healthcare professionals and participants undergoing screening?

c. What is the interobserver variability in the histological diagnosis of serrated neoplasia in expert pathologists and how does this compare to local pathologist reporting in real world surveillance programs?

2. If there is standardisation of technique, monitored quality indicators for operators, implementation of technology that may help to improve detection i.e., chromocolonoscopy, reduction of variation of the reporting of serrated neoplasia by the intervention of pathologists then What would be the prevalence of serrated neoplasia in a screened population that uses a stool-based tests as the initial test to offer further colonoscopy?

3. What is the malignant potential of serrated neoplasia?

    a. What would be the appropriate surveillance interval for proximal sessile serrated lesions with and without coexisting CRC and coexisting adenomas or if high risk serrated lesions such as SSL with dysplasia or TSA LGD are found.

    b. Are there risk factors associated with serrated neoplasia and are there chemo preventative measures that would reduce the development and growth of sessile serrated lesions and associated cancers?

    c. What molecular markers and mutational signatures can predict the future malignant potential of sessile serrated lesions?

4. Finally, If by adopting strategies that can accurately help to detect, diagnose, manage, and identify the risk of serrated neoplasia in the proximal colon and thereby reduce their incidence help to reduce the incidence of interval cancers/post colonoscopy CRC in the proximal colon?

1.11 The Aims of the Research:

1. Chromocolonoscopy:

a) The impact of chromocolonoscopy on the detection of serrated colorectal lesions b) The acceptability of chromocolonoscopy to colonoscopists and participants and c) The feasibility of chromocolonoscopy for wider application in a population based CRC screening programme.

2. Histopathology:

a) To understand the prevalence of serrated neoplasia in the proximal colon in a FOB positive screened population following endoscopic removal and rigorous histopathological assessment and review by expert pathologists using both WHO and AGA criteria b) To report the inter-observer variability of serrated neoplasia between expert GI pathologists and the interobserver variability between the local pathologists and expert pathologists.

3. Mutational Signatures:

To explore the mutational signatures in serrated neoplasia (exploratory dataset) and explore the feasibility of using Fixed formalin paraffin embedded (FFPE) samples for next generation sequencing (NGS) in a selected cohort.

Chapter 2: Feasibility and acceptability of chromocolonoscopy for the detection of proximal serrated neoplasia in a population-based screening programme (CONSCOP study)

This study was conducted as a part of my MD and a paper resulting from this work was published in the Lancet Gastroenterol Hepatol 2019; 4: 364-75 (Appendix A)

Feasibility and economic assessment of chromocolonoscopy for detection of proximal serrated neoplasia within a population-based colorectal cancer screening programme (CONSCOP): an open-label, randomised controlled non-inferiority trial.

Authors: The details on author contribution are listed in Appendix A.

R Ramaraj*, C Hurt*, A Farr, M Morgan, N Williams, CJ Philips, GT Williams, G Gardner, C Porter, J Sampson, S Hillier, H Heard, S Dolwani

*Joint first authors

2.1 Introduction

In the United Kingdom (England and Wales) the population-based bowel cancer screening programme implemented between 2006-2008 initially invited people aged between 60 and 74 years. It was based on gFOBT followed by colonoscopy for patients deemed fit for the procedure after a positive screening test result. This has recently in 2019 changed to quantitative FIT testing with an aim to reduce age thresholds for invitation to screen to 50 years.

The **CONSCOP** study (Feasibility of reduction of right sided bowel cancer through **CON**trast Enhanced colono**SCOP**y.

This was a feasibility randomised open controlled trial (RCT) comparing dye enhanced colonoscopy (chromocolonoscopy) to standard white light colonoscopy. It was conducted at index procedures in the bowel cancer screening programme in Wales, United Kingdom between 2014-2017. An index procedure is the first colonoscopy that is performed after a positive screening stool test which was the gFOBT used at the time of the study.

The data obtained from this study informed the assessment of feasibility and design of a future RCT that would be powered to look for reduction in bowel cancer mortality.

This chapter will begin with an introduction that provides essential background information to enable understanding of the rationale for the CONSCOP study. This will be described considering the following points:

2.1.1 The challenges in detecting proximal colon neoplasia and serrated neoplasia.

2.1.2 The influence of colonoscopy quality on the detection of proximal serrated neoplasia.

2.1.3 The known and potential impact of chromocolonoscopy in the detection of colorectal neoplasia.

2.1.4 The current understanding of methods in improving colonoscopy quality and finally,

2.1.5 The rationale and aims of the CONSCOP study.

The following sections thereafter will describe the study methodology, results, and definitive discussion of findings of the study.

2.1.1 The challenges in detecting proximal colon neoplasia and serrated neoplasia

As described in the introductory chapters, serrated polyps can be responsible for around 15 to 30% of sporadic cases of CRC (123) and have distinct differences from adenomas by way of their morphology, endoscopic appearance, histopathological features and biology (306). Serrated lesions pose multiple challenges in clinical practice particularly in relation to detection (224). SSLs are an important subset of serrated neoplasia and usually located in the right side of the colon. They are very subtle and difficult to detect flat lesions hence detection by any diagnostic modality including colonoscopy is a challenge.

In addition to this SSLs are associated with synchronous and metachronous advanced neoplasia (238) and have a higher risk of incomplete resection (307). The detection and accurate characterisation of proximal colon serrated neoplasia have several challenges which can impact screening, surveillance and management strategies for CRC prevention and also directly relates to the reduction and prevention of PCCRC.

The challenges in detection of proximal colon neoplasia and serrated neoplasia have been described below by the diagnostic modalities used to detect colorectal polyps and cancer. In a population-based screening programme this would include faecal occult blood stool tests (gFOBT, FIT), colonoscopy, CT Colonography, and capsule endoscopy.

2.1.1.1 Stool Based tests

The National Health Service (NHS) bowel cancer screening programme started using gFOBT in 2006 for England and full roll-out in 2010 supported by evidence from multiple large RCTs that showed a reduction in CRC mortality of 15 to 33% (33-39).

The gFOBT was used in the UK until recently and this has been replaced by a more sensitive quantitative Faecal immunochemical test (FIT) that directly assays the presence of Haemoglobin (Hb) in the stools. FIT is superior to FOBT and has a much higher sensitivity (308) (73.3% in comparison to 33% in FOBT). FIT has been in use in the bowel cancer screening programme in Wales since June 2019 by a phased implementation process based on age.

FIT sensitivity for CRC varies between 71-91% depending on the Hb cut off used with a specificity of 90-95% (48) however the sensitivity for advanced adenomas reduces to 25-40% (55, 56). The sensitivity reduces further in the detection of serrated lesions including larger ones (23).

For example in one study, the sensitivity for any sessile serrated lesion was 6.2% and for advanced adenomas 20.9% with a FIT threshold of 20 μg Hgb/g faeces (24). In another study EQuIPE (290) (evaluating quality indicators of the performance of endoscopy) showed a high adenoma detection rate of 45% in a FIT positive sample but they also observed a very low serrated lesion detection rate of 1.8%. This is probably because serrated lesions are less likely to bleed than conventional adenomas (23).

In a multicentre retrospective series of 70,000 colonoscopies reporting significant association of sessile serrated lesions with caecal intubation rate, presence of at least one adenoma and ADR. The study showed no association between FIT and the detection of sessile serrated lesions (217). There is some promise in using multitarget stool DNA testing alongside FIT testing which may improve the sensitivity of detection of serrated polyps (97). The large study in average risk cohort using FIT versus FIT plus multitarget stool DNA testing, the latter outperformed (42.4% versus 5.1% respectively, $p < 0.001$) just using a FIT test for the detection of serrated polyps of >1 cm in size (216).

Strategies such as the use of flexible sigmoidoscopy as a primary screening tool may not detect these polyps and hence are likely to be missed in a screening programme that use faecal blood tests.

Moreover, in stool-based screening programmes particularly where the threshold for FIT values may be set slightly higher alongside the lower sensitivity anyway with lower thresholds, the prevalence of serrated lesions and proximal adenomas may not be accurate and variable in comparison to countries such as USA that adopt primary colonoscopy as a screening strategy (34).

2.1.1.2 Colonoscopy

The main colonoscopy related factor that poses a challenge in detecting proximal colonic adenomas and serrated neoplasia is poor quality colonoscopy. This includes operator factors such as those endoscopists with a poor ADR and technique. Procedural factors that impede adequate mucosal visualisation of the colon thereby leading to missed polyps. This can occur due to poor bowel preparation, anatomical factors, and difficult visualisation of flat and subtle polyps.

The caecum and proximal colon are challenging areas to achieve complete visualization during colonoscopy due to anatomical factors such as angulation, looping, and inadequate bowel preparation, which can limit the detection of neoplastic lesions (309). In addition to this proximal serrated neoplasia, can be difficult to detect due to the endoscopic appearance appearing subtle or flat with indistinct borders and a pale surface and can be concealed by a mucous cap or stool debris. They are often located in haustral folds or behind flexures and predominantly found in the right colon where bowel preparation can be poor (162, 306, 310, 311).

Hence these polyps can be easily missed or incompletely resected which then could result in PCCRCs (17, 258, 291).

Suboptimal Imaging Techniques: Conventional white light endoscopy (WLE) may have limitations in detecting subtle or flat serrated neoplasia, especially in the proximal colon. High definition colonoscopes and image enhancing modalities such as chromoendoscopy (virtual or dye spray) techniques have shown promise in improving lesion detection, but their routine use and standardised application in clinical practice are still evolving (312, 313).

Histopathological variation has been described in the nomenclature of serrated lesions in different countries and more recently the WHO criteria developed in 2019 has addressed these issues. However, preceding the WHO 2019 criteria, the lack of consensus has been one of the challenges in the accurate identification, detection and in reporting prevalence of proximal serrated neoplasia.

2.1.1.3 CT Colonography (CTC) and Colon Capsule Endoscopy (CCE)

The sensitivity for CTC to pick up flat lesions was thought to be low in earlier studies (249, 250) which may partly be due to lack of recognition of their existence and clinical importance. A Japanese national CTC trial (314) reported 65% sensitivity for > 1 cm non-polypoidal lesions and in a Dutch randomised screening trial (252) there was significantly lower detection rate of high risk serrated polyps. However one study (251), showed that CTC based screening can detect sessile serrated lesions in the proximal colon with a non-diminutive (6 mm) prevalence of 3.1%.

The authors claimed that the adherent contrast material coating on these polyps aided the detection despite them having a flat appearance.

Whilst detecting serrated neoplasia can be challenging for CTC perhaps with meticulous bowel preparation, distension, and scrutiny along with the above could potentially help to improve sensitivity of detection of serrated neoplasia.

A prospective single centre RCT (253) that compared CCE with CTC for FIT positive patients in a screening programme showed that CCE was more sensitive than CTC for detecting significant lesions (96.1% vs 79.3%).

In summary, there are diagnostic challenges in detecting proximal colon neoplasia particularly serrated neoplasia. The following section describes the influence of colonoscopy quality in the detection of serrated neoplasia.

2.1.2 The influence of colonoscopy quality on the detection of proximal serrated neoplasia

Colonoscopy is gold standard for the detection and prevention of CRC through the identification and removal of precursor lesions such as adenomas and serrated neoplasia. However, despite advancement in technology, it is recognised that it is not a perfect detection tool for serrated neoplasia and detection rates are variable (224).

There are various interventions at colonoscopy that may influence colonoscopy quality and thereby have an impact on serrated lesion detection rates (97). These include a). operator factors such as the Colonoscopists adenoma detection rate (ADR) and withdrawal technique and time, proximal colon retroflexion (224)

b). Intra procedural quality assurance factors such as the quality of the bowel preparation and c). Other factors to help improve mucosal visualisation. This includes the use of devices or techniques such as high definition colonoscopes, chromoendoscopy, narrow spectrum endoscopy.

2.1.2.1 Operator factors

The skill and experience of the endoscopist plays a crucial role in the detection of proximal serrated neoplasia. The ADR is an accepted and important quality indicator of a colonoscopy for both screening colonoscopy as well as for general or symptomatic colonoscopy (97, 270). Colonoscopists with a high ADR had a reduced risk of developing PCCRC (258, 266, 276) compared to those who have an ADR below 20% where there was an increased risk of PCCRC noted. There is some evidence to suggest that ADR correlates with the sessile serrated lesion detection rate (222) and proximal serrated lesion detection rate. (225, 240). The overall detection rates for serrated neoplasia i.e., the percentage of colonoscopies that have at least one serrated neoplastic lesion have variable reports in the average risk screening populations and range between 4%-35% (221, 227, 229, 231, 234, 238, 315, 316). However in both screening and symptomatic population this has been reported to be 13 to 21% (315, 317), and 10% in the surveillance patient group (229).

Along with this a good withdrawal technique that enables adequate visualisation of the mucosa around colonic folds is likely to minimise the risk of missing polyps that could be in “blind spots”. This would also include dynamic position change of the patient, adequate insufflation, suctioning of excess liquid, and good colonoscopy technique enabling adequate withdrawal time.

A longer withdrawal time of around 8 minutes has been shown to be associated with a high ADR (283) and studies (225) have shown the detection rate of serrated neoplasia increases each minute of withdrawal time above 6 minutes with maximum benefit noted at 9 minutes as demonstrated in one study (284) (incident rate ratio 1.77; 95% CI, 1.15- 2.72).

An observational study showed the benefits of increased detection of serrated polyps with a second look after caecal intubation and retroflexion in the right colon (caecum to hepatic flexure) with meticulous mucosal examination and cleaning for enhanced detection of adenomas and SSLs (97, 318).

The quality of bowel preparation is also an important factor as a higher quality bowel preparation also significantly improves ADR (277).

2.1.2.2 Quality of Bowel preparation in colonoscopy

There have been very few studies that have looked at the quality of bowel preparation with the detection of serrated neoplasia as the primary outcome (30). One screening study in the Netherlands (225) showed that there was no association with the quality of bowel preparation with lower proximal serrated lesion detection rates. Another cross-sectional US registry based study that looked into the effect of bowel preparation quality on adenoma and serrated polyp detection and did not show significant differences in overall of proximal area or serrated polyp detection rate between colonoscopies with fair versus optimal bowel preparation however concluded that poor preparation may reduce proximal ADR (319). This may be explained by the effect of thicker mucous cap on serrated lesions which may be evident with lower preparation quality that may assist with detection (97, 319).

However a study thereafter (320) showed the detection rate of serrated lesions with intermediate quality bowel preparation versus high quality bowel preparation respectively was reported to yield an overall detection of 4.6% versus 12% (odds ratio, 0.37; 95% CI, 0.15-0.87) and in the proximal colon 1.5% versus 7.9% (OR, 0.19; 95% CI, 0.05-0.81). The study showed that any bowel preparation that was below the level of high quality showed a significant decrease in the detection of sessile serrated lesions whereas intermediate quality preparation was still adequate for adenoma detection. Hence high quality bowel preparation is important for optimal detection of conventional adenomas as well as serrated neoplasia (320).

2.1.2.3 Other factors to help improve mucosal visualisation

In addition to the above factors, other devices and techniques can help to improve mucosal visualisation in the proximal colon and thereby improve the detection of proximal serrated neoplasia.

High definition (HD) colonoscopy, in a recent retrospective study of average risk screening population, HD colonoscopy significantly improved sessile serrated lesion detection rate (321). In another prospective randomised study (322) that assigned three endoscopy systems (Fujinon, Olympus and Pentax) in combination with 4 modalities i.e., conventional white light endoscopy (WLE), high-definition white light endoscopy (HD WLE), virtual chromoendoscopy (CE) and HD virtual CE, there were no significant differences noted between the three endoscopy systems.

There was also no difference found in the ADR between the four imaging modalities however HD WLE resulted in significantly higher detection of sessile serrated lesions (8.2% versus 3.8%; p <0.01) compared with conventional WLE (322).

However, some trial studies (205) have not shown any difference between HD and conventional WLE for high-risk adenomas and hence though HD WLE may be beneficial the trial results are not consistent. A RCT (323) that investigated the use of narrowband imaging (NBI) compared to HD WLE for detecting serrated lesions showed that though more proximal serrated lesions were detected by NBI compared to HD WLE this did not achieve statistical significance. A subsequent randomised multicentre trial found no difference in polyp miss rates using HD-WLE or NBI in patients with serrated polyposis syndrome (324).

Interventions such as narrow spectrum colonoscopy (NBI, FICE, iSCAN) (97, 325), underwater colonoscopy have not shown clear benefit in improving detection rates of proximal serrated neoplasia (30). Dye-based chromoendoscopy has consistently shown to improve ADR particularly flat adenomas compared to standard definition or HD WLE (157, 285, 286).

Endoscopic detection of serrated neoplasia can be assisted by HD scopes, chromoendoscopy with or without image enhancement, adoption of quality criteria for colonoscopy and possibly the use of ancillary devices (307).

In a study that looked at chromocolonoscopy with indigo carmine (0.4%) versus standard colonoscopy for the detection of neoplastic lesions (157) using high definition (HD) colonoscopes in both groups, the overall detection of adenomas (0.95 versus 0.66 per patient) and serrated lesions (1.19 versus 0.49 per patient) was found to be higher in the chromocolonoscopy group. The difference between the two groups was statistically significant. This study was a two-centre trial with the primary aim to detect adenomas.

In summary, studies have shown that the endoscopists ADR, longer withdrawal times, the use of techniques and technology that can improve mucosal visualisation that include withdrawal technique, proximal colon retroflexion, the use of high definition colonoscopes and enhancing mucosal visualisation with the use of dye can enhance the visualisation of proximal neoplasia and can potentially improve serrated neoplasia detection rates. However other techniques such as narrow spectrum imaging have not shown promising results.

2.1.3 The known and potential impact of chromocolonoscopy in the detection of colorectal neoplasia

Chromocolonoscopy (CC) or Chromoendoscopy is an image enhanced endoscopy technique that improves detection and characterisation of subtle mucosal abnormalities by providing a detailed contrast enhancement of the surface of the colonic mucosa. It can either be topical dye-based or electronic/virtual chromocolonoscopy.

Dye-based chromoendoscopy involves spraying the surface of the colon with absorptive dyes (methylene blue, cresyl violet) or contrast dye such as indigo carmine which is a food colouring agent and is not absorbed by the mucosa.

Indigo carmine is usually diluted by water to 0.2- 0.4% and acts by pooling in the mucosal crevices and thereby highlighting various patterns over the surface of the polyp (Figure 17). This technique was first described in 1976 and has been used to detect dysplasia in ulcerative colitis where the dysplastic polyps are flat and very subtle (180, 326).

Virtual chromoendoscopy (VCE) (327) is an electronic endoscopy imaging technology that provides detailed contrast enhancement of the mucosal surface and blood vessels. This includes optical technologies such as narrowband imaging (NBI-Olympus), flexible spectral imaging colour enhancement (FICE, Fujinon), blue light imaging (BLI) and iSCAN (Pentax).

The new generation image enhancing endoscopy (IEE) has incorporated special filters along with laser technology to help in visualisation of the mucosa such BLI and LCI (Linked Colour Imaging).

The role of dye-based CC is established in detecting flat dysplastic colorectal lesions in inflammatory bowel disease and is one of the recommendations to be used as for surveillance in longstanding IBD in Europe and UK (53, 205, 328-331). Virtual CC with or without HD has not shown consistent evidence yet (332-334).

A meta-analysis in 2011 showed that CC was significantly better than WLE in detecting dysplasia in experienced centres with an increased procedure time of 11 minutes overall in comparison to standard colonoscopy (335).

In another study in colitis, withdrawal time decreased as experience with CC increased (336).

In hereditary non polyposis colorectal cancer syndrome (HNPCC) or Lynch syndrome, though studies have shown that indigo carmine based chromoendoscopy with WLE helps in the detection of flat diminutive polyps in the proximal colon for lynch syndrome (337, 338) however one multicentre randomised controlled trial did not show that dye-based CC was superior to WLE (339) and another study showed that high definition WLE was not inferior to pancolonic CC (340). However, in both these studies experienced high detector endoscopists using HD scopes could have shown a decrease in the advantageous effect of dye-based CC (205).

In serrated polyposis syndrome, there has been a recent single RCT study in 2019 (341) that evaluated dye-based CC (using indigo carmine) using HD WLE in comparison to just HD WLE and found that there were significantly higher additional serrated lesion detection rate in the group where dye-based CC was used (40% vs 24 %, $P<0.001$).

Hence based on this one RCT, the use of HD scopes with dye-based CC is associated with an increase in detection of polyps and serrated lesions in the colon in SPS. The role of virtual CC in SPS has not been proven yet (324, 342).

A Cochrane systematic review in 2016 (343) that analysed 7 RCTs that assessed the role of dye-based CC in the detection of colorectal neoplasia outside the setting of colitis or polyposis, found that pan-colonic CC significantly increased the number of patients with at least one polyp detected that was either adenomatous in nature or cancer.

One of the limitations of the systematic review was that there was significant heterogeneity between studies and lack of blinding between studies.

A study from the United States (286) that looked at high definition chromocolonoscopy versus high-definition white light colonoscopy for average risk CRC screening. It found a marginal increase in overall adenoma detection and a modest increase in flat adenomas and small adenoma detection compared with HD WLE. The findings did not support the routine use of HD chromocolonoscopy for CRC screening in average risk patients. It was hypothesised that the high adenoma detection rate observed in the study could have been due to HD technology used in both groups (286).

Another study from Germany (157), looked at pan colonic chromoendoscopy with indigo carmine versus standard colonoscopy in a prospective two centre randomised controlled trial and showed that chromoendoscopy increased the overall detection rate of adenomas (0.95 versus 0.66 per patient), flat adenomas (0.56 versus 0.28 per patient) and serrated lesions (1.19 versus 0.49 per patient) with $P = <0.001$. The study showed that there was a significant trend towards increased detection of advanced adenomas and the withdrawal time was significantly higher in the chromoendoscopy group (11.6 vs 10.1 min). The study included not just average risk population but also surveillance and symptomatic patients.

Virtual chromoendoscopy such as narrow spectrum endoscopy (NBI (324, 342), FICE (344), I-SCAN (345)), have not shown any clear benefit in improvement in adenoma or polyp detection rates and hence unlikely to benefit in the detection of serrated lesions according to meta-analysis studies (97, 325).

A study that combined HD colonoscopy with I-SCAN found that this was superior in detecting advanced adenomas >= 10 mm compared to HD colonoscopy (346). However, it was not clear as to whether the increased sensitivity of ADR was due to HD colonoscopy or I-SCAN.

The data on IEE technology such as BLI and LCI for the detection of colorectal lesions are preliminary (205). Recent RCTs and LCI have shown an increased per patient ADR compared to HD-WLE (37% versus 28%) (347) and a reduction in miss polyp rate in the proximal colon (348). A RCT on BLI have shown an increased mean adenoma per patient rate but no increase in ADR or PDR compared to HD-WLE (349).

The implementation of the widespread use of dye based chromocolonoscopy in a screening population maybe associated with limitations or barriers as discussed below.

2.1.3.1 Limitations or Barriers to dye based chromocolonoscopy

Though dye-based CC may provide some benefit in the detection of colorectal neoplasia, there may be several perceived limitations associated with implementation particularly on a large-scale in population-based screening programs.

Time and resources: The additional time and resources required for chromoendoscopy may not be perceived to be feasible in busy clinical settings and there is also a learning curve associated with the use of chromoendoscopy.

The equipment that is used to perform CC includes one of the dye stains (absorptive or contrast reactive stains) and whilst these dye stains are relatively inexpensive, ensuring availability at all times can occasionally be challenging. In addition to this the dye can be dispensed either by a spray catheter or via a pump that dispenses through the colonoscope. These costs need to be factored in along with the cost of additional time to perform high-quality CC which can be much harder to quantify (350).

Additional time to undertake the procedure may discourage colonoscopists to perform this routinely. It can be quite messy which may not be acceptable to the endoscopist or assistant and inadequate training in the use of dye-based CC may result in a variable baseline.

The question on whether CC offers a cost savings when used in real-world surveillance programs remains unanswered and more longitudinal studies are required to clarify whether it actually reduces cancer incidence or improves survival (350).

In summary, dye-based chromocolonoscopy increases the adenoma detection rate and polyp detection rate and suggests some advantage in inflammatory bowel disease and serrated polyposis syndrome.

Virtual chromocolonoscopy has not shown consistent and reproducible results in the same setting as yet. Although CC has been shown to improve detection rates (343). The effectiveness of such an intervention in a national screening programme is not known particularly in increasing the yield of serrated lesions in the proximal colon.

Hence it is important to assess whether interventions such as CC can help to improve outcomes by improving detection and removal of serrated neoplasia and thereby reduce the incidence of PCCRC in the long-term. As serrated CRC may account for up to 15% of all CRCs, perhaps better detection, and removal of these might help to reduce the incidence of PCCRC considerably.

The following section describes the current methods used and available to help to improve colonoscopy quality.

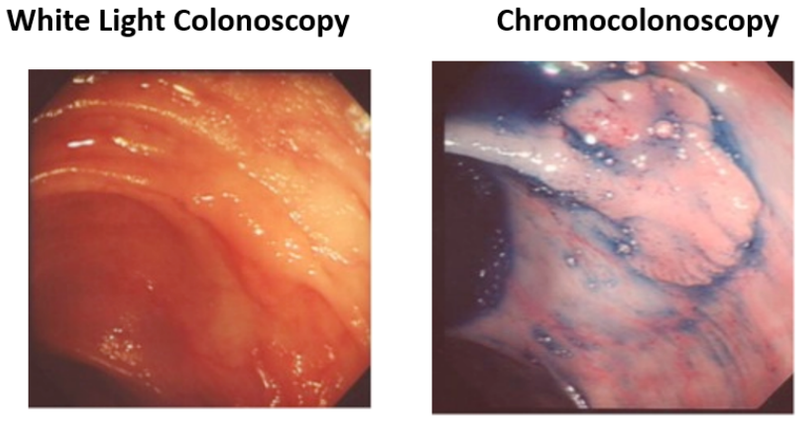


Figure 17: Chromocolonoscopy can highlight flat lesions like serrated lesions.

Endoscopy pictures used with permission from training slides.

2.1.4 The current understanding of methods in improving colonoscopy quality

The current methods used to improve colonoscopy quality are described under the following categories:

1. Technical factors that include improving the intra procedural quality and visualisation of the mucosa in colonoscopy.

2. Devices that are used as an adjunct to aid in mucosal visualisation and

3. Training of the operator that enables improvement of their key performance indicators.

2.1.4.1 Technical factors

In order to improve the visualisation of the colonic lumen and thereby improve the detection of colonic polyps and serrated lesions, the following may help to improve colonoscopy quality. These include good quality bowel preparation, good withdrawal technique, slower withdrawal time, the use of antispasmodics and the use of techniques such as chromoendoscopy and water exchange colonoscopy.

2.1.4.1.1 Bowel preparation

Good bowel preparation is essential for visualisation of mucosa in detection of lesions. Poor bowel preparation has been associated with an adenoma miss rate of 43% (351).

There is an increase in ADR of almost 35% and an associated increase in caecal intubation rate (93.6- 95.5%) with good bowel preparation and the use of split dose preparation for colonoscopy (352).

2.1.4.1.2 Withdrawal Technique

The essential components of a good withdrawal technique include adequate luminal distension or insufflation, cleaning and suctioning of fluid and solid debris and repetitive inspection behind the folds (353). Adequate luminal distension enables good visualisation and thereby increases polyp detection. Dynamic position change helps to open the angles of the flexures and shifts the fluid away from the area of interest (354).

Studies (353-355) including a systematic review and meta-analysis have shown that dynamic position changes during colonoscopy withdrawal helps to improve luminal distension and therefore increases adenoma and polyp detection rate compared to a static left lateral position.

2.1.4.1.3 Withdrawal time

Analysis of data from the English national bowel cancer screening programme have shown that a low ADR was associated with withdrawal times of less than seven minutes and a high ADR is associated with withdrawal time of 9-11 minutes (41, 225, 277, 283, 284)

2.1.4.1.4 Antispasmodic agent

Hyoscine butylbromide (Buscopan) is a relatively safe antispasmodic that is routinely used in colonoscopy(1). A study conducted in the bowel cancer screening programme in England showed that it did improve ADR (277) and in another RCT showed an increased polyp detection rate in the right colon (0.43 vs 0.31, P=0.01 (97, 356).

However two meta-analysis studies, one that reviewed five RCTs (357) and the other that reviewed eight RCTs (358) conducted in three continents (Europe, Asia, and Australia) did not show any significant increase in ADR with the use of hyoscine.

2.1.4.1.5 Water infusion techniques

Water infusion colonoscopy, which involves using water infusion either in conjunction with or as a replacement for air insufflation, is primarily employed as an innovative method to ease caecal intubation and minimize patient discomfort (1).

A systematic review revealed varying ADR patterns when comparing water infusion with traditional colonoscopy, with a higher ADR observed proximal to the splenic flexure when using water exchange (359).

Additionally, a single-centre observational study demonstrated an increased detection of adenomas during colonoscopy when the water exchange technique was combined with cap-assisted colonoscopy (P = 0.002). However, despite the higher ADR in this group, the difference was not statistically significant (360).

2.1.4.2 Device factors

These include the following technology and techniques to:

1. Enhance optical images inbuilt into the endoscope and

2. ancillary devices to improve mucosal visualisation and polyp detection (dye spray, distal attachment devices, accessory video processing devices).

2.1.4.2.1 Technology used to enhance optical Images

These include 1. wide field White Light Endoscopy (WLE) which includes High Definition (HD) colonoscopes, near focus/dual focus. 2. virtual chromoendoscopy (NBI, RDI, BLI, I-SCAN, FICE, LCI). 3. Fluorescence Endoscopy (Autofluorescence- AFI, Infra-Red Imaging)

High-definition colonoscopy (HDC): HDC enhances the ADR, particularly for colonoscopists with a low ADR (<20%); however, there is no improvement in detecting high-risk, flat polyps or proximal lesions for those with a high ADR (>20%) (361). Other research (362) has demonstrated that HDC, when compared to standard colonoscopy, significantly improves detection rates, particularly for flat and sessile polyps. A recent retrospective study from 2022 (321) revealed that HDC significantly increased the detection rate of sessile serrated lesions during population-based average risk group screening colonoscopy compared to standard colonoscopy.

Narrow spectrum endoscopy includes narrowband imaging (NBI) on Olympus scopes, Fujinon intelligent colour enhancement (FICE) and I- scan on Pentax scopes.

Meta-analysis studies that have looked at RCTs evaluating conventional white light endoscopy versus narrowband imaging has shown no increase in ADR nor does it decrease the miss rates of adenomas in patients undergoing screening surveillance colonoscopy(363-366).

Autofluorescence imaging (AFI): A meta-analysis (367) assessed Autofluorescence Imaging (AFI) for detecting colorectal neoplasia in average-risk patients and found no difference between AFI and White Light Endoscopy (WLE) in terms of ADR or Polyp Detection Rate (PDR).

A randomised controlled trial (368) that examined the updated AFI for detecting flat lesions showed a significant increase in the detection of right-sided flat lesions, primarily adenomas and carcinomas, but not sessile serrated lesions. Furthermore, there was no overall increase in ADR or PDR. Consequently, AFI appears to offer no substantial additional value for polyp detection in the average-risk population (205).

2.1.4.2.2 Ancillary technology and techniques to improve mucosal visualisation and polyp detection

These include the following (307), 1. dye based chromocolonoscopy (as discussed above). 2. distal attachment devices: distal attachment devices are disposable attachments at the end of the colonoscope such as transparent caps and Endocuff device. 3. specialised colonoscopes such as full-spectrum endoscopy, extra wide-angle view colonoscope, G-EYE balloon colonoscope. 4. accessory video processors-Third Eye Retroscope and Third Eye Panoramic

Standard distal attachment caps have not consistently demonstrated improvements in polyp detection (369). However the Endocuff device may result in an increase in ADR (370) particularly for polyp detection the right colon (35.4% to 53.5%). A multicentre RCT in the UK (371) showed that Endocuff significantly improves ADR in bowel cancer screening patients (36.2% to 40.9%; P = 0.02).

A network meta-analysis (372) in 2019 compared the various devices and endoscopy techniques used to help to increase ADR reviewed 74 randomised controlled trials to compare the efficacies of different approaches. These studies compared the efficacies of add-on devices such as the use of a cap, Endocuff, Endo rings, G-EYE, enhanced imaging techniques such as chromoendoscopy, NBI, FICE, BLI and newer endoscopes such as full-spectrum endoscopy, extra wide-angle view colonoscopy, dual focus and low-cost optimising existing resources such as water aided colonoscopy, second observer, dynamic position change alone or in combination with HD colonoscopy or each other.

The results of this network meta-analysis showed that the low-cost optimising of existing resources (OR, 1.29; 95% CI, 1.17-1.43), enhanced imaging techniques (OR, 1.21; 95% CI, 1.09-1.35) and add-on devices (OR, 1.18; 95% CI, 1.07-1.29) were associated with a moderate increase in ADR compared with HD colonoscopy. The use of newer endoscopes was not associated with significant increases in ADR compared with high-definition colonoscopy (OR, 0.98; 95% CI, 0.79-1.21).

In the comparative efficacy analysis, there was no single specific technology for increasing ADR that was superior to others. There was also no significant difference found between the technologies in the detection of advanced ADR, polyp detection rate or the number of adenomas per patient.

The conclusion was that low-cost optimisation of existing resources is as effective as enhanced endoscopy imaging or add-on devices and increasing ADR during high-definition colonoscopy.

2.1.4.3 Training

In order to maximise the detection and visualisation of these flat polyps the following factors need to be taken into consideration which inadvertently helps to improve the quality of colonoscopy and thereby improve proximal and serrated neoplasia detection. This includes improving the cognitive knowledge of the endoscopist by means of training. Studies have shown that there is a learning curve (280-282) associated with recognising flat polyps that include serrated neoplasia and ADR.

A study examining the impact of screening colonoscopy quality discovered that a colonoscopists volume and life experience did not influence ADR. Interestingly, those who frequently attended continuing medical education meetings exhibited the highest ADR (373). Another study showed that simple educational efforts can improve ADR (281). In a study called the Endoscopic Quality Improvement Program (EQUIP), colonoscopists who participated in an additional training program were randomly assigned to the EQUIP program, and their baseline and post-training ADRs were measured. Following the training, the ADR of endoscopists in the EQUIP group increased to 47%, while the ADR for those who did not receive the training remained constant at 35% (281).

In summary, the key to improving colonoscopy quality and optimising serrated neoplasia detection seems to include high quality bowel preparation, meticulous examination technique and endoscopists with high ADR along with the use of HD scopes (307).

Endoscopists with high ADR are less likely to gain significant improvements in ADR by using additional technologies and ancillary devices currently available (307).

2.1.5 The Rationale and Aims of the CONSCOP Study

2.1.5.1 Rationale

Bowel cancer screening started in 2006 in the UK subsequent to evidence from large-scale randomised trials on stool-based faecal occult blood testing followed by colonoscopy for those individuals testing positive (34, 36, 39, 374). The strategy has been proven to reduce mortality from bowel cancer by 15% however its effectiveness seems to be variable and seems to have a limited impact in the reduction of proximal colon cancers (15, 269, 375).

Some studies have cast doubt over the effectiveness of colonoscopy reducing the incidence and mortality of proximal colorectal carcinomas with a relatively high incidence rate of PCCRC in the proximal colon (14, 15). As discussed in the preceding chapter, the factors that can contribute to the occurrence of PCCRC include technical factors which include the quality of the procedure, operator factors and biology dependent factors, for example failure to detect flat lesions such as serrated polyps in the proximal colon (258, 376).

There has been a wide variation in the reporting of the prevalence of serrated lesions in the proximal colon and this has been mainly based on retrospective studies during the time of this study (255, 303, 377). Serrated polyps are thought to account for a significant proportion of missed polyps that eventually can lead to PCCRC (303).

Unlike conventional colonic adenoma surveillance appropriate surveillance interval for serrated polyps is uncertain and is mainly based on expert and consensus recommendation with a low evidence-base (287).

The technique of chromocolonoscopy with the use of dye spray has been investigated in a number of studies in different settings and all studies have consistently demonstrated increase in polyp detection rates (157, 285, 378, 379). In contrast methods of digital contrast enhancement though very helpful to characterise polyps morphologically have failed to conclusively demonstrate an increase in polyp detection rates when controlled for other variables (380).

Most of the evidence of adjunctive devices were published after the completion of this study. Digital contrast enhancement technology also varies between commercial processes for colonoscopy unlike dye spray technique that is universally applicable. A Cochrane review (285) of chromocolonoscopy has also analysed studies using dye spray technique though not in the setting of screening and found a positive correlation between polyps detected and technique. Pancolonic chromocolonoscopy already forms a part of standard practice in other settings such as surveillance for high-risk cases and IBD in this part of the national guidelines and the setting (328).

Various studies as described in the preceding subchapters have demonstrated that there are a number of variables that can potentially affect adenoma, polyp, and cancer detection rate such as the quality of bowel preparation, quality of the procedure including withdrawal times, withdrawal technique and training and experience of the colonoscopist.

In the national bowel cancer screening programme, colonoscopists in England and Wales have to undergo a rigorous assessment and accreditation process in order to achieve and maintain a high-quality minimum standard for KPIs (381) such as ADR, caecal intubation, withdrawal time which are closely monitored. Patient -related outcomes that include quality of bowel preparation, comfort scores, lesion detected, and plans and outcomes of management are also strictly monitored.

Therefore, the UK bowel cancer screening programme provides the appropriate setting to investigate and provide real-life data, whether colonoscopy (chromocolonoscopy) can improve detection and removal of serrated lesions in the proximal colon and in the long-term the incidence of post-colonoscopy interval cancers with the best current standardisation of these variables.

However, prior to committing resource to any intervention to potentially reduce PCCRC, this study is a feasibility study to justify the clinical and cost effectiveness of chromocolonoscopy in the screening programme. If deemed feasible the outcomes would help to inform a larger study to be able to assess outcomes for PCCRC and inform surveillance programs for serrated neoplasia. This study aims to test the feasibility of undertaking such an intervention within the bowel cancer screening programme in Wales in order to inform the above.

Research question: Is it feasible and acceptable to participants, professionals, and the screening programme to undertake an enhanced colonoscopy technique during bowel screening and is the time taken and resource needed for this likely to be considered feasible and acceptable by organisations in charge of screening?

2.1.5.2 Aims of the study

1. To assess feasibility of implementation of Chromocolonoscopy within a population wide screening programme (Uptake by both participants and screeners).
2. To assess whether the Chromocolonoscopy takes an acceptable length of additional time to conduct within the screening programme.
3. To estimate the proximal serrated polyp detection rate in the intervention arm (with standardised and monitored operator and procedure quality and rigorous histopathology assessment) in the trial arms to inform the sample size calculation of a future trial by allowing an assessment of the possible magnitude of improvement in cancer detection.
4. To assess the feasibility of incorporating an economic evaluation into a larger scale trial by exploring issues relating to collection of data relating to resource utilization, costs, and outcome measures.

Once the feasibility of the trial has been established, this will help in the design of a future trial that will allow the estimation of the proportion of samples needed for long-term longitudinal studies and to also inform the design of the translational element of a future trial.

2.2 Methodology

2.2.1 Funding and ethical approval

The CONSCOP trial was funded by the National Institute for Social Care and Health Research (NISCHR), research for patient and public benefit Wales and hence was a part of the NIHR/NISCHR portfolio of clinical trials. The trial was supported by Cancer research UK core funding at the Wales Cancer trials unit (WCTU). The trial protocol was approved by a UK Multi-Centre Research Ethics Committee Appendix B (reference 14/WA/0004) and was sponsored by Cardiff University.

The main trial management group consisted of the trial manager, statistician, patient representative, chief investigator, and clinical research fellow (RR). The research fellow was responsible for completion of the application for approval through the integrated research application system (IRAS), design of the database for data collection, design of the case report form (CRF), the trial protocol, participant information sheet (PIS), material for the training days, (Appendix F,G) individual site initiation and troubleshooting with the individual sites alongside the trial manager and data collection of polyp histopathology, coordination and chair for the pathology meetings to discuss polyp histology and achieve consensus opinion including recording data from the pathology meetings.

2.2.2 Study Design and Participants

The study was a multicentre, randomised, open-label, non-inferiority, feasibility trial of dye enhanced colonoscopy (chromocolonoscopy) versus standard white light colonoscopy in the bowel screening programme in Wales recruiting for a period of 18 months.

All Bowel Screening Wales (BSW) centres (N=14), all bowel cancer accredited screening colonoscopists (N=23) around Wales, and eligible participants (between the age 60 and 74 years) were encouraged to participate in the trial.

2.2.2.1 The Inclusion criteria for participants

This included participants between the age of 60 and 74 years who tested positive on the faecal occult blood test (FOBT) in the bowel cancer screening programme in Wales who were eligible for an index screening colonoscopy procedure.

2.2.2.2 Exclusion criteria

The following participants were deemed ineligible to participate in the trial:

1. Participants with a known diagnosis of hereditary polyposis syndrome (Lynch syndrome, polyposis syndrome) and those with known chronic inflammatory bowel disease
2. Any participant not deemed fit for colonoscopy in the screening programme and/or undergoing alternative investigations such as CT Colon or minimal prep CT scan in place of their index procedure.
3. Participants who had previously undergone colorectal surgery were excluded from the study however their standard management in the screening programme was unaltered.

4. Any participant with a known allergy to a food colouring agent

2.2.3 Recruitment Process

The following describes the recruitment process for bowel screening sites, practitioners, and participants.

2.2.3.1 Colonoscopists and local assessment bowel screening centres in Wales

At least 3 training days were organised and screening colonoscopists and specialist screening practitioners (SSPs) from all over Wales were invited to participate. The day-long training event which was delivered by the chief investigator and research fellow included quizzes of images and videos before and after the training, a training resource (Appendix D) for reference, and lectures and video tutorials on technique and lesion detection with and without indigo carmine dye spray. A refresher training on Paris classification, Kudo classification, and lesion characterisation with virtual and dye-based chromocolonoscopy was also included.

The purpose of the training days for colonoscopists and screening practitioners, was to ensure standardisation of technique of dye dilution and spray, and detection, identification, and removal of polyps under indigo carmine dye.

Although colonoscopists who undertake bowel cancer screening in this cohort were all accredited to the same standard and some had previous experience of pancolonic dye spray use in the context of chronic inflammatory bowel disease and Lynch syndrome. This was not standard practice in the symptomatic service and there were some who did not have this experience. Hence it was necessary to ensure standardisation of technique prior to the initiation of the trial. The protocol used for dye dilution to 0.2% was provided in the training pack (Appendix H)

The sites that agreed to participate then had local ethical approval process in place and thereafter each site had a trial initiation day where the chief investigator, research fellow and trial manager visited each site to relay and re-emphasise information and also be able to allow access to troubleshoot any issues in the sites.

Participating sites were also provided with resources such as irrigation pumps, supply of dye and stop watches. SSPs were offered further support by training days, trial initiation days and email support if any further queries.

2.2.3.2 Recruitment process of participants

A summary of this process has been described in the trial schema (Figure 18).

2.2.3.2.1 Before the procedure

The SSP conducts a telephone interview for those participants who have a positive faecal occult blood test as routine prior to organising a colonoscopy test. (Appendix I).

Eligible people had the study described to them and, if they were interested in participating, were sent more information (Appendix F,L,M,) along with standard information about the screening colonoscopy. Informed consent was taken by an SSP when the patient attended for colonoscopy, prior to the patient being told which trial arm they had been allocated to (Figure 18). No consent was taken at this point and the participant was allocated to a list as usual.

Around 2-3 days before the designated list, the SSP checked that the list was complete and online randomisation was done by a web link. The SSP did not share this information with the participant or the colonoscopist until the day of the procedure just before the commencement of the procedure. (Appendix J)

Consent was confirmed with the participant on the day of the procedure. Information regarding which trial arm was not shared with the participant or colonoscopist until the start of the procedure. Colonoscopists were made aware of the trial arm only when the procedure was commenced. The endoscopy staff had diluted dye prepared in advance.

2.2.3.2.2 During the procedure

Participants randomly assigned to the standard group had a colonoscopy performed as per standard practice. For participants randomly assigned to the chromocolonoscopy group, once the caecum was reached, indigo carmine dye (0·2% as used in standard clinical practice; manufactured by Diagmed, Thirsk, UK) was sprayed on the surface of the proximal colon (caecum to splenic flexure) with a pump-assisted spray through the colonoscope on withdrawal.

In participants allocated to the chromocolonoscopy group, with inadequate bowel preparation on the day (Appendix K) dye was used at the subsequent adequately prepared colonoscopy, otherwise repeat procedures in the standard arm used standard white light colonoscopy.

Colonoscopists were allowed to use the irrigation pump with water for washing colonic mucosa, without any restriction in both trial groups. Ten sites used high definition colonoscopes (not mandated), one used a high-resolution colonoscope, and one used a standard definition colonoscope. Polyps retrieved from all index colonoscopies and at associated index associated clearance procedures up to 1 year after were included in the analysis. Surveillance procedures (including high risk one year surveillance as per previous BSG 2009 guidelines) were not included. Polyps found on CT colonography, performed for incomplete procedures, were excluded from the analysis. Data collection and process during the procedure and after has been described in the following section.

2.2.4 Randomisation and masking

Randomisation and masking all potential participants were randomly assigned (1:1; with the use of minimisation stratified by centre with an 80:20 random element) to either standard white light colonoscopy (the standard group) or chromocolonoscopy (the chromocolonoscopy group) for their index procedure with the use of a secure, internet-based, computerised, randomisation system that used centralised, dynamic allocation.

It was not possible to mask either the patient or colonoscopist to trial group, but the expert panel of three gastrointestinal pathologists who classified every proximal colon polyp were blinded to the local pathologists reports and to each other's reports (Appendix E).

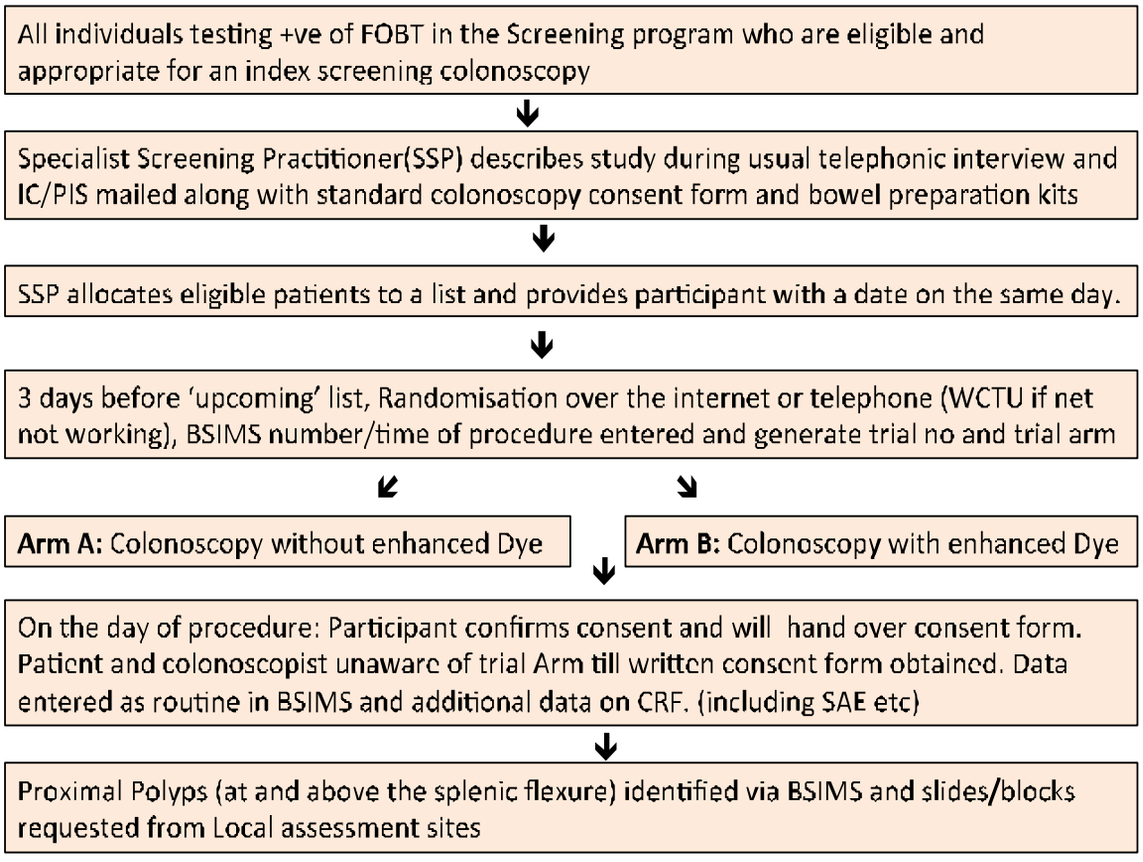


Figure 18: Trial Schema.

Abbreviations: F.O.B-faecal Occult Blood; PIS-Patient Information Sheet, IC-informed consent form; SSP-Specialist Screening Practitioner; WCTU-Wales Cancer trials unit; BSIMS-Bowel Screening Information Management System; CRF- Case Report Form; SAE-Serious Adverse Events

2.2.5 Data Collection and Process

The Bowel Screening Information Management System (BSIMS) is a database that contains all the clinical information of a participant that has undergone a procedure or participated in the programme by returning a positive FOB test. Each bowel screening participant had an identifier number assigned to them called a BSW number. The SSPs in usual practice entered details of the participant onto BSIMs from the point of contact or consultation.

The SSP was also responsible for recording details of their procedure, outcome, and management. SSPs were advised to upload scanned histopathology reports onto the BSIMS system as a part of the trial.

This was done for the research fellow (RR) to be able to extract accurate polyp histology information from a final report which included the unique histology number, data regarding location and size of the polyp, pathology report and details of the assessment centre and reporting pathologist. If the pathological size was not available (in case of piecemeal removal of polyp or fragmentation) then the endoscopic size of the polyp was recorded. This has been described in detail in Chapter 3 which describes the histopathological aspects of the trial (Appendix C).

The time taken to perform the colonoscopy along with the data on colonoscopy outcomes, polyps found, bowel preparation, sedation, and technical quality indicators were collected by SSPs as part of routine data collection. Additional data that was needed for the trial was recorded on a trial specific case report form (CRF) that included smoking, family history of bowel cancer, endoscopist assessment of procedural difficulty and details on dynamic position change.

The participant comfort levels were recorded as scores (1-no discomfort, 2-minimal, 3-mild, 4-moderate, 5-severe discomfort) by the SSP as per the modified Gloucester comfort scale (382).

In addition to this data on resource use during index colonoscopy (e.g., probes, coagraspers, clips, snares, specimen pots) which were not routinely collected in clinical practice, was collected from one site and recorded on the CRF (Appendix N).

CRFs were completed by the SSP, and copies faxed to WCTU within a 4- week period. Complications that occurred immediately or within 30 days were highlighted by SSPs or clinicians and the clinical trials management group completed serious adverse events (SAE) and AE as per protocol within the time frame. Data monitoring was managed by the trial management group on a periodic basis. All SAEs were reported until 30 days after colonoscopy.

All proximal polyps were included in the analysis, regardless of initial reported histology and were collected from local centres and assigned a unique polyp number by the research fellow (RR) and presented for central review by an expert panel of three gastrointestinal consultant pathologists. The process of data collection and methodology of the histopathological aspect of the study has been detailed in chapter 3.

All three pathologists were part of the national referral pathways for the bowel cancer screening programme reviews of pathology and were involved in pathologist training and accreditation and regular review of second opinion lesions as part of a national pathology expert panel.

In order to avoid variation in the final reports, predefined standard diagnostic criteria based on WHO 2010 (153) classification and the AGA criteria (162) for serrated lesions were agreed.

In accordance with the UK guidance, the term sessile serrated lesion was used for lesions, which are described elsewhere as sessile serrated adenomas or polyps. The expert panel reviewed all slides independently and were masked to the original report. Cases without diagnostic agreement were re-reviewed by all three pathologists to reach a consensus diagnosis. If an agreement was not achieved, the lesion was deemed unclassifiable.

An advanced adenoma was defined as a conventional adenoma with either high-grade dysplasia, more than 25% villous histology, or measuring 10 mm or larger in size. (153) Significant serrated lesions included either sessile serrated lesion with dysplasia, non-dysplastic sessile serrated lesions measuring >=10 mm, and all traditional serrated adenomas. The term advanced neoplasia incorporated all advanced adenomas and significant serrated lesions.

The health economics aspect of the trial was performed by the health economics team in Swansea.

A cost-consequence analysis was done to evaluate the costs associated with colonoscopy procedures within the study to compare resource use. The costs were assessed from the perspective of the UK National Health Service in two parts: the additional costs of providing new resources required to implement chromocolonoscopy and resources used during routine practice.

Implementation costs of chromocolonoscopy included additional resources in the form of staff time (both trainee and trainer) to train in the new procedure, and the cost of the contrast dye and dispersion equipment.

Resource use data regarding staff time performing the procedure and medications or bowel preparation administered during a procedure were collected from all participating screening sites. The data on resources and consumables used were collected from only one site during index colonoscopies. Details of resource use analysis methods can be found in Appendix N.

2.2.6 Statistical Analysis

This was performed by the statistician in the trial management group with clinical advice from the PI and research fellow. This feasibility study was powered to assess non-inferiority of time taken to perform the colonoscopy procedure. The experience from previous studies had suggested that chromocolonoscopy might take 12 minutes longer than standard colonoscopy however may not exceed 15 minutes.

The power calculation was performed on the assumption that for a common standard deviation (SD) of 15 min (normally distributed based on BSW data), 858 patients would be required for the trial (90% power, one-sided $\alpha=0{\cdot}05$) based on a two-group t test.

The protocol initially aimed to recruit 1052 patients to allow for about 18% loss to follow-up for any reason. However, the trial management group decided to stop recruitment when 741 participants had been recruited because set up of some centres took longer than anticipated. There were no patients who were lost to follow-up after consent, and 741 patients still gave a power of 86%.

The statistical analysis of data was performed by the statistician of the trials management group according to a prespecified analysis plan using the Stata SE 14 statistical package.

The analysis included the primary outcome, analyses of colonoscopy performance, and technical quality indicators (Appendix N) An intention-to-treat population was used for all other analyses. The primary endpoint was assessed by calculating the 95% CIs around the mean difference and comparing them to the non-inferiority margin.

The proportions were compared using chi-square (χ^2) tests. For detection rates, univariable logistic regression was used to calculate the odds ratios for the trial group effect and important prognostic variables (smoking, obesity, sex, family history of cancer). These variables were used in all multivariable models, and screening centres as a random effect, using multilevel mixed-effects logistic regression. Patients found to have cancer also had polyps removed if found and we included these patients in the analyses of polyp detection rates.

The cost analysis and health economics was done by the College of Human and Health Sciences, Swansea centre for Health Economics in Swansea University (Appendix N).

2.3 Results

2.3.1 Recruitment and participation

The trial was open for recruitment over an 18-month period between November 20, 2014, and June 16, 2016. Figure 20 demonstrates the trial profile and CONSORT diagram.

During this time, 86% (12/14) bowel cancer screening centres in Wales (Figure 19) and 87% (20/23) bowel screening colonoscopists agreed to participate in the trial following attendance at the pre-initiation training days.

1031 participants from 12 screening sites who were gFOBT positive and expected to proceed to colonoscopy after discussion with a SSP were offered participation in the trial. Of these participants, (Figure 20), 903/1031 (88%) were considered eligible for the trial (958/1031-93% met the eligibility criteria, N=55 was excluded due to nonparticipating colonoscopist) and 741/903 (82%) consented to participate in the trial.

Following randomisation, the consent rates were similar in each group with 87% (360/416) in the standard colonoscopy (SC) group and 90% (381/424) in the chromocolonoscopy (CC) group.

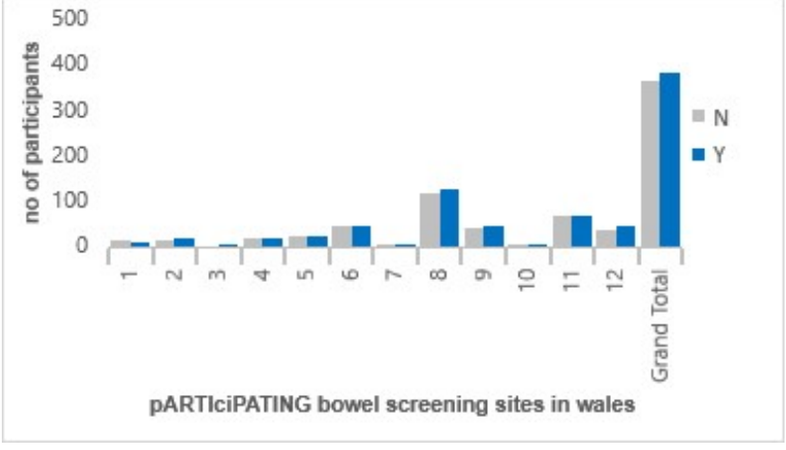


Figure 19: Participating bowel cancer screening sites with distribution of eligible participants into different arms.

(N = no Dye or standard colonoscopy; Y = use of dye or Chromocolonoscopy)

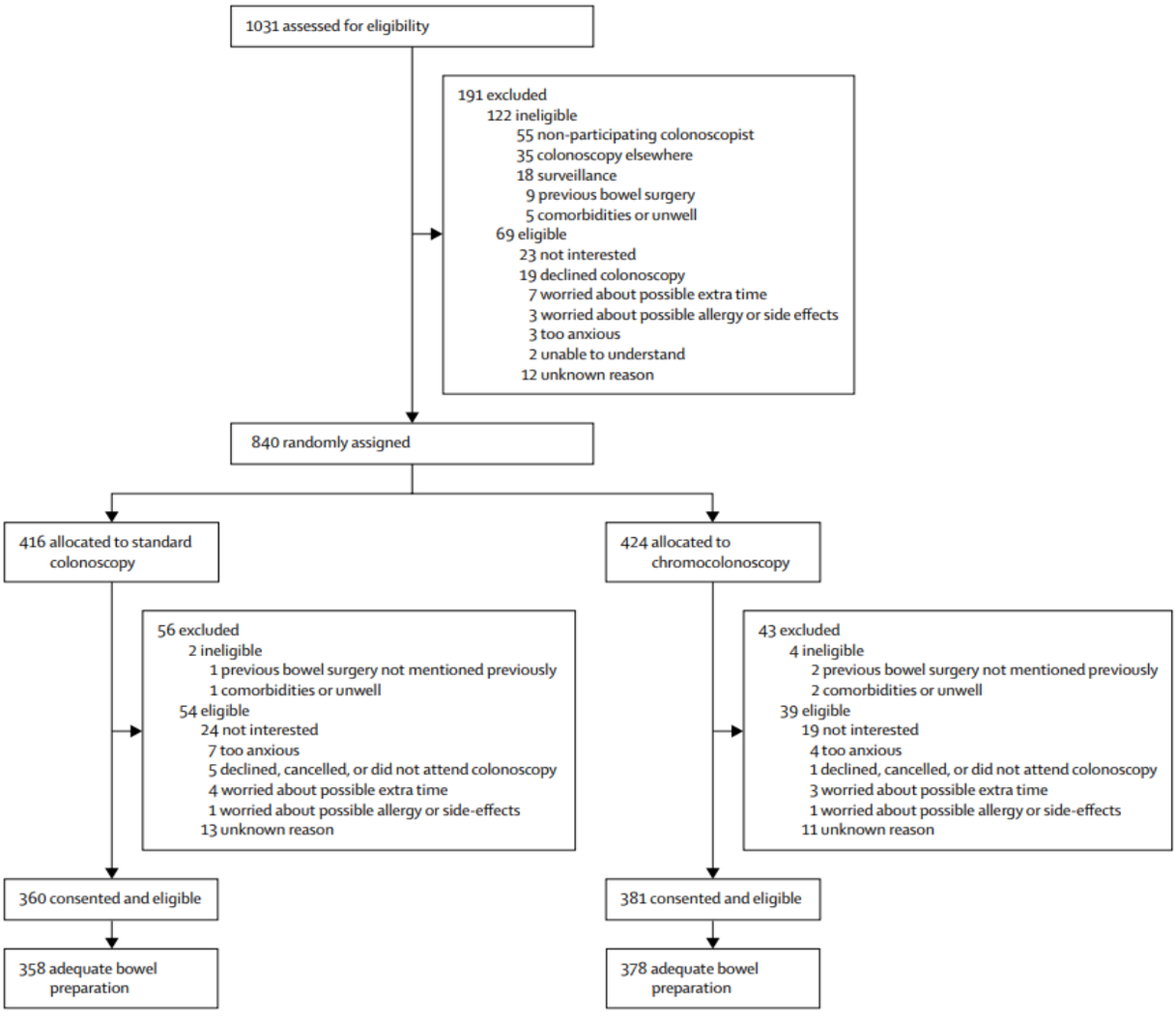


Figure 20: CONSORT diagram-Trial Profile

2.3.2 Baseline demographics and pre-procedure characteristics

The baseline demographics of the participants between the trial groups has been detailed in Table 4.

The median age in both arms for both sexes was 68 years. There was a preponderance of males (65%, 67%) versus females (35%, 33%) in both arms over this was well balanced. Similarly, BMI, family history of bowel cancer, presence of diverticular disease, distribution of smoking status and aspirin use were well balanced between the two arms of the trial.

The following demographic characteristics were noted in the standard colonoscopy (SC) versus the chromocolonoscopy (CC) trial arms. The mean BMI was 28.8 vs. 28.9 with 37.2% vs 33.3% of participants that had a BMI over 30. Those participants that had a family history of bowel cancer was 12.5% vs. 12.6%. Those that had previous abdominal and pelvic surgery and diverticular disease were 26.7% vs. 28.3% and 55.8% vs. 51.2%.

The current smoking status that was recorded on the CRF demonstrated that majority of the participants had stopped smoking (ex-smokers) with current smokers (10.3% vs. 11.8%), ex-smokers (50% vs. 51.4%), those who had never smoked (39.7% vs. 36.5%). The median pack year history for both smokers and ex-smokers was 20 versus 16 in the SC and CC arms respectively.

Aspirin data was only collected after the first 210 participants (N =531) with similar participants noted in both arms (SC vs. CC, 254 vs. 277). The majority of participants (71.3% vs. 70.8%) had never taken aspirin and 20.5% versus 20.6% were currently taking aspirin, and 95.4% in both groups were taking aspirin at a dose of 75 mg once a day.

Table 4: Baseline Demographics of the participants

Abbreviation: BMI-body mass index, SD-standard deviation, IQR-into quartile range (median).

Demographics		**Standard colonoscopy (N=360)**	**Chromocolonoscopy (N=381)**
Age	Median (IQR)	67.6 (62.6-70.7)	67.7 (62.7-70.8)
Sex	Male	234 (65.0%)	256 (67.2%)
	Female	126 (35.0%)	125 (32.8%)
BMI	mean (SD); Obese ≥30 - n (%)	28.8 (5.1); 134 (37.2)	28.9 (5.6); 128 (33.6)

	Missing	2 (0.6%)	5 (1.3%)
Family history of bowel cancer	No	302 (83.9%)	318 (83.5%)
	Second degree	9 (2.5%)	13 (3.4%)
	First degree	45 (12.5%)	48 (12.6%)
	Both	3 (0.8%)	0 (0.0%)
	Missing	1 (0.3%)	2 (0.5%)
Family history of bowel polyps	No	340 (94.4%)	349 (91.6%)
	Second degree	2 (0.6%)	2 (0.5%)
	First degree	15 (4.2%)	29 (7.65)
	Missing	3 (0.8%)	1 (0.3%)
Previous abdominal/pelvic surgery	Yes	96 (26.7%)	108 (28.3%)
	Missing	5 (1.4%)	4 (1.0%)
Presence of diverticular disease	Yes	201 (55.8%)	195 (51.2%)
	Missing	6 (1.7%)	5 (1.3%)
Current smoking status	Smoker	37 (10.3%)	45 (11.8%)
	Ex-smoker	180 (50.0%)	196 (51.4%)
	Never smoker	143 (39.7%)	139 (36.5%)
	Missing	0 (0.0%)	1 (0.3%)
	Pack years for smoker/ex-smoker – median (IQR, n, missing data)	20 (10-39, n=205 ,12)	16 (8-34, n=231, 10)
Aspirin data was only collected after the first 210 patients			
		N=254	**N=277**
Does the patient take daily aspirin?	Currently	52 (20.5%)	57 (20.6%)
	Previously	21 (8.3%)	24 (8.7%)
	Never	181 (71.3%)	196 (70.8%)
If currently taking aspirin, what is daily dose?	75mg	49/52 (94.2%)	55/57 (96.5%)
	>75mg	3/52 (5.8%)	1/57 (1.8%)
	Missing	0/57 (0.0%)	1/57 (1.8%)

2.3.3 Intra-procedural characteristics for index colonoscopy

The mean volume of fluid sprayed (diluted indigo carmine dye 0.2%) in the chromocolonoscopy group was 165.8 mL (SD 62.3). No participants withdrew consent during the colonoscopy procedure.

2.3.3.1 Quality Indicators

The bowel preparation scores, completion rates, endoscopist assessment of procedural difficulty, and comfort scores were similar in each arm. Technical quality indicators, percentage of participants who had a position change and other manoeuvres during the procedure, and use of antispasmodic and sedation at first colonoscopy were also well balanced between the trial groups.

The following intraprocedural characteristics for index standard colonoscopy (SC) versus chromocolonoscopy (CC) were noted. In over 99% of participants in both groups had excellent to adequate bowel preparation (99.7% vs. 99.2%). The caecal intubation rate was 96.4% vs. 96.8%. In terms of technical quality indicators, colonoscopist demonstrated a high-quality procedure in over 95% of participants in both arms. This included dynamic position change (96.4% vs. 95.8%, adequate insufflation (98% vs. 97.1%), repetitive examination of colonic segment (95.8% in both arms), examination of flexures and proximal sides of folds (96.9% vs. 96%), the use of torque to flatten folds (96.9% vs. 95.2%) and suctioning of excess liquid (97.8% vs. 96.3%).

The patient comfort score, rated as per the modified Gloucester comfort score showed that 64.5% vs. 66.6% of the participants in SC vs.CC groups no discomfort or minimal discomfort.

The use of conscious sedation that included a benzodiazepine (midazolam) along with an opiate (fentanyl or pethidine) in participants was 48.9% vs.47.6% in SC vs.CC arm respectively. Entonox was used in 22.3% vs. 24.3% and buscopan and was used in 43.3% vs. 44.4% of participants in both groups.

In about 78% of participants in both groups, the colonoscopist found the procedure easy to average to perform however the procedure was rated difficult in 16.2% vs.17.5% and 3.6% vs. 3.2% the procedure was not completed in SC vs.CC groups.

Table 5: Quality indicators including Bowel Preparation quality, technical factors, sedation, procedural difficulty, and patient comfort score.

		Standard colonoscopy	**Chromocolonoscopy**
Index colonoscopy with adequate bowel preparation		358	378
Inadequate bowel preparation at index then no further colonoscopies		2 (0.6%)	3 (0.8%)
Bowel preparation score	Adequate	237 (66.2%)	240 (63.5%)
	Excellent	120 (33.5%)	135 (35.7%)
	Missing	1 (0.3%)	3 (0.8%)
Completion rate	Complete (caecum/ileum)	345 (96.4%)	366 (96.8%)
	Incomplete (other)	13 (3.6%)	12 (3.2%)
Colonoscopy technical quality indicators	Patient position change required	345 (96.4)	362 (95.8)
	Missing	1 (0.3)	3 (0.8)
	Adequate insufflation	351 (98.0)	367 (97.1)
	Missing	5 (1.4)	10 (2.6)
	Repetitive examination of colonic segment	343 (95.8)	362 (95.8)
	Missing	5 (1.4)	11 (2.9)

	Examination of flexures/proximal sides of folds	347 (96.9)	363 (96.0)
	Missing	5 (1.4)	11 (2.9)
	Use of torque to flatten folds	347 (96.9)	360 (95.2)
	Missing	5 (1.4)	11 (2.9)
	Suctioning of excess liquid	350 (97.8)	364 (96.3)
	Missing	5 (1.4)	10 (2.6)
	Use of mucolytic / simethicone over polyp	172 (48.0)	186 (49.2)
	Missing	6 (1.7)	11 (2.9)
Type of bowel prep	Moviprep	225 (62.8)	228 (60.3)
	Kleenprep	127 (35.5)	146 (38.6)
	Picolax	2 (0.6)	1 (0.3)
	Missing	4 (1.1)	3 (0.8)
Sedation	Entonox	80 (22.3)	92 (24.3)
	Missing	5 (1.4)	7 (1.9)
	Midazolam	175 (48.9)	180 (47.6)
	Missing	1 (0.3)	0 (0.0)
	Pethidine	17 (4.7)	22 (5.8)
	Missing	2 (0.6)	1 (0.3)
	Fentanyl	159 (44.4)	162 (42.9)
	Missing	1 (0.3)	0 (0.0)
Other medication	Buscopan	155 (43.3)	168 (44.4)
	Missing	2 (0.6)	1 (0.3)
Endoscopist assessment of procedural difficulty	Easy	108 (30.2%)	106 (28.0%)
	Average	172 (48.0%)	190 (50.3%)
	Difficult	58 (16.2%)	66 (17.5)
	Unable to complete	13 (3.6%)	12 (3.2%)
	Missing	7 (2.0%)	4 (1.1%)

Procedure comfort score (Gloucester)	1- No discomfort	73 (20.4%)	72 (19.0%)
	2- Minimal	158 (44.1%)	180 (47.6%)
	3- Mild	107 (29.9%)	101 (26.7%)
	4 -Moderate	17 (4.7%)	23 (6.1%)
	5- Severe	2 (0.6%)	2 (0.5%)
	Missing	1 (0.3%)	0 (0.0%)

N.B. Numbers in cells are n (%)

2.3.3.2 Procedure time and withdrawal time:

In the first (index) colonoscopy with adequate bowel preparation, the procedure time was longer in the chromocolonoscopy arm (mean 36.8 vs 30.6 minutes) (Table 6). However, the difference did not exceed the 15 minutes specified *a priori* as the non-inferiority margin (mean difference 6.3 minutes, 95% CIs: 4.2-8.4). The data showed some evidence of positive skew, but bootstrapping produced the same estimate for the confidence interval.

The magnitude of this difference was reflected in the withdrawal times (mean 24.1 vs 18.7 minutes). The difference in withdrawal times was smaller when no polyps were removed (mean 28.6 vs 24.2 minutes) compared to when polyps were removed (mean 41.3 vs 35.2 minutes).

The procedure time was also longer in the CC arm when the colonoscopist assessed the procedure as being difficult (66 vs.58 minutes) with a mean difference of 6 minutes.

Table 6: Procedure and withdrawal time by mean, median and inter quartile range (IQR).

Procedure Time							
		No of procedures	Mean	SD	Median	Min IQR	Max IQR
Average procedure time in minutes	WLE	358	30.6	13.7	28	22	36
	CC	378	36.8	15	34	27	45
When polyps removed	WLE	207	35.2	14.2	33	25	42
	CC	244	41.3	15.2	39	30	50
When no polyps removed	WLE	151	24.2	9.8	24	17	28
	CC	133	28.6	10.5	28	21	34
When endoscopist assessed procedure as difficult	WLE	58	41.4	14.8	40	31	48
	CC	66	47.4	15.9	44	36	58
Withdrawal Time							
Average withdrawal time in minutes	WLE	358	18.7	11.3	16	11	22
	CC	378	24.1	12.7	21	15	31
When polyps removed	WLE	204	23	12.2	20	15	28
	CC	242	28.5	12.8	25.5	19	35
When no polyps removed	WLE	141	12.5	5.6	11	8	16
	CC	123	15.4	6.6	15	10	18

2.3.4 Post- Procedure findings

This included repeat procedures (repeated due to previous incomplete procedure, poor bowel preparation and for polyp clearance) and polyp detection rate following confirmation from histological reports and expert consensus view of the proximal polyps.

2.3.4.1 Repeat Procedures

Participants in the chromocolonoscopy arm had more procedures (477 vs 427) than in the standard arm (Table 7). This was due to more repeat procedures to remove polyps (therapeutic indication) or check completeness of previous excisions in line with the BSG guidelines (Figure 21). In the chromocolonoscopy arm, more participants (76/381 (19.9%) vs 48/360 (13.3%); post hoc χ^2=5.812, p=0·016) had a final outcome of requiring high risk surveillance as per the previous BSG guidelines (53) 2009 which had recommended a 12 month surveillance.

Fewer participants had an outcome of discharge back to routine FOBT testing (159/381 (41.7%) vs 162/360 (45.0%)).

Figure 21: Repeat procedures following index colonoscopy-white light endoscopy (WLE) versus Chromocolonoscopy

Participants that required a further polyp clearance procedure continued to 1 year after the last participant had their index procedure.

Table 7: Repeat Procedures for the different trial arms

Procedure outcome		Standard colonoscopy	Chromocolonoscopy
Number of participants		**360**	**381**
Number of procedures	Total number	427	477
	Per person rate	1.19	1.25
	Number of people receiving >1 procedure	53 (14.7%)	65 (17.1%)
Repeat Procedures	Check Procedure after piecemeal removal	26	33
	Incomplete previous procedure	2	0
	Poor bowel preparation at previous procedure	11	8
	Therapeutic indication	28	55
Type of repeat procedure	Colonoscopy	399	430
	Flexible sigmoidoscopy[A]	28	47
Final outcome	Repeat	21 (5.8%)	20 (5.2%)
	Discharge back to routine FOBT screening	162 (45.0%)	159 (41.7%)
	No further colonoscopies required due to age limit/other bowel condition	22 (6.1%)	19 (5.0%)
	3-year surveillance – intermediate risk	64 (17.8%)	63 (16.5%)
	12-month surveillance – high risk	48 (13.3%)	76 (19.9%)
	Refer to surgery for non-cancer indication	2 (0.6%)	4 (1.0%)
	Cancer[B]	41 (11.4%)	40 (10.5%)

2.3.4.2 Polyp and Cancer Detection

Table 8 shows the WHO classification of all polyps retrieved at index colonoscopy and associated polyp clearance procedures up to one year afterwards. All but five proximal polyps were reviewed centrally by the expert panel. More polyps overall (903 vs 570), and more polyps of each type were found in the chromocolonoscopy arm. The polyp detection rate in the whole colon was 67.7% (258/381) in the CC arm vs. 60.3% (217/360) in the standard arm.

The number of cancers (Table 8) that were detected were similar in both groups (SC vs.CC) with 11.4% (N = 41) vs. 10.5% (N=40). Predominantly proximal CRC was detected in both groups (33 vs. 26).

None of the patients had serrated polyposis as defined by WHO criteria though it was thought that it would be likely that some cases may fulfil these criteria at subsequent colonoscopy.

2.3.4.3 Adenoma detection rate

Higher adenoma detection rates anywhere in the colon (Table 8, Figure 22) was found in the chromocolonoscopy arm 60.9% (232/381) vs 56.4% (203/360). Advanced adenomas defined as those adenomas of size 10 mm or above or with villous features or high-grade dysplasia were similar in both the arms of the study. Advanced adenomas located anywhere in the colon was 30.3% (109) vs. 33.6% (128) and in the proximal colon 8.3% (30) vs. 7.1% (27) in the chromoendoscopy and standard arm respectively.

2.3.4.4 Serrated neoplasia detection rate

The detection rate of serrated neoplasia which includes all types of serrated neoplasia i.e., serrated lesions (SL), significant serrated lesions and sessile anywhere in the colon and the proximal colon between chromocolonoscopy and standard colonoscopy groups. The findings are also summarised in Figure 22,Table 8 and Table 10.

The univariate and multivariable logistic regression for serrated neoplasia which analyse the variables by gender, BMI, smoking history, and a family history of bowel cancer has been presented in Table 10,11 and 12 which is described in the following sections.

2.3.4.4.1 Serrated Lesion (SL)

A significantly higher detection rate in the chromocolonoscopy arm for SLs was found anywhere in the colon: 81/381 (21.3%) vs 51/360 (14.2%), multivariable OR 1.66, 95% CI: 1.12-2.46, p=0.012.

The detection rates for proximal SLs were significantly higher in the chromocolonoscopy arm than in the standard arm in both univariable and multivariable analyses: 45/381 (12%); vs 23/360 (6%) univariable OR 1.96, 95% CI: 1.16-3.32, p=0.012; multivariable OR 2.04, 95% CI: 1.18-3.50, p=0.010) (Table 10) (Figure 22).

SLs were more common in smokers (for proximal SLs multivariable OR 1.79, 95% CI: 1.00-3.22, p=0.050) and for all serrated lesions in the colon; multivariable OR 1.58, 95% CI: 1.03-2.42, p=0.038).

2.3.4.4.2 Significant SL

Secondary regression analyses compared other rates of polyp detection. While absolute polyp numbers are small there is a suggestion that detection rates of "significant" SLs anywhere in the colon were higher in the chromocolonoscopy arm (Table 11) (multivariable OR 2.18, 95% CI: 0.88-5.37, p=0.092) and also in males (multivariable OR 3.23, 95% CI: 0.94-11.2, p=0.063).

When the AGA definition of SSL was used, 13 proximal hyperplastic polyps were re-classified as SSLs (one ≥10mm in the chromocolonoscopy arm). This marginally increased the detection rate of "significant" SLs in the chromocolonoscopy arm: 17/381 (4.5%) vs 7/360 (1.9%); multivariable OR: 2.31, 95% CI: 0.94-5.67, p=0.066).

2.3.4.4.3 Sessile Serrated Lesion (SSL)

The detection rate of proximal SSLs was significantly higher in the chromocolonoscopy arm (Table 12) (multivariable OR 1.91, 95% CI: 1.02-3.59, p=0.045), but this difference disappeared when the AGA definition of SSL was used: 34/381 (8.9%) vs 22/360 (6.1%), multivariable OR 1.58, 95% CI: 0.90-2.78, p=0.114.

Of the 85 SSLs identified in both arms of the study combined, six of the ten polyps with dysplasia were ≥10mm compared with only 13 of the 75 without dysplasia. Interestingly, the 4 proximal SSLs with dysplasia were diminutive with size being <=5mm while all 6 distal SSLs with dysplasia were ≥10mm.

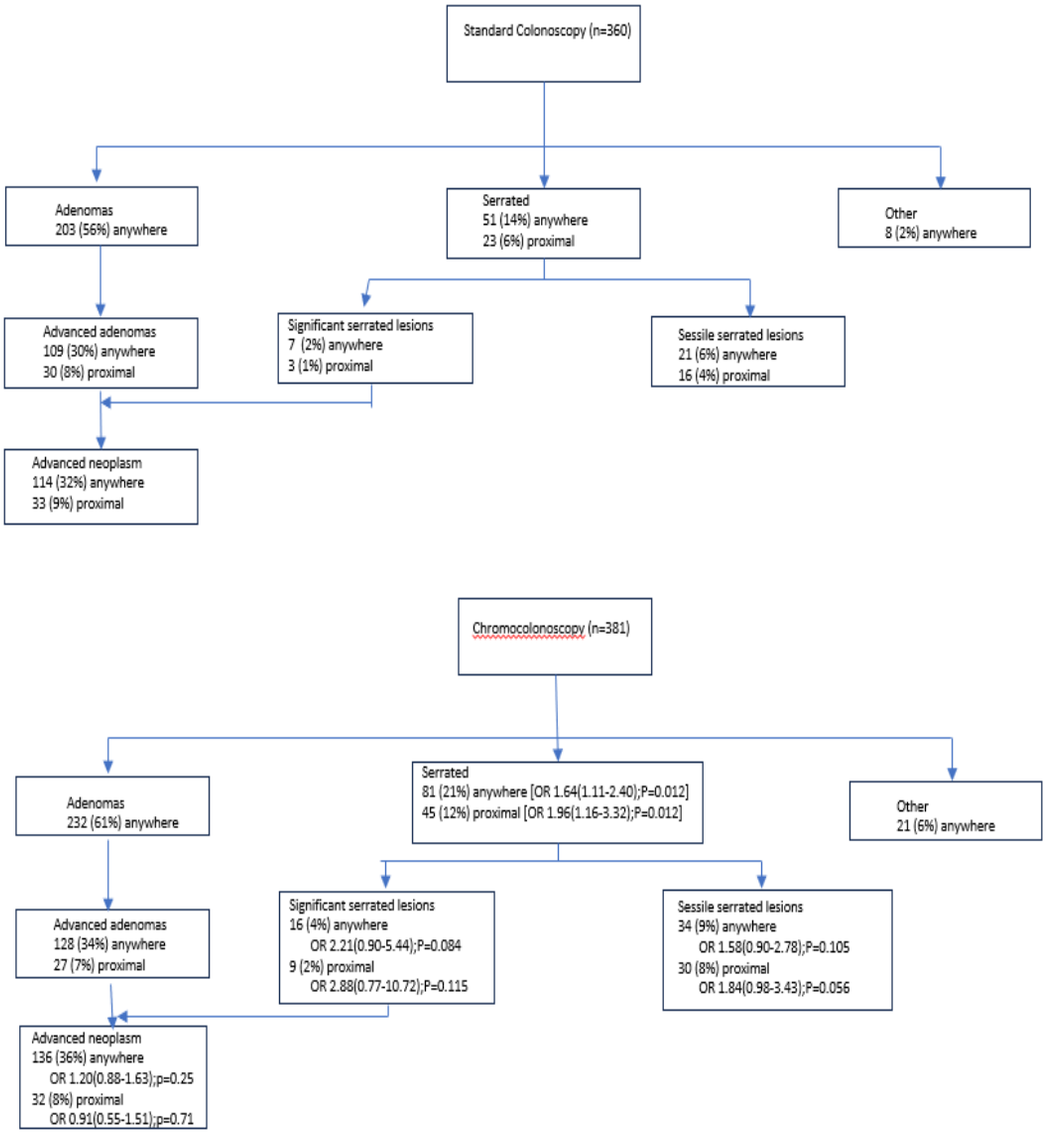


Figure 22: Key Polyp detection rates.

Number of individuals with one or more lesion detected by treatment group. Uni variable ORs are given with 95% CIs, with standard colonoscopy as the reference group. OR = odds ratio.

	Standard colonoscopy (N=360)				Chromocolonoscopy (N=381)			
	Number of participants		**%**		**Number of participants**		**%**	
No polyps or cancer	116		32.2		98		25.7	
Cancers	41		11.4		40		10.5	
Proximal	33		9.2		26		6.8	
Distal	8		2.2		14		3.7	
	Number of polyps		**Polyp detection rate**		**Number of polyps**		**Polyp detection rate**	
	n	**per patient**	**n**	**%**	**n**	**per patient**	**n**	**%**
Polyps (any)	570	1.583	217	60.3	903	2.370	258	67.7
Adenomas	482	1.339	203	56.4	734	1.927	232	60.9
1. HGD or villous features	36	0.100	33	9.2	39	0.102	34	8.9
2. Other	446	1.239	193	53.6	695	1.824	220	57.7
a. Other ≥ 10mm	122	0.339	85	23.6	152	0.399	105	27.6
Serrated lesions (SL)	78	0.217	51	14.2	141	0.370	81	21.3
1. Any SSL	24	0.067	21	5.8	61	0.160	34	8.9
a. SSL no dysplasia	20	0.056	17	4.7	55	0.144	31	8.1
ai. SSL no dysplasia ≥ 10mm	2	0.006	2	0.6	11	0.029	8	2.1
b. SSL with dysplasia	4	0.011	4	1.1	6	0.016	5	1.3
bi. SSL with dysplasia ≥ 10mm	2	0.006	2	0.6	4	0.010	3	0.8
2. TSA	1	0.003	1	0.3	5	0.013	5	1.3
3. HP	53	0.147	37	10.3	75	0.197	54	14.2
Other	8	0.022	8	2.2	27	0.071	21	5.5
1. Mixed polyp [A]	2	0.006	2	0.6	4	0.010	4	1.0
2. Inflammatory	3	0.008	3	0.8	14	0.037	11	2.9
3. Dysplasia and inflammation	0	0.000	0	0.0	3	0.008	1	0.3
4 .Unclassifiable	3	0.008	3	0.8	6	0.016	6	1.6
Proximal SLs	28	0.078	23	6.4	60	0.157	45	11.8
1. Any SSL	18	0.050	16	4.4	39	0.102	30	7.9
a.. SSL no dysplasia	16	0.044	14	3.9	37	0.097	28	7.3

ai. SSL no dysplasia ≥ 10mm	1	0.003	1	0.3	9	0.024	7	1.8
b. SSL with dysplasia	2	0.006	2	0.6	2	0.005	2	0.5
bi. SSL with dysplasia ≥ 10mm	0	0.000	0	0.5	0	0.000	0	0.0
2. TSA	0	0.000	0	0.0	1	0.003	1	0.3
3. HP	10	0.028	9	2.5	20	0.052	19	5.0
"Advanced neoplasia" [B]								
Overall	164	0.456	114	31.7	214	0.562	136	35.7
Proximal	45	0.125	33	9.2	57	0.150	32	8.4
"Advanced adenomas" [C]								
Overall	156	0.433	109	30.3	190	0.499	128	33.6
Proximal	42	0.117	30	8.3	43	0.113	27	7.1
"Significant SLs" [D]								
Overall	7	0.019	7	1.9	22	0.058	16	4.2
Proximal	3	0.008	3	0.8	12	0.031	9	2.4
At least one SL and adenoma								
Overall			39	10.8			61	16.0
Proximal			13	3.6			28	7.3

Table 8: Polyps (WHO classification) retrieved over first and repeat procedures.

[A]One polyp in standard arm was advanced, two in chromo colonoscopy arm were advanced ;

[B] Advanced adenoma or "Significant SL" or advanced mixed polyp; [C]HGD or villous features or ≥10mm ; [D]SSL with dysplasia or any SSL≥10mm or TSA

2.3.4.4.4 Serrated lesion and synchronous advanced adenoma

An univariable logistic regression analysis (Table 9) identified statistically significant associations between the finding of any SSL and the presence of synchronous advanced adenoma(s) (OR 2.42, 95% CI: 1.19-4.93, p=0.015) and between any proximal significant SL and advanced adenoma(s) (OR 4.10, 95% CI: 1.01-16.7, p=0.049) in the chromocolonoscopy arm but not in the standard arm.

2.3.4.4.5 Advanced neoplasia

This group included those participants that had either (or synchronous) advanced adenoma and significant serrated lesion. Advanced neoplasia was similar in both arms of the study where the detection rate being 36% (136) versus 32% (114) anywhere in the colon and in the proximal colon being 8% (32) versus 9% (33) in the chromoendoscopy and standard colonoscopy arm respectively. (Table 8).

A univariate (OR 1.98, 95% CI 1.19-3.31; P =0.009) and multivariate (N = 730, OR = 1.87, 95% CI 1.10-3.16, p = 0.020) logistic regression analysis for advanced neoplasia detection rate in the proximal colon suggest that obesity may be an important risk factor (*Table 13*). The univariate (OR 1.50, 95% CI 1.08-2.08; P =0.016) and multivariate (N = 730, OR = 1.45, 95% CI 1.03-2.05, p = 0.033) also showed that male gender may be an important risk factor for advanced neoplasia located anywhere in the colon.

Aspirin: A sensitivity analysis was conducted in the subset of patients with aspirin data (n=521) and the trial arm effect in the multivariable regression was still found to be significant (OR 1.98, 95% CI: 1.05-3.74, p=0.036), but the effect of aspirin was not (OR 1.79 in favour of taking aspirin, 95% CI: 0.72-4·50, p=0.212).

A further multivariable analysis of advanced neoplasm detection rates conducted in the subset of patients with aspirin data (n=521) in both arms of the trial combined found a significant protective effect of aspirin (23/103 (22.3%) vs 153/418 (36.6%), OR 2.11 95% CI: 1.27-3.51, p=0.004).

Table 9: Univariable logistic regression for advanced adenoma detection rates by categories of serrated lesions

Variable	Category	Standard				Chromocolonoscopy			
		n (%) with advanced adenoma	Univariable			n (%) with advanced adenoma	Univariable		
			OR	95% CIs	p		OR	95% CIs	p
Any SSL	No	103/339 (30.4)				110/347 (31.7)			
	Yes	6/21 (28.6)	0.92	0.35-2.43	0.861	18/34 (52.9)	2.42	1.19-4.93	0.015
Any significant SL	No	106/353 (30.0)				120/365 (32.9)			
	Yes	3/7 (42.9)	1.75	0.38-7.94	0.470	8/16 (50.0)	2.04	0.75-5.57	0.164
Any proximal significant SL	No	108/357 (30.3)				122/372 (32.8)			
	Yes	1/3 (33.3)	1.15	0.10-12.8	0.908	6/9 (66.7)	4.10	1.01-16.7	0.049
Any SSL or significant SL	No	102/338 (30.2)				110/343 (32.1)			
	Yes	7/22 (31.8)	1.08	0.43-2.73	0.871	18/38 (47.4)	1.91	0.97-3.75	0.061

Table 10: Univariable and multivariable logistic regression for Serrated Lesions

a. Serrated lesions

Variable	Category	N	Proximal							Anywhere						
			n (%) with lesion	Univariable			Multivariable (N=730)			n (%) with lesion	Univariable			Multivariable (N=730)		
				OR	95% CIs	p	OR	95% CIs	p		OR	95% CIs	p	OR	95% CIs	p
Trial arm	Standard	360	23 (6.4)							51 (14.2)						
	Chromocolonoscopy	381	45 (11.8)	1.96	1.16-3.32	**0.012**	2.04	1.18-3.50	**0.010**	81 (21.3)	1.64	1.11-2.40	**0.012**	1.66	1.12-2.46	**0.012**
Sex	Female	251	21 (8.4)							37 (14.7)						
	Male	490	47 (9.6)	1.16	0.68-1.99	0.585	1.06	0.60-1.87	0.854	95 (19.4)	1.39	0.92-2.11	0.119	1.30	0.84-2.01	0.238
Obese	No	472	36 (7.6)							77 (16.3)						
	Yes	262	31 (11.8)	1.63	0.98-2.70	0.060	1.58	0.93-2.68	0.092	54 (20.6)	1.33	0.91-1.96	0.146	1.31	0.87-1.96	0.193
Smoker	Never	282	20 (7.1)							39 (13.8)						
	Ever (current and ex- smokers)	458	48 (10.5)	1.53	0.89-2.64	0.123	1.79	1.00-3.22	0.050	93 (20.3)	1.59	1.06-2.39	**0.026**	1.58	1.03-2.42	**0.038**
First degree relative with bowel cancer	No	642	56 (8.7)							113 (17.6)						
	Yes	96	12 (12.5)	1.49	0.77-2.90	0.235	1.56	0.77-3.14	0.214	19 (19.8)	1.16	0.67-1.99	0.602	1.17	0.66-2.05	0.590

Table 11: Univariable and multivariable logistic regression for Significant Serrated Lesions

b. Significant SL

Variable	Category	N	Proximal							Anywhere						
			n (%) with lesion	Univariable			Multivariable (N=730)			n (%) with lesion	Univariable			Multivariable (N=730)		
				OR	95% CIs	p	OR	95% CIs	p		OR	95% CIs	p	OR	95% CIs	p
Trial arm	Standard	360	3 (0.8)							7 (1.9)						
	Chromocolonoscopy	381	9 (2.4)	2.88	0.77-10.7	0.115	2.81	0.75-10.5	0.125	16 (4.2)	2.21	0.90-5.44	0.084	2.18	0.88-5.37	0.092
Sex	Female	251	2 (0.8)							3 (1.2)						
	Male	490	10 (2.0)	2.59	0.56-11.9	0.221	2.17	0.46-10.2	0.327	20 (4.1)	3.52	1.04-12.0	**0.044**	3.23	0.94-11.2	0.063
Obese	No	472	7 (1.5)							15 (3.2)						
	Yes	262	5 (1.9)	1.29	0.41-4.11	0.664	1.42	0.44-4.57	0.559	8 (3.1)	0.96	0.40-2.29	0.926	1.06	0.44-2.55	0.904
Smoker	Never	282	2 (0.7)							6 (2.1)						
	Ever	458	10 (2.2)	3.13	0.68-14.4	0.143	2.68	0.57-12.5	0.211	17 (3.7)	1.77	0.69-4.55	0.234	1.46	0.56-3.81	0.434
First degree relative with bowel cancer	No	642	11 (1.7)							21 (3.3)						
	Yes	96	1 (1.1)	0.60	0.08-4.73	0.631	0.63	0.08-5.06	0.665	2 (2.1)	0.63	0.15-2.73	0.536	0.70	0.16-3.07	0.635

Table 12: Univariable and multivariable logistic regression for Sessile Serrated Lesions

c. SSL

Variable	Category	N	Proximal							Anywhere						
			n (%) with lesion	Univariable			Multivariable (N=730)			n (%) with lesion	Univariable			Multivariable (N=730)		
				OR	95% CIs	p	OR	95% CIs	p		OR	95% CIs	p	OR	95% CIs	p
Trial arm	Standard	360	16 (4.4)							21 (5.8)						
	Chromocolonoscopy	381	30 (7.9)	1.84	0.98-3.43	0.056	1.91	1.02-3.59	**0.045**	34 (8.9)	1.58	0.90-2.78	0.111	1.60	0.91-2.82	0.105
Sex	Female	251	14 (5.6)							15 (6.0)						
	Male	490	32 (6.5)	1.18	0.62-2.26	0.611	1.12	0.57-2.20	0.743	40 (8.2)	1.40	0.76-2.58	0.284	1.31	0.70-2.46	0.405
Obese	No	472	25 (5.3)							33 (7.0)						
	Yes	262	21 (8.0)	1.56	0.85-2.84	0.148	1.54	0.83-2.87	0.169	22 (8.4)	1.22	0.70-2.14	0.489	1.24	0.70-2.20	0.457
Smoker	Never	282	14 (5.0)							15 (5.3)						
	Ever	458	32 (7.0)	1.44	0.75-2.74	0.271	1.51	0.77-2.96	0.235	40 (8.7)	1.70	0.92-3.14	0.089	1.64	0.87-3.11	0.125
First degree relative with bowel cancer	No	642	39 (6.1)							47 (7.3)						
	Yes	96	7 (7.3)	1.22	0.53-2.80	0.646	1.21	0.51-2.86	0.664	8 (8.3)	1.15	0.53-2.52	0.725	1.15	0.52-2.56	0.728

Table 13: Univariable and multivariable logistic regression for advanced neoplasm detection rates

Variable	Category	N	Proximal							Anywhere						
			n (%) with lesion	Univariable			Multivariable (N=730)			n (%) with lesion	Univariable			Multivariable (N=730)		
				OR	95% CIs	p	OR	95% CIs	p		OR	95% CIs	p	OR	95% CIs	p
Trial arm	Standard	360	33 (9.2)							114 (31.7)						
	Chromocolonoscopy	381	32 (8.4)	0.91	0.55-1.51	0.712	0.91	0.54-1.54	0.733	136 (35.7)	1.20	0.88-1.63	0.247	1.16	0.85-1.58	0.357
Sex	Female	251	21 (8.4)							70 (27.9)						
	Male	490	44 (9.0)	1.08	0.63-1.86	0.780	1.06	0.60-1.87	0.842	180 (36.7)	1.50	1.08-2.08	**0.016**	1.45	1.03-2.05	**0.033**
Obese	No	472	32 (6.8)							161 (34.1)						
	Yes	262	33 (12.6)	1.98	1.19-3.31	**0.009**	1.87	1.10-3.16	**0.020**	85 (32.4)	0.93	0.67-1.28	0.647	0.94	0.68-1.31	0.716
Smoker	Never	282	21 (7.5)							89 (31.6)						
	Ever	458	44 (9.6)	1.32	0.77-2.27	0.315	1.26	0.72-2.22	0.415	161 (35.2)	1.18	0.86-1.61	0.316	1.11	0.80-1.54	0.546
First degree relative with bowel cancer	No	642	53 (8.3)							214 (33.3)						
	Yes	96	11 (11.5)	1.44	0.72-2.86	0.301	1.35	0.67-2.75	0.404	34 (35.4)	1.10	0.70-1.72	0.687	1.11	0.70-1.75	0.671

2.3.5 Serious Adverse Reactions (SARs)

Six SARs were reported in the trial, two in the standard arm and four in the chromocolonoscopy arm of the trial with five of these being incidences of post polypectomy bleeding and one case of anxiety and hyperventilation). The rates of post-polypectomy bleeding were: 1/358 (0·3%) vs 2/378 (0·5%) in the standard and chromocolonoscopy arms respectively. None of these cases required any further interventional procedures related to the bleeding. There were no allergic reactions or deaths.

2.3.6 Health Economic Evaluation (HEE)

The HEE analysis was done by the HE team based in Swansea with clinical input and data provided by the research fellow (RR), statistician and PI.

The economic evaluation case analysis included 899 procedures (904 index and associated non-surveillance repeat procedures conducted within one year (Table 14) minus five procedures (four from the chromocolonoscopy arm and one from the standard arm) with missing data). 183 (20%) of these (91 standard arm and 92 chromocolonoscopy arm) were first procedures conducted at the site that documented the use of consumables constituted the complete case analysis. The mean training cost per procedure was £4.94 and mean equipment cost £47.99 (a total implementation cost per procedure of £52.93). A spray catheter attached to the pump was used in only 30% of procedures with a higher cost of £40 per colonoscopy.

This compared to the technique used in 70% of procedures of adapting existing pumps with tubing and a valve which added £8.88 to the cost of the colonoscopy.

Table 14 and 15, show the higher costs associated with chromocolonoscopy. This is primarily due to the extra time required by staff to perform the chromocolonoscopy (£26.15 per procedure) and additional implementation costs (£52.93 per procedure).

The cost incurred (ICER) in securing a 1% likelihood increase in additional SL retrieved due to the chromocolonoscopy was £11.41. Subgroup ICERs produced the following results: Any SSL: a 1% likelihood increase in additional polyp retrieval would cost £26.13. Significant SLs overall: a 1% likelihood increase in additional polyp retrieval would cost £35.22.

Table 14: Cost analysis of all index and repeat procedures (£ per procedure)

Component costs of the procedure:	**Standard colonoscopy**	**Chromocolonoscopy**	**Mean Cost difference (95% CI) £**	**P value**
Staff cost Mean cost (SD) Min, max	n=425 114.27 (61.78) 7.48, 561.00	n=471 140.42 (75.95) 11.22, 546.04	26.15 (17.02 to 35.29)	<.0001
Medication Mean cost (SD) Min, max	n=426 0.68 (0.64) 0, 2.49	n=473 0.73 (1.42) 0, *28.39	0.05 (-0.10 to 0.20)	.511
Bowel prep Mean cost (SD) Min, max	n=415 9.00 (1.55) 3.39, 10.83	n=464 9.24 (1.43) 3.39, 10.83	0.24 (0.05 to 0.44)	.015
Consumables Mean cost (SD) Min, max	n=91 66.65 (84.39) 0, 435.32	n=92 78.57 (79.72) 0.19, 458.22	11.92 (-12.02 to 35.86)	.327
Implementation Mean cost (SD) Min, max	N/A	n=110 52.93 (14.77) 40.91, 91.38	N/A	N/A
Overall Total Cost				
****Available cases** Mean cost (SD) Min, max	N=426 190.60 (78.89) 81.01, 655.88	N=473 271.60 (89.42) 99.03, 713.31	81.00 (69.91 to 92.09)	<.0001
*1 patient received 200mcg flumazenil cost 13.50 per 100mcg, **mean imputation for implementation and consumables variables where data collection only occurred at one site. 5 procedures excluded due to missing data across all cost variables.				

Table 15: Cost analysis of all repeat procedures only (£ per procedure)

Component costs of the procedure:	Standard colonoscopy	Chromocolonoscopy	Mean Cost difference (95% CI)	P value
Staff cost Mean cost (SD) Min, max	N=66 119.23 (105.00) 18.70, 561.00	N=92 155.66 (126.39) 11.22, 546.04	**36.43** **(-1.15 to 74.01)**	.057
Medication Mean cost (SD) Min, max	N=66 0.56 (0.70) 0, 2.49	N=92 0.62 (0.66) 0, 2.29	**0.06** **(-0.15 to 0.28)**	.562
Bowel prep Mean cost (SD) Min, max	N=59 9.07 (2.34) 3.39, 10.83	N=85 9.80 (1.53) 3.39, 10.83	**0.73** **(0.09 to 1.36)**	.026
Consumables Mean cost (SD) Min, max	66.65	78.57	**11.92**	
Overall Total Cost				
****Available cases** Mean cost (SD) Min, max	N=66 195.49 (105.66) 94.35, 637.21	N=92 244.60 (126.97) 99.03, 635.73	**49.11** **(11.33 to 86.88)**	.011
**mean imputation for consumables variables where data collection only occurred at one site. 5 procedures are excluded due to missing data across all cost variables				

2.4 Discussion

This multicentre, open-label, non-inferiority RCT study demonstrated the feasibility of implementation of chromocolonoscopy within a population-based CRC screening programme in Wales. The study showed a recruitment of 82% of those eligible patients, acceptability among 86 % of screening centres and 87% of colonoscopists.

Some screening colonoscopists are already familiar with the concept of chromocolonoscopy from their IBD surveillance procedures and will consequently have fewer training requirements (383). With 20 of 23 colonoscopists from 12 of 14 screening centres in the BSW programme participating in the current study, the study has shown the feasibility and results from a real-world programme-wide roll out of chromocolonoscopy. Conversely, previous studies have largely focused on expert centres and expert colonoscopists (384).

The study also demonstrated that quality standards for the technical aspects and patient factors including comfort scores and the quality of bowel preparation during the colonoscopy procedure exceeded the national benchmarks. This indicated high quality procedures and performance that was similar in each trial group.

The dye (indigo carmine) was found to be safe and consequent polyp detection and resection was associated with a very low rate of post-polypectomy bleeding, similar to the standard group.

Although the procedure took approximately 6 min longer in the chromocolonoscopy group, the 95% CI suggested that chromocolonoscopy would be unlikely to increase procedure times by more than 10 min. There were more repeat procedures in the chromocolonoscopy arm which was mainly due to a therapeutic indication for polyp removal and to check the polypectomy site as part of surveillance.

The chromocolonoscopy group showed higher detection rates for proximal serrated lesions and sessile serrated lesions and all serrated lesions in the colon. In addition to this, more advanced neoplasias and significant serrated lesions were found in this group than the standard group.

The health economics evaluation reported that the additional costs of adopting the chromocolonoscopy technique would be £81.00 per procedure. However further follow-up work would be required in order to assess the extent of further costs involved in screening surveillance as a result of improved detection.

With the lack of reduction in mortality from proximal colon cancer with screening, an intervention that improves detection of proximal serrated lesions must be feasible within a screening programme and any increase in the proportion of significant proximal precursor lesions detected must be of the order that might affect surveillance and outcomes in the longer term. This study also identified several other interesting findings.

First, although distal SSL with dysplasia (SSLD) occurred only in lesions >=10 mm, all proximal SSLD were diminutive with an average size of <=5 mm. This finding was consistent with another study (127) which found that most proximal SSLDs to be smaller than 10 mm and hence suggests the need for caution in setting guidelines for clinical significance based solely on the size of serrated lesions.

Second, in the chromocolonoscopy group (but not in the standard group), synchronous advanced conventional adenomas were more common in individuals with SSLs. There were significant associations for synchronous advanced adenomas with an SSL located anywhere (OR 2.42, 95% CI: 1.19-4.93, p=0.015) or with a proximal significant SL (OR 4.10, 95% CI: 1.01-16.7, p=0.049) in the chromocolonoscopy arm but not in the standard arm.

These findings are consistent with a meta-analysis (242) that showed that there was a strong risk relationship between the presence of proximal serrated polyps and synchronous advanced neoplasia (OR = 2.77, 95% CI 1.71-4.46). Patients with proximal serrated polyps and larger serrated polyps were found to be associated with a threefold increase in the detection of advanced neoplasia (OR = 3.35, 95% CI, 2.51-4.46).

Larger serrated polyps have been found to be strongly associated with synchronous CRC (239) and sessile serrated lesions to be associated with an increased risk of metachronous CRC (240, 241).

Hence though the reasons for this finding are unclear, but the improved identification of otherwise occult serrated lesions by chromocolonoscopy might go some way in explaining the appearance of post-colonoscopy interval cancers in conventional screening programmes.

Thirdly, some evidence suggested that aspirin protects against advanced neoplasia. Chromocolonoscopy is perceived to be time consuming, and this study provides quantification of the additional time taken per procedure and of the additional costs associated with chromocolonoscopy. The cost-consequence analysis provides an indication of the additional resources required to adopt this technique and shows that additional costs are primarily due to implementation.

Previous estimates of prevalence of serrated lesions have shown significant variation possibly partly due to inconsistency in histopathological categorisation of these lesions.(296, 305, 385). To address this variation, unlike the previous randomised controlled trials involving chromocolonoscopy, this study included an expert gastrointestinal central pathology panel reviewing all slides of proximal colonic polyps (343). This will be described in detail in Chapter 3.

Randomisation was stratified by centre to ensure that any centre effects were balanced across trial groups. There was very little difference between groups in technical factors affecting mucosal visualisation and consequent polyp detection and addressed most major sources of bias in previous studies due to procedure quality. This study is also the first to estimate the resource use associated with training and implementation of this intervention in routine clinical practice.

It is difficult to completely remove bias in chromocolonoscopy because it is impossible to mask assessors. Withdrawal times in both groups, even where polyp resection was not required, were higher than the prespecified minimum withdrawal time of 7 min in the quality assurance criteria for BSW.

Previous studies (225),(41) suggest that longer withdrawal times might improve detection rates for serrated polyps. It could be that the dye promotes longer withdrawal times, which in turn led to the higher detection rates. However, none of the previous studies suggest that a withdrawal time greater than 11 min would be effective in independently achieving a significant improvement in detection rates for both adenomas and serrated lesions, supporting the findings of an independent and significant positive effect of the chromoendoscopy (284).

The use of high definition (HD) colonoscopes was not specified as a prerequisite, but the majority of centres and colonoscopists used HD colonoscopes in this study and some data from previous studies suggest that impact of HD colonoscopes would be uncertain in influencing the results of this study (386),(235).

The use of Aspirin was not specified, and data was collected for only a subset of patients and hence the results should be treated with caution, especially in this selected screening population— although sensitivity analysis in that subset supported the main finding of the study in proximal serrated polyp detection rate. This study was a feasibility study not powered to find differences in detection rates and a definitive trial with longer follow-up and high-definition colonoscopy mandated in both groups is planned. Finally, some variables were subject to recall bias—e.g., smoking and family history of cancer or polyps.

In summary, Index chromocolonoscopy is safe and feasible within the CRC screening setting, with an acceptable increase in procedure time of approximately 6 minutes. It is also feasible (in terms of safety, recruitment rates, procedure time, and trial logistics) to do a larger randomised trial comparing chromocolonoscopy to standard white light colonoscopy. Such a trial could be powered to find a difference in significant sessile serrated lesion detection rate at index colonoscopy as a useful surrogate end point since a study powered to detect a difference in PCCRC would require tens of thousands of participants. The higher proximal serrated polyp detection rates and advanced neoplasia found with chromocolonoscopy in this study contribute data to the discussion around its effect on colonoscopy quality and PCCRC.

Chapter 3: Histopathological aspect of serrated neoplasia (CONSCOP study)

Chapter 3 overview:

The **CONSCOP** study (Feasibility of reduction of right sided bowel cancer through **CON**trast Enhanced colono**SCOP**y was a feasibility RCT that compared chromocolonoscopy to standard white light colonoscopy. It was conducted at index procedures in the bowel cancer screening programme in Wales following a positive faecal occult blood test. Chapter 2 has described the rationale for the study and the findings from the endoscopic aspect of the study. This chapter describes the second part of the study i.e., the histopathological aspect which involved the collection and central review of the histology of all proximal colon polyps by expert pathologists. It aims to report on the interobserver variability of serrated neoplasia between the pathologists and understand the prevalence of serrated neoplasia in the proximal colon following rigorous histopathological assessment.

This chapter will start with an introduction that will describe the background and rationale along with the varying terminologies used and the histopathology findings in serrated neoplasia and then the aim of the study. This will be followed by the methodology used and discussion on the findings of the study.

3.1 Introduction

It is widely accepted that the adenoma carcinoma sequence is the main pathway for the evolution of adenomatous polyps to CRC. However, there are different pathways involved in the development of CRC which include the serrated neoplasia precursor pathway that may be responsible for up to 20% of all the sporadic CRCs particularly in the proximal colon (123). In this pathway the precursors responsible for CRC include sessile serrated lesions (SSLs) and the traditional serrated adenoma (TSA) (123, 387).

SSLs are endoscopically challenging to detect because of their flat and non-polypoidal morphology. There is also a wide variation in the histopathological interpretation of serrated polyp subtypes that can affect the accurate categorisation of the potential precursors to the serrated pathway (226, 292).

In view of this it is important to accurately identify these lesions endoscopically by improving detection techniques but also there needs to be a consistent and accurate pathological diagnosis in order to accurately report the prevalence of these premalignant polyps (388).

In addition to the variability in recognising and reporting SSLs, there have been two different pathological descriptions and criteria used to diagnose SSLs. One described by the World Health Organisation (WHO) 2010 (153) and the other by the American Gastroenterological Association (AGA) (162). Therefore, due to inconsistent nomenclature and terminology reported prevalence rates in studies may vary depending on the criteria used (97, 305, 389).

3.1.1 Terminology and histological features of serrated neoplasia

There has been significant evolution in the nomenclature of serrated colorectal polyps over the last two decades with some differences of opinion between the UK, European and US pathologists regarding the optimal terminology and pathological features required to make a diagnosis of a serrated lesion (97).

In the past all flat polyps were referred to as hyperplastic polyps until a full appreciation of the pathological differences was established. Hyperplastic polyps were in fact thought to be inconsequential when first described in 1971 by Lane et al (390).

It was only in 1990 that Longacre recognised that they may have some neoplastic potential associated with serrated glandular pattern seen in hyperplasia and coined the term serrated adenoma (117). The WHO 2000, first used the terminology for hyperplastic polyps as encompassing all serrated polyps without overt nuclear dysplasia and serrated adenomas were described as superficial dysplasia in a serrated polyp.

In 2010, WHO re-classified serrated colorectal lesions into three categories (153). The classification included 1. Hyperplastic polyp (HP) 2. Sessile serrated adenoma/polyp (SSA/P) with or without cytological dysplasia and 3. Traditional serrated adenoma (TSA).

The feature that distinguishes SSL from a hyperplastic polyp is the distortion in architecture as a result of alteration in the proliferative zone of the crypts. The 2010 WHO diagnostic criteria defined SSA/P that satisfy the criteria of overall distortion and normal architecture in 2 to 3 contiguous crypts.

This classification has recently been updated by the WHO in 2019 which is consistent with the UK guidance for pathological reporting of serrated lesions of the colorectum (391). The updated WHO 2019 diagnostic criteria describes the presence of a single unequivocally distorted crypt as being diagnostic for an SSL (119) which is similar to the AGA criteria which describes that only one crypt showing the characteristic features is sufficient for the diagnosis of SSLs.

The updated WHO 2019 classification summarised in Table 16, includes the following categories (119): 1.Hyperplastic polyp (HP) which encompasses microvesicular type (MVHP) and goblet cell-rich type (GCHP) (in the 2010 WHO criteria this also included another subtype which was mucin poor type (MPHP), this is now deleted from the terminology) 2. SSL and SSL with dysplasia (SSLD) 3. traditional serrated adenoma (TSA) 4. serrated adenoma unclassified (this is now a new entity).

It is recognised that there is significant interobserver variability with the pathological reporting of serrated lesions which is also not helped by variation in terminology and diagnostic criteria between hyperplastic polyps and SSLs (97, 221, 293, 294).

The key histological characteristic of serrated neoplasia is summarised in

Table 16 with images of these features demonstrated in Figure 23.

Types of serrated lesions (as per WHO 2019)	Histological features
1. Hyperplastic polyp (HP)	
A). Microvesicular HP (MVHP)	Narrow, uniform basal crypt serration in the upper crypt Eosinophilic mucin droplets in the cytoplasm
B). Goblet cell HP (GCHP)	Less serration than MV HP predominantly goblet cells in the epithelium
Sessile serrated lesion (SSL)	Dilatation and serration at the crypt bases branching of the crypts horizontal extension of crypt bases (L-shaped or inverted T shaped crypts)
Sessile serrated lesion with dysplasia (SSLD)	eosinophilic cytoplasm and tightly packed small glands nuclear atypia
Traditional serrated adenoma (TSA)	Pseudo-stratification villous pattern with stretched or pencillate nuclei eosinophilic predominance ectopic crypts
Serrated adenoma unclassified	

Table 16: Histological types and features of serrated lesions (WHO 2019)

In particular, the histological features described for SSL in Table 16 and Figure 23 are: Irregular distribution of crypts, dilatation of crypt bases, serration present at the crypt bases, branched crypts, horizontal extension of crypt bases ('L' shaped or inverted 'T' shaped crypts), dysmaturation of crypts, herniation of crypts through muscularis mucosa.

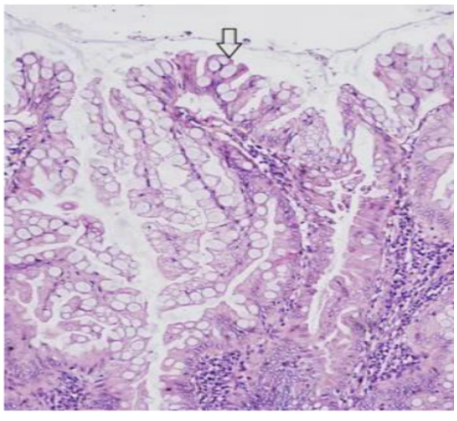


Fig A: Haematoxylin eosin stain magnification 200x of a hyperplastic polyp with arrow highlighting mucin vesicles

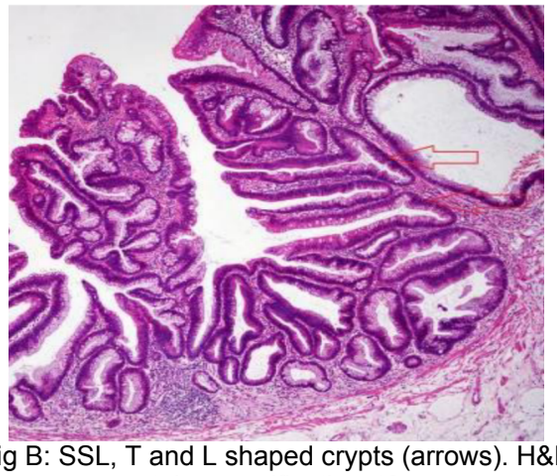


Fig B: SSL, T and L shaped crypts (arrows). H&E stain magnification 40x

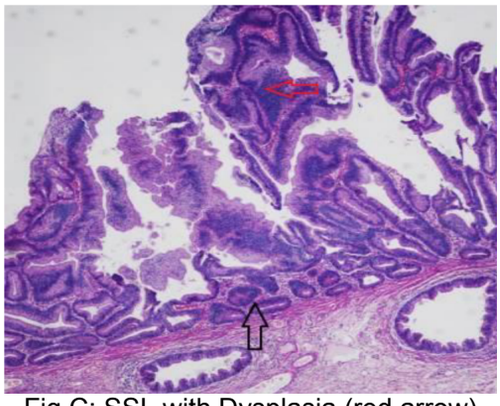


Fig C: SSL with Dysplasia (red arrow) and pseudoinversion pattern (black arrow). H&E, Magnification 40x

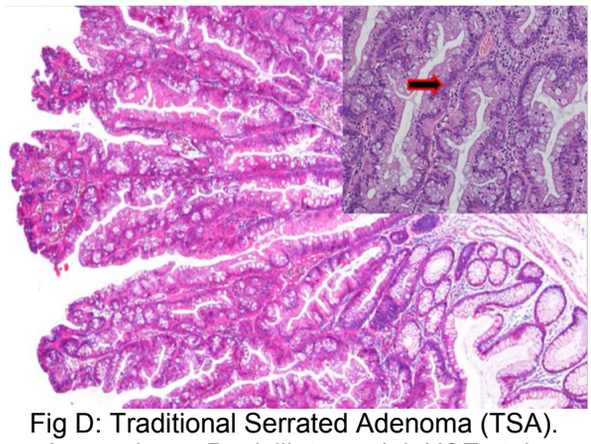


Fig D: Traditional Serrated Adenoma (TSA). Arrow shows Penicillate nuclei. H&E stain magnification 20x

Figure 23: Histopathological images of serrated lesions adapted from [122]

The WHO 2010 criteria (392) states that at least three crypts or at least two adjacent crypts must show one or more of these features to enable a diagnosis of SSL. On the other hand , the AGA criteria (393), required only one crypt showing the characteristic features above to sufficiently diagnose an SSL.

The prevalence of serrated neoplasia has been underestimated for many years and has a wide reported variation in the literature from 1% to 14.7% (124, 218-220). Since the identification of morphological and molecular pathways that indicate an accelerated pathway to carcinogenesis i.e., the serrated pathway, it is important to accurately differentiate the histology of these lesions. Moreover, the malignant potential differs for the different subtypes of serrated neoplasia. A study (237) that estimated the 10-year CRC risk for different polyp subtypes showed that non-dysplastic SSLs carries 2.56% risk. Dysplastic SSL and TSA are those subtypes that have a significant malignant potential with a 10-year CRC risk of 4.43% and 4.5% respectively (Table 17).

Hence accurate histological diagnosis is important due to different prognosis, follow-up and response to treatment when compared to traditional CRCs (394).

Table 17: Estimated 10-year risk of CRC for polyp subtypes [237]

	Cases/controls	Adjusted OR (95% CI)	Estimated 10-year risk[a]
SSA/P with synchronous conventional adenomas	30/61	2.66 (1.70–4.16)	2.47%
SSA/P without synchronous conventional adenomas	49/81	3.40 (2.35–4.91)	3.16%
SSA/P with cytologic dysplasia	20/25	4.76 (2.59–8.73)	4.43%
SSA/P without cytologic dysplasia	59/117	2.75 (1.99–3.80)	2.56%
Conventional adenomas without SSA/P	727/1631	2.50 (2.24–2.80)	2.33%
Traditional serrated adenomas overall	14/17	4.84 (2.36–9.93)	4.50%
Hyperplastic polyps only	55/235	1.30 (0.96–1.77)	1.21%

a The number of colorectal cancers among individuals without polyps (1155) divided by the total number of patients without polyps (209,744) and divided by the mean follow-up period (5.90 y) estimates the annual colorectal cancer risk (r). The 10-year risk for patients without polyps is estimated as 1- (1 - r)^{10} and equals 0.93%. The 10-year risk of colorectal cancer for each polyp type then is estimated as the 10-year risk for patients without polyps times the OR for the relevant polyp type.

"Reprinted from Gastroenterology. 2016;150(4):895-902 e5., Erichsen R et al., Increased Risk of Colorectal Cancer Development Among Patients With Serrated Polyps. 150, (4):895-902 e5.Copyright (2016);with permission from Elsevier"

3.1.2 Aim of the study

1. To report the interobserver variability (IOV) of serrated neoplasia between expert gastrointestinal pathologists and the IOV between the local and expert pathologists.
2. To understand the prevalence of serrated neoplasia in the proximal colon in a screening population following rigorous histopathological assessment by expert gastrointestinal pathologists using both the WHO 2010 and AGA criteria.

3.2 Methodology

The study included all participants who underwent an index screening colonoscopy procedure and had polyps removed as a part of the CONSCOP study. This study was a prospective, multicentre, randomised population-based feasibility trial of dye enhanced chromocolonoscopy versus standard white light colonoscopy involving 12 participating bowel cancer assessment sites. All proximal colonic (defined as polyps at or above the splenic flexure) polyps were included in the study and collected from the local assessment centres (LAC) for central pathology review by an expert panel. All proximal polyps reported by the local pathologists were included regardless of the initial reported histology.

The expert panel formed part of the central review team and consisted of three expert gastrointestinal pathologists (A, B and C). All three experts were nationally and internationally recognised in their work in gastrointestinal histopathology. Pre-defined standard diagnostic criteria were agreed by the experts to avoid any variation in the final reports.

Polyp histology was based on the revised Vienna criteria and categorised into adenomas including the grade of dysplasia and the presence of a villous component (395). Sessile serrated lesions that fulfilled both the 2010 WHO criteria (392) and American Gastroenterology Association (AGA) criteria (393) were categorised separately. In accordance with UK guidance,(97) the term ‘sessile serrated lesion’ (SSL) was used for lesions described as ‘sessile serrated adenoma/polyp’ (SSA/P).

The following terminology was used as pre-defined criteria for pathological diagnosis:

1. Hyperplastic micro-vesicular (MVHP) type
2. Hyperplastic goblet cell type
3. SSL type 2 (WHO 2010 criteria-a serrated lesion where distortion in 2 to 3 contiguous crypts demonstrate the features of an SSL)
4. SSL type 2a (AGA criteria-a serrated lesion with distortion in one crypt)
5. Mixed polyp
6. Traditional serrated adenoma (TSA)
7. Tubular adenoma (TA)
8. Tubulovillous adenoma (TVA)
9. Villous adenoma (VA)
10. Carcinoma
11. Other (includes Normal, fibro epithelial polyps, inflammatory polyps)
12. Other-Unclassifiable

The above was further classified by the type of dysplasia into:

a) No dysplasia

b) Low-grade dysplasia (LGD)

c) High-grade dysplasia (HGD)

d) Dysplasia not otherwise specified (NOS) i.e., unclassifiable.

Exclusions:

Any polyp that was reported as normal deemed by consensus (there were some slides reported as normal initially that were reclassified as having a pathology and these were included), inadequate specimen, specimen too damaged to characterise due to diathermy, carcinoma, slides that were not obtained from the centre due to being lost in file or unable to retrieve were excluded from the analysis.

Inflammatory polyps were not excluded from the original report (because some pathologists from the local centre thought they were Inflammatory polyps whilst the expert pathologists did not necessarily think so).

3.2.1 Data collection method:

The methodology used to collect and process histopathology data has been summarised in Figure 24 and Figure 25.

The case report forms (CRF) from the assessment sites were sent into the trials unit (WCTU) and the trial manager (TM) then collated this information onto a database. Thereafter on a weekly basis, the TM sent data with three identifiers containing the trial number, Bowel Screening Wales (BSW) number and date of birth to the clinical research fellow (RR) who was part of the central study team and not a pathologist.

The Bowel Screening Information Management System (BSIMS) is a database that contains all the clinical information of a participant that has undergone a procedure or participated in the programme by returning a positive gFOBT test.

Each bowel screening participant has an identifier number assigned to them called a BSW Number. The Specialist Screening Practitioners (SSPs) in usual practice input details of the participant onto BSIMs from the point of contact or consultation. The SSP is also responsible for recording details of their procedure, outcome, and management. SSPs were advised to upload scanned histopathology reports onto the BSIMS system as a part of the trial. This was done to be able to extract accurate polyp histology information from a final report which included the unique histology number, data regarding location and size of the polyp, pathology report and details of the assessment centre and reporting pathologist. If the pathological size was not available (in case of piecemeal removal of polyp or fragmentation) then the endoscopic size of the polyp was recorded (97).

By using the BSW number provided on the weekly excel spreadsheets, RR then retrieved information from the BSIMS that included polyp data from endoscopy reports and histopathology data of all polyps extracted from the scanned reports and recorded this on to an Excel database. For those reports that were not uploaded, the research fellow (RR) contacted the individual SSPs by email requesting them to provide this data.

All right-sided or proximal polyps were identified as those located from the caecum to the splenic flexure. Each histology report had a unique identifying histology number which was required for the pathology administrator (PA) to request slides or blocks from the local assessment centre. RR designated a unique polyp identifier number (P number) and assigned this to the individual proximal polyps.

On a weekly basis, RR sent a list of identified proximal polyps which included details of the BSW number, trial number, histology specimen identifier number, bowel screening assessment centre detail and the “P” number to the PA who then requested for the haematoxylin and eosin-stained slides that were then obtained from any of the 12 bowel screening centres centrally in the pathology dept at Cardiff and Vale UHB.

Once the slides were received in Cardiff, the PA labelled the slides with the “P” number along with the trial number, this was to ensure blinding was complete and to prevent any bias or influence pathological diagnosis that may potentially arise due to recognition of the centre or patient by the individual expert histopathologists. The PA would then update the database for the slides that were received and anonymised and set this aside in a file ready for expert review. RR would then collate the list on a Microsoft Excel spreadsheet which would include the P number and the trial number with columns for the expert pathologists (EP) to report based on pre-agreed criteria described above and sent this to the individual EP for independent reporting.

The expert panel reviewed all slides independently and were blinded to the original report. The panels individual reports were recorded on a Microsoft Excel spreadsheet that had only the polyp number (P-which was uniquely assigned to each slide) along with pre-populated drop-down boxes of the pre-defined criteria. Once RR received the assessments from the 3 individual EPs, all the reports were collated, and the database was updated to reflect those polyps that had a consensus report and those that had differing reports.

At regular intervals (6-8 weekly), RR organised a 'non-consensus' face to face meeting during the 2-year period (2015-2017) where these slides were discussed using multi-view microscopes till a consensus was obtained. For those polyps where diagnostic agreement could not be obtained, these were either discussed again at another meeting or deemed as 'unclassifiable'.

For those slides that required to be discussed again further endoscopic images or clinical detail regarding how the polyp was removed (for e.g., diathermy was used or if there was underlying inflammation in the colon) was obtained to help come to a final decision.

The slides that were eventually selected for the subsequent genetic study for extraction of DNA and sequencing for mutational signatures, had the areas of interest marked on the slides.

The methodology involved with this has been discussed in chapter 4.

3.2.2 Data collection and statistical analysis

Data collection included the original pathology report of all available right and left sided polyps from the local assessment centres, expert reports of right sided polyps as described above, location of the polyps within the right colon, size of the polyps (pathological size recorded unless fragmented in which case endoscopy size used), demographic data including gender, local assessment centres in Wales (anonymised by numbers) and the number of local pathologists.

Data was collected onto a Microsoft Excel spreadsheet and further statistical analysis performed using IBM SPSS Version 27. The Fleiss Kappa statistic was used to assess and determine the interrater reliability (IRR) or inter-observer variability (IOV) between multiple observers (experts and local pathologists) for the polyp type, dysplasia grade and final conclusion.

Landis and Koch (1977) (396) described Kappa value interpretation to evaluate agreement between raters such as the following: <0 = no agreement, 0-0.20 = slight agreement, 0.21-0.40 = fair agreement and 0.41-0.60 = moderate agreement, 0.61-0.81 = substantial agreement and 0.81-1.0 =almost perfect agreement and 1 = perfect agreement.

Any additional unusual features noted by the expert pathologists were also described as “novel” findings from the review.

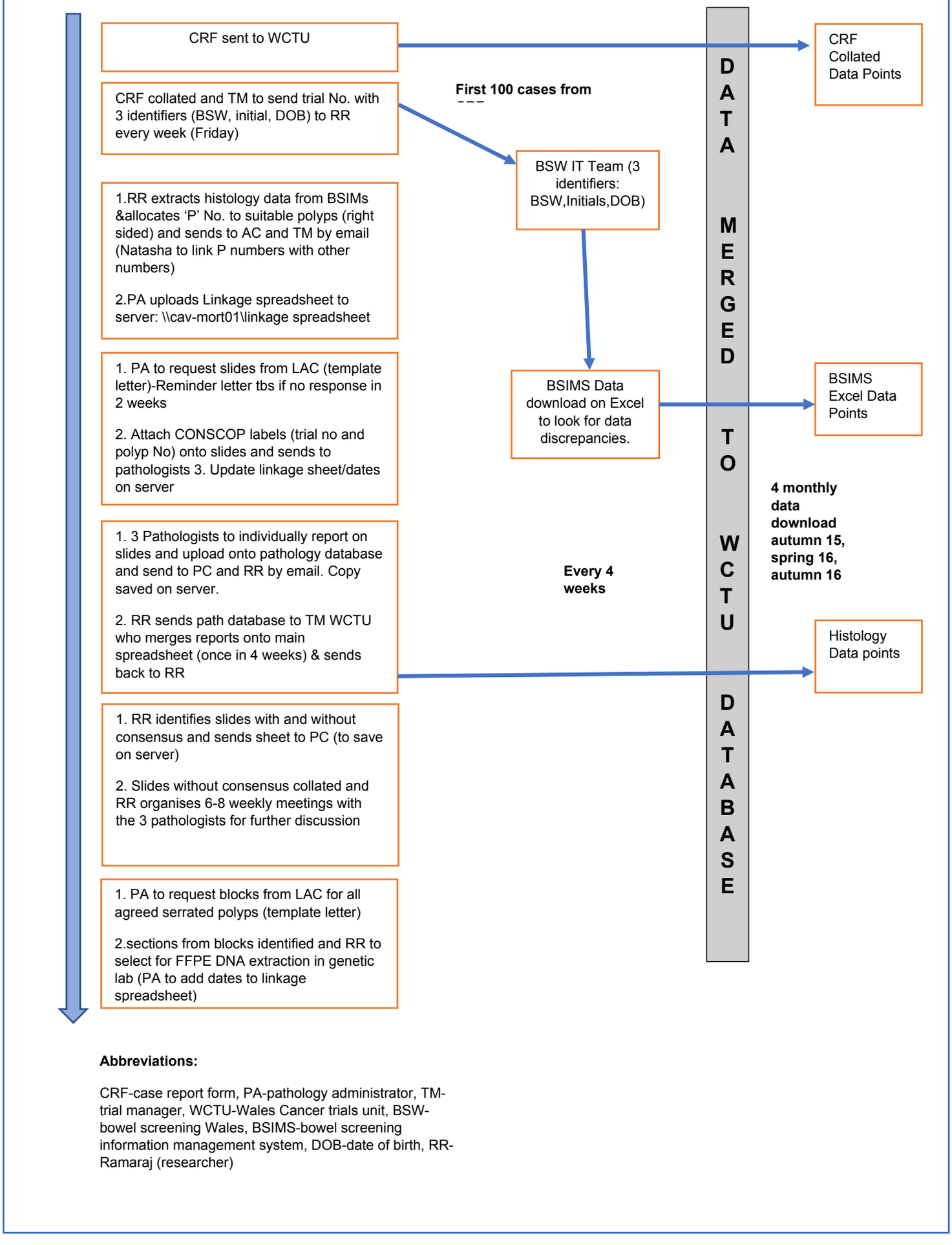
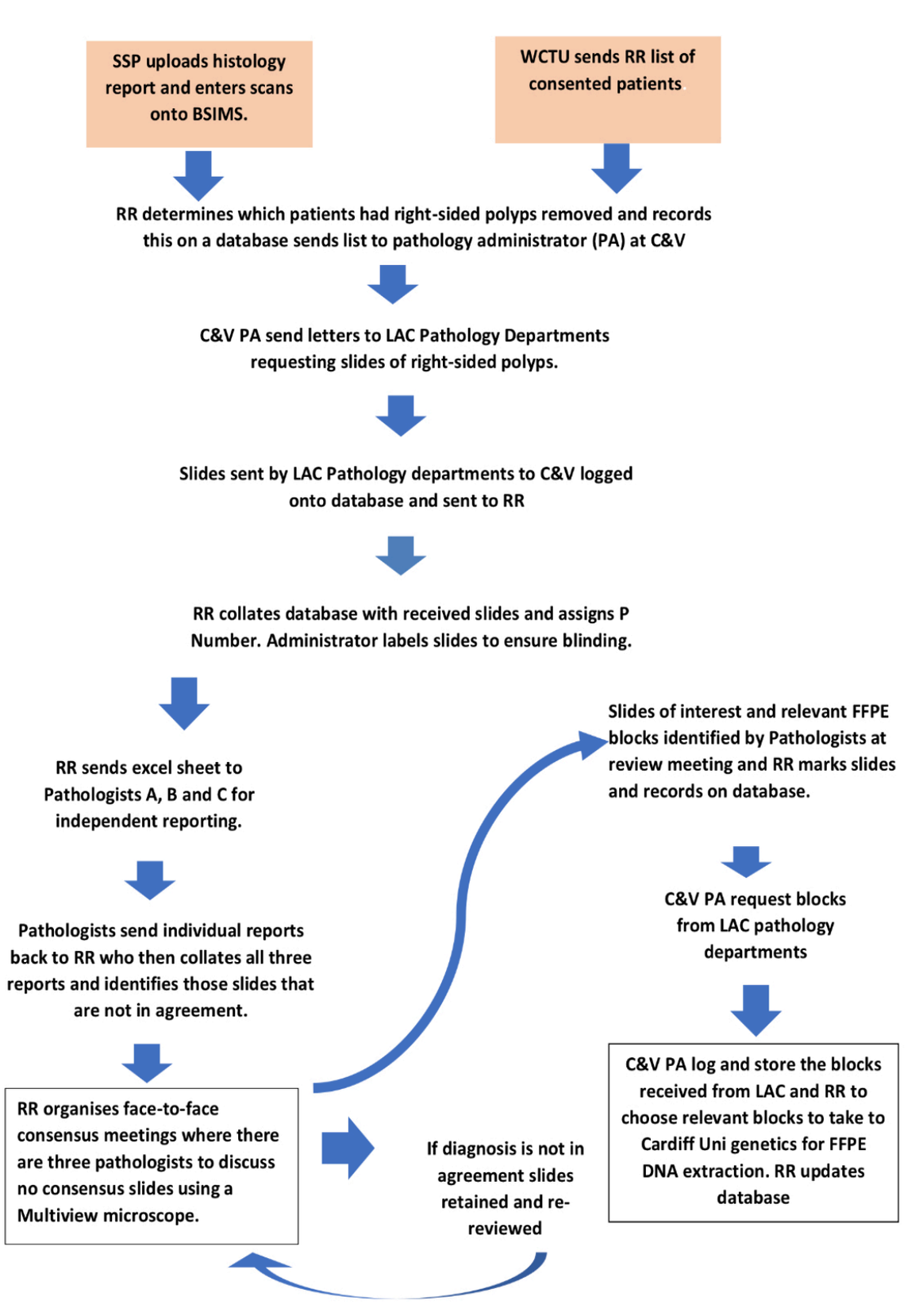


Figure 24: Flow diagram to demonstrate the methodology used to collect histopathology data

Figure 25: CONSCOP-Polyp Processing Flowchart



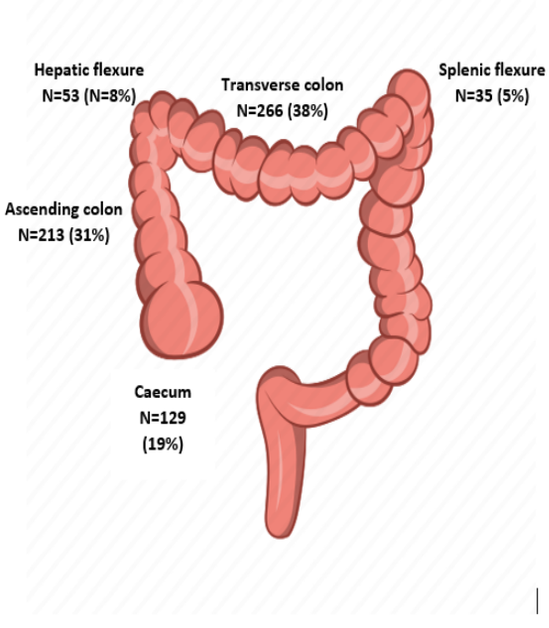
3.3 Results

3.3.1 Demographics

The total number of participants in both arms of the study (white light and chromocolonoscopy) was 736. Of these 303/736 participants had proximal colon polyps and 367 participants had 711 distal polyps (excluding cancers, inflammatory polyps, unclassifiable and normal reported polyps). The following describes the participant characteristic of those who had proximal colon polyps.

Around two thirds (73%) of the participants were male (N = 220) and approximately one third (27%) were identified as female (N = 83). With respect to smoking history, 13% were smokers (N = 38), 51% were ex-smokers (N = 154) and 36% had never smoked (N = 110) (Table 21). The distribution of polyps as per location in the colon is summarised in Figure 26.

Figure 26: Distribution of polyps by location in the proximal colon



3.3.2 Central Pathology review of proximal polyps

A total of 772 slides of proximal colon polyps were requested from the local assessment centres of which 757 slides were received at the centre in Cardiff. Each of the 757 slides that were received were reported by 35 histopathologists at the 12 local assessment centres.

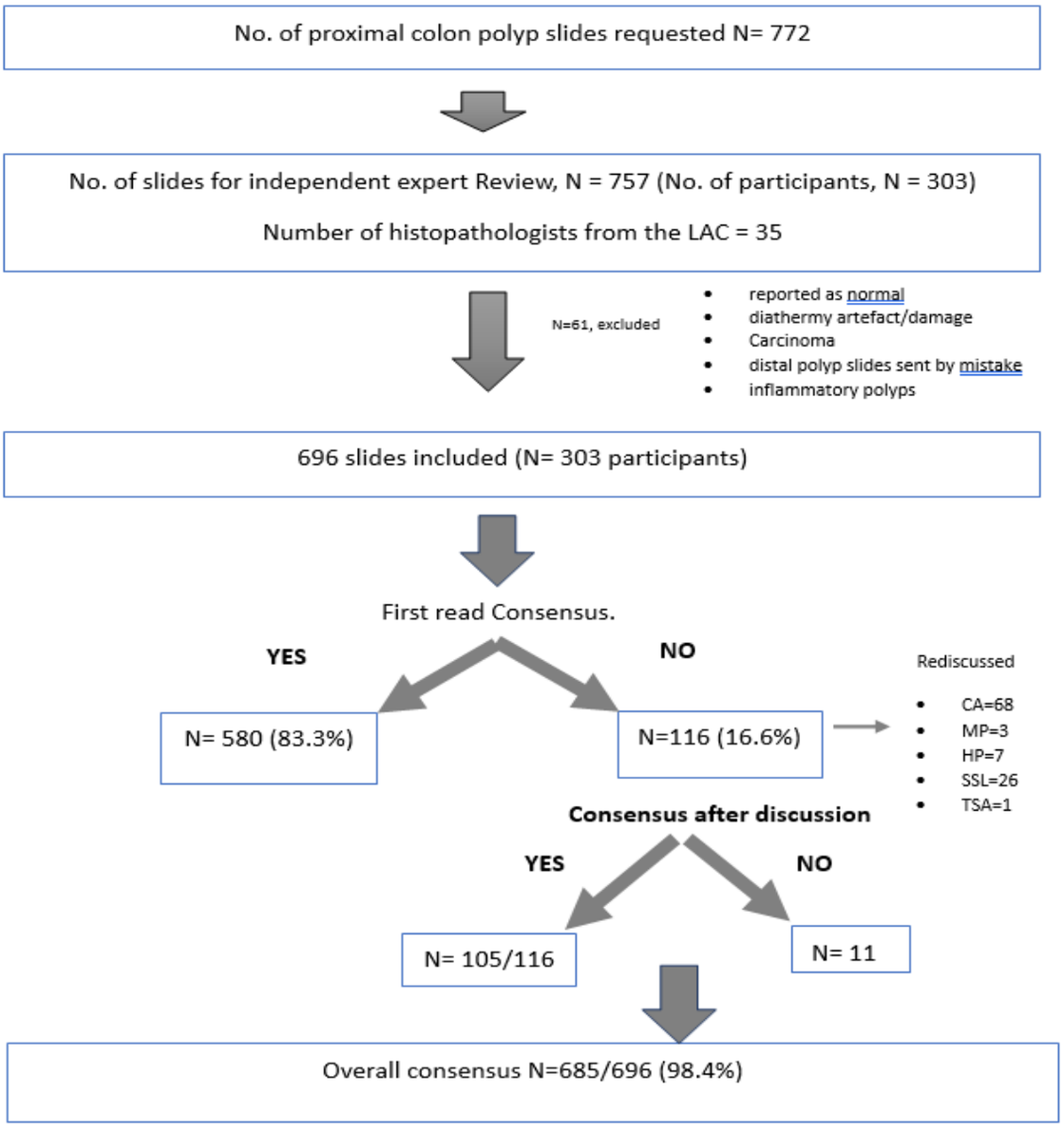
Over a period from July 2015 to March 2017, 11 face-to-face meetings were organised to discuss polyps that failed to reach a consensus on diagnosis alongside reviewing all serrated lesions on a 6 to 8 weekly interval basis.

A total of 61/757 slides were excluded from the analysis for reasons such as being reported as normal, unable to characterise due to diathermy artefact or it being a damaged specimen, presence of carcinoma, distal polyp slides sent by mistake and inflammatory polyps (N =12). Hence in total 696 slides of proximal colon polyps were included in the final analysis, and this was in 303 participants.

A consensus in diagnosis was obtained in 83.3% (N = 580) of the slides at the first reading in assessment by the experts. The remaining 116/696 (16.6%) that did not have an immediate consensus were discussed at meetings and on further discussion a consensus was obtained in 105/116 whilst in 11/116 a consensus could not be obtained despite repeated discussions.

Hence in total following first and repeated reads and consensus meetings 685 out of 696 (98.4%) of the experts had an agreement with the final diagnosis. Please refer to Figure 27 for a summary.

Figure 27: Flowchart demonstrating slides discussed among 3 GI Experts (LAC-local assessment centre)



Around 39% of serrated lesions (N = 34) needed a repeated discussion to obtain consensus amongst experts and interestingly this included traditional serrated adenoma (N = 1) and all of the sessile serrated lesions with dysplasia (N = 4). Table 18 illustrates the consensus in diagnosis amongst experts broken down by polyp type.

Polyp Type	Consensus at first read	Consensus after discussion		Total
		No	Yes	
Adenoma	526		68	594
Mixed Polyp			3	3
HP	10		7	17
SSL	44		26	70
TSA			1	1
Other-UC		11		11

Table 18: Consensus in polyp diagnosis among 3 GI Expert Pathologists.

Around 37% (26/70) SSLs needed to be rediscussed (Table 19) for a consensus opinion and this included 100% of the dysplastic SSLs (N=4).

SSLs that needed to be re-discussed	
SSL Type 2 (WHO) No Dysplasia	10
SSL Type 2 (WHO) with Dysplasia	4
SSL Type 2a No dysplasia	12

Table 19: SSLs that needed discussion at the consensus meeting.

Of the 11 polyps where a consensus could not be reached, (N = 5) were unclassifiable due to inadequate specimen and artefact damage. In over half (N = 6) there was at least one expert who reported a serrated lesion but were unable to agree on a final diagnosis (Table 20).

	LAC Report	Expert A	Expert B	Expert C	After discussion
1	SSL LGD	SSL Type 2a No dysplasia	SSL Type 2 (WHO) LGD	Tubular Adenoma LGD	Serrated tubular adenoma
2	HP	Hyperplastic Microvesicular	SSL Type 2 (WHO) No Dysplasia	Hyperplastic Microvesicular	Hyperplastic Microvesicular (with SSL features-difficult to say) hence unclassifiable
3	Other	Other	Dysplasia NOS	Dysplasia NOS	Other-exclude inadequate specimen
4	Other-diathermy	Tubular Adenoma LGD	Other-damage	Normal	Other-inadequate
5	Adenoma	Normal	Other-damage	Hyperplastic Microvesicular	Other-inadequate
6	Other-diathermy	Tubular Adenoma LGD	Other-damage	Tubular Adenoma LGD	Other-inadequate
7	Adenoma	Other	Other-? SSL	Hyperplastic Microvesicular	Other-unclassifiable too much damage
8	HP	Normal	Other	Hyperplastic Microvesicular	Other-Unclassifiable
9	HP	Normal	SSL Type 2a No dysplasia	SSL Type 2a No dysplasia	Other-Unclassifiable
10	Normal	Hyperplastic Microvesicular	SSL Type 2a No dysplasia	Normal	Other-Unclassifiable
11	Adenoma	Other	Tubular Adenoma LGD	Other	Other-Unclassifiable

Table 20: Proximal Polyps that did not reach a consensus after review by the Experts

Total No of Participants in the study ***		736	
No of participants with		Proximal **colon** Polyps	Distal Colon Polyps
		303	367
Characteristics of participants with proximal polyps			
Gender			
	Male	220	
	Female	83	
Smoking History	Smoker	38	
	Ex- Smoker	154	
	Never	110	
	Unknown	1	
No of Proximal Colon Polyps included		696	
Consensus obtained by Expert Pathologists	First Read	580	
Total No. with expert consensus-final read		685	
Distribution by Site in the Proximal Colon			
	Ascending Colon	213	
	Caecum	128	
	Hepatic Flexure	53	
	ICV	1	
	Splenic Flexure	35	
	Transverse Colon	266	
Average size of Polyps in mm		5.5	
No of Polyps by Size in mm	<10mm	604	
	10-19mm	73	
	>20mm	18	
	Unknown	1	
Polyp Type (Proximal Colon)	Dysplasia	Experts	LAC
Adenoma (Total)		594	573
Adenoma	HGD	3	1
Adenoma	LGD	591	570
Adenoma	LGD+HGD	0	2
Mixed Polyp (MP)		3	6
MP	LGD	2	6
MP	LGD+HGD	1	
Serrated Lesion (SL) Total	88	96	
SL		LGD	4
	No Dysplasia	83	24
Other-UC		11	16
Serrated Lesions – subtype	Hyperplastic	1	63
	Hyperplastic Goblet Cell	5	3

5

	Hyperplastic Microvesicular	11	0
	SSL Type 2 (WHO)	57	24
	SSL Type 2a	13	N/A
	TSA	1	1
Advanced adenoma*		84	
Advanced SSL*		17	

* An advanced adenoma' was defined as a conventional adenoma with either high grade dysplasia (HGD), >25% villous histology, or measuring >10mm in size. 'Serrated lesions' (SLs) incorporated hyperplastic polyps, SSLs and traditional serrated adenomas (TSAs). 'Significant SLs' incorporated SSLs with dysplasia of any size, SSLs measuring >10mm and all TSAs. The term 'advanced neoplasia' incorporated all advanced adenomas and all significant SLs.***Participants with adequate bowel preparation

Table 21: Summary of Polyp characteristics including demographics, location and histology

3.3.3 Reclassification of Hyperplastic Polyps and SSLs:

The histopathological distinction between hyperplastic polyps and sessile serrated lesions can be quite challenging (397) and there is often discrepant classification interchanging the two types of serrated neoplasia. Of the 13 polyps that had a final histological diagnosis of SSL type 2a, 12 polyps needed to be rediscussed to obtain a consensus opinion. 2 out of 3 experts were of the opinion that 6/12 polyps were hyperplastic micro vesicular type and the remaining expert reported 1/12 polyps to be hyperplastic (subtype not specified).

There was only 1/13 polyp that had concordance. However, In comparison to the local assessment centre, all 13 polyps were reported to be hyperplastic polyps. It also appears that the local assessment centres reported a larger number of hyperplastic polyps in comparison to the expert centres.

Table 22 demonstrates that the expert centre reported 70 SSLs (both WHO 2010 and ACG which is the current WHO 2019) in comparison to 30 SSLs at the local centres.

However, 65 hyperplastic polyps were reported at the local centres in comparison to 17 at the expert centre. Of the 65 hyperplastic polyps that were initially reported by the LPs (Figure 28), when reviewed by the EPs, 8 were reclassified as adenomas, 30 (46.1%) were reclassified as SSLs (Figure 28) and 3 were reclassified as other or unclassifiable. There were 2 SSLs from the LP that were reclassified as hyperplastic polyps by the EP.

		Local pathologists						Total-expert report
	Polyp type	Adenoma	MP	HP	SSL	TSA	UC	
Final read expert pathologists	Adenoma	566	3	8	2	0	15	594
	MP	2	1	0	0	0	0	3
	HP	1	0	12	2	0	2	17
	SSL	1	2	42	25	0	0	70
	TSA	0	0	0	0	1	0	1
	UC	3	0	3	1	0	4	11
Total LAC		573	6	65	30	1	21	696

Table 22: Comparison of individual polyp type reports between the experts and LAC

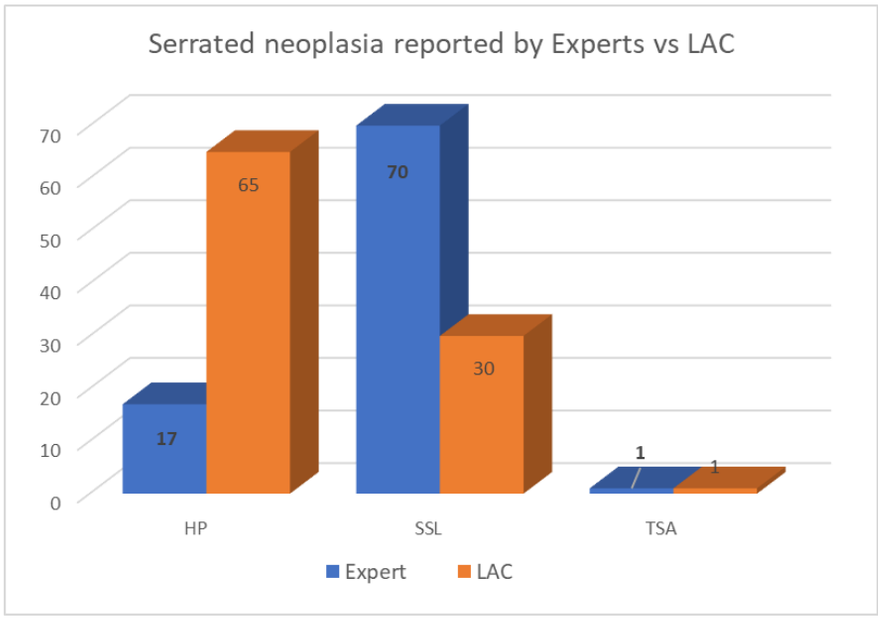


Figure 28: Serrated neoplasia reported by Local assessment centre (LAC) vs. Experts

3.3.4 Inter-observer variability (IOV) of serrated neoplasia

Three expert pathologists independently reported 696 slides/polyps as per stringent criteria and their opinions were blinded to each other as well as blinded to the local histopathology report. There were no identifying factors such as patient ID or origin from hospital on the slide and this was masked by a label that had the trial number and the unique Polyp ID assigned. On the first read between the experts there was 83.3% overall concordance that improved to 98.4% concordance on subsequent discussions and reads.

3.3.4.1 IOV by polyp type

Polyp type included the following categories: tubular adenoma, tubulovillous adenoma, villous adenoma, hyperplastic, SSL type 2 (WHO), SSL type 2a and TSA.

3.3.4.1.1 Between expert pathologists

The overall Kappa coefficient for the above categories on first read was 0.70 (95% CI 0.67-0.73) indicating an overall substantial agreement between the experts in the categorisation of all polyp types. When this was applied to the individual polyp type categories the following observations were made (Table 23). The mean interobserver agreement was almost perfect with a Kappa value of 0.83 (95% CI 0.78-0.86) for tubular adenoma, substantial agreement in tubulovillous adenoma (K=0.61; 95% CI 0.56-0.65) and fair agreement in the reading of villous adenoma (K=0.33; 95% CI 0.28-0.37).

For serrated neoplasia the following observations were made. For hyperplastic polyp type there was moderate agreement (K=0.53; 95% CI 0.48-0.57), almost perfect agreement for SSL type 2 WHO polyps 0.86 (95% CI 0.81-0.90), fair agreement for SSL Type 2a (K=0.33; 95% 0.28-0.37) and TSA (K=0.30; 95% CI 0.25-0.34).

There was fair agreement in the reading of SSL type 2a (ACG criteria) amongst the experts This study predated the updated WHO 2019 criteria. Hence if the WHO 2019 criteria were used to categorise SSLs (includes both SSL Type 2 and 2a) then there would be almost perfect agreement (K = 0.88; CI 0.83-0.92).

3.3.4.1.2 Between Experts and Local Pathologists

The overall Kappa coefficient for polyp type between the final read of the experts and the Local Pathologists (LP) was 0.78 (95% CI 0.70-0.85) which was a substantial agreement. However, when applied to individual polyp types (Table 23), for adenomas, there was substantial, fair, and moderate agreement for tubular adenomas (K=0.83; 95% CI 0.78-0.86), tubulovillous adenomas (K 0.61, 95% CI 0.56-0.65) and villous adenomas (K=0.33, 95% CI 0.28-0.37) respectively.

For serrated neoplasia, as the LAC did not have pre-agreed criteria as the experts, IOV was analysed for hyperplastic, SSL type 2 and TSA polyp types. The IOV was fair for hyperplastic polyps (K=0.27, 95%CI 0.20-0.34), moderate for SSL Type 2 (WHO) (K=0.55, 95% CI 0.48-0.63) and perfect for TSAs (K=1, 95% CI 0.93-1.07, N=1).

Polyp type	Total no of polyps that at least one expert classified in this category	IOV of Polyp type between 3 EPs on first Read (Fleiss Kappa)		Percentage agreement between EP	IOV between final read of EP and LP	
		First Read IOV Kappa (95% CI)	Interpretation of Kappa score	First Read %	EP and LP Kappa (95% CI)	Interpretation of Kappa score
Tubular Adenoma	558	0.83 (0.78-0.86)	Almost perfect	92.1	0.71 (0.64-0.79)	Substantial
Tubulovillous Adenoma	31	0.61 (0.56-0.65)	Substantial	35.5	0.39 (0.32-0.47)	Fair
Villous Adenoma	5	0.33 (0.28-0.37)	Fair	20	0.42 (0.35-0.50)	Moderate
Hyperplastic	17	0.53 (0.48-0.57)	Moderate	58.8	0.27 (0.20-0.34)	Fair
SSL Type 2 (WHO)	57	0.86 (0.81-0.90)	Almost perfect	75.4	0.55 (0.48-0.63)	Moderate
SSL Type 2a	13	0.33 (0.28-0.37)	Fair	7.7	NA	NA
TSA	1	0.30 (0.25-0.34)	Fair	0	1 (0.93-1.07)	Perfect
SSL WHO 2019	70	0.88 (0.83-0.92)	Almost Perfect	83.1	NA	NA

Table 23: IOV of Polyp type between Expert Pathologists (EP) and IOV between EP and Local Pathologists (LP)

3.3.4.2 IOV of grade of dysplasia:

3.3.4.2.1 Between the experts

There was strong (almost perfect) agreement for the interpretation of no dysplasia and low-grade dysplasia and moderate agreement for high-grade dysplasia (Table 24) amongst the experts.

3.3.4.2.2 Experts and LAC

The overall agreement between the final read of the experts and LAC for the grade of dysplasia was substantial (K=0.78 (95% CI 0.71-0.85).

When this was analysed further, no dysplasia and low-grade dysplasia had substantial agreement and high grade had a fair agreement (K=0.28, 95% CI 0.21-0.36).

Dysplasia type	No. of slides that at least one expert classified in this category	Dysplasia IOV first read EP		Dysplasia IOV final read EP and LP	
		Kappa (95% confidence interval)	Interpretation of K value	Kappa (95% confidence interval)	Interpretation of K value
None	94	0.88 (0.84-0.92)	Almost perfect	0.80 (0.73-0.87)	Substantial
Low grade	598	0.87 (0.82-0.91)	Almost perfect	0.78 (0.70-0.85)	Substantial
High grade	4	0.53 (0.49-0.57)	Moderate	0.28 (0.21-0.36)	Fair

Table 24: Based on Dysplasia grade, IOV between EP and IOV between EP and LP

3.3.4.3 IOV between the subtypes of serrated lesions:

Figure 29 shows the shows the interobserver variability (IOV) between the different subtypes of serrated lesions between the three expert pathologists (EP). There was almost perfect agreement for SSL type 2 (WHO) no dysplasia (K= 0.87, 95% CI 0.83-0.91), there was fair agreement for hyperplastic goblet cell type (K= 0.36, 95% CI 0.32-0.40), TSA (K = 0.30, 95% CI 0.25-0.34) and SSL type 2 (WHO) with dysplasia (K= 0.22, 95% CI 0.18-0.26). There was moderate agreement for hyperplastic Microvesicular type (K= 0.51, 95% CI 0.47-0.56).

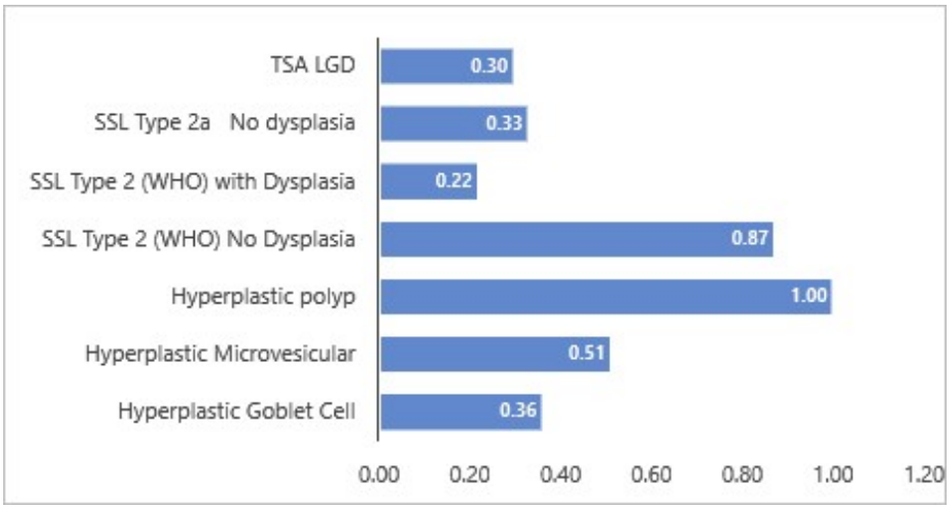


Figure 29: Interobserver variability of serrated neoplasia subtypes between the 3 experts

The IOV between the final read of the EPs and the LPs revealed a fair agreement for hyperplastic and SSL with dysplasia (Figure 30, Table 25), perfect agreement for TSA and moderate agreement for SSL with no dysplasia.

IOV between the final read of the EP vs. LP		
Histology	Kappa	95% CI
HP	0.248	0.17 – 0.32
SSL without dysplasia	0.424	0.35 – 0.49
SSL with dysplasia	0.217	0.14 – 0.29
TSA	1.000	0.92 – 1.07

Table 25: Interobserver variability of serrated neoplasia between the expert and local pathologists

Abbreviations: IOV-interobserver variability; HP-Hyperplastic Polyp; SSL-sessile serrated lesion; TSA-traditional serrated adenoma

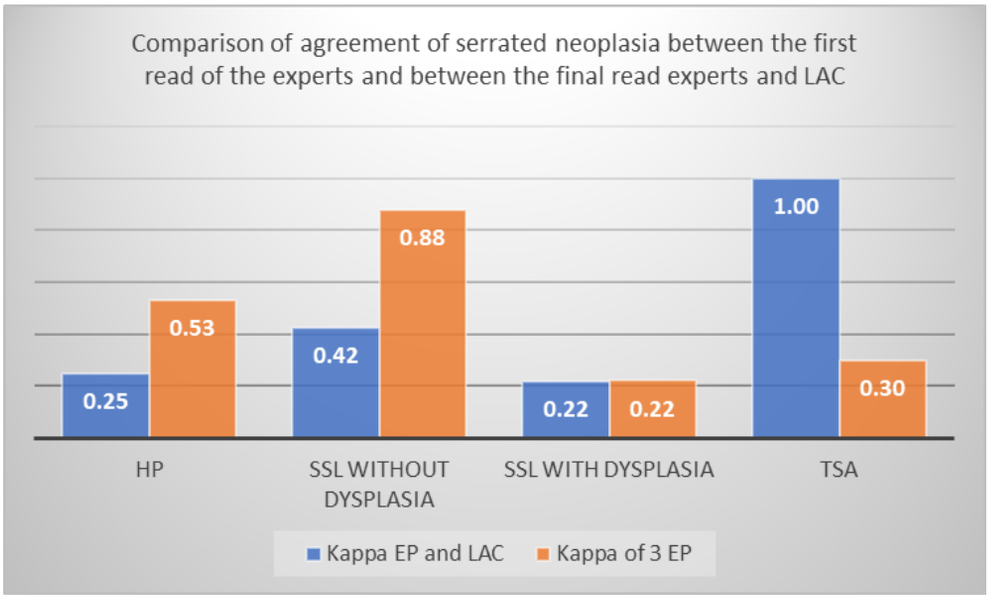


Figure 30: Comparison of Kappa agreement of serrated neoplasia between expert pathologists (EP) and EP vs local assessment centre (LAC) pathologists

3.3.5 Prevalence of serrated neoplasia

Following a review by the expert pathologists (Table 26), the total number of serrated lesions (HP, SSL, TSA) was N = 88 in 68 participants. Hence the prevalence of serrated lesions in the proximal colon where a participant had at least one serrated lesion was estimated to be 9.2%.

$$\text{Prevalence of SL proximal colon} = \frac{No\ of\ participants\ \ with\ Serrated\ Lesions\ (N=68)}{Total\ No\ of\ particpants\ \ in\ the\ study\ (N=736)} = \mathbf{9.2\%}$$

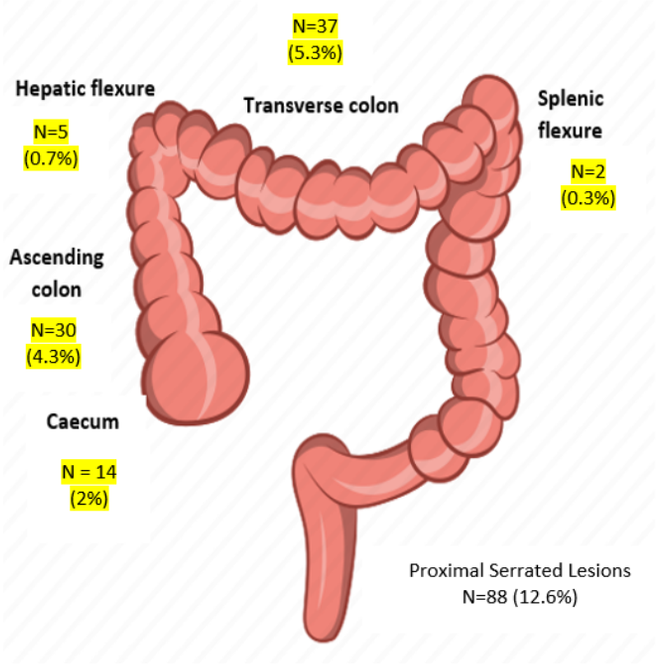


Figure 31: Distribution of serrated lesions in the proximal colon

(88/696)

However, if hyperplastic polyps were to be discounted and only significant serrated neoplasia was to be included i.e., SSL type 2/2a, SSLD and TSA then this would be N= 56, with a prevalence of significant serrated lesions in the proximal colon calculated to be 7.6% (56/736).

Without rigorous histopathological assessment by the expert pathologists, if the prevalence of serrated neoplasia (HP, SSL, TSA) in the proximal colon was based on the local pathology reports then this would be estimated to be 7.9% (58/736).

However, the prevalence of significant serrated lesions in the proximal colon (SSL with and without dysplasia and TSA) would be reduced further to 3.3% (number of participants with a significant serrated lesion= 24).

This would not have altered surveillance intervals as there were concomitant proximal colon adenomas present in this cohort.

	Expert Pathology Review			Local Pathology Review		
Polyp Type	Pts with > 1 SL	Pts with 1 SL	Total	Pts with > 1 SL	Pts with 1 SL	Total
Hyperplastic	1		1	42	8	50
Hyperplastic Goblet Cell	1	4	5			
Hyperplastic Microvesicular	3	8	11			
SSL Type 2 (WHO) with and without dysplasia	13	44	57	16	7	23
SSL Type 2a	1	12	13			
TSA	1		1	1		1
Total	20	68	88	59	15	74

Table 26: Distribution of serrated neoplasia in participants based on expert and local pathology review

3.3.6 Novel findings:

There have been several suggestions regarding the morphological variation in serrated lesions in the recent years (145). There have been minor morphological variants with distinct molecular features that have been defined recently. This includes (145) serrated tubulovillous adenoma, mucin rich variant of traditional serrated adenoma and superficially serrated adenoma. Additionally, dysplasia not otherwise specified (NOS) has been added to the dysplasia subtypes of SSLs. The above morphological variants have not been added to the recent WHO 2019 criteria as further clinicopathological and molecular data is required of the above for it to be included.

During the period when the above study which was conducted between 2015-2017, we were aware that there was description of serrated tubulovillous adenoma (sTVA) in the literature published in 2016 (398). Bettington et al from Brisbane, described a subset tubulovillous adenomas with prominent serrated architecture along with ectopic crypt formations (ECF) with distinct morphological features from TSA (for which these polyps can be misdiagnosed as however the absence of typical TSA type cytology and slit light serration is key to make the correct diagnosis) and conventional tubulovillous adenomas.

The group demonstrated that with set criteria for diagnosis of sTVA reliability and reproducibility of diagnosis was feasible and the authors hypothesised that KRAS mutation is associated with the development of morphological serration in these polyps.

In addition to this they found that sTVA was most commonly found in the proximal colon and larger in size with presence of advanced histology compared to conventional TVAs. They also harboured KRAS mutation more frequently and showed more frequent CIMP in comparison with conventional TVA. There were no cases that showed MLH1 loss or BRAF mutation in the sTVA. Hence the group collectively hypothesised that these polyps may be precursors of KRAS-mutated, microsatellite stable (MSS) colorectal carcinomas.

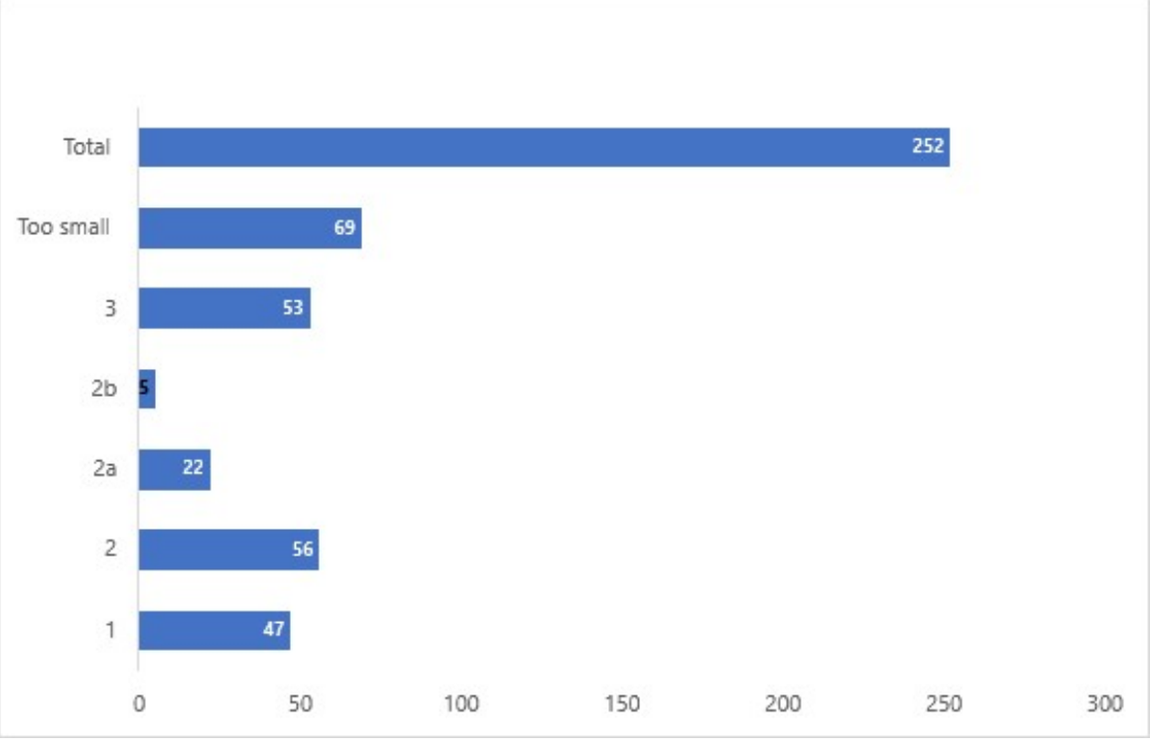
Our group were unaware of any other morphological descriptions of serration in conventional adenomas apart from what has been described as above in the literature at the time of our study.

It was observed in the cohort analysed that in addition to serrated TVA ('Brisbane lesion') that there were some tubular adenomas (TA) that also had some serrated features which varied from conventional TAs. This included presence of serrated features either greater than or less than 50%, occasional or prominent ectopic crypts and a grade of mucinous component present as well. Around 252 slides of tubular adenomas were reviewed by the group again to attempt to categorise these tubular adenomas for subsequent analysis for their molecular characterisation. Hence one of the novel findings in this study was an attempt to describe another morphological entirety called serrated tubular adenoma (sTA) or the 'Cardiff' Lesion.

Based on this the following diagnostic criteria was recommended for both sTVA and sTA.

Scoring of mucinous components for both Cardiff and Brisbane Lesions	
Grade	% Mucinous component
1	0-25%
2	25-50%
3	50-75%
4	76-95%
5	>95%
Categorisation of Tubular Adenomas for molecular characterisation	
1	Conventional Tubular Adenoma
2	Tubular Adenoma with serrated features <50%
2a	with occasional/ seldom Ectopic crypts
2b	Prominent Ectopic Crypts
3	Tubular Adenoma with serrated features >50% (Cardiff lesion)- %mucinous
Categorisation of Tubulovillous Adenomas for molecular characterisation	
1	Conventional Tubulovillous Adenoma
2	TVA <50% Serrated features
3	TVA >50% Serrated features

Based on the above, categorisation the expert pathologists reviewed 252 tubular adenomas and attempted to categorise them based on the above classification system. These slides were also marked for future molecular and genetic studies.



Categorisation of serrated TVA: there were 22 TVA polyps reviewed of which,

1. Conventional TVA (N = 2)

2. TVA with less than 50% serrated features (N= 6) and

3. Serrated TVA (N=22)

Since our study which has not been published yet, there has been a group that has describe superficially serrated adenomas (N = 20) (399) however these lesions are mostly located in the distal colon and there was no diagnostic criteria for categorisation used however further studies on these polyps have shown high frequencies of KRAS mutations similar to precursors of KRAS-mutated MSS CRC. There was in fact a case report in 2019 that reported a sigmoid colon cancer with KRAS mutation originating from a superficially serrated adenoma (400).

In summary, there appear to be distinct morphological variants of adenomas with serrated features that appear to have features that might contribute to CRC. This is an observation from the study and the significance of this would require additional research including a larger study that would include further validation and analysis of the above alongside immunohistochemistry and molecular genetic studies that may add more information to inform cancer risk that may contribute to the literature.

3.4 Discussion

This chapter attempts to explore the histopathological and morphological features of colorectal polyps and in particular serrated neoplasia and tries to firstly, understand the prevalence of serrated neoplasia in the proximal colon in an organised CRC screening programme in Wales. This is following rigorous histopathological assessment by expert pathologists using both WHO 2010 and AGA categorisation.

Secondly to understand the interobserver variability in the histological diagnosis of serrated neoplasia and other colorectal polyps between pathologists (both expert and local pathologists) following rigorous review of polyp histology in proximal colon. This is important, as variation in histopathological classification of colorectal polyps by pathologists could result in a variation or inconsistencies in the surveillance interval recommendation given to participants (295-299).

In this study, a consensus in histopathological diagnosis was obtained in 83.3% of the 696 polyp slides that were discussed. Around one third of the slides that were rediscussed (34/116) were serrated lesions that included all SSLD (N = 4) and TSA (N =1). The polyp slides whereby a consensus could not be obtained (11/696), majority (9/11) a consensus could not be obtained as there was too much damage to the specimen however in 2/11, a consensus could not be obtained despite repeated discussion and deemed to be unclassifiable. These 2/11 included serrated tubular adenoma and a hyperplastic Microvesicular polyp with serrated features.

Even though this is a smaller cohort, it demonstrates the difficulty to obtain a histological diagnosis in serrated lesions even amongst expert pathologists.

The study highlights the role of clear and unambiguous criteria in the assessment of serrated neoplasia. The IOV using kappa values between the three expert pathologists showed a fair concordance rate with TSA, SSL type 2a however an almost perfect concordance with the diagnosis of SSL type 2 (WHO 2010).

Whilst there was fair agreement in the reading of SSL type 2a (ACG criteria) amongst the experts which is the involvement of at least one crypt as per the criteria used by the AGA and incorporated into the current study. This study predated the updated WHO 2019 criteria. Hence if the WHO criteria 2019 was used to categorise SSLs (includes both SSL Type 2 and 2a) then there would be almost perfect agreement (K = 0.88; CI 0.83-0.92).

When the experts were compared to the local pathologists, the concordance was fair for hyperplastic polyps, moderate for SSL type 2 (WHO) and perfect for TSA (N=1). The poor concordance in hyperplastic polyps has been described in the literature and it is estimated that 20 to 30% of lesions previously classified as hyperplastic polyp currently correspond to SSLs and TSAs (394, 401).

In the study, there were only 4 dysplastic SSLs, and the concordance was fair amongst the experts and the local pathologist. Interestingly all these polyps occurred in males and the average size of these polyps was 5 mm. The review of the additional comments that were made by the individual expert pathologists reveal the diagnostic difficulty with these polyps. These included a differential diagnosis of traditional serrated adenoma with low grade dysplasia, a mixed polyp with both adenomatous and serrated features with low grade dysplasia and hyperplastic micro vesicular polyp. This may be reflective of the histological heterogeneity of sessile serrated lesions with dysplasia (402).

We also observed in this study that around 46% of hyperplastic polyps that were reported at the local assessment centre were re-classified as sessile serrated lesions. Though as a result of reclassification this did not significantly impact surveillance intervals of these participants.

The prevalence of significant serrated neoplasia in the proximal colon with rigorous review by the experts revealed a rate of 7.6% versus 3.3% if stringent criteria and review were not applied as in the local pathologists' report. In this study, this would not have led to a change in surveillance strategy given that all the participants who had significant proximal serrated lesions also had synchronous proximal adenomas.

Hence although this did not lead to a change in surveillance due to the presence of concomitant adenomas in this study, it has significant implications for those who may not have synchronous adenomas.

According to the literature (292-294, 391, 394, 403, 404) there remains a difficulty related to the adoption and interpretation of the classification of serrated lesions by pathologists probably related to a degree of subjectivity of existing histological criteria but also due to the different classification systems (previously ACG and WHO 2010 which has now been unified by the recent WHO 2019 criteria). In addition to this there might be unique polyp characteristics that might lead to a potential diagnostic dilemma in all types of colorectal polyps.

The interobserver variability in different series of studies show that most report a poor to fair concordance for serrated neoplasia in studies from 2009 (293, 405, 406), 2011(407), 2014 (295). In a few studies (2008) there was good interobserver concordance (408) and fair to good in a study in 2014 (292). Ensari et al (294) also reported very good concordance for serrated lesions, hyperplastic and TSA.

Sessile serrated lesions with dysplasia (SSLD) are also thought to be precursors of interval CRC as they are rapidly progressive, difficult to detect endoscopically and can sometimes be incompletely resected (291). Dysplastic SSL and TSA also have a significant malignant potential with a 10-year CRC risk (237) of 4.43% and 4.5% respectively (Table 17).

Histologically, these lesions have also been described to have different patterns of dysplasia including subtle architectural and cytological changes and often requires immunohistochemical studies to identify the MLH1 loss of expression (402) which is the critical molecular event underpinning lesion progression.

Discrepancies in classification of those polyps that have a higher risk of developing CRC particularly those with high grade dysplasia or dysplastic serrated lesions or TSA could lead to a recommendation of a longer surveillance interval that could contribute to the development of interval cancers due to the underlying biology of these polyps.

Surveillance guidelines following the removal of serrated polyps have different recommendations in the UK, Europe and the USA. For example a sessile serrated lesion less than 10 mm without dysplasia has no recommended surveillance if in the UK, 10 years if in Europe and five years if in USA. For high risk lesions such as SSLD it is a one of colonoscopy at three years for all three continents (200).

Hence the strategies for surveillance of these polyps can only be adequately used after accurate histological diagnosis (394).

Strategies that may help to improve the accuracy of histological diagnosis of SSLD and TSA could include a second gastrointestinal pathology review. In addition to this, addition to this MLH1 immunohistochemistry could be a useful ancillary test to support the diagnosis of dysplasia in SSLs however this should only be recommended in certain situations that include equivocal cytological atypia secondary to inflammation, those with mild morphological changes and minimal deviation dysplasia (402) however this may not be useful in those lesions that have unequivocal architectural and cytological dysplasia as MLH1 may not alter the final diagnosis.

The other factor that could potentially help in accurate identification of these high risk lesions is endoscopic recognition of these lesions and with the advent of AI could potentially improve in the future and a close collaboration between the endoscopist and histopathologist to ensure accurate reporting (291).

The fair concordance of at-risk lesions between the centre and local centres could be reflective of training. However, all pathologists in Wales who report polyps in the bowel screening programme undergo regular specialist training. Setting key performance indicators for diagnosis of these lesions in pathology units similar to colonoscopy may help to understand standards and improvement of diagnosis.

We also observed in this study that around 46% of hyperplastic polyps that were reported at the local assessment centre were re-classified as sessile serrated lesions. This has also been widely reported in the literature and one large retrospective study done in 2013 (409) over a four-year period reclassified 30 to 60% of hyperplastic polyps into sessile serrated lesions. This study did not include inter observer variability.

Due to the inherent difference in the risk of malignant transformation of serrated polyps located in the proximal colon and the consequence on colonoscopic surveillance (294, 410, 411), distinguishing between hyperplastic polyps and sessile serrated lesions is important (294). SSLs are more common in the proximal colon and hyperplastic polyps are more common in the distal colon (97). Reclassifying hyperplastic polyps as serrated lesions has important clinical implications such as interval cancer risk depending on presence of dysplasia and surveillance guidelines for the individual. Patients with serrated lesions, especially SSLs, may have a higher risk of synchronous or metachronous colorectal neoplasia (412) compared to those with conventional adenomas.

Accurate pathological diagnosis is important to help to identify risk to the individual but also to inform surveillance intervals particularly in those participants who isolated advanced serrated neoplasia or those who have hyperplastic polyps misclassified as serrated lesions which can potentially reduce the frequency of surveillance intervals or in the vice versa increase surveillance intervals thereby reducing the potential for developing interval cancers.

In this cohort, as a result of the reclassification this did not change surveillance interval significantly due to small numbers and presence of concurrent polyps. However, if the current UK surveillance guidance is applied for high-risk criteria, then N=5 would have changed to 3-year surveillance from the previously recommended one year surveillance.

The possible future solutions to improve interobserver concordance of serrated neoplasia could include the following:

1. Access to training, Although there are national guidelines and training days for histopathologists perhaps it would be helpful if each unit were to have nationally agreed key performance indicators based on population prevalence and method of ascertainment of these lesions

2. There is a need to reduce subjectivity and introduce standard criteria for improving the diagnostic accuracy of serrated neoplasia (for example the presence of ectopic crypts). Perhaps the development of diagnostic tools to help like microscopic criteria for diagnosis of serrated lesions and practical application could be helpful.

3. Diagnostic reproducibility of serrated neoplasia improves when diagnostic criteria have been adopted. For example, increased recognition of criteria like crypt distortion or whole length serration leads to increase agreement (297). In addition to this this also improves with time and educational emphasis on criteria.

4. The use of immunohistochemistry in appropriate cases such as MLH1, BRAF, Annexin 10, A10 and molecular markers such as methylation assays could potentially help in the future.

5. The use of artificial intelligence (AI) augmented digital system could potentially help with the improvement and accuracy in classification of colorectal polyps and reproducibility by pathologists compared with a standard microscopic assessment (413).

A study (413) demonstrated that pathologists using the microscope digital system did not outperform the stand-alone deep learning model and recognise the importance of pathologist knowledge and experience to bring value especially in challenging cases where a deep learning model without adequate training and validation may be inaccurate.

Hence future directions could include refinement of both underlying deep learning models and development of further pathological diagnostic criteria that could potentially lead to gains when an AI augmented system is utilised.

The limitations of the study were: 1. Reproducibility or intraobserver variability amongst the experts was not assessed. This would have helped to understand whether specific additional criteria would have been needed to ensure reproducibility of diagnosis particularly in those difficult to diagnose polyps such as the sessile serrated lesions with dysplasia and traditional serrated adenomas. Though these slides were discussed at consensus meetings it involved discussion as a group as opposed to review of these the slides individually. When strictly defined and standardised diagnostic criteria is used there is considerable improvement in the diagnostic reproducibility of reporting serrated neoplasia (294).

2. The second limitation was that the local pathologists did not have the standard pre-defined criteria that was agreed amongst the expert pathologists. This could potentially explain significant variation and the fair concordance for hyperplastic polyps as local reporting had to be standardised retrospectively for analysis.

3. Thirdly – the study (CONSCOP) was set up for proximal polyps only and perhaps rigorous histological process should have been included distal polyps as well which may have helped to answer any difference in surveillance intervals.

4. The other limiting factor to take into consideration in determining the prevalence of serrated neoplasia in this particular study of a screened population is the low sensitivity of gFOBT which was the screening test used during this study. Faecal blood testing i.e. either by gFOBT (55, 213) or FIT (214, 215) demonstrates low detection rates for adenomas especially those located in the proximal colon. Studies have shown that gFOBT. has a negligible or minimal effect on CRC incidence (20, 21) and data is limited as to the sensitivity of detecting proximal serrated neoplasia. The sensitivity of FIT for SSLs using a threshold of 20ug Hgb/g faeces was reported in one study to be 6.2% (in comparison to 20.9% for advanced adenomas) (24).

In conclusion, this prospective study demonstrated the interobserver variability in the reporting of serrated neoplasia and also explores the possible factors that can help to improve concordance. It also reports the prevalence of serrated neoplasia in a screening population. The future direction could include the use of digital technology and artificial intelligence alongside molecular studies to help to reduce subjectivity of interpretation and recognition of morphological variants that can potentially help in the further determination of risk of these polyps alongside molecular genetic analysis.

Chapter 4- Mutational Signatures in Serrated Neoplasia

4.1 Introduction:

CRC is a heterogenous disease, and two thirds of CRCs arise through the classical chromosomal instability pathway however 15 to 30% arise through the serrated neoplasia pathway(71, 81, 123). This results in CRC that occurs from precursor serrated lesions with different histopathological, morphological and molecular differences from the traditional adenomas (414).

In the last two decades the landscape of CRC has been modified by the explosion of molecular biology techniques (415).

The molecular pathogenesis of serrated neoplasia has been described in detail in chapter 1.4 and includes the lifestyle, environmental factors that influence serrated neoplasia development through the interplay with epigenetics.

This chapter will describe the concept of mutational signatures in relation to serrated neoplasia and thereafter describe the aims, methodology, results and conclusions of the study that was undertaken.

4.1.1 Mutational signatures:

The metamorphosis from a normally functioning colorectal cell to an invasive carcinoma is fuelled by the acquisition of somatic mutations (416). The majority of somatic mutations found in a cancer genome are considered passenger mutations as they do not play a direct role in carcinogenesis however there are a small number of somatic mutations called driver mutations that are positively selected across cancer genomes and confer a clonal growth advantage (416, 417).

There are specific patterns of somatic mutations which are called mutational signatures that are imprinted by different mutational processes operative through the lineage of a cancer cell (416, 417).

Somatic mutations that are present in cancer genomes may arise due to various factors such as an interplay among DNA damage, DNA repair and DNA replication(418). These somatic mutations can be attributed to specific exposures such as tobacco smoking and lung cancer and ultraviolet light in skin cancers (418, 419) or to abnormalities of DNA maintenance such as defective DNA mismatch repair in some CRCs (418, 420). Buried within the somatic mutation spectrum of a cancer genome lies signatures of biomarkers of the mutational process that resulted in the formation of the tumour.

Different mutational mechanisms can result in a variety of combination of different mutational types that can produce a mutational signature.

Each mutational signature is derived from compositional changes of single base substitutions (SBS), indel (ID) and doublet base substitutions (DBS) (421).

A landmark study analysed millions of somatic mutations from thousands of different cancer types using the latest next-generation sequencing techniques and revealed the existence of 20 distinct mutational signatures (418).

In the last few years, large amount of cancer sequencing data has been produced and the reference set of mutational signatures was developed and provided to users within the catalogue of somatic mutations and cancer (COSMIC) database (417, 422). This database is the most detailed and comprehensive resource for the exploration of the effect of somatic mutations in human cancer.

4.1.2 Methods of detecting mutational signatures:

Next-generation sequencing (NGS), also known as high-throughput sequencing, is a modern and powerful technology that enables rapid and accurate sequencing of DNA and RNA molecules. NGS allows for the parallel sequencing of millions of DNA fragments simultaneously, making it faster and more efficient than traditional Sanger sequencing (423).

The recent advances in next-generation sequencing (NGS) have enabled the identification and characterisation of mutational signatures and cancer. The two main methods to detect mutational signatures include whole Genome Sequencing (WGS) and whole Exome Sequencing (WES).

NGS involves several major steps in DNA sequencing. This involves DNA fragmentation, library preparation, massive parallel sequencing, bioinformatics analysis and variant annotation and interpretation (424).

DNA fragmentation is used to break targeted DNA into shorter segments to measure 100-300 bp in length and the different methodologies include polymerase chain reaction (PCR) amplification (424). In this method primer pairs are used to amplify targeted DNA using PCR and the PCR products then serve as short segments of targeted DNA. The DNA segments are then used for library preparation (424).

In the library preparation process, DNA segments are modified so that each sample has a unique specific index code allowing sequencing adapters to be added to the DNA segments. This allows the sequencing primers to find all the DNA segments and thereafter enables massive parallel sequencing (424).

Massive parallel sequencing is usually performed by a NGS specific sequence whereby the library is loaded onto a sequencing metrics in a certain sequence of. There are different types of sequencers and methods available. Illumina NGS sequencer uses flow cells and another sequencer called the Ion Torrent uses chips instead.

The data that is generated from sequencing is then analysed by bioinformatics analysis which is a process involving base calling, read alignment, variant identification and annotation (425)

4.1.3 Whole genome sequencing (WGS):

WGS provides a comprehensive view of the entire genome enabling the identification of both coding and non-coding mutations. This method allows for the detection of all types of mutations including single nucleotide variants, insertions and lesions and structural variations. It is less frequently used in cancer somatic mutation because the average depth used here is limited detection and different mutations with different allelic frequencies often need deep sequencing which can be challenging to do using this method (424). whole genome sequencing: this is quite expensive with coverage achieved 30 times. The DNA input is 1 µg.

4.1.4 Whole Exome Sequencing (WES):

WES focuses on the protein coding regions of the genome which represents approximately 1 to 2% of the entire genome. Although less comprehensive than WGS, WES is more cost-effective and has been widely used to identify mutational signatures. WES is likely to be more expensive than targeted DNA sequencing with the coverage achieved to be around 100 times greater. Typically, the DNA input for WES is usually 0.5-1 µg.

Targeted panel sequencing also offers an opportunity for greater depth of study in these regions due to reduce sequencing burden.

NGS platforms, such as Illumina's HiSeq and MiSeq, have revolutionized genomics research by providing a cost-effective and high-throughput approach to study the genetic basis of diseases.

Hiseq- A comprehensive gene panel for CRC on the HiSeq platform would typically contain a larger set of genes compared to a targeted gene panel on the MiSeq platform. These comprehensive panels are designed to provide a more in-depth analysis of the genomic landscape of CRC, including genes associated with development, progression, treatment response, and resistance.

MiSeq- A targeted gene panel for CRC on the MiSeq platform typically contains a set of genes that are known or suspected to be associated with the development, progression, or treatment response of colon cancer.

There are several computational tools and databases that have been made available which has a catalogue of mutational signatures such as the catalogue of somatic mutations and cancer (COSMIC) (422) and MutaGene which is an online computational framework that explores DNA context dependent mutational patterns and underlying somatic cancer mutagenesis whilst analysing mutational profiles of cancer samples also identifies other endogenous and exogenous mutagenic factors (426).

4.1.5 FFPE samples:

The type of specimen used for NGS depends on what sort of tumours assayed. For example in a haematological malignancy fresh tissue samples are more likely to be used however for a solid tumour FFPE samples are widely used. FFPE samples are usually provided as an stained slides with the haematoxylin and eosin stained slide that gives a microscopic review of abnormal areas and enables of these areas for DNA extraction.

DNA preparation from FFPE tissue for next-generation sequencing is associated with several challenges. The yields are often limited due to the precious nature of the sample and the compromise state of the DNA. In addition to this artefacts introduced by fixation and embedding conditions and long-term storage are most prevalent in sequencing results when starting with limited material. One particular problem is the deamination of cytosine bases to deoxyuracil. This can lead to C-T conversion in sequencing reactions. The Generead DNA FFPE kit allows for purification of high yield of DNA from small amounts and includes the removal of deaminated cytosine to prevent false results in DNA sequencing.

The challenges of NGS lies in the interpretation of the results as many variants can be identified.

The strategies for minimisation of sequence artefacts from FFPE DNA (427) include at the :

DNA extraction stage: pre-analytical assessment of FFPE DNA by pathologists to identify tumour enriched areas, macrodissection of the tumour enriched areas, heat treatment to remove formaldehyde induced cross links to facilitate tissue digestions with proteinase and extended proteinase K treatment digestive issue and remove cross links to DNA. DNA assessment: ensuring there is adequate assessment of double-stranded DNA using fluorometry and quantification of amplifiable templates using q PCR.

In the sample library preparation phase: ensuring there is in vitro removal of uracil prior to PCR amplification of FFPE DNA, use of assays that generate short amplicons to increase the number of templates for PCR, ensuring that specific primers are used for each strand of DNA template in amplicon - based target enrichment approach. The use of high fidelity DNA polymerise to reduce polymerase errors in PCR amplification.

4.1.6 Aim of the study:

The aim of the study was to –

1. Explore the feasibility of extracting DNA from formalin-fixed paraffin-embedded (FFPE) samples in a selected cohort for next-generation sequencing (targeted gene panel and comprehensive panel)
2. To evaluate whole Exome sequence data from a small number of patient samples collected as part of the CONSCOP feasibility study to assess the known mutation burden and identify mutational signatures and its association with smoking

4.2 Materials and Methods:

4.2.1 Reagents and Equipment:

The materials and reagents used for this study are as follows:

Supplier	Reagent /Product
Agilent®	Agilent® 2100 Bioanalyzer®
	Agilent High Sensitivity DNA Kit (Agilent cat. no. 5067-4626)
Beckman Coulter cat. no. A63880	Agencourt® AMPure® XP Kit
Fisher brand	Microcentrifuge tube conical snap cap natural 2.0mL
	50-Well Microtube Rack Assorted
	12603145 - Cryobox 133mm x 133mm base, 50mm/75mm height 2-in-1 slip lid adjustable for height natural
illumina	Illumina Nextera Rapid Capture Enrichment kit
Life technology	Qubit® dsDNA HS Assay Kit (Life technologies/catalogue no: Q32851)
	Qubit® assay tubes are 500 µl thin-walled polypropylene tubes for use with the Qubit® Fluorometer. (Life technologies catalogue no: Q32856)
Qiagen	GeneRead DNA FFPE Kit
	For 50 preps: QIAamp MinElute Columns, Collection Tubes (2ml), Deparaffinization Solution, Uracil-N-Glycosylase, RNase-Free Water, RNase A, and Buffers
	GeneRead DNAseq Targeted Panels
	High-quality, nuclease-free water
	GeneRead DNA QuantiMIZE Array or Assay Kit (QIAGEN cat. nos. 180642/180654)
	Microcentrifuge 1.5 ml LoBind tubes
	0.2 ml PCR tubes, 96-well reaction plates, or PCR strips and caps
	DNase-free pipet tips and
	0.2 ml 96-well PCR plate
	GeneRead DNA Library L Core Kit (QIAGEN cat. no. 180462)
	GeneRead DNA L Amp Kit (QIAGEN cat. no. 180485)

	GeneRead Adapter L Set 12-plex (QIAGEN cat. no. 180994)
	GeneRead DNAseq Library Quant Kit for Ion PGM Sequencer (QIAGEN cat. no. 180601)
Rainin LTS Sterile Tips (anachem website)	Part No: 17014961 Model No: TR-L10F Tips 0.1-20uL Sterile Racked pk960 LTS
	Part No: 17014963 Model No: TR-L200F Tips 5-200uL Sterile Racked pk960 LT
	Part No: 17014967 Model No: TR-L1000F Tips 100-1000uL Sterile Racked pk768 LTS
Sigma-Aldrich	Tough-Tags™ microcentrifuge tube labels
Thermo Fisher Scientific	Sterile disposable Swann Morton Blade No 10
	Swann-Morton-Handle No.3 surgical stainless steel
	Eppendorf® Safe-Lock microcentrifuge tubes, volume 1.5 mL, natural
	12688336 - Slide box to store 76mm x 26mm slides, 100 slide capacity 210mm x 160mm x 33mm (w x d x h)
	Marker pen secure line marker II, permanent, nontoxic black
	DynaMag™-96 Side Magnet (cat no. 12331D)
	Ethanol
	Multichannel pipette, Single channel pipettor
Misc. (Cardiff University)	Ethanol
	Thermocycler
	Water bath
	Single and multi-channel pipettes

4.2.2 Case Cohort

This study was part of the CONSCOP trial and as described in chapter 3, all proximal polyp FFPE slides were reviewed by the expert gastrointestinal histopathologists. Of all the slides that were reviewed, 50 slides were selected from this cohort based on four groups described in the case cohort below.

The selection of groups was based on the following polyp information:

Group 1- participants who had only SSLs without dysplasia (without the presence of any other type of polyp), N=11 , group 2- Dysplastic SSL (with or without adenomas), N=5, Group 3-Non dysplastic SSL with concomitant and matched adenoma, N=17, Group 4- Adenomas only (N=17).

The selection of slides from these groups also included information on gender and smoking status. The aim was to obtain aspirin data as well however this was excluded as majority of this data for the below groups were missing.

Prior to application of the following described method to extract and purify DNA from the selected groups FFPE samples, five random adenomas were chosen and implementation of the following described DNA extraction method was performed successfully. It was following confirmation of this that it was then applied to the main study samples.

The selected H&E slides were examined under a microscope by Dr Meleri Morgan and abnormal areas were highlighted with a marker pen (Figure 32). These slides were then photocopied by me and filed with the view to use after blocks obtained from these polyps (from the local assessment centres) and were sectioned by microtome

Figure 32: Marking of areas of interest on H&E slides.

4.2.3 Workflow of FFPE sample DNA extraction and sequencing:

4.2.9.1 Identification of abnormal areas on the FFPE sectioned slide:

The area of interest on each of the unstained slides was identified by overlaying with the stained section (Figure 32) that was photocopied.

4.2.9.2 Macro dissection of tissue from FFPE sections:

A scalpel was used to dissect the abnormal areas on the slide. These scrapings were then collected in a clean micro centrifuge tube (1.5ml Eppendorf) and labelled.

4.2.10 DNA extraction, purification, and quantification from FFPE samples:

GeneRead DNA FFPE Kit (by QIAGEN) was used to extract and purify DNA from the FFPE samples.

The kit uses silica-based membrane technology in combination with optimized buffers and reagents to efficiently isolate high-quality DNA from FFPE samples. The purified DNA is suitable for downstream applications, such as PCR, qPCR, and next-generation sequencing (NGS).

The workflow for purifying DNA from FFPE samples using the GeneRead DNA FFPE Kit involves sample preparation, deparaffinisation, lysis, cross-linked reversal, binding, washing, elution and finally quantification and quality assessment using fluorometer (Qubit dsDNA assay and 2.0 fluorometer).

This was undertaken by myself with assistance from a lab technician, Dr Gareth Marlow. Figure 33 broadly demonstrates the workflow and overall aim of the study to obtain purified DNA from FFPE samples and thereafter prepare this for next-generation sequencing using Illumina, for comprehensive gene panel testing (HiSeq) and targeted/small panel testing (MiSeq) to explore whether this is feasible to do to determine mutational signatures. The following will describe the steps involved in the above process.

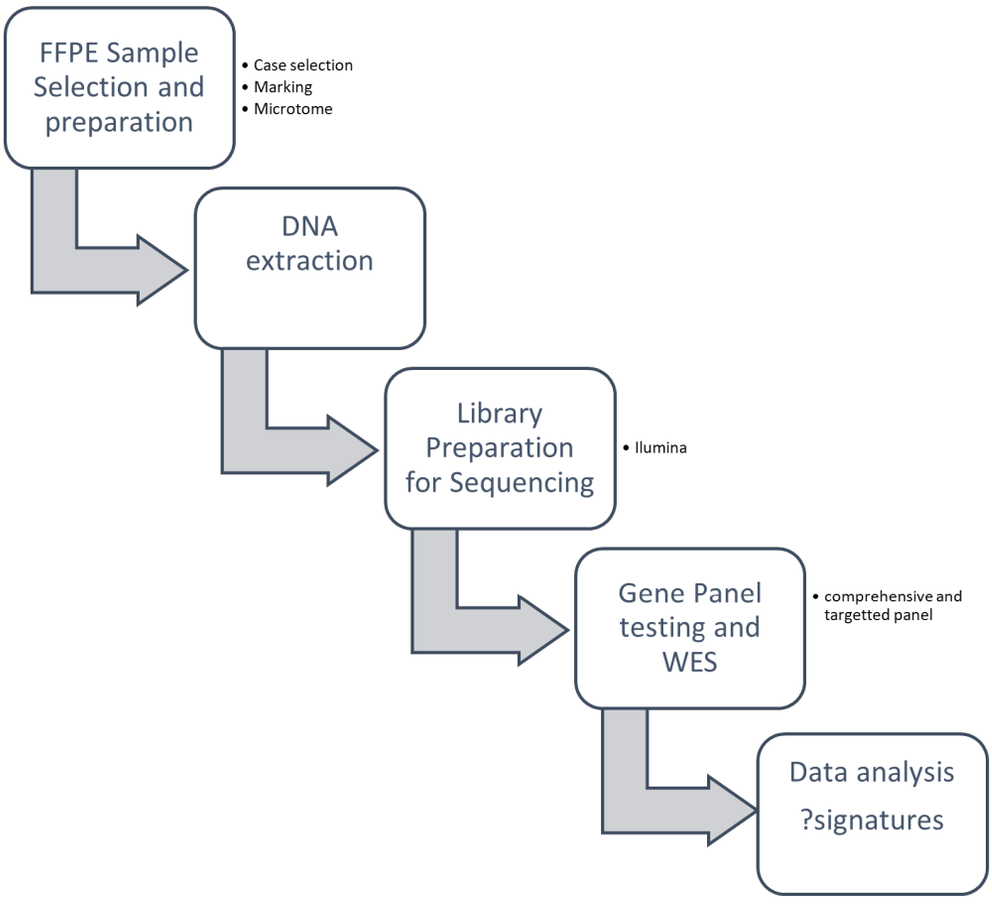


Figure 33: Workflow for FFPE DNA extraction and sequencing

4.2.17 Preparation of slides from FFPE Blocks

4.2.17.1FFPE Block sectioning:

This involved preparing fresh slides from FFPE blocks which was done by the team in the pathology Department which I observed.

At least five sections of 10 µm thickness was taken from each block. Each section was flattened by floating in warm water and then fixed to a microscope slide by air drying at room temperature overnight. The lab technician was unable to do thinner sections (5 µm) as the biopsy specimens were very small.

4.2.17.2Identification of abnormal areas on the FFPE sectioned slide:

The area of interest on each of the unstained slides was identified by overlaying with the stained section (Figure 32) that was photocopied.

4.2.17.3Macro dissection of tissue from FFPE sections:

A scalpel was used to dissect the abnormal areas on the slide. These scrapings were then collected in a clean micro centrifuge tube (1.5ml Eppendorf) and labelled.

4.2.18 DNA extraction, purification, and quantification from FFPE samples:

GeneRead DNA FFPE Kit (by QIAGEN) was used to extract and purify DNA from the FFPE samples.

The kit uses silica-based membrane technology in combination with optimized buffers and reagents to efficiently isolate high-quality DNA from FFPE samples. The purified DNA is suitable for downstream applications, such as PCR, qPCR, and next-generation sequencing (NGS).

The workflow for purifying DNA from FFPE samples using the GeneRead DNA FFPE Kit involves sample preparation, deparaffinisation, lysis, cross-linked reversal, binding, washing, elution and finally quantification and quality assessment using fluorometer (Qubit dsDNA assay and 2.0 fluorometer).

This was undertaken by myself with assistance from a lab technician, Dr Gareth Marlow.

4.2.18.1DNA extraction and purification:

Prior to commencing the experiments, the buffers were prepared (as per the protocol-appendix O) and equilibrated in advance at room temperature. The deparaffinization solution needed to be incubated at 30° in order to ensure liquidation.

To each of the micro centrifuge tube that contained FFPE tissue, 160 µl of deparaffinization solution was added and vortexed for 10 seconds and then centrifuged at 13,000 rpm for 1 minute in order to bring the sample to the bottom of the tube.

The samples were then incubated at 56°C in a water bath for 3 minutes and then allow to cool at room temperature. For those samples that still had a waxy appearance, more deparaffinization solution was added and the incubation continued at 56°C as described above.

To the samples then 55 µL of RNase-free water, 25 µL Buffer FTB, 20 µL proteinase K (a master mix comprising of the above was prepared in advance). This was briefly vortexed and centrifuged for five seconds. Then incubated at 56°C for 1 hour.

The samples then further incubated at 90°C for 1 hour. Thereafter to remove the droplets from inside the lid brief centrifugation at 13,000 rpm was done for five seconds.

Following this, the lower clear phase in the micro centrifuge tube is transferred into a new 1.5 ml micro centrifuge tube and 115 µL of RNase free water is added and mixed. To this 35 µL of UNG (uracil N-glycosylase which is stored at - 20°C) is added and briefly vortexed and incubated at 50°C for 1 hour in a thermomixer.

Following a brief centrifuge of the above sample, 2 µL RNase A is added and further incubation for two minutes at room temperature. 250 µl of buffer AL was added and mixed thoroughly by Vortex, 250 µL of ethanol (100%) was added and vortexed. This mixture was then centrifuged briefly.

Each sample (700 µL) was then transferred to a QIAamp MinElute column, placed inside a 2 mL collection tube and then centrifuged at maximum speed for 1 minute. The supernatant was discarded and the MinElute columns were placed in a clean 2 mL collection tubes. 500 µl of buffer AW 1 was added to the MinElute column and centrifuged at 6000 rpm for 1 minute.

The supernatant was then discarded and the MinElute columns placed inside a clean 2 mL collection tube and 500 µL of buffer AW2 was added to the column and centrifuged at 6000 rpm for one minute. The supernatant was discarded and to the collection tube 250 µL of 100% ethanol was added to the column and further centrifuged for one minute. The supernatant and the collection tube was then discarded.The MiniElute column was then placed in a clean 2 mL collection tube and centrifuged for 1 minute.

The QiAmp MinElute column was then placed in a clean 1.5 mL micro centrifuge tube discarding the collection tube that contained the supernatant. To the column, 22 µL of buffer ATE was then added to the centre of the membrane and incubated at room temperature for 5 minutes and this was then centrifuged at 14,000 rpm for 1 minute.

The volume of DNA obtained from the above process was 20 µL. From this 1 µL was used to quantify DNA using a fluorometer (Qubit 2.0) immediately and another 1 µL stored in a separate tube to quantify DNA again just prior to library preparation. Hence the final volume obtained and was 18 µL that were stored at -20°C.

4.2.18.2DNA quantification:

For quantification of DNA, prior to storage, the Qubit dsDNA HS (high-sensitivity) as a kits were used along with the Qubit 2.0 Fluorometer. The protocol (Figure 34) was as follows. 1 µL of the Qubit reagent and 199 µL of the Qubit Buffer were mixed to make the Qubit working solution.

Two volumes of the qubit working solution was prepared and 199 µL was mixed in 1 µL of the DNA sample and in another tube, 190 µL of the working solution was added to 10 µL of the standard solution from the kit as control.

The assay tubes were then vortexed for two seconds and incubated at room temperature for 2 minutes. The tube was then placed in the Qubit 2.0 fluorometer to obtain a DNA quantification reading.

For each sample, the polyp number, trial number, group, yield of DNA, total volume, size in millimetres square of the area removed from the slide, number of slides, thickness of the slide, protocol notes (including whether there was any deviation from protocol) was recorded in my laboratory work and then transcribed onto Excel (appendix P). Those samples that did not have enough DNA quantity (<0.5 ng/ µL) were not included for the following step i.e. library preparation.

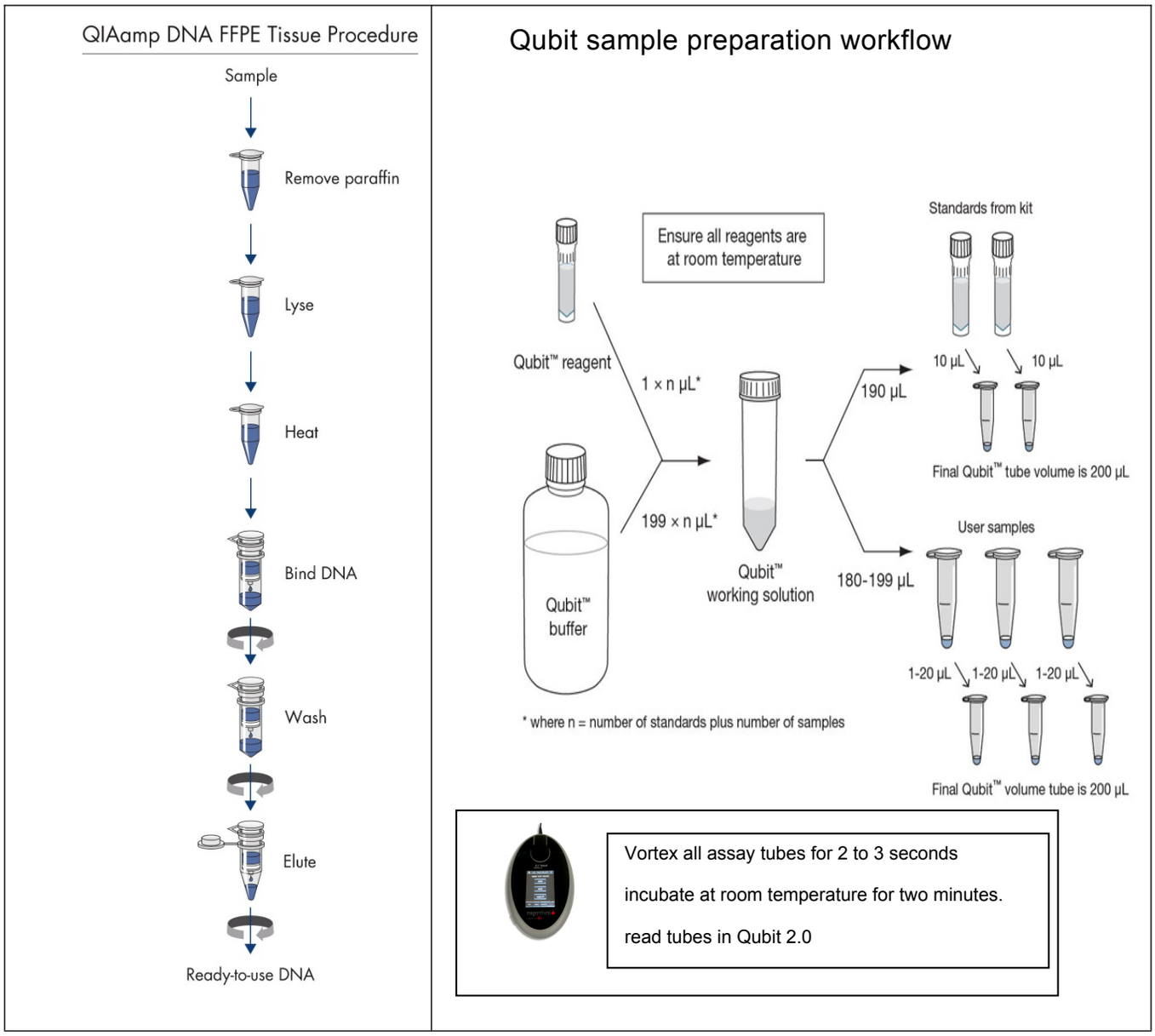


Figure 34: Workflow of purification of DNA from FFPE samples using gene read and quantification of DNA using Qubit assay.

4.2.19 DNA Library preparation for next-generation sequencing:

Following the extraction and purification of DNA, this would need to be converted into a sequencing library which involves several enzymatic and chemical steps. The objective of library construction is to achieve the highest coverage depth and ensure uniformity of DNA. The protocol that ensures low fragmentation and amplification bias offers the highest library yields.

Prior to this, targeted enrichment technology enables next-generation sequencing platform uses to sequence specific regions of interest instead of the entire genome, effectively increasing sequencing depth and throughput with low cost. Gene read DNA seq targeted panel V2 uses multiplex PCR-based targeted enrichment technology in combination with a sophisticated primer design algorithm to enable amplification and enrichment of any gene or targeted region in the human genome in order to detect genetic variations using NGS.

The protocol used for this study was GeneRead DNAseq targeted panel for targeted enrichment prior to next-generation sequencing (appendix Q).

This was conducted by Dr Gareth Marlow whilst I mainly observed and assisted when possible during the library preparation process.

The following steps were involved (Figure 35):

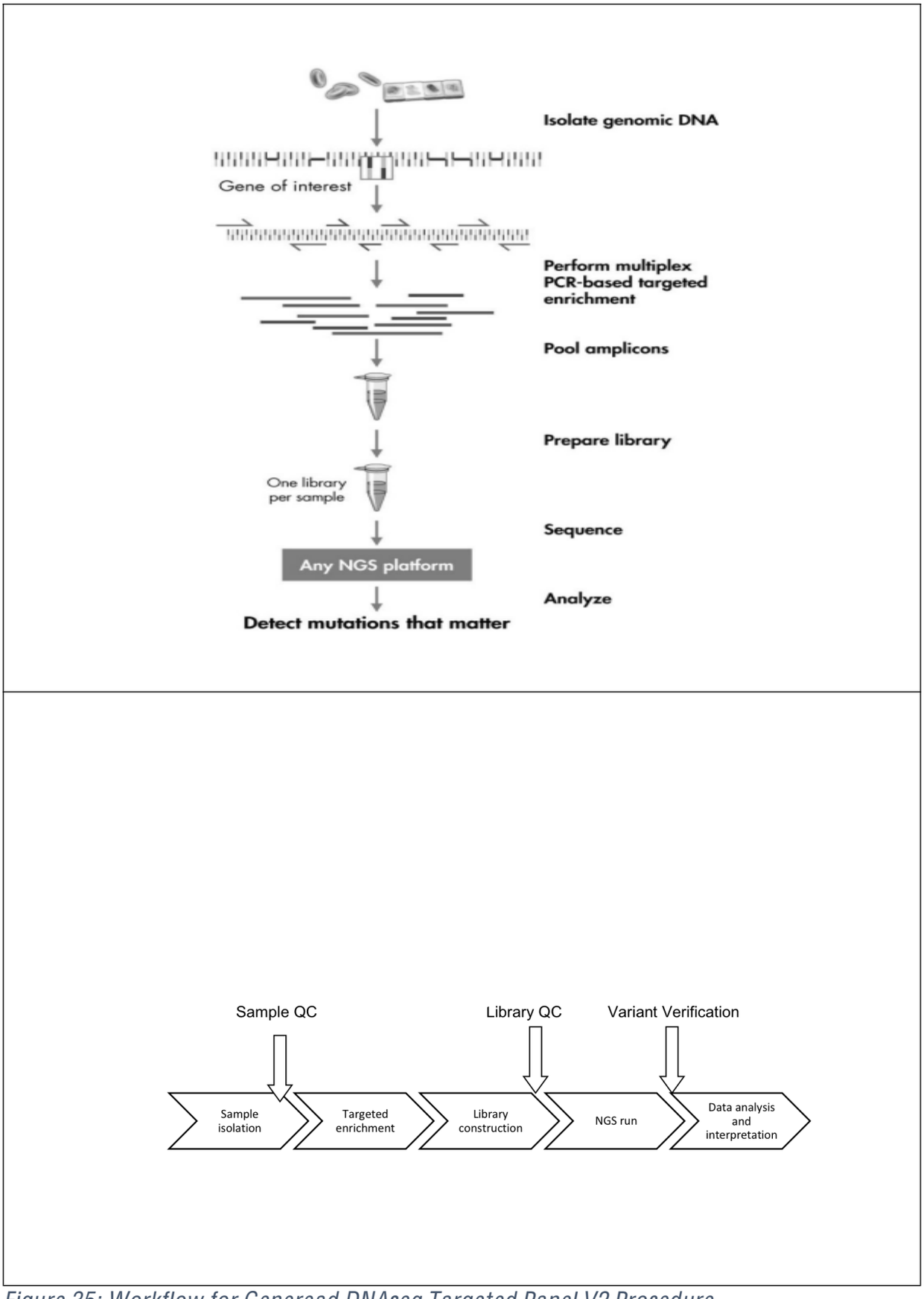


Figure 35: Workflow for Generead DNAseq Targeted Panel V2 Procedure

4.2.19.1Target enrichment:

This consists of two processes 1. PCR set up and 2.Sample pooling and purification

4.2.19.1.1PCR set up:

DNA from the sample was diluted to 5 ng/µL with DNase-free water in a tube after quantifying each DNA sample. A 4 pool panel was used for each sample. Each PCR plate contained 96 wells. Hence 24 samples could be used per plate. A PCR mix (Figure 36) was prepared for each primer (A, B, C, D) by the addition of the following volume per sample: GeneRead DNAseq panel PCR buffer (4.4 µL), primer mix pool (11 µL), GeneRead HotStar Taq DNA polymerase (1.5 µL) and DNase-free water (2.7 µL). Once the primer mix pool is prepared, aliquot 18 µL of the PCR primer mix into each of the wells and and 2 µL of DNA. Hence each available contained 20 µL volume equivalent to 10 ng/µL of DNA (Figure 37).

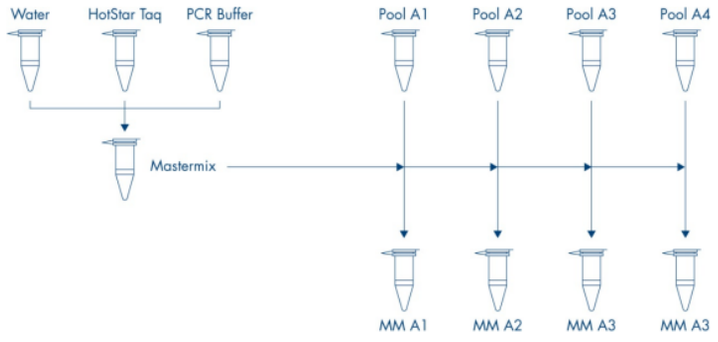


Figure 36: Preparation of PCR Master mix for each primer mix pool

MM = master mix

Following this the wells were sealed with PCR strips to prevent evaporation and thereafter placed in a thermocycler. The PCR program (Table 27) was then set by a preprogrammed cycle and allowed to run for 2.33 hours.

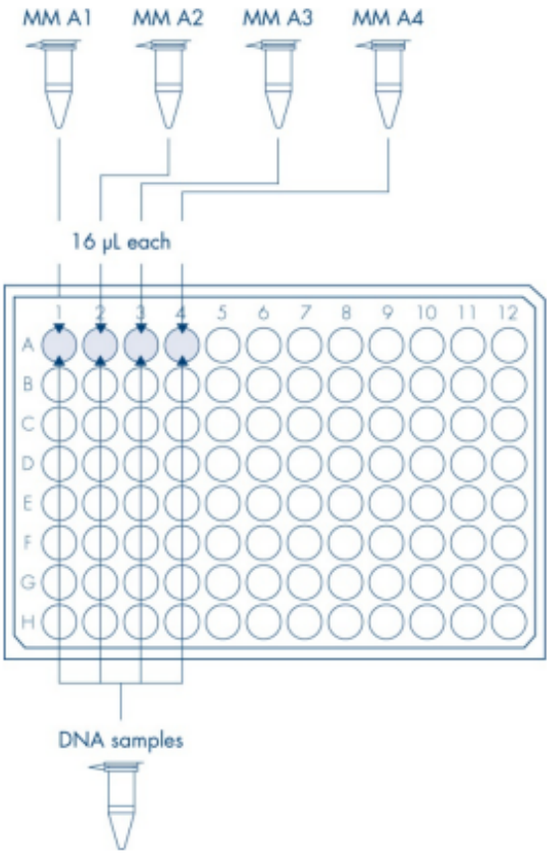


Figure 37: 4- pool panel

Note that unlike the diagram 18 µL master mix (MM) added and 2 µL of DNA.

Cycle	Temperature	Time
1	95°C	15 minutes
26	95°C 60°C	15 seconds 4 minutes
1	72°C	10 minutes
1	4°C	Till Completion

Table 27: PCR program

PCR- Polymerised Chain Reaction

4.2.19.1.2 Sample pooling and purification using AMPure XP beads:

The PCR plate was removed from the thermocycler and 40 µL from each sample was transferred to a 96 well PCR plate for purification (the remaining 40 µL from each sample was stored as extra at -20°C). 36 µL of AMPure XP beads were added to each sample and mixed well by pipetting. This was incubated at room temperature for five minutes.

Thereafter the PCR plate was placed on a magnetic rack for 5 minutes and the beads separated from the supernatant. 70 µL of the supernatant was then transferred to another plate and the beads are discarded.

Thereafter 64 µL of AMPure XP beads was added again to the supernatant and mixed well by pipetting and incubated for 5 minutes at room temperature. The plate was placed on a magnetic rack for 5 minutes and thereafter was carefully removed and the supernatant was discarded. The remaining beads are not disturbed as they contain the DNA target.

The beads were then washed by adding 200 µL of 80% ethanol to each well whilst on the magnetic track and was moved from side to side to mix and wash the beads. The supernatant was then carefully discarded. This process was repeated one more time. Thereafter ethanol was completely removed and the beads were dried for 15 minutes on the plate on the rack.

The DNA target beads were then eluted in 28 µL of nuclease free water which was mixed well by pipetting. The plate is placed on the magnetic rack until the solution was clear.

Thereafter 25 µL of supernatant was transferred to a clean PCR plate. The samples were checked for DNA quantity by Qubit fluorometer technique. The aim of quantification is to ensure that there is at least 10 to 200 ng PCR enriched DNA present as any quantity that is less than 10 or over 200 ng decreases the efficiency of the library construction. This was then stored in a freezer at -20°C prior to library construction. (pre and post lib qubit excel) Appendix P

4.2.19.2Library construction:

For library construction the following steps were followed as per the protocol (appendix Q):

4.2.19.2.1End repair of DNA:

A 25 µL (per sample volume) reaction mix consisting of 20.5 µL of PCR enriched DNA, 2.5 µL of end repair buffer and 2 µL of end repair enzyme mix was prepared for all the samples. This 25 µL reaction mix was added to each sample on the PCR plate and thoroughly mixed by pipetting up and down several times. This was then incubated in a thermocycler for 30 minutes at 25°C followed by 20 minutes at 75°C. The plate was pulse spun and returned to ice.

4.2.19.2.2A-addition:

31 µL of the reaction mix for capital a-addition was prepared by the addition of 25 µL of end-repaired DNA (from the previous step), 3 µL of A-addition buffer and 3 µL of Klenow fragment (3’-5’ exo-).

This was mixed by pipetting up-and-down several times and then incubated in a thermocycler for 30 minutes at 37°C followed by 10 minutes at 75°C.

4.2.19.2.3 Adapter ligation:

The 90 µL of reaction mix for the adapter ligation was prepared by mixing 31 µL of DNA from the previous step, 45 µL of ligation buffer, 5 µL of the adapter, 4 µL of T4 DNA Ligase and 5 µL of DNase-free water. The reaction mix was then mixed thoroughly by pipetting up-and-down and then incubated in a thermocycler at 25° for 10 minutes.

4.2.19.2.4 Clean-up of adapter ligated DNA with AM Pure XP beads:

To the 90 µL DNA solution on the PCR plate, 108 µL of AMPure XP beads was added and mixed well by pipetting and incubated for five minutes at room temperature. This was then placed in a magnetic rack to separate the beads from the supernatant for 10 minutes. The supernatant was then discarded. To the beads, 200 µL of freshly made 80% ethanol was added whilst on the magnetic rack and then mixed by moving the plate from side to side. The supernatant was then discarded and this step was repeated again.

Following the removal of ethanol, the beads were dried for 10 minutes whilst on the rack. The DNA target beads was then eluted in 19 µL of nuclease-free water and mixed well by pipetting. 17 µL of supernatant was then transferred to a clean PCR plate for the step of PCR amplification.

4.2.19.2.5 Amplification of purified library:

The 50 µL reaction mix for PCR amplification was made by mixing the following components-PCR Master mix 25 µL, Primer mix 1.5 µL, RNase-free water 6.5 µL and library DNA 17 µL in the PCR plate. This is then set up in the cycler for amplification of the DNA library.

The cycling conditions are as follows which was preset on the thermocycler (A). Initial denaturation at 90°C for 2 minutes, B) 5 cycles of 90°C at 20 seconds, 60°C at 30 seconds, 72°C and 30 seconds C). 1 cycle at 72° for one minute and D). To hold for 4°C till completion on the thermocycler. Thereafter cleanup process occurs by the following.

4.2.19.2.6 Clean-up of amplified library with AM Pure XP beads:

40 µL of AMPure XP beads was added to 50 µL of PCR solution and mixed and incubated for 5 minutes at room temperature. This was then placed in a magnetic rack and the supernatant discarded.

86 µL of the supernatant was then transferred to the plate without disturbing the beads and beads are then discarded as they contain unwanted large DNA fragments.

20 µL of AMPure XP beads was added to the supernatant and mixed well and incubated for five minutes at room temperature. This was then placed on the magnetic rack and allow to rest for five minutes till the solution was clear. The beads contained the DNA target and hence not discarded. 200 µL of fresh 80% ethanol was then added to each well and carefully rotated and placed in the magnet. The supernatant was then discarded and the step was repeated again. Following drying the beads for 10 minutes, 30 µL of nuclease-free water was then added and placed in the magnetic rack till clear. 28 µL of supernatant was then transferred to a PCR tube. The library was stored at -20°C prior to performing quantity and quality check using Qubit 2.0 and agilent bioanalyser.

4.2.19.2.7 Library quality control (QC):

The DNA quantity was checked using Qubit 2.0 fluorometer pre and post library construction. The bio analyser was used to validate the fragment size and concentration. A peak (Figure 38) around 280bp would be aimed for being adequately prepared for sequencing. If the peak was <280bp then this would represent adapter dimers which would then require further cleanup to remove this. Hence a pre-and post cleanup check was done prior to sequencing.

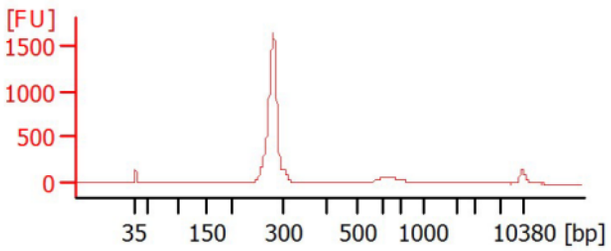


Figure 38:Sample Agilent bioanalyzer image of a MiSeq sequencer library for illumina. A peak of around 280 bp is observed.

4.2.19.3NGS Run:

This will be discussed into sections-as part of targeted and comprehensive gene panel testing using MiSeq and HiSeq illumina platform done with the help of Dr Gareth Marlow. And in a separate section on whole Exome sequencing which was performed by the Wales gene Park team.

4.2.19.3.1Targeted and Comprehensive gene panel:

Following library construction, this was then run on illumina platform for MiSeq initially and then HiSeq. This was performed and analysed by Dr Gareth Marlow and I was not present during this process.

The library preparation for the targeted small panel and the comprehensive panel was completed and attempted sequencing through MiSeq was performed and the data obtained put through the Mutagene programme to look at mutational signatures. Ensembl Variant Effect Predictor (VEP) programme was also used to determine the effect of variants (SNP, insertions, deletions,CNV or structural variants) on genes. The percentage coverage per targeted gene was recorded along with a preliminary report generated for possible mutational signatures using the MutaGene programme.

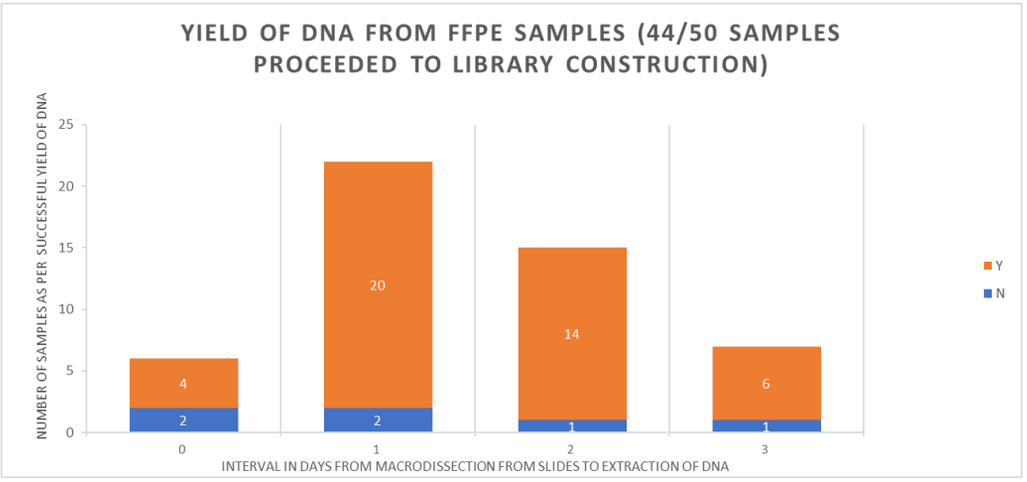
Panel	Targeted genes
Targeted 24 gene Panel (MiSeq)	ALK, RET, PDGFRA, MAP2K1, IDH1, IDH2, PTEN, BRAF, EGFR, AR, NRAS, STK11, PIK3CA, GNA11, GNAQ, ERBB2, KIT, AKT1, CTNNB1, KRAS, FGFR3, MET, TP53, DDR2
HiSeq- Comprehensive Panel	experiment failed

4.2.19.3.2Data analysis

Data analysis was done by uploading the files that were obtained from sequencing onto a mutagene program and somatic signatures were referenced to the known COSMIC curated mutational signatures. This was performed by Dr Gareth Marlow and subsequently reviewed by Bioinformatician Dr Kevin Ashelford.

4.3 Results:

1. DNA purification and quantification from FFPE blocks: DNA was extracted from 50 FFPE blocks using the methodology described above. In 44/50 samples, a sufficient yield of DNA (>0.5 ng/μL) was obtained. The table below summarises this.



There was no significant deviation from the protocol. The average time taken from the macrodissection of DNA from FFPE slides to DNA extraction was 1.46 days (range 0-3). All sections were taken at a 10 µm thickness. The average area of DNA obtained from the paraffin-embedded slide was 33.07 mm² (9-160). In those samples that did not have a successful adequate DNA yield (N = 6), the average interval from macro dissection to extraction was 1.16 days and average area on the slide from where DNA was obtained was 21.8 mm².

2. Library Construction and QC:

The table in appendix p summarises the DNA quantification obtained following library construction and bioanalyser info for MiSeq and the demonstration of adequate fragment size and concentration. An example of pre-and post cleanup during bioanalyser process has been demonstrated in Figure 39.

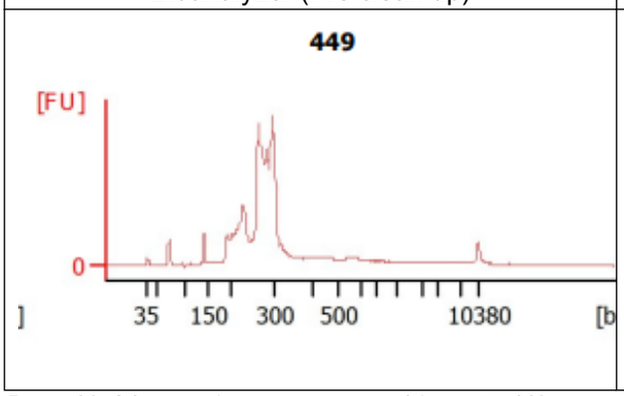
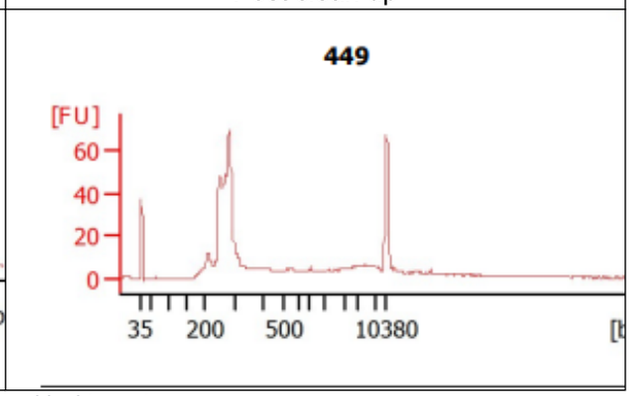
Bioanalyzer (Pre clean up)	Post clean up
	

Figure 39: Adequate fragment size noted for polyp 449 measured by bioanalyser.

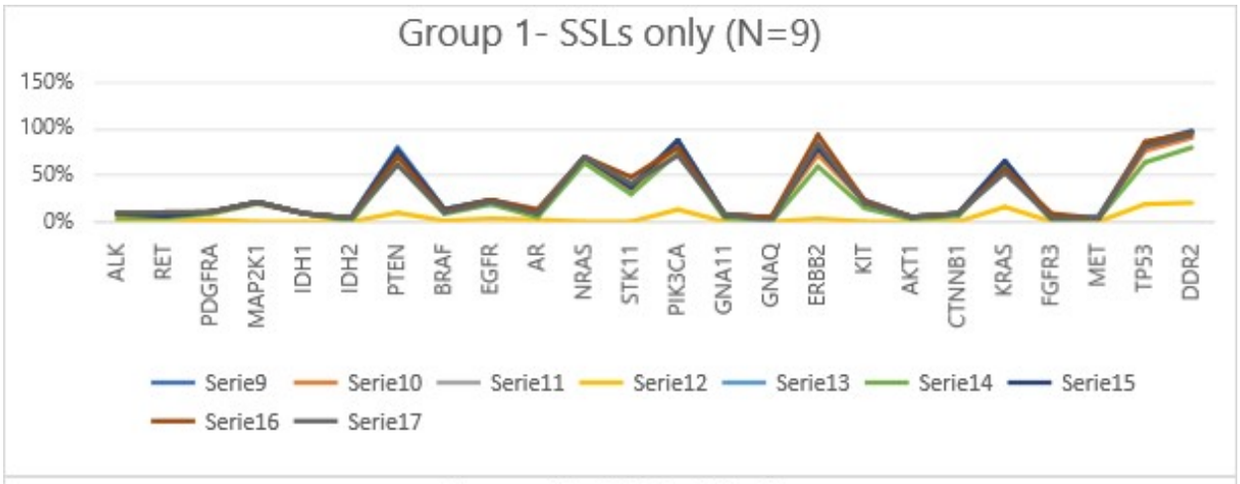
There was no deviation in the protocol used for library construction. The 44 samples underwent bioanalyser review and all samples had adequate fragment size and quantity required for further NGS run.

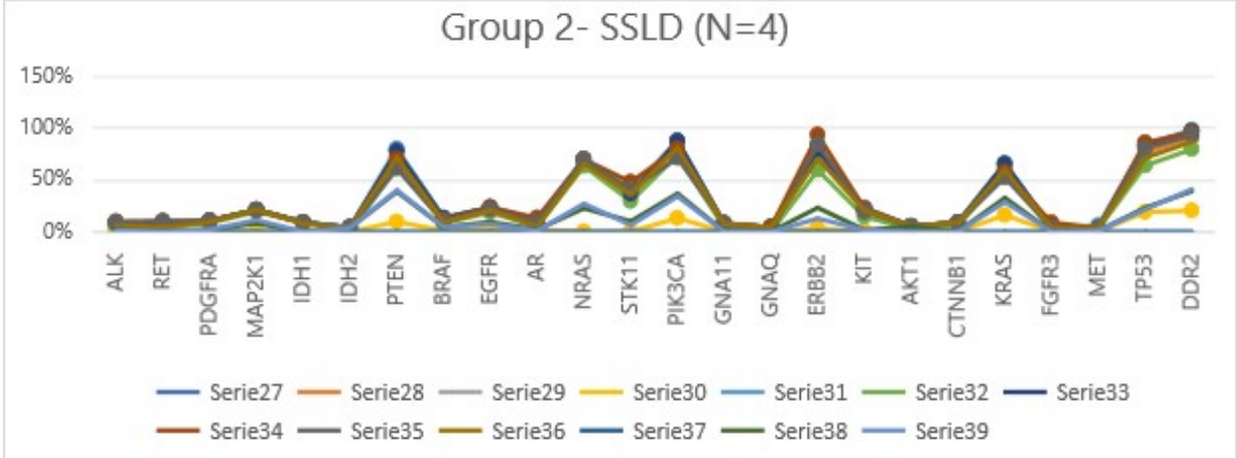
3. NGS run : the 44 samples were then prepared for MiSeq run of the targeted small panel and the comprehensive gene panel. The library preparation involved ensuring concentration of DNA as per protocol (final library concentration of between 6-9 ng/μL), QC prior to the run and use of primers. This was successful with some preliminary data obtained from the MiSeq run.

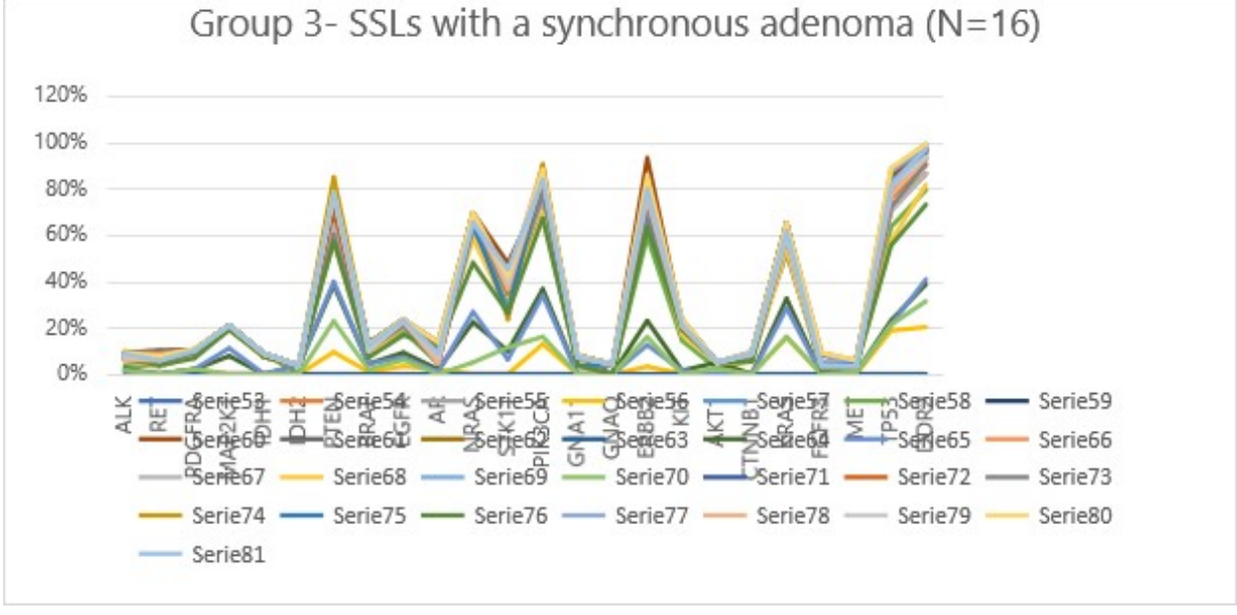
   The samples were put through a sequencing QC run on a MiSeq nano which is the usual process to check on performance. However the samples failed to pass this process and hence HiSeq was unable to be performed for the comprehensive panel.

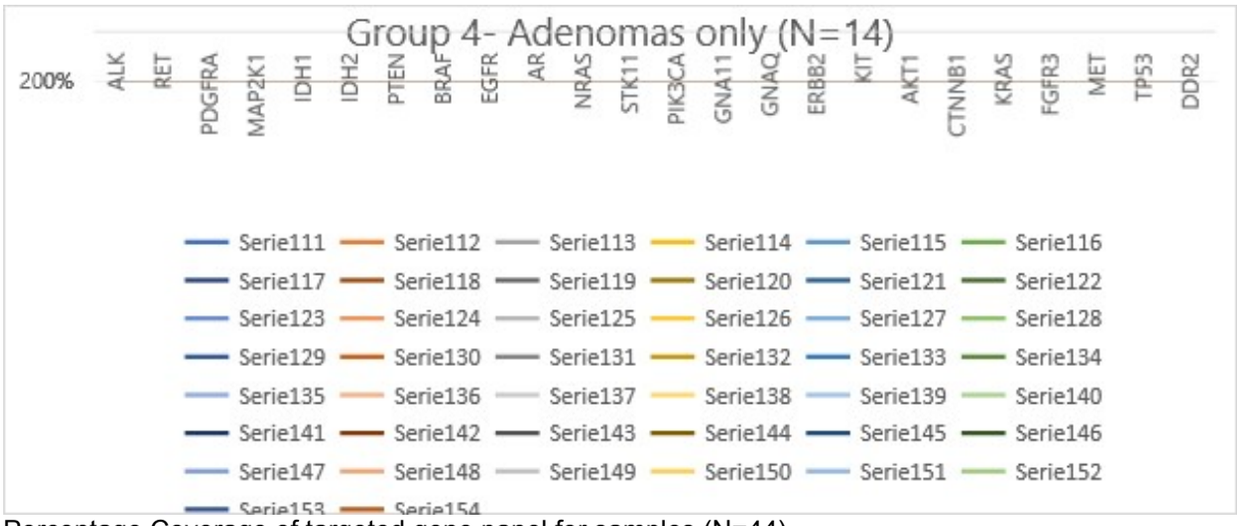
   The preliminary results of the targeted small panel MiSeq run revealed that there was adequate percentage coverage in all samples except in two (sample 72, 491) and there were mutational signatures demonstrated when this was put through the online Mutagene programme.

   The percentage coverage for the small panel was as follows-


Group 1- SSLs only (N=9)
150%
100%
50%
0%
ALK RET PDGFRA MAP2K1 IDH1 IDH2 PTEN BRAF EGFR AR NRAS STK11 PIK3CA GNA11 GNAQ ERBB2 KIT AKT1 CTNNB1 KRAS FGFR3 MET TP53 DDR2
Serie9 Serie10 Serie11 Serie12 Serie13 Serie14 Serie15
Serie16 Serie17


Group 2- SSLD (N=4)
150%
100%
50%
0%
ALK RET PDGFRA MAP2K1 IDH1 IDH2 PTEN BRAF EGFR AR NRAS STK11 PIK3CA GNA11 GNAQ ERBB2 KIT AKT1 CTNNB1 KRAS FGFR3 MET TP53 DDR2
Serie27 Serie28 Serie29 Serie30 Serie31 Serie32 Serie33
Serie34 Serie35 Serie36 Serie37 Serie38 Serie39


Group 3- SSLs with a synchronous adenoma (N=16)
120%
100%
80%
60%
40%
20%
0%
Serie53 Serie54 Serie55 Serie56 Serie57 Serie58 Serie59
Serie60 Serie61 Serie62 Serie63 Serie64 Serie65 Serie66
Serie67 Serie68 Serie69 Serie70 Serie71 Serie72 Serie73
Serie74 Serie75 Serie76 Serie77 Serie78 Serie79 Serie80
Serie81



Percentage Coverage of targeted gene panel for samples (N=44)

Group	Gender	Smoking	Sample	Mutational Signatures												
1	M	Ex Smoker	**55**	3	20	12	28				23			7		
1	F	Ex Smoker	**85**	3	20	12	28		26		23					
1	F	Never Smoked	**103**	3	20	12	28									
1	F	Never Smoked	**168**		20	12	28	1								
1	F	Never Smoked	**169**	3	20	12	28	1								
1	M	Ex Smoker	**217**	3	20	12	28	1		15						
1	M	Ex Smoker	**374**	3	20	12				15						
1	M	Ex Smoker	**505**	3	20	12			26							
1	M	Ex Smoker	**506**	3	20				26							
2	M	Never Smoked	**71**	3	20	12	28	1								16
2	M	Never Smoked	**72**								23					
2	M	Never Smoked	**393**		20	12	28					24		5		
2	M	Current smoker	**654**	3	20	12	28	1		15				5		
3	F	Current smoker	**17**	3	20	12		1								
3	F	Never Smoked	**128**	3	20	12	28	1				24				
3	F	Never Smoked	**128.1**	3	20	12	28	1				24				
3	F	Current smoker	**151**	3	20	12		1								
3	M	Current smoker	**214**	3	20		28		26			24				
3	M	Current smoker	**399**	3	20	12				15						
3	M	Current smoker	**400**	3	20	12	28									
3	M	Ex Smoker	**447**	3	20	12	28	1								
3	M	Ex Smoker	**449**	3	20	12	28	1								
3	M	Ex Smoker	**450**	3	20	12		1								
3	F	Ex Smoker	**624**	3	20	12	28	1				24				
3	F	Ex Smoker	**625**	3	20	12	28	1				24				
3	M	Ex Smoker	**673**	3	20			1	26	15						

3	M	Ex Smoker	**677**	3	20	12		1	26	15	23					
3	M	Current smoker	**708**	3	20	12						24				
3	M	Ex Smoker	**715**	3	20	12	28	1		15		24				
4	F	Ex Smoker	**172**	3	20	12		1		15						
4	M	Never Smoked	**247**	3	20	12	28		26		23		29			
4	M	Ex Smoker	**251**	3	20	12		1								
4	F	Current smoker	**264**	3	20	12	28			15	23					
4	M	Ex Smoker	**329**	3	20	12	28		26			24				
4	M	Ex Smoker	**336**	3	20	12	28	1							6	
4	F	Ex Smoker	**409**	3	20	12	28	1								
4	M	Ex Smoker	**435**		20		28		26				29			
4	M	Ex Smoker	**462**	3	20	12	28	1								
4	M	Current smoker	**491**				28	1		15						
4	M	Never Smoked	**493**	3	20	12	28									
4	F	Never Smoked	**503**	3	20	12	28	1	26				29			
4	M	Ex Smoker	**517**	3	20	12	28	1								
4	F	Never Smoked	**634**	3	20	12	28	1								
4	M	Current smoker	**686**	3	20	12			26	15						

Signature	Aetiology	Mutational Features
1	endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine	associated with small numbers of small insertions and deletions in most tissue types.
3	associated with failure of DNA double-strand break-repair by homologous recombination.	associates strongly with elevated numbers of large (longer than 3bp) insertions and deletions with overlapping microhomology at breakpoint junctions

7	Based on its prevalence in ultraviolet exposed areas and the similarity of the mutational pattern to that observed in experimental systems exposed to ultraviolet light Signature 7 is likely due to ultraviolet light exposure	associated with large numbers of CC>TT dinucleotide mutations at dipyrimidines. Additionally, Signature 7 exhibits a strong transcriptional strand-bias indicating that mutations occur at pyrimidines (viz., by formation of pyrimidine-pyrimidine photo dimers) and these mutations are repaired by transcription-coupled nucleotide excision repair.
12	The aetiology of Signature 12 remains unknown	exhibits a strong transcriptional strand-bias for T>C substitutions.
15	associated with defective DNA mismatch repair.	associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.
20	believed to be associated with defective DNA mismatch repair	is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.
23	The aetiology remains unknown	exhibits very strong transcriptional strand bias for C>T mutations
24	has been found in cancer samples with known exposures to aflatoxin. Additionally, the pattern of mutations exhibited by the signature is consistent with that previous observed in experimental systems exposed to aflatoxin.	exhibits a very strong transcriptional strand bias for C>A mutations indicating guanine damage that is being repaired by transcription-coupled nucleotide excision repair.
26	believed to be associated with defective DNA mismatch repair	is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.
29	The aetiology of Signature 28 remains unknown	exhibits transcriptional strand bias for C>A mutations indicating guanine damage that is most likely repaired by transcription-coupled nucleotide excision repair. Signature 29 is also associated with CC>AA dinucleotide substitutions.
5	Signature 29 has been found in cancer samples from individuals with a tobacco chewing habit.	exhibits transcriptional strand bias for T>C substitutions at ApTpN context.
6	The aetiology of Signature 5 is unknown	associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.
16	Signature 6 is associated with defective DNA mismatch repair and is found in microsatellite unstable tumours.	exhibits an extremely strong transcriptional strand bias for T>C mutations at ApTpN context, with T>C mutations occurring almost exclusively on the transcribed strand.
	The aetiology of Signature 16 remains unknown	

However when this was further scrutinised by Dr Kevin Ashelford, bioinformatician it was thought that mutational signatures that were provided in the Mutagene Profile was unreliable.

His opinion based on the limited information that was provided for further analysis was as follows.

The data was processed, filtered variant calls from 44 samples. Each VCF file contained, on average, 73 somatic calls (min 2, max, 102). Due to filtering of the data it was not possible to assess the level of background noise. The variants have been called with the Somatic Variant Caller supplied with the MiSeq Reporter software and have been annotated with the Ensembl Variant Effect Predictor. This means each variant has been labelled in a range of ways that could be used to assess potential pathogenic impact. A brief exploration of the variants suggest that they may be a plausible calls however are too few in number to determine mutational signatures.

It was also thought that the mutational signatures that were determined by the MutaGene tool were not reliable with a few errors. For example, the allocation of ‘Signature 23’ to sample 72 is based on two SNVs alone. (Signature 23 exhibits high transcriptional strand bias for C>T mutations; the two SNVs from sample 72 are both C>T. This was thought to be a poor justification for allocation of a mutational signature.

The variants provided appear plausible but are far too few in number to determine mutational signatures. The variants have been filtered heavily and so lack information that could be used to assess FFPE-derived background noise. In addition to this the signatures provided through the Mutagene tool were unreliable.

Hence it was decided to process the remaining samples for WES performed by the next-generation sequencing laboratory (HiSeq) in the Wales gene Park. Bioinformatics was done by Dr Kevin Ashelford.

4.4 Whole Exome Sequencing:

Whole Exome Sequencing (WES) on 18 samples was performed by the Wales Gene Park in October 2019 for the CONSCOP study.

Bioinformatic Analysis Following Whole Exome Sequencing Bioinformatic analysis was performed by Dr. Kevin Ashelford (WGP).

The remaining 44 samples where DNA had been purified from FFPE samples was further subjective quantification. In order to commence the Exome procedure 100 ng of each DNA sample was diluted into 50 µL. The requirement for each preparation was 100 ng. Hence only 18/44 samples met the above criteria for further tests.

The TruSeq DNA exome protocol was used. It was noted that the concentrations were lower than normally needed and the libraries were queued for a sequencing QC run on a MiSeq nano which is the usual process that precedes in order to check the samples are performing properly. The samples that passed this were then queued for main sequencing run.

4.4.1 Methodology

Briefly, 50 ng of gDNA was used as the input template. Sequencing libraries were prepared using the Illumina Nextera Rapid Capture Enrichment kit. Subsequent steps included tagmentation of the gDNA, clean-up of the tagmented DNA, amplification of DNA, clean-up of amplified DNA, hybridisation of probes, capture of the hybridised probes, second hybridisation of probes, second capture, clean-up of the captured library, amplification of enriched library, clean-up of the enriched library, validation of the complete library.

The manufacturer's instructions were largely followed with extra quantitation steps prior to the hybridisation of the probes to ensure that close to 50 ng of each sample was pooled. The libraries were validated using the Agilent 2100 Bioanalyser and a high-sensitivity kit (Agilent Technologies) to ascertain the insert size, and the Qubit 2.0 Fluorometer was used for quantitation. Following validation, the libraries were normalised to 4 nM, pooled together and clustered on the cBot™2 following the manufacturer's recommendations.

The pool was sequenced using a 75-base paired-end (2x75bp PE) dual index read format on the Illumina HiSeq 2500 in highoutput mode according to the manufacturer's instructions.

4.4.1.1 Quality control

Sequencing quality was high: across the eighteen samples. Between 94.6 and 98.6% of reads had an average per base quality score of 30 or greater.

Mapping quality was consistently high across all samples. Between 98.8 and 99.9 % of reads mapped to the hg38 human reference.

Read duplication was low, and within acceptable limits, with between 12.8 and 18.7 % of mapped reads redundant (mean, 15.4 %).

Median insert sizes for paired-end reads were appropriate with inserts ranging from 136 to 160 bp (mean, 145 bp).

Overall, the quality of all sequencing was good and suitable for further analysis.

4.4.1.2 Pre-processing

Reads were trimmed for adapter sequence and low quality reads using Trimmomatic version 0.39.

4.4.1.3 Mapping

Reads were mapped to human reference sequence Grch38/Hg38, standard version as provided by the GATK resource bundle.

Mapping was performed with BWA software, version 0.7.17-r1198-dirty, using the MEM algorithm and default settings with secondary hits marked (-M flag). Mapping output was converted into sorted index BAM files using samtools version 1.9.

4.4.1.4 Post-processing

Following best-practice guidelines, base Quality Score Recalibration (BQSR) was performed on all samples with the aid of the Genome Analysis Toolkit (GATK, version 4.1.3.0), using tools BaseRecalibrator, ApplyBQSR, and AnalyzeCoveriates, to remove likely systematic errors in base quality scores.

Duplicate reads were marked using the MarkDuplicates tool from the Picard toolkit (version 2.20.5).

4.4.1.5 Variant discovery — identification of somatic variants

The Mutect2 tool, part of GATK 4.1.3.0, was used to identify putative somatic variants in the absence of matched normal samples as per GATK best-practice guidelines (https://gatkforums.broadinstitute.org/gatk/discussion/24057/how-to-call-somatic-mutations-using-gatk4-mutect2#latest).

In the absence of matched normal data, Mutect2 was run as per instructions, with genome aggregation database data, as provided by the GATK resource bundle, used as a suitable germline resource.

GATK tools LearnReadOrientationModel, GetPileupSummaries, CalculateContamination, and FilterMutectCalls were applied as per instructions to produce final variant calls, in VCF format, annotated for variant quality.

Resultant VCF files were then filtered to produce final variant files containing only SNVs that passed all Mutect filters and located on canonical human chromosomes chr1-22, X, Y, and M.

The samples varied greatly in the number of putative somatic mutations detected, ranging from a low of 40,618 (sample 493) and a high of 182,688 (sample 447). On average, 75,934 somatic mutations were detected per sample (Table 28).

4.4.1.6 Inputs

Eighteen samples were provided (Table 28). The samples were categorised into one of three smoking groups:

1. Smoker (n = 7)
2. Never smoked (n = 3)
3. Ex-smoker (n = 8)

The samples were sequenced on the Illumina NovaSeq platform. Sufficient sequence data were generated with between 35.5 to 73.7 million 2 x 150 bp paired-end reads produced (mean, 53.9 million).

Table 28: Samples processed, categorised by smoking status, with raw DNA yield from sequencing specified.

Wales Gene Park sample ID	Investigators' sample ID	Smoking status	Total number of paired-end reads generated (x 10^6)	Total yield (Mb)
E045-H-001	634	Never smoked	46.4	14,016.4
E045-H-002	393	Never smoked	42.0	12,687.1
E045-H-003	654	Smoker	63.9	19,300.1
E045-H-004	17	Smoker	64.5	19,475.8
E045-H-005	151	Smoker	56.7	17,125.0
E045-H-006	399	Smoker	66.8	20,172.0
E045-H-007	400	Smoker	61.3	18,514.7
E045-H-008	447	Ex-smoker	51.6	15,596.1
E045-H-009	450	Ex-smoker	73.7	22,272.2
E045-H-010	625	Ex-smoker	57.0	17,210.5
E045-H-011	708	Smoker	55.9	16,871.4
E045-H-012	715	Ex-smoker	46.6	14,082.3
E045-H-013	172	Ex-smoker	39.7	11,988.0
E045-H-014	435	Ex-smoker	55.1	16,633.9
E045-H-015	462	Ex-smoker	57.5	17,367.8
E045-H-016	491	Smoker	35.5	10,726.6
E045-H-017	493	Never smoked	54.9	16,591.2
E045-H-018	517	Ex-smoker	40.9	12,353.7
Mean			53.9	16,276.9

Table 29: Outcome of Mutect2 analysis.

Wales Gene Park sample ID	Investigators' sample ID	Smoking status	Raw variant count	Raw SNV count	PASS SNV count — putative somatic mutations
E045-H-001	634	Never smoked	206,822.00	137,701.00	50,141
E045-H-002	393	Never smoked	197,042.00	130,555.00	43,020
E045-H-003	654	Smoker	378,006.00	252,200.00	86,599
E045-H-004	17	Smoker	406,723.00	270,560.00	87,036
E045-H-005	151	Smoker	415,805.00	274,224.00	81,182
E045-H-006	399	Smoker	519,321.00	342,895.00	105,059
E045-H-007	400	Smoker	511,407.00	341,614.00	112,736
E045-H-008	447	Ex-smoker	1,224,206.00	798,682.00	182,668
E045-H-009	450	Ex-smoker	389,573.00	258,774.00	88,471
E045-H-010	625	Ex-smoker	390,016.00	262,702.00	95,705
E045-H-011	708	Smoker	303,528.00	199,217.00	61,670
E045-H-012	715	Ex-smoker	392,053.00	248,083.00	58,404
E045-H-013	172	Ex-smoker	259,195.00	168,970.00	49,557
E045-H-014	435	Ex-smoker	187,671.00	124,235.00	44,283
E045-H-015	462	Ex-smoker	350,394.00	231,408.00	74,174
E045-H-016	491	Smoker	248,552.00	163,149.00	50,452
E045-H-017	493	Never smoked	187,925.00	121,715.00	40,618
E045-H-018	517	Ex-smoker	230,141.00	154,036.00	55,038

4.4.1.7 Mutational signatures inferred.

Mutational signatures were inferred from the filtered VCF files using the SomaticSignatures package (version 2.20.0) running on R version 3.6.1.

The samples were processed (i) grouped according to the smoking status of patient, and (ii) separately. In each case, mutational spectrum was calculated and then mutational signatures inferred using both non-negative matrix factorisation (NMF) and principal component analysis (PCA).

4.4.2 Results and discussion

4.4.2.1 A. Inferring mutational signatures by group

In this section we considered the eighteen samples in terms of the three 'smoking status' groups they represent: 'never-smoker', 'smoker', and 'ex-smoker'. This was a two stage process in which the first stage determined the frequency of all possible trinucleotide mutational motifs (the so- called mutational spectrum). During the second stage, the mutational spectrum was decomposed mathematically to infer possible mutational signatures.

4.4.2.1.1 Stage 1. Determine mutational spectrum.

First, for each group, the frequency of all 96 possible trinucleotide motifs was determined, thereby producing a matrix of of motifs, which were sometimes called the catalog matrix, *C*. The catalog matrix defines the mutational spectrum of the samples, grouped by smoking status, and can be visualised as a bar chart as shown in Figure 40.

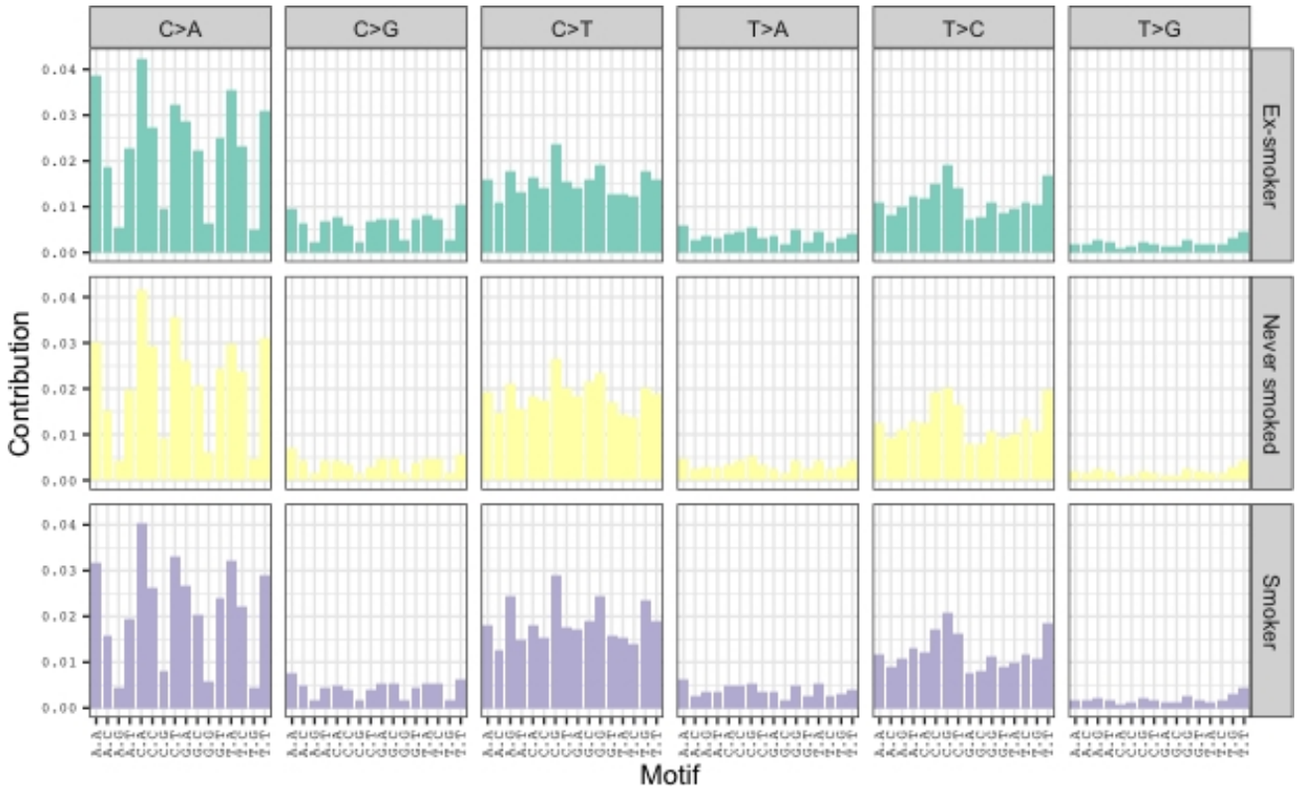


Figure 40:Observed motif frequency for each of the three patient groups, collectively referred to as the mutational spectrum of the data.

The mutational spectrum provided a first impression of the data and revealed little variation among the three groups. It was thought that if there was any difference in the somatic profile among the three groups then it was too subtle to be interpreted by visualisation of the graphs. Hence in order to determine if there was any difference could be detected, hierarchical clustering of the mutational spectrum was performed. This is demonstrated in Figure 41.

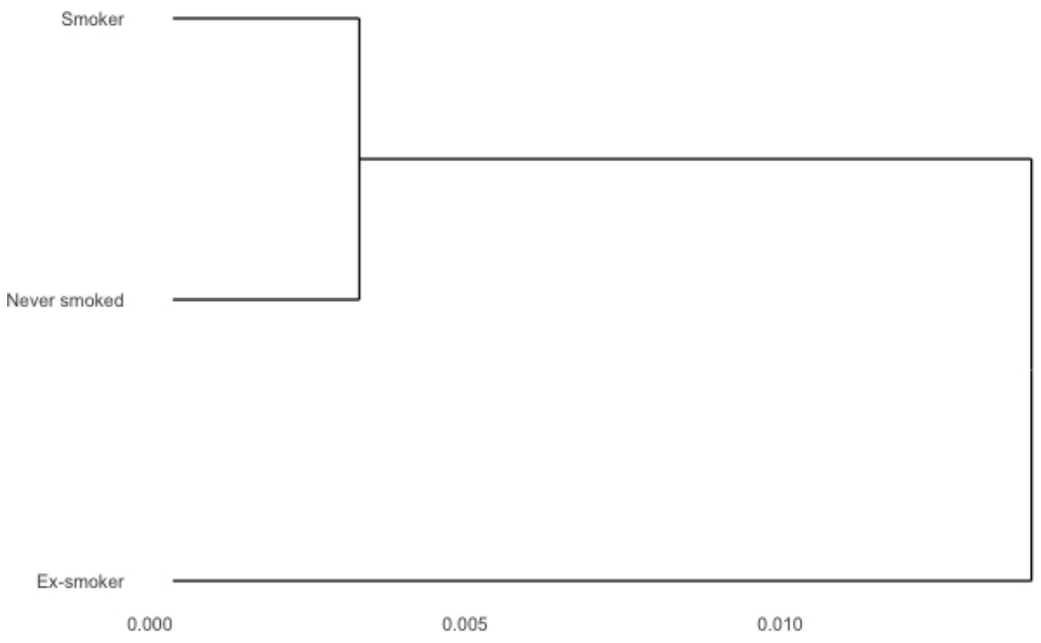


Figure 41: Hierarchical clustering of the mutational spectrum, according to motif.

Hierarchical clustering Figure 41, visually reveal the differentiation between the three groups with ex-smokers standing out more from the other two groups.However the Euclidean distances were not large, reflecting the bar chart results. Hence the stage was to mathematically decompose these frequencies in order to infer possible mutational signatures that might better reveal differences among the three groups.

4.4.2.1.2 Stage 2: Identifying mutational signatures.

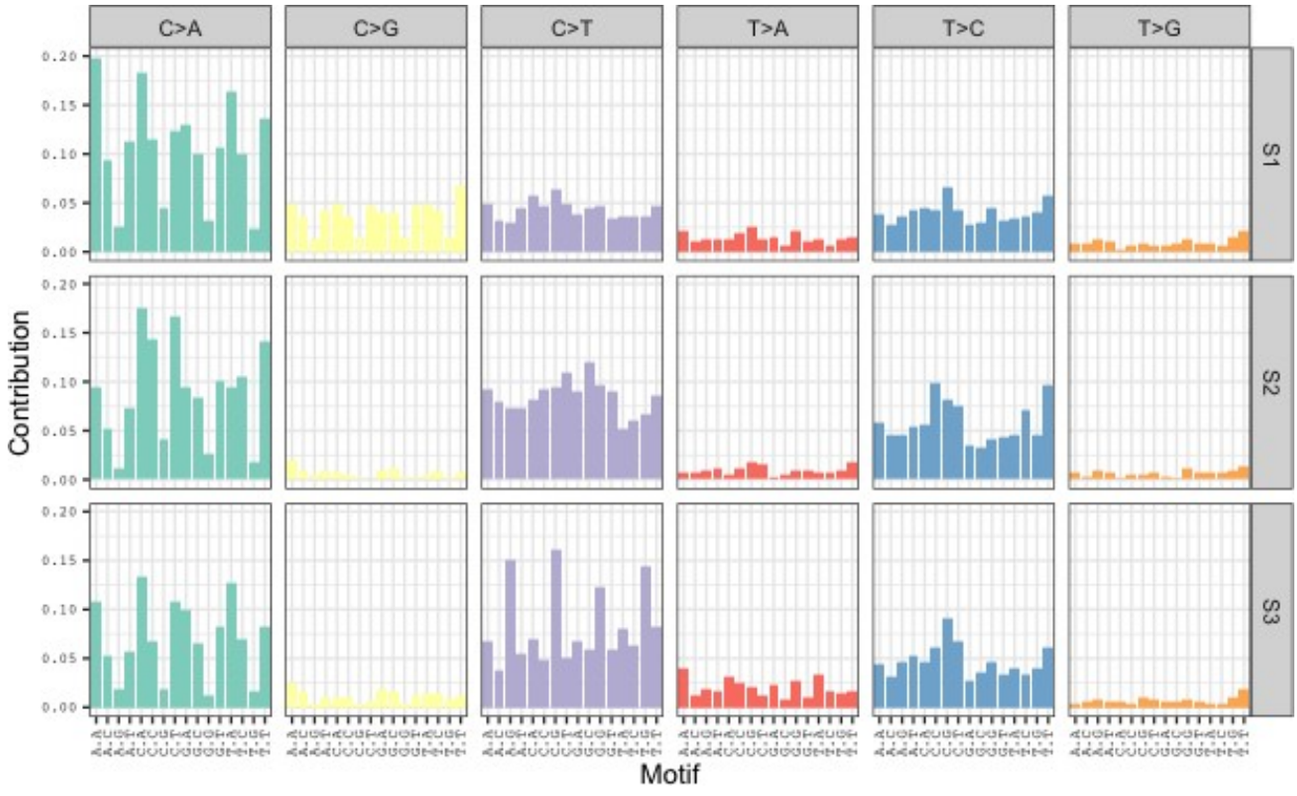
There are various mathematical approaches that are available to decompose a sample's mutational spectrum in order to infer possible mutational signatures. In this study two alternative approaches were used: (i) non- negative matrix factorisation, NMF and (ii) principal component analysis, PCA.

A first step was to determine *r*, the number of signatures expected. With the three groups, the methodology was limited to a maximum of three signatures. (Ideally, this would need prior biological knowledge or other experimental data in order to estimate the likely number of signatures, in this case, inferring *r* from the data suggested that three will fully account for the variation observed).

The next step involved was to attempt decomposing the mutational spectrum by non-negative matrix factorisation, NMF.

This resulted in the identification of three mutational signatures, labelled S1, S2, and S3, as illustrated in Figure 42.

A. Inferring mutational signatures by group



B

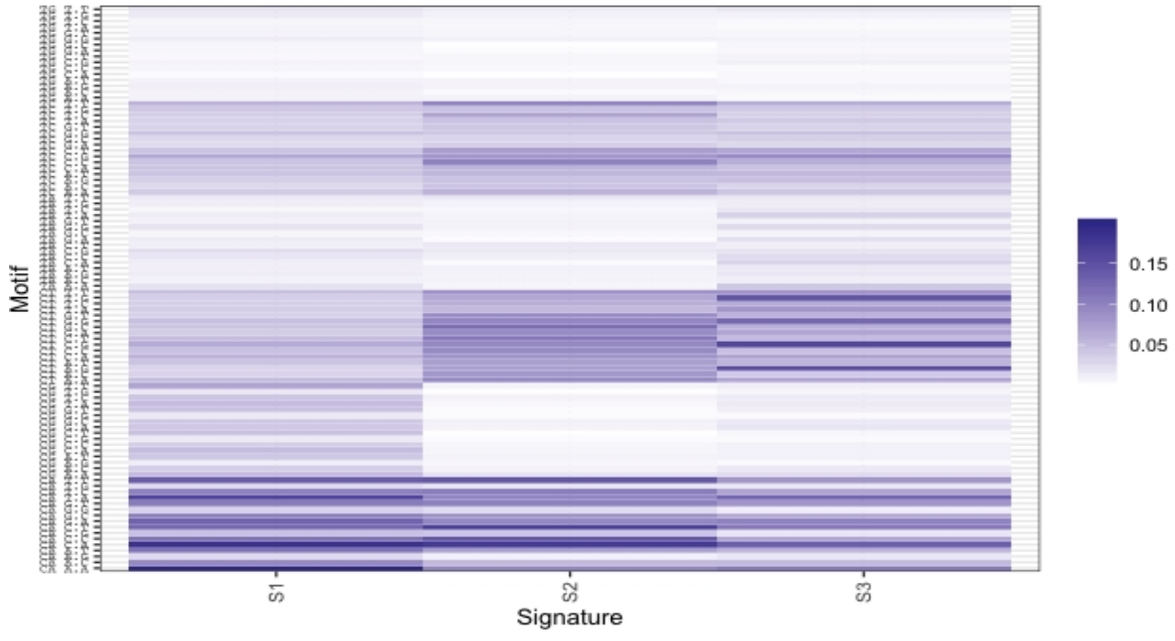


Figure 42: Mutational signatures inferred with the NMF method, represented both as a bar chart (Panel A) and a heat map (Panel B)

The three signatures do not differ, greatly nor do they correspond to any known mutational signature as documented by the Catalogue Of Somatic Mutations In Cancer, COSMIC. As such they must be treated with caution — differences are detected through the likely background noise (? artefact)

The contribution of the three signatures to each patient group can be visualised as a heat map or bar chart as illustrated in *Figure 43*

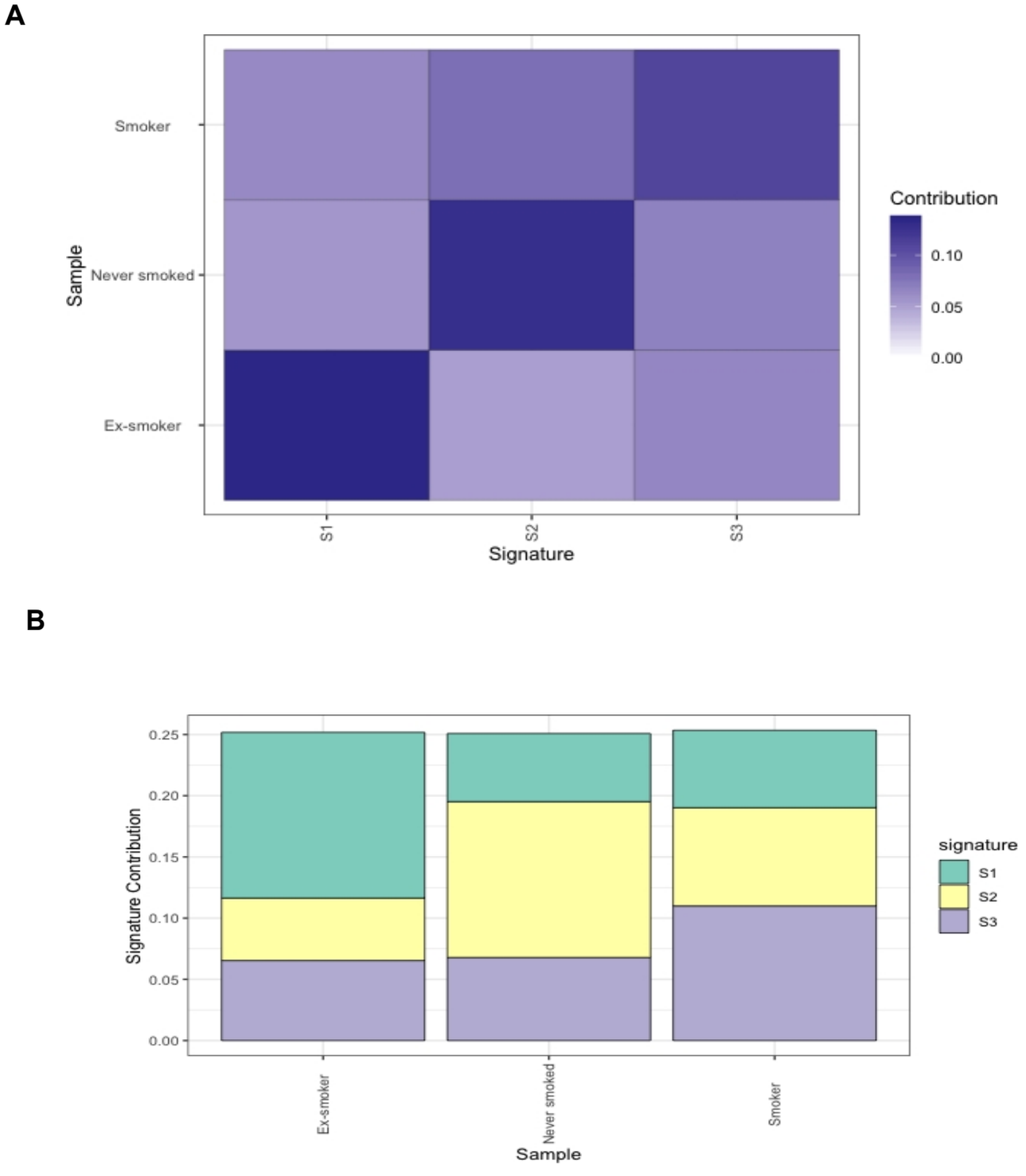
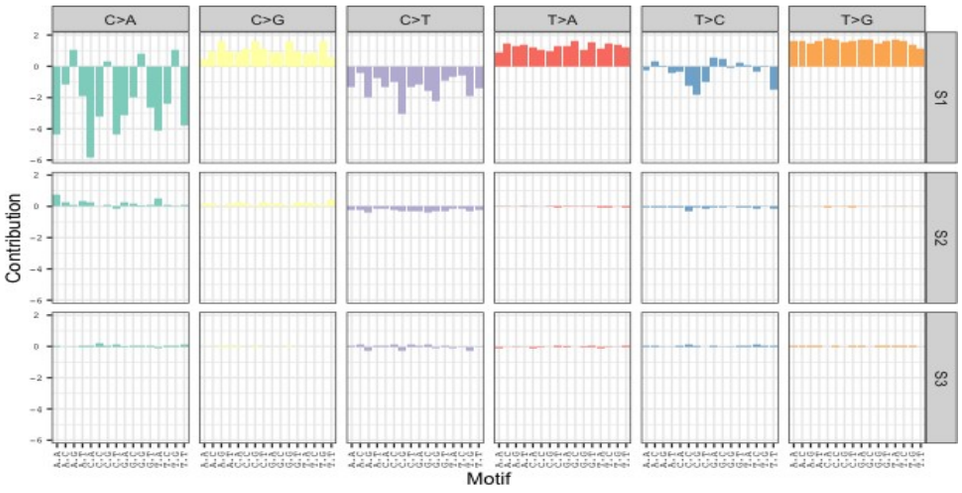


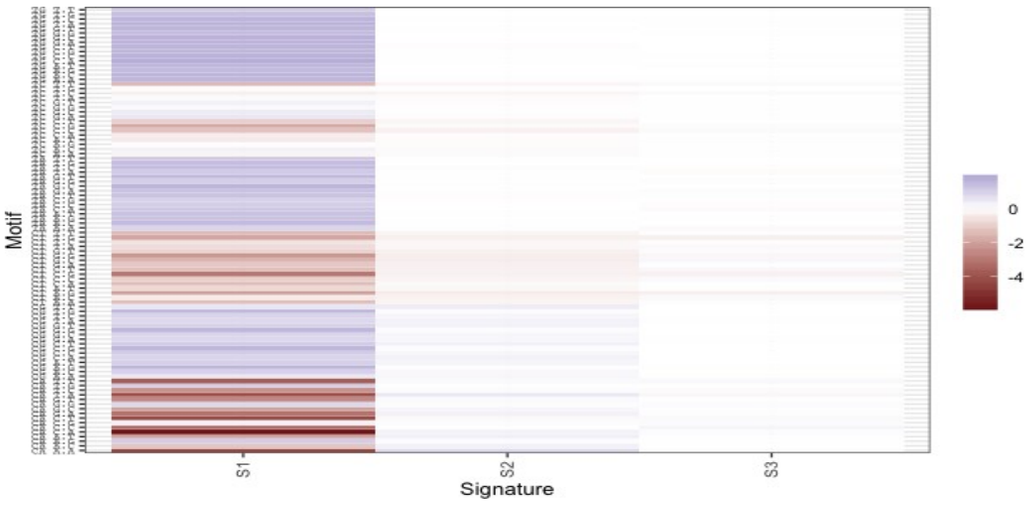
Figure 43: Contribution of the three inferred mutational signatures to observed mutational spectrum, visualised as a heat map (Panel A) and a bar chart (Panel B)

All three signatures contribute to each group but mutational signature S1 contributes most to the ex-smoker mutational spectrum, S2 to the never-smoked spectrum, and S3 to the smoker spectrum.

The alternative approach to decomposition was to infer signatures with principal component analysis, PCA. This resulted in again (due to methodological limits) to the identification of a maximum of three mutational signatures, labelled S1, S2, and S3, as illustrated in *Figure 44*. The contributions each signature made is illustrated in *Figure 45* .



A

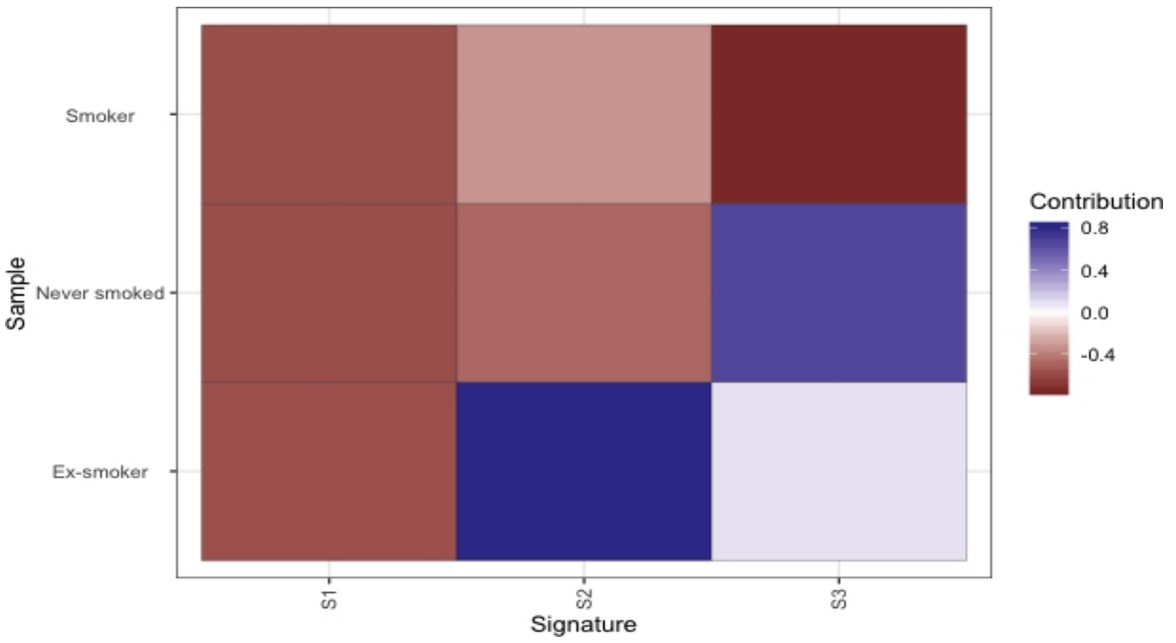


B

Figure 44: Mutational signatures inferred with PCA, represented as a bar chart (Panel A) and a heat map (Panel B)

Signature S1 is seen to dominate. Does this represent background noise due to limitations of our experimental approach? No signature corresponds to any known mutational signature as documented by the Catalogue Of Somatic Mutations In Cancer, COSMIC.

A



B

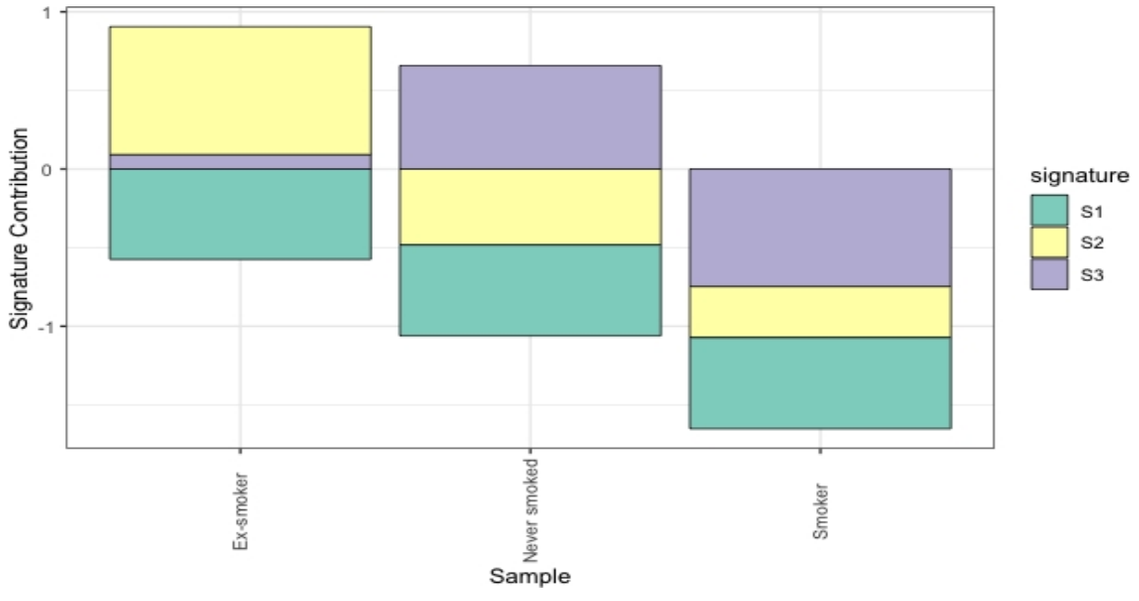


Figure 45: Contribution of the three inferred mutational signatures to observed mutational spectrum, visualised as a heat map (Panel A) and a bar chart (Panel B)

Note how the dominant S1 signature contributes substantially to all three groups. Signatures S2 and S3 reveal differences between the three groups but given the weak signal for these signatures are, this must be treated with caution.

As with NMF, a distinction was made among the three groups, but on this occasion the signatures appeared to be very different. Ex-smokers appeared to be most different with a larger contribution from signature S2 and a far smaller contribution from signature S3. However signature S1 appeared in all three groups which suggested that there was a background signature.

Hence there were different results obtain depending on the decomposition method used however there was a consistent result in that the three groups were distinguishable, with ex-smokers particularly prominent in this group. Due to a lot of background noise it was difficult to infer as to the nature of the difference however a faint discriminating signal was possibly seen.

4.4.2.2 B. Inferring mutational signatures by sample

By grouping the samples in the above methods there is a possibility of introduction of unexplored confounding variables such as (e.g., age, duration of smoking, purity of tumour samples, etc.) that would add to the anticipated background noise arising from lack of paired-normals and reliance on FFPE samples. Hence the following analysis explores whether by ignoring the groups whether individual samples would provide more information on mutational spectrum.

4.4.2.2.1 Stage 1: Determine mutational spectrum for individual samples

As was done previously, the mutational spectrum of the data was determined by calculating the frequency of the 96 motifs according to each sample as illustrated in *Figure 46*.

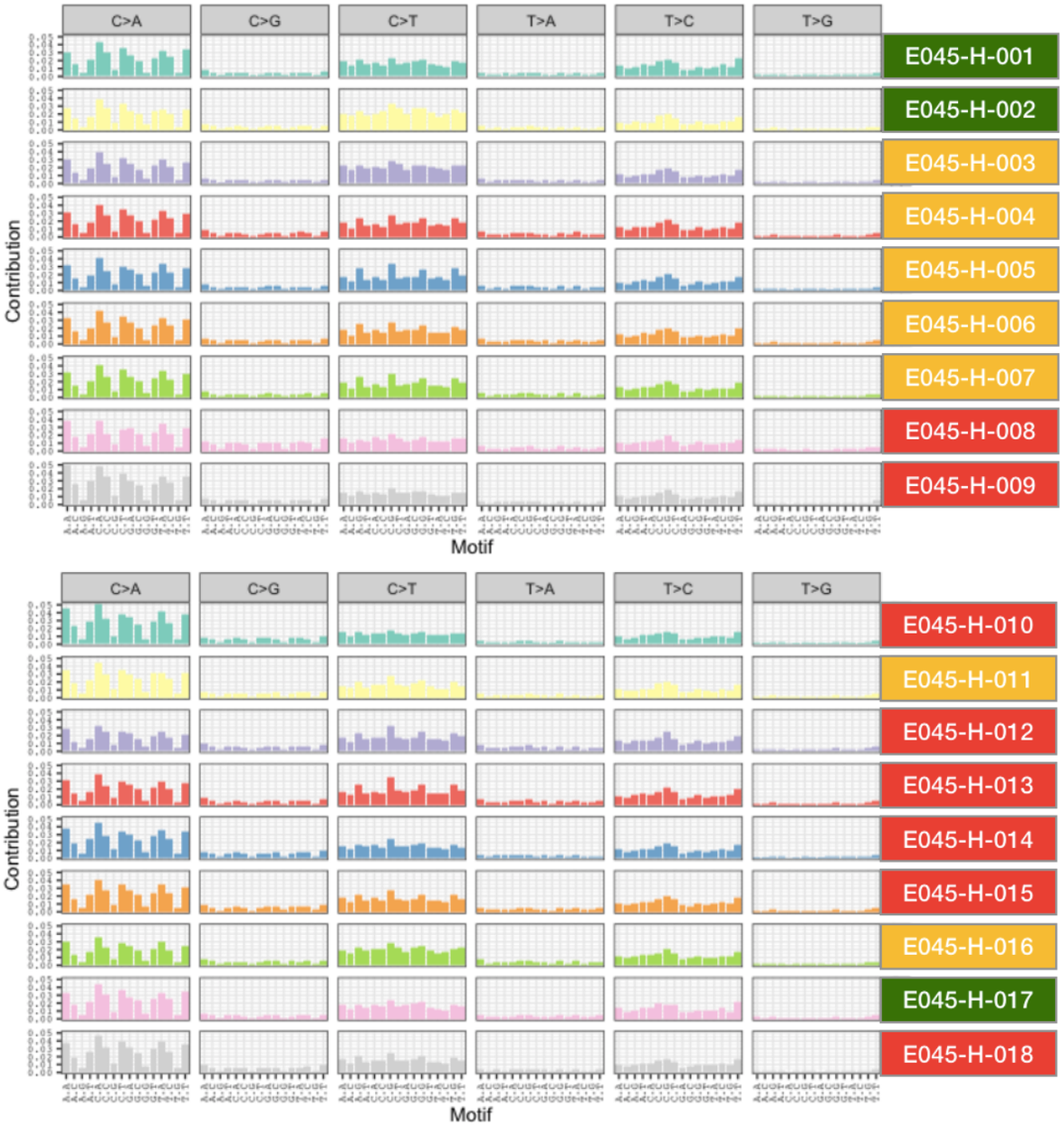


Figure 46: Observed motif frequency for each of the eighteen samples, collectively referred to as the mutational spectrum of the data

Samples are labelled with WGP ids, colour-coded according to smoking history (green, no- smoker; amber, smoker; red, ex-smoker). Modest differences are observed but overall, the pattern of motif frequency is essentially the same. Motif patterns cannot be grouped to smoking category by eye.

The mutational spectrum suggests that there are possibly mutational signature differences among the samples however there was no obvious trend that was visualised. This was further subject to hierarchical clustering that also confirmed a lack of obvious correlation with the smoking group when motifs were clustered, as illustrated in Figure 47.

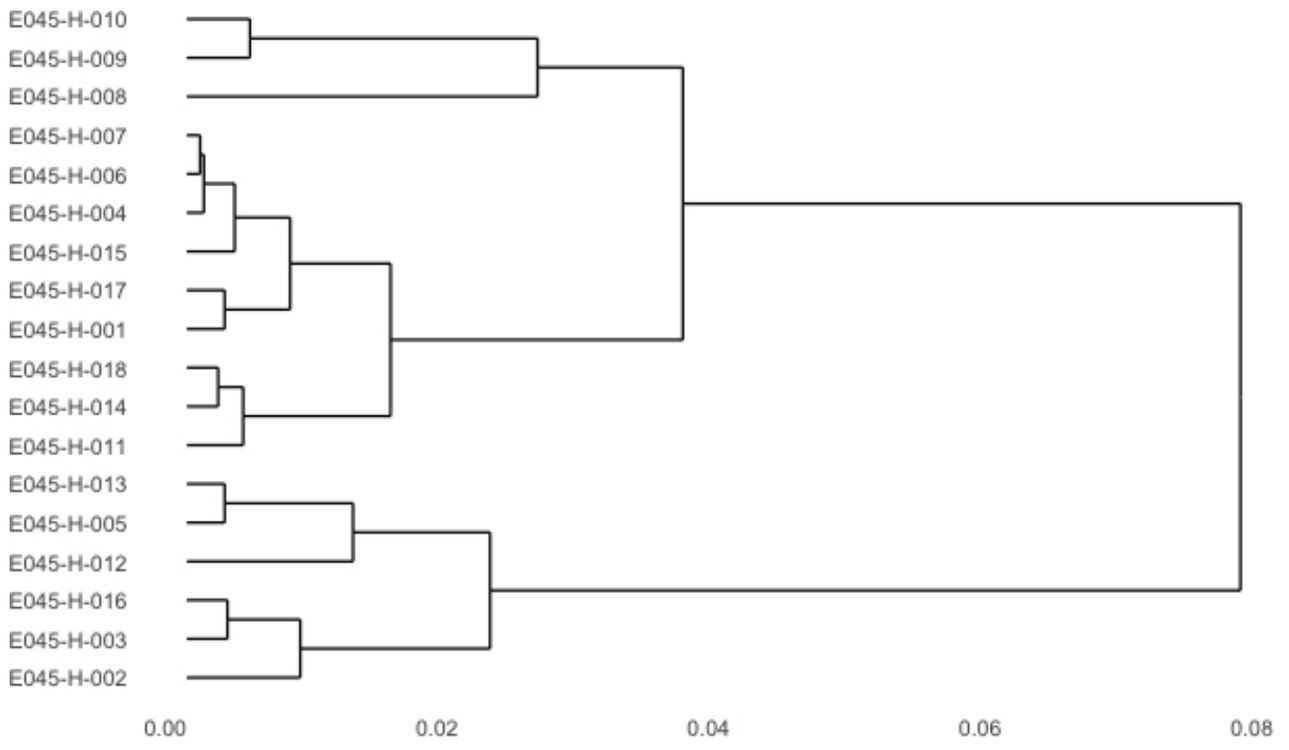


Figure 47: Hierarchical clustering of the mutational spectrum per sample, according to motif

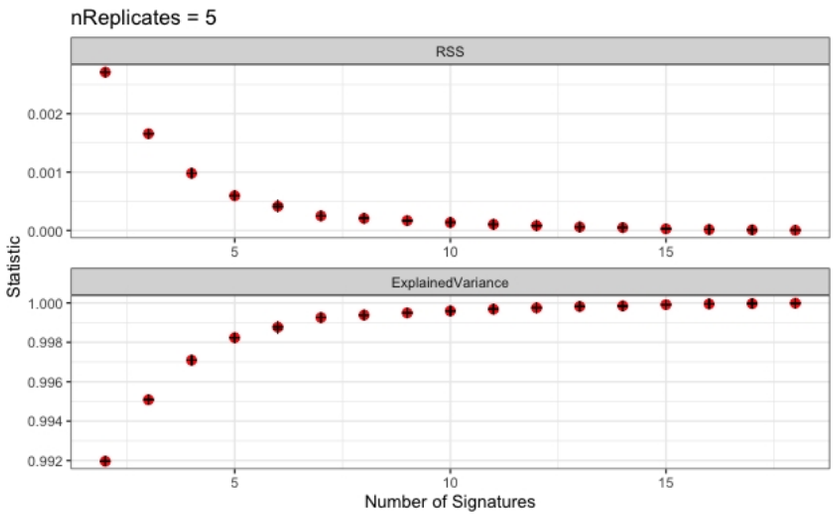
Hence though there were some differences noted, none of these differences obviously corresponded to the smoking group. Hence the overall impression was that the background noise that was present in all the samples obscured any possible signal.

4.4.2.2.2 Stage 2: Identifying mutational signatures for individual samples.

Similar to previously when identifying mutational signatures as per groups, a mutational signature analysis was performed. As was done in the previous analysis, it was necessary to estimate *r*, which is the likely number of expected mutational signatures. With the availability of 18 samples, the methodology allowed for a far wide-ranging potential signatures (between 2 and 18). Although it was preferable to estimate the likely number of signatures from prior biological knowledge or previous experimental data, it was possible to estimate *r* statistically from the data and the results of the analysis is shown in Figure 48.

By performing this analysis, there were seven or eight signatures that were predicted to explain the data. This appeared to be a plausible signal however without the guidance of a priori data, there remains uncertainty in the definitive interpretation which suggested a difficulty in detection of a strong signal. By acknowledging this uncertainty, the data was explored further and decomposition was performed with *r*=8, and 3 for both NMF and PCA analysis (Figure 49, Figure 50, Figure 51,Figure 52).

A



B

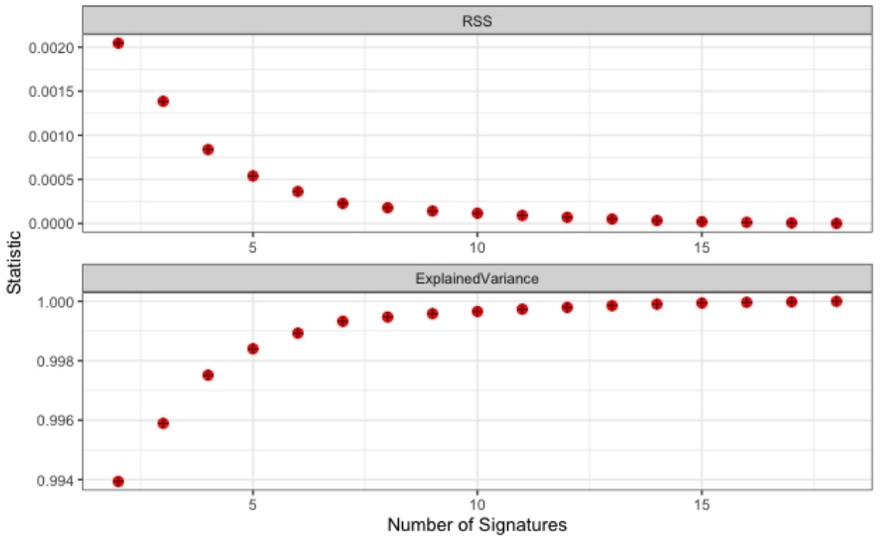


Figure 48: Summary statistics for selecting the number of signatures, determined for NMF (panel A) and PCA (panel B)

Here we are looking for the number of signatures that best approximates the data. In this case, eight signatures seem to explain the bulk of the observed mutational spectrum. NMF-non-negative matrix factorisation. PCA-principal component analysis.

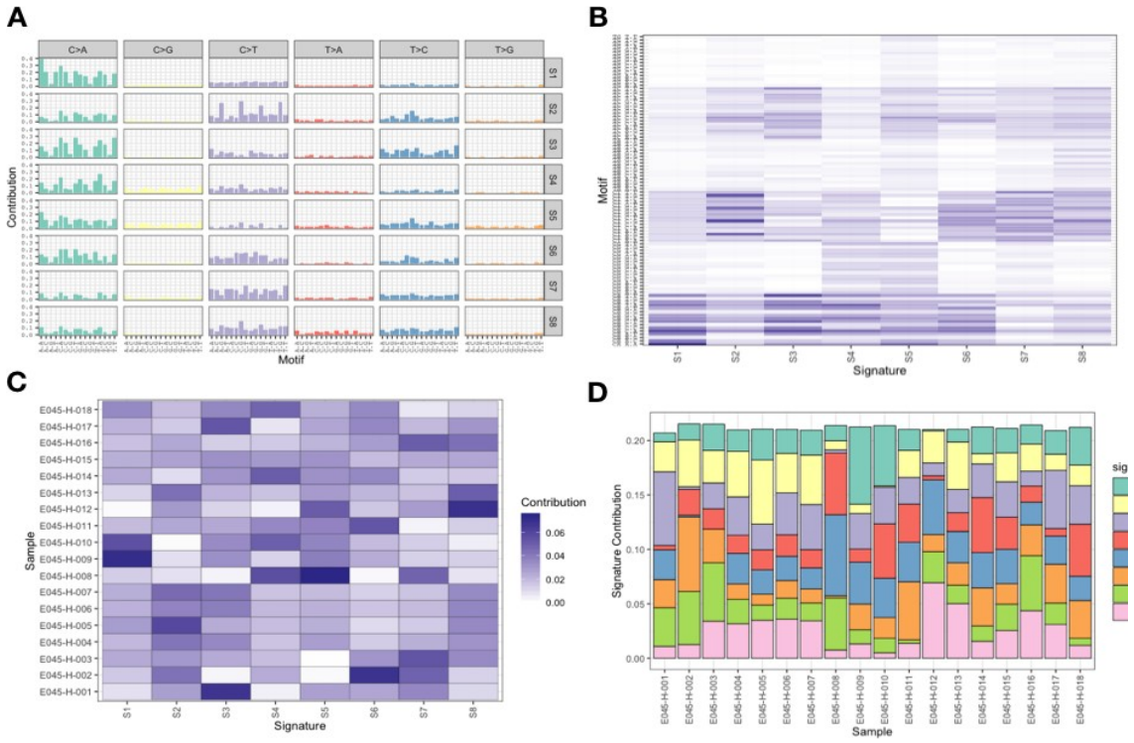


Figure 49: Mutational signatures inferred with NMF assuming 8 signatures.

Represented as a bar chart (A) and a heat map (B). Contribution of each signature to the eighteen samples is shown as a heat map (C) and bar chart (D). NMF-non-negative matrix factorisation

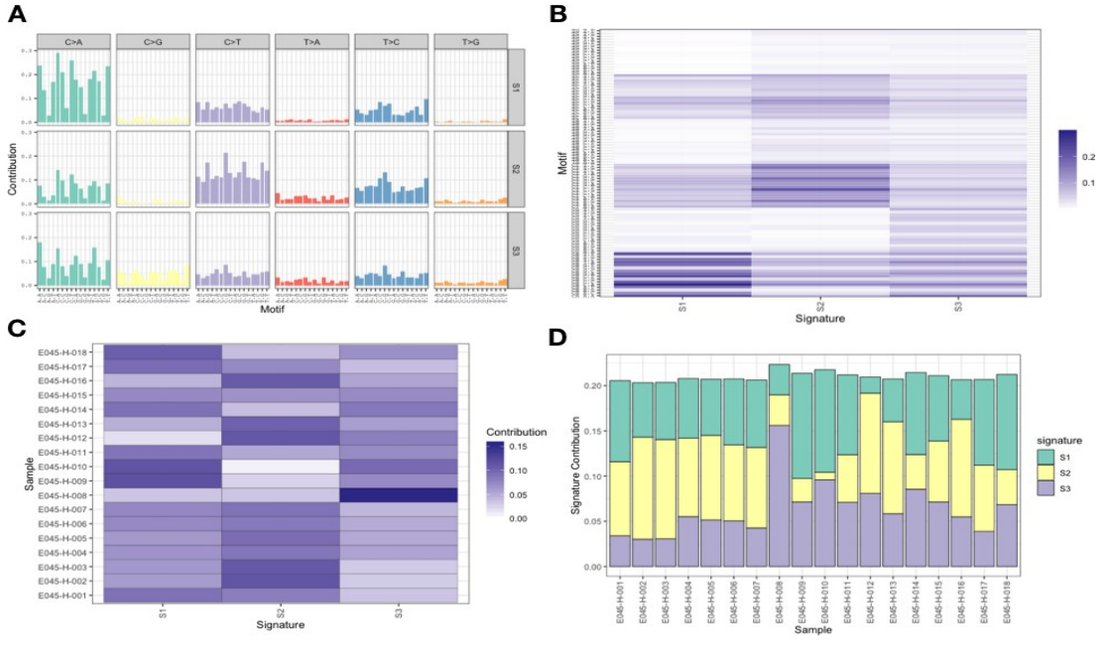


Figure 50:Mutational signatures inferred with NMF assuming 3 signatures.

Represented as a bar chart (A) and a heat map (B). Contribution of each signature to the eighteen samples is shown as a heat map (C) and bar chart (D). NMF-non-negative matrix factorisation

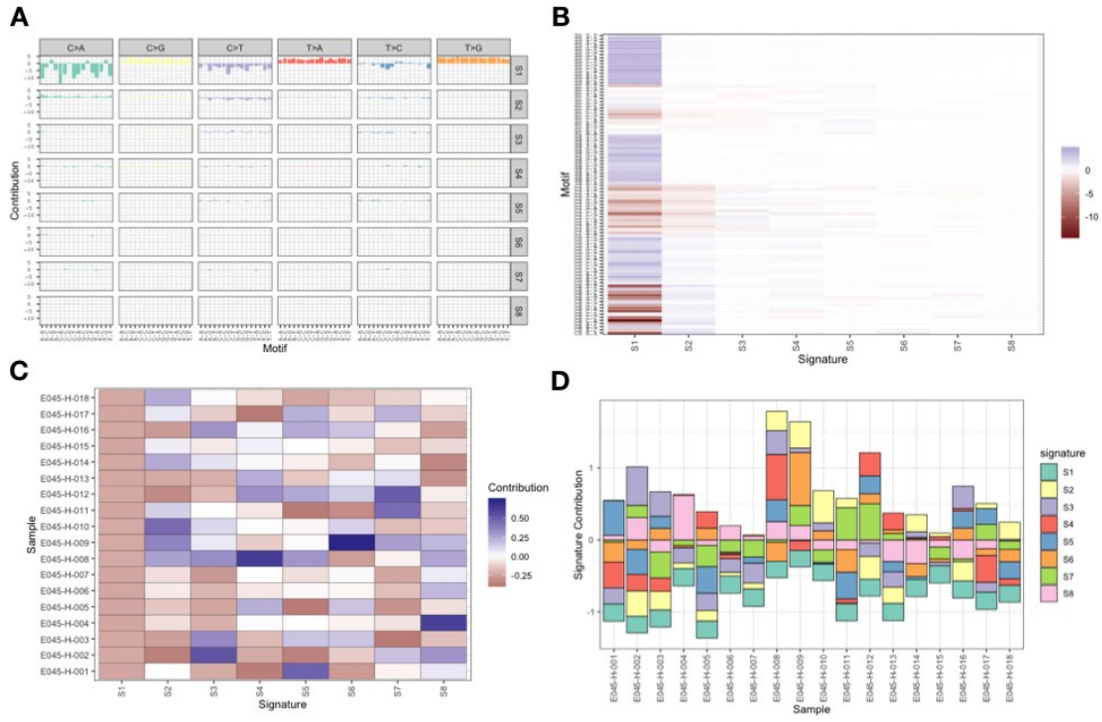


Figure 51: Mutational signatures inferred with PCA assuming 8 signatures.

Represented as a bar chart (A) and a heat map (B). Contribution of each signature to the eighteen samples is shown as a heat map (C) and bar chart (D). PCA-principal component analysis

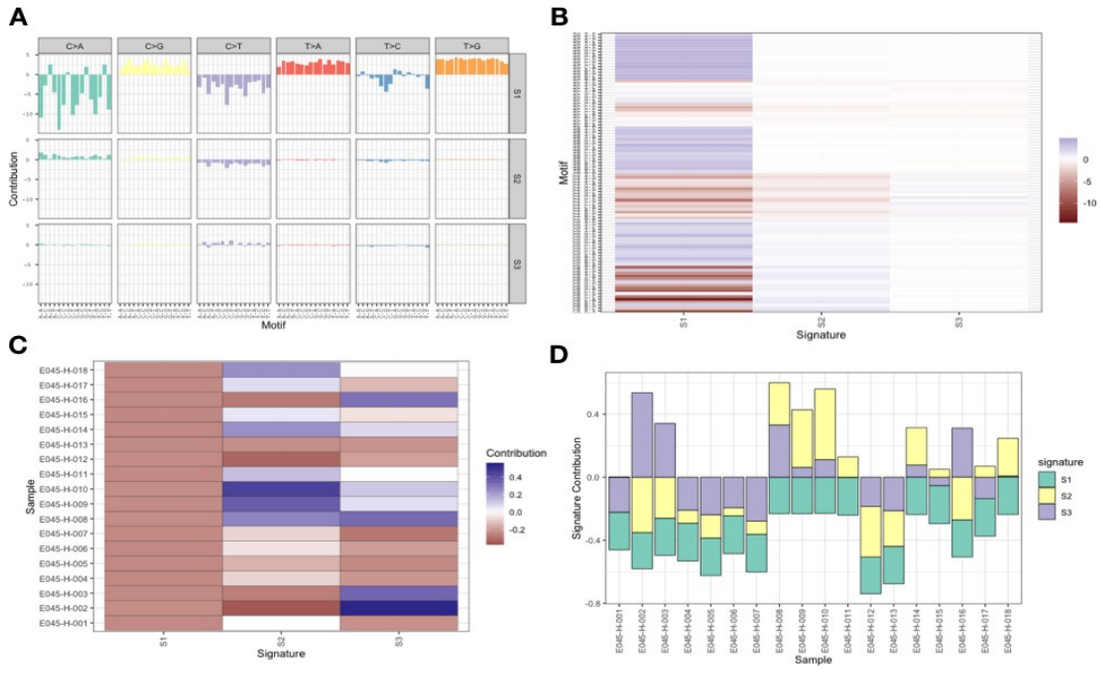


Figure 52: *Mutational signatures inferred with PCA assuming 3 signatures.*

Represented as a bar chart (A) and a heat map (B). Contribution of each signature to the eighteen samples is shown as a heat map (C) and bar chart (D). PCA-principal component analysis

4.4.3 Conclusion from WES results:

Inferring mutational signatures from individual samples in this experiment generated preliminary data however this had several limitations. There was insufficient data to be able to distinguish the smoking category from the other categories. The 18 samples that were analysed individually had high levels of background noise however there was some signal that was apparent that could potentially be biologically informative but this could not be distinguished in the smoking category. This was because there was high levels of background noise from the FFPE sampling.

However by using the methodology of limiting the signatures to 3, this suggested that there were mutational signature profiles that aligned with these smoking groups however this interpretation is far from conclusive.

Sample E045-H-008 (id 447, an ex-smoker), stood out as being different and NMF analysis and in addition to this sample also had the highest number of putative somatic SNVs detected. Hence there may be further merit in exploring the reasons for the clarity of this signal in this sample as opposed to others.

4.5 Discussion:

FFPE samples are commonly used for samples taken from the colon and this method is an invaluable biobank for retrospective research with molecular methods such as PCR and next-generation sequencing. Once a polyp is removed from the colon, this is then placed in a specimen port that contains formalin which then gets sent to the lab for further processing where the polyp gets embedded in paraffin and fixed into blocks.

However the use of FFPE material in next-generation sequencing has several challenges that include a limitation in quantity and quality of DNA that is obtained. FFPE tissues sustain DNA damage such as formaldehyde induced cross links, DNA fragmentation, deamination of cytosine bases leading to C >T mutations (427). In addition to this fixation time with formalin is essential for tissue preservation and either over fixation or under fixation can result in degradation of nucleic acids, protein, change in gene expression that can affect the NGS analysis (428, 429).

In order to minimise this, specialised kits (GeneRead Qiagen) that help to optimise the DNA yield for subsequent NGS was used and there was no significant deviation from the protocol that was used. In addition to this the protocol used for purification of DNA for downstream NGS workflow includes a strict protocol to enable fragmentation, size selection and library preparation for further sequencing (MiSeq/HiSeq).

From the study, from 44/50 samples a sufficient DNA yield was obtained to enable further library construction for next-generation sequencing from manual microdissection.

The reasons for a low yield in FFPE samples apart from the natural degradation that occurs from formalin, could be related to other factors which could include the type of formalin (429) used (buffered versus unbuffered), processing of samples at the local assessment centres (delayed processing) either at the time of endoscopy (when the polyp is removed) pathology laboratory.

In addition to this the quality of DNA extracted from FFPE samples tends to degrade over time with a study showing that FFPE samples used within seven years is suitable for NGS (429).

The other reason speculated could be related to the size of the polyp, the number of sections taken from the polyp, loss of DNA material from sectioning of the blocks, the interval taken from macrodissection to commencing the process of DNA purification.

For subsequent library construction for next-generation sequencing, sufficient yield of DNA was obtained from 44 samples to enable NGS run for the targeted gene panel sequence. However the limitations of this study was the variants have been filtered heavily and so lack information that could be used to assess FFPE-derived background noise. In addition to this the signatures provided through the Mutagene tool were unreliable.

The advantage of using targeted DNA sequencing is that more relevant data obtained, it is more cost-effective and the coverage achieved is higher compared to the other methods. Hence there is increased confidence in sequencing results. There is usually a lower DNA requirement (10 ng) and it has higher multiplexing capabilities.

Although better quality DNA can be obtained from fresh frozen tissue samples, the disadvantage is that they can degrade sooner than FFPE samples. FFPE samples on the other hand can be stored at room temperature for long periods of time and is convenient and cost-effective, in addition to be being made available for retrospective analysis.

The study has demonstrated that adequate quantities of DNA can be extracted from FFPE samples with strategies used to minimise FFPE artefacts and the factors that would need to be taken into consideration for quality control. In order to ensure that there is optimisation of the yield of DNA produced from this method factors such as careful sample selection, review of processing of samples done in the endoscopy unit in pathology laboratory along with the implementation of strict protocols that are used in the NGS stream for DNA purification and isolation.

A recent study (430), has shown promising results to reduce the background noise from FFPE samples by using a computational algorithm called FFPEsig which has been designed to rectify the formalin induced artefacts that occurs in the mutational catalogue.

In the whole Exome sequencing performed on the FFPE samples, there was significant background noise due to artefacts from FFPE sampling however there was a weak mutational signature associated with one sample where the individual was an ex-smoker.

This is encouraging for future studies where samples will need to be carefully selected with enough numbers per group. In addition to this a larger study with blood samples available for whole genome sequencing would help to reduce the noise associated with FFPE and help to increase accuracy of interpretation. The use of computational programs that are designed to rectify formalin induced artefacts may also help in this regard.

The study was an exploratory dataset and has many weaknesses however there are a lot of strengths in the study that highlight areas needed to be considered for a future study.

5. Summary of Research and Future directions

My research set out to address the following objectives:

1. To assess the feasibility of implementation of chromocolonoscopy within a population-based screening programme.
2. To estimate the proximal serrated neoplasia detection rate in a population-based CRC screening programme with chromocolonoscopy.
3. To understand the prevalence of serrated neoplasia in the proximal colon following rigorous histopathological assessment by expert pathologists using both WHO 2010 and AGA criteria.
4. To report the interobserver variability of serrated neoplasia between experts and compared to local pathologists.
5. To explore the feasibility of using FFPE samples for DNA extraction and whole exome sequencing through next-generation sequencing and assess as to whether mutational signatures relevant to serrated polyp pathogenesis can be identified.

The following summarises the research methodology and findings from the studies of this trial that were undertaken as a part of my thesis. It answers the above research questions and describes the implications, limitations, and future direction for research.

5.1 Overview of chapter structure:

The first part of my research is described in Chapter 2 i.e., the **CONSCOP** study (Feasibility of reduction of right sided bowel cancer through **CON**trast Enhanced colono**SCOP**y).

This was a multicentre feasibility open labelled randomised controlled trial (RCT) of dye enhanced colonoscopy using indigo carmine (chromocolonoscopy) in the proximal colon versus standard white light colonoscopy in an index procedure in the bowel cancer screening programme in Wales, United Kingdom between 2014-2017. This study assessed the feasibility of implementation of chromocolonoscopy within a population-based screening programme and also estimated the detection rate of proximal serrated neoplasia along with significant serrated lesions and advanced adenomas in this cohort selected via an initial screening faecal occult blood test.

Chapter 3 described the second part of the study that involved the rigorous histopathological assessment of proximal colon polyps by three expert GI pathologists using both WHO 2010 and AGA criteria (the current WHO 2019 criteria) and reported on the interobserver variability of serrated neoplasia between the experts and the local pathologists. This study also helped to understand the prevalence of serrated neoplasia in the proximal colon and its potential implications in clinical practice.

Chapter 4 described the third part of the study that involved exploring the mutational signatures of serrated neoplasia. This involved selecting a proportion of serrated polyps based on the presence and absence of dysplasia, known non genetic factors thought to contribute to the development of serrated neoplasia such as smoking history along with adenomas. It explored the feasibility of being able to extract DNA from FFPE blocks and assessed whether mutational signatures could be identified using next-generation sequencing methods.

The following section describes the research methodology used in each of the studies.

5.2 Research methodology:

5.2.1 Chapter 2

This was a feasibility study implemented in the bowel screening programme with the view to assessing the feasibility of a larger more definitive trial by examining factors such as recruitment, compliance, acceptability, and attrition rates. The advantage of having a multicentre design was to see if results could be generalisable to a broader population, to increase the sample size and hence come to more robust conclusions.

In order to compare interventions a randomised controlled trial is a considered best practice. Randomly assigning participants to treatment groups can help to control confounding variables and ensure that results are less likely to be biased. The random variables included demographic factors and randomisation was done electronically by the computer on a list basis whereby only a maximum of two patients on a list were randomised to the dye arm.

An open label design meant that both the patient and the investigators are aware of which treatment is being administered. However, to minimise this bias, both the colonoscopist and the patient were not made aware of the trial arm until just before commencement of the colonoscopy procedure.

During the consent process, participants were counselled in detail regarding the rationale of the study and that they would not be able to choose which arm of the study they would be allocated to. The need for equipoise was emphasised during the informed consent process.

Use of a central randomisation process ensured unbiased allocation of participants to the intervention groups. The participant was only told of which arm they were participating in just prior to the commencement of the procedure thereby minimising selection bias.

Around 13.7% and 11% in the standard arm and chromocolonoscopy arm respectively refused consent on the day of the procedure however none of these patients were aware of the trial arm that they were selected into.

There was no apparent report of participants withdrawing consent during the procedure. Hence there was no attrition bias.

The Hawthorne effect is a phenomenon whereby individuals may modify their behaviour in response to being observed of being aware of being part of a study. For colonoscopist knowing the intervention arm could potentially lead to changes in their examination technique, vigilance of interpretation of findings due to the awareness of the performance being evaluated. However, it could be argued that this performance bias was minimal as strict performance evaluation in the bowel screening programme is a standard practice.

The colonoscopists participating in the screening programme already have high adenoma detection rates as they are rigorously assessed before entering into a screening programme alongside stricter monitoring of KPI at regular intervals to ensure maintenance of standard.

To further minimise this performance, detection and ascertainment bias, training days had been organised with attendance by all the participating Colonoscopists and specialist screening practitioners for one full day to ensure that there was a consistency in examination techniques, dye spray and interpretation of findings along with a clear definition of objectives.

During a bowel cancer screening list there is always a specialist screening practitioner who assists with the procedure which includes documentation and confirmation of findings. This is part of ensuring high standard during bowel cancer screening procedures. Standardised data collection was done by the specialist screening practitioner (SSP) who recorded details of the colonoscopy during a procedure on the IT management system for bowel screening. The trial specific data was collected on a case report form which I (RR) had designed with the trial group and this data was collated by the SSP. In addition to this the collection of data in terms of confirmation of histology and selection of polyps was done by RR who was blinded to the intervention arm.

Data monitoring and quality control was done on a periodic basis which was managed by the trial management group for the trial.

5.2.2 Chapter 3:

This part of the study involved the histopathological aspect of the trial.

As part of the trial the histology reports were uploaded onto the IT management system of the bowel screening programme (BSIMS). The trial manager on a weekly basis sent trial numbers alongside the participating site detail to the research fellow (RR). Histopathology of the individual polyps was collected by RR (myself) and collated onto a spreadsheet. The proximal colon polyps were assigned unique ID numbers, which were given to individual expert GI pathologists. Expert pathologists were blinded to the local pathologists' report independently reported on the pathology slides.

In order to minimise bias, there were clear definitions of the terminology used that was agreed and decided prior to the trial. There was blinding of the local pathology report between the expert pathologists. There was no prior knowledge of the arm of the trial to any of the pathologists and the researcher presenting the data. Any disagreement between the expert pathologists was assessed independently by the research fellow (RR) and a meeting was then organised to discuss the disagreement with a view to obtaining consensus.

5.2.3 Chapter 4:

The selection of the FFPE samples was based on four groups: those patients that had 1. Non-dysplastic SSLs only, 2. Dysplastic SSLs (with or without adenomas), 3. Non-dysplastic SSLs with a matched adenoma, 4. Adenomas only. The aim of the selection of the groups was to include adequate representation of males, females, smokers, non-smokers, and ex-smokers along with aspirin data distributed equally amongst all the four groups.

DNA was then extracted from the FFPE blocks following sectioning and macro-dissection which then underwent library construction for targeted gene panel sequencing and comprehensive gene panel sequencing in order to identify existing and novel genes involved alongside whole exome sequencing to identify mutational signatures in these groups.

5.3 Summary of research findings:

The CONSCOP study recruited 741 patients with a participation of 82% of patients and 87% of colonoscopists that agreed to participate in the trial. The participation, baseline demographics and pre-procedure characteristics were balanced between both the arms of the trial.

As expected, the procedure time was longer in the chromocolonoscopy arm with a mean difference of 6.3 minutes. There was no difference found in the degree of difficulty of the procedure or difference in patient discomfort between the two arms.

The following findings of polyps in the chromocolonoscopy arm versus standard colonoscopy was as follows: there was an increase in the proximal sessile serrated lesion detection rate (11.8% vs 6.4% (P = 0.157)), Significant serrated lesion detection rate-4.2% vs 1.9% (p = 0.05), proximal significant serrated lesion detection rate (2.4% versus 0.8%; P = 0.03). There was also an overall increase in the number of adenomas in the chromocolonoscopy group in comparison to the standard group.

Hence this answered the research objectives set out in the study which was that it is feasible for chromocolonoscopy to be implemented in a bowel cancer screening programme with acceptability from colonoscopists and patients.

The study also detected more proximal serrated neoplasia particularly significant serrated lesions. This is significant as sessile serrated lesions with dysplasia i.e., the significant SLs have an increased 10-year risk of CRC 4.43% (Adjusted OR 4.76, 95% CI 2.59-8.73) (237) and hence recognition is quite important in order to reduce the risk of CRC.

This is the first study to demonstrate that with a rigorous trial design, chromocolonoscopy can be implemented within a UK-based population bowel screening programme that has specifically looked at the detection of proximal serrated neoplasia along with estimating the additional time and resources associated with this and minimisation of bias due to colonoscopy or pathology related factors.

A further trial is warranted to look at the difference in significant proximal serrated lesion detection with full economic evaluation. Longitudinal studies to follow-up and assess clinical effectiveness based on longer term outcomes of chromocolonoscopy over time and the impact on clinical practice for surveillance are necessary.

The second part of the study i.e., the histopathology showed that following rigorous review of proximal polyps by the expert pathologists that the prevalence of significant serrated neoplasia in the proximal colon was 7.6% versus 3.3% if reviewed by local pathologists. This study also showed that though there was good interobserver concordance for non-dysplastic SSLs, this was fair for dysplastic SSLs between the expert pathologists and also when compared with the local pathologists. Interestingly all of these dysplastic SSLs were diminutive with an average size of 5 mm.

As a result of the rigorous review by the experts, 46% of hyperplastic polyps were reclassified as SSLs.

The novel part of the study included the description of a morphological variant of serrated neoplasia i.e., serrated features in tubular adenoma that had distinctly different morphological features compared to conventional tubular adenoma. This will need further exploration and characterisation in future studies.

The third part of the study i.e., The exploratory dataset looking at the mutational signatures of serrated neoplasia showed that it was feasible to obtain quantifiable DNA from FFPE blocks. Whole exome sequencing potentially showed a weak mutational signature in samples where the individual was an ex-smoker. There was however significant background noise associated with FFPE samples. This is encouraging for future studies where improved techniques could be developed to extract DNA from FFPE samples.

The use of matched germline blood samples for whole genome sequencing can potentially help to reduce the noise associated with FFPE and increase the accuracy of interpretation.

5.4 Limitations of the Studies:

The strengths of the study have been described in the research methodology which describes a rigorous trial design that has endeavoured to minimise bias and ensured blinding where feasible.

There are a few limitations to this study however as described below:

The study was conducted between 2014-2016 and during this time gFOBT was used in the bowel screening programme in Wales. This has recently been replaced by the more sensitive faecal immunochemical test (FIT) since 2019. The sensitivity of stool tests to detect advanced adenomas and serrated lesions is very low. For example, for FIT for advanced adenomas reduces to 25-40% (55, 56). The sensitivity reduces further in the detection of serrated lesions including larger ones(22, 23). The sensitivity for detecting advanced adenoma was using gFOBT is lower than FIT and has shown to vary between 6 to 14% in some studies (431) and hence likely to be even lower for serrated neoplasia. Hence the prevalence of the reported proximal serrated neoplasia rate may be influenced by this.

The second limitation is that though strategies were used to minimise the impact of the Hawthorne effect, it would have helped to measure if there was a sustained effect of detection of adenomas and serrated polyps amongst colonoscopists.

In order to do this, a sub study that measured the KPIs of individual colonoscopists prior to the trial, during the first half of the trial and towards the end of the trial would have helped to inform this.

In addition to this comparing their KPI data on all their procedure in the symptomatic and bowel screening service would have been able to partly measure this effect.

Though there was a significant uptake from patients and colonoscopists in the study, in order to qualify the acceptability levels, qualitative studies involving questionnaires could have helped.

In the study involving the histopathologists, intraobserver variability or reproducibility was not assessed amongst the experts. This would have been helpful to understand whether specific additional criteria would have been needed to ensure reproducibility of diagnosis particularly in those difficult to diagnose polyps such as dysplastic SSLs and TSA where the inter observer concordance was only fair. In addition to this though there was strict pre-agreed criteria for the expert histopathologists, this was not used for the local pathologists. During the time of the study the criteria for serrated neoplasia included the WHO 2010 and the AGA criteria. This is the strength of the trial as it holds relevance currently as subsequent to this the WHO 2019 criteria have now incorporated both of these.

This study was specifically set up for proximal polyps and hence distal polyps were not reviewed as part of the protocol. However inclusion of distal polyps for rigorous pathology review would have helped to answer any difference in surveillance intervals.

In the genetic study, the groups were selected based on the type of serrated neoplasia along with demographic characteristics including smoking, gender and aspirin and the presence of matched adenomas. However, the groups were not equally represented in the final dataset due to the available samples, loss of samples as a result of inadequate DNA being extracted and failure of targeted and comprehensive panel sequencing. It would have also helped to have included some of the morphological variants of serrated neoplasia that were found during the study i.e., serrated Tubulovillous polyps and tubular adenomas with serrated features in the groups above.

From 50 samples where DNA was attempted to be extracted from FFPE blocks, only 18 samples were feasible to use for whole exome sequencing. In addition to this there was significant background noise to interpret any mutational signatures and having blood samples and whole genomic sequencing would have helped to reduce this background noise. In addition to this, refinement of DNA extraction methods from FFPE blocks would need to be considered for future studies.

Recently a study (430) has recognised that there is major missed assignment of signature activities by the use of uncorrected mutational catalogues of FFPE. In order to overcome this, a computational algorithm called FFPEsig has been designed to rectify the formalin induced artefacts that occur in the mutational catalogue. The study demonstrated that the use of this algorithm could enable accurate mutational signature analysis in simulated and whole genome sequenced FFPE cancer samples. Hence this algorithm could potentially be used to explore future FFPE samples.

5.5 Future directions and research:

This study has shown that chromocolonoscopy is feasible to implement in a population-based screening programme and clearly helps to improve the detection of proximal significant serrated lesions and advanced adenomas. The main value of this study is to improve our understanding of proximal colon cancer and PCCRC in the proximal colon and if this can be reduced by increasing the detection of proximal serrated neoplasia.

In addition to this there is lack of high-quality longitudinal studies that examine the malignant potential of serrated neoplasia that includes determining the cancer risk factors and molecular factors such as biomarkers in high-risk significant serrated lesions (dysplastic SSL, TSA).

The current surveillance guidelines for serrated polyps are based on expert opinion and further studies are required to look at the evidence-based surveillance programme that determines the exact risk of developing CRC after removing these lesions. The surveillance strategy of serrated lesions in the absence of synchronous adenomas also needs to be determined.

In order to find out what the true prevalence of serrated neoplasia is in the screening population and to be able to understand the epidemiological factors and risk factors associated with serrated neoplasia, there is a need for high quality evidence, research collaboration and long-term longitudinal studies that can factor in the above to know the burden of serrated neoplasia i.e., the prevalence of serrated neoplasia. In order to understand the above there needs to be a reduction in variation in polyp detection and categorisation in colonoscopy and histopathology. In addition to this there also needs to be methods to improve detection that include benchmarking serrated polyp detection rate as a key performance indicator, improving the sensitivity of stool-based tests, improving techniques needed for detection of these polyps and incorporating new technology to aid this.

Serrated polyp detection rate (SPDR) should ideally be included as one of the parameters to assess key performance indicators (KPI).

The American Gastroenterology Association (AGA) is the first society in 2021 to recommend SPDR as a parameter to measure KPIs. The recommendation is ~7% (however to aim for 10%) (262). This is an important KPI to aspire in all societies/ countries.

Anderson et al in 2022 (432) showed that the PCCRC rate was statistically lower in those endoscopists who had a high clinically significant serrated polyp detection rate. The CONSCOP study was completed in 2017 and data from the study published in 2019, the above data was not available at the time.

Since the CONSCOP study, there is already CONSCOP2 that is underway involving multiple centres (¬20) from three nations in UK (Wales, England and Scotland). This study is a randomised controlled trial which is set up within the bowel cancer screening programme recruiting for two years with the primary aim to assess the effectiveness of chromocolonoscopy within index screening colonoscopy and assess its impact on development of further colorectal neoplasia in this cohort (through routine data linkage). This study is currently looking at whether chromocolonoscopy is more effective in achieving improved proximal significant serrated polyp i.e., advanced forms of serrated polyps with detection of the initial procedure and thereafter looking at whether chromocolonoscopy is more effective and cost-effective and reducing the numbers of polyps and cancer is found at the subsequent surveillance colonoscopy. CONSCOP2 aims to do this by modelling the results of the study including results from follow-up in surveillance procedures and link it to routine healthcare data patient characteristics to determine optimal follow-up frequency for different groups of patients.

The aim is also set at a higher threshold in comparing detection rates between high-definition white light colonoscopy and chromocolonoscopy.

The secondary aims include comparing the overall lesion detection rates and to assess the impact of FIT thresholds on serrated lesion detection rate in three nations that have different FIT thresholds.

This study will also assess the longer-term economic impact of chromocolonoscopy within the screening setting and assess the association between demographic and lifestyle factors of serrated lesions at index and surveillance colonoscopy.

The longer-term follow-up of these participants will continue for three years thereafter. In addition to this a qualitative study that looks into the experiences of chromocolonoscopy, and training provided prior to the trial is planned to be undertaken with a view to obtaining opinions for the implementation of chromocolonoscopy into the screening programme. This will include a questionnaire that is done prior to training, during the study and post-trial.

The study also aims to incorporate artificial intelligence into the trial to (FORE-AI study) enable comparison with AI assisted chromocolonoscopy and High-Definition White light colonoscopy with corroborative data from histopathology.

Other ongoing research initiated as a result of CONSCOP include translational research into serrated neoplasia including the consideration of prospective studies for dietary influences and the gut microbiome in these patients (CRUK Early Detection grant award).

CONSCOP2 has also incorporated digital pathology slides to evaluate the impact of these on variation of interpretation and to test feasibility of this approach in a large screening program cohort.

Artificial intelligence-based digital pathology is an emerging area and promises to increase both the accuracy and quality in the interpretation of pathology (433).

Finally, this study helps in understanding the methods and challenged involved in obtaining mutational signatures from FFPE samples for serrated neoplasia. The CONSCOP2 study will be able to overcome these challenges and further translational research into molecular markers, mutational patterns and signatures associated with serrated neoplasia along with attempting to understand the carcinogenic risk, epigenetics, and epidemiological risk factors.

Bibliography

1. Bond A, Sarkar S. New technologies and techniques to improve adenoma detection in colonoscopy. World Journal of Gastrointestinal Endoscopy. 2015;7(10):969.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424.
3. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-91.
4. Cancer research UK, Bowel cancer incidence statistics [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence#heading-One. Accessed [January] [2024].
5. Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJY, Young GP, et al. Colorectal cancer screening: a global overview of existing programmes. Gut. 2015;64(10):1637-49.
6. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. Nat Rev Dis Primers. 2015;1:15065-.
7. Arhin N, Ssentongo P, Taylor M, Olecki EJ, Pameijer C, Shen C, et al. Age-standardised incidence rate and epidemiology of colorectal cancer in Africa: a systematic review and meta-analysis. BMJ Open. 2022;12(1):e052376.
8. Office for National statistics-cancer registration statistics 2021 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/cancerregistrationstatisticsengland/previousReleases. Accessed [October] [2021].
9. Tadros M. The rise of proximal colorectal cancer: a trend analysis of subsite specific primary colorectal cancer in the SEER database. Ann Gastroenterol. 2021.
10. National Cancer Institute (NIH), Surveillance, Epidemiology and End Results Program (SEER) [Available from: https://seer.cancer.gov/statfacts/html/colorect.html. [October] [2021].
11. Brenner H, Stock C, Hoffmeister M. Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ (Clinical research ed). 2014;348:g2467.
12. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, et al. Long-Term Colorectal-Cancer Incidence and Mortality after Lower Endoscopy. New England Journal of Medicine. 2013;369(12):1095-105.
13. Adler J, Robertson DJ. Interval Colorectal Cancer After Colonoscopy: Exploring Explanations and Solutions. Official journal of the American College of Gastroenterology | ACG. 2015;110(12):1657-64.
14. Singh H, Turner D, Xue L, Targownik LE, Bernstein CN. Risk of developing colorectal cancer following a negative colonoscopy examination: evidence for a 10-year interval between colonoscopies. JAMA : the journal of the American Medical Association. 2006;295(20):2366-73.
15. Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of colonoscopy and death from colorectal cancer. Ann Intern Med. 2009;150(1):1-8.
16. Pohl H, Robertson DJ. Colorectal cancers detected after colonoscopy frequently result from missed lesions. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2010;8(10):858-64.
17. Pohl H, Srivastava A, Bensen SP, Anderson P, Rothstein RI, Gordon SR, et al. Incomplete polyp resection during colonoscopy-results of the complete adenoma resection (CARE) study. Gastroenterology. 2013;144(1):74-80.e1.
18. Arain MA, Sawhney M, Sheikh S, Anway R, Thyagarajan B, Bond JH, et al. CIMP status of interval colon cancers: another piece to the puzzle. The American journal of gastroenterology. 2010;105(5):1189-95.
19. Sawhney MS, Farrar WD, Gudiseva S, Nelson DB, Lederle FA, Rector TS, et al. Microsatellite instability in interval colon cancers. Gastroenterology. 2006;131(6):1700-5.

20. Rees CJ, Bevan R, Zimmermann-Fraedrich K, Rutter MD, Rex D, Dekker E, et al. Expert opinions and scientific evidence for colonoscopy key performance indicators. Gut. 2016;65(12):2045-60.
21. Rutter MD, Beintaris I, Valori R, Chiu HM, Corley DA, Cuatrecasas M, et al. World Endoscopy Organization Consensus Statements on Post-Colonoscopy and Post-Imaging Colorectal Cancer. Gastroenterology. 2018;155(3):909-25 e3.
22. Mowat C, Digby J, Strachan JA, Steele RJC, Fraser CG. Low Sensitivity of Fecal Immunochemical Tests (FIT) for Detection of Sessile Serrated Adenomas/Polyps Confirmed Over Clinical Setting, Geography, and FIT System. Dig Dis Sci. 2019;64(10):3024-6.
23. Anderson JC, Robertson DJ. Serrated Polyp Detection by the Fecal Immunochemical Test: An Imperfect FIT. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(6):880-2.
24. Chang L-C, Shun C-T, Hsu W-F, Tu C-H, Tsai P-Y, Lin B-R, et al. Fecal Immunochemical Test Detects Sessile Serrated Adenomas and Polyps With a Low Level of Sensitivity. Clinical Gastroenterology and Hepatology. 2017;15(6):872-9.e1.
25. Lansdorp-Vogelaar I, Knudsen AB, Brenner H. Cost-effectiveness of Colorectal Cancer Screening. Epidemiologic Reviews. 2011;33(1):88-100.
26. Colorectal cancer screening committee 2005-2022 [Available from: https://www.worldendo.org/about-us/committees/colorectal-cancer-screening/.Accessed [October][2022]
27. Miles A, Cockburn J, Smith RA, Wardle J. A perspective from countries using organized screening programs. Cancer. 2004;101(S5):1201-13.
28. IARC. International agency for research on Cancer handbooks of cancer prevention: World Health Organisation; 2005.
29. Benson VS, Atkin WS, Green J, Nadel MR, Patnick J, Smith RA, et al. Toward standardizing and reporting colorectal cancer screening indicators on an international level: The international colorectal cancer screening network. International Journal of Cancer. 2012;130(12):2961-73.
30. Benson VS, Patnick J, Davies AK, Nadel MR, Smith RA, Atkin WS. Colorectal cancer screening: A comparison of 35 initiatives in 17 countries. International Journal of Cancer. 2008;122(6):1357-67.
31. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, et al. Prospective evaluation of methylatedSEPT9in plasma for detection of asymptomatic colorectal cancer. Gut. 2014;63(2):317-25.
32. Koo S, Neilson LJ, Von Wagner C, Rees CJ. The NHS Bowel Cancer Screening Program: current perspectives on strategies for improvement. Risk Management and Healthcare Policy. 2017;Volume 10:177-87.
33. Shaukat A, Mongin SJ, Geisser MS, Lederle FA, Bond JH, Mandel JS, et al. Long-Term Mortality after Screening for Colorectal Cancer. New England Journal of Medicine. 2013;369(12):1106-14.
34. Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. Lancet. 1996;348(9040):1472-7.
35. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. The New England journal of medicine. 1993;328(19):1365-71.
36. Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. Lancet. 1996;348(9040):1467-71.
37. Kewenter J, Brevinge H, Engarás B, Haglind E, Ährén C. Results of Screening, Rescreening, and Follow-up in a Prospective Randomized Study for Detection of Colorectal Cancer by Fecal Occult Blood Testing: Results for 68,308 Subjects. Scandinavian Journal of Gastroenterology. 1994;29(5):468-73.

38. Towler B, Irwig L, Glasziou P, Kewenter J, Weller D, Silagy C. A systematic review of the effects of screening for colorectal cancer using the faecal occult blood test, Hemoccult. BMJ (Clinical research ed). 1998;317(7158):559-65.
39. Scholefield JH, Moss S, Sufi F, Mangham CM, Hardcastle JD. Effect of faecal occult blood screening on mortality from colorectal cancer: results from a randomised controlled trial. Gut. 2002;50(6):840-4.
40. Selby K, Jensen CD, Lee JK, Doubeni CA, Schottinger JE, Zhao WK, et al. Influence of Varying Quantitative Fecal Immunochemical Test Positivity Thresholds on Colorectal Cancer Detection. Annals of Internal Medicine. 2018;169(7):439-47.
41. Shaukat A, Rector TS, Church TR, Lederle FA, Kim AS, Rank JM, et al. Longer Withdrawal Time Is Associated With a Reduced Incidence of Interval Cancer After Screening Colonoscopy. Gastroenterology. 2015;149(4):952-7.
42. Shaukat A, Oancea C, Bond JH, Church TR, Allen JI. Variation in Detection of Adenomas and Polyps by Colonoscopy and Change Over Time With a Performance Improvement Program. Clinical Gastroenterology and Hepatology. 2009;7(12):1335-40.
43. Hernandez LV, Deas TM, Catalano MF, Guda NM, Huang L, Ketover SR, et al. Longitudinal assessment of colonoscopy quality indicators: a report from the Gastroenterology Practice Management Group. Gastrointestinal Endoscopy. 2014;80(5):835-41.
44. Senore C, Basu P, Anttila A, Ponti A, Tomatis M, Vale DB, et al. Performance of colorectal cancer screening in the European Union Member States: data from the second European screening report. Gut. 2019;68(7):1232-44.
45. Kaminski MF, Robertson DJ, Senore C, Rex DK. Optimizing the Quality of Colorectal Cancer Screening Worldwide. Gastroenterology. 2020;158(2):404-17.
46. Giorgi Rossi P, Vicentini M, Sacchettini C, Di Felice E, Caroli S, Ferrari F, et al. Impact of Screening Program on Incidence of Colorectal Cancer: A Cohort Study in Italy. The American journal of gastroenterology. 2015;110(9):1359-66.
47. Vicentini M, Zorzi M, Bovo E, Mancuso P, Zappa M, Manneschi G, et al. Impact of screening programme using the faecal immunochemical test on stage of colorectal cancer: Results from the IMPATTO study. International journal of cancer Journal international du cancer. 2019;145(1):110-21.
48. Ferlizza E, Solmi R, Sgarzi M, Ricciardiello L, Lauriola M. The Roadmap of Colorectal Cancer Screening. Cancers. 2021;13(5):1101.
49. Brenner H, Stock C, Hoffmeister M. Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ : British Medical Journal. 2014;348:g2467.
50. Grobbee EJ, van der Vlugt M, van Vuuren AJ, Stroobants AK, Mallant-Hent RC, Lansdorp-Vogelaar I, et al. Diagnostic Yield of One-Time Colonoscopy vs One-Time Flexible Sigmoidoscopy vs Multiple Rounds of Mailed Fecal Immunohistochemical Tests in Colorectal Cancer Screening. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2020;18(3):667-75.e1.
51. Holme Ø, Løberg M, Kalager M, Bretthauer M, Hernán MA, Aas E, et al. Long-Term Effectiveness of Sigmoidoscopy Screening on Colorectal Cancer Incidence and Mortality in Women and Men: A Randomized Trial. Ann Intern Med. 2018;168(11):775-82.
52. Jodal HC, Helsingen LM, Anderson JC, Lytvyn L, Vandvik PO, Emilsson L. Colorectal cancer screening with faecal testing, sigmoidoscopy or colonoscopy: a systematic review and network meta-analysis. BMJ Open. 2019;9(10):e032773.
53. Cairns SR, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, et al. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). Gut. 2010;59(5):666-89.
54. Rutter MD, East J, Rees CJ, Cripps N, Docherty J, Dolwani S, et al. British Society of Gastroenterology/Association of Coloproctology of Great Britain and Ireland/Public Health England

post-polypectomy and post-colorectal cancer resection surveillance guidelines. Gut. 2020;69(2):201-23.
55. Imperiale TF, Gruber RN, Stump TE, Emmett TW, Monahan PO. Performance Characteristics of Fecal Immunochemical Tests for Colorectal Cancer and Advanced Adenomatous Polyps: A Systematic Review and Meta-analysis. Ann Intern Med. 2019;170(5):319-29.
56. Gies A, Cuk K, Schrotz-King P, Brenner H. Direct Comparison of Diagnostic Performance of 9 Quantitative Fecal Immunochemical Tests for Colorectal Cancer Screening. Gastroenterology. 2018;154(1):93-104.
57. Cock C, Anwar S, Byrne SE, Meng R, Pedersen S, Fraser RJL, et al. Low Sensitivity of Fecal Immunochemical Tests and Blood-Based Markers of DNA Hypermethylation for Detection of Sessile Serrated Adenomas/Polyps. Digestive Diseases and Sciences. 2019;64(9):2555-62.
58. Church J. Molecular genetics of colorectal cancer. Seminars in Colon and Rectal Surgery. 2016;27(4):172-5.
59. Kastrinos F, Syngal S. Inherited Colorectal Cancer Syndromes. The Cancer Journal. 2011;17(6):405-15.
60. Fearnhead NS, Wilding JL, Bodmer WF. Genetics of colorectal cancer: hereditary aspects and overview of colorectal tumorigenesis. British Medical Bulletin. 2002;64(1):27-43.
61. Bogaert J, Prenen H. Molecular genetics of colorectal cancer. Ann Gastroenterol. 2014;27(1):9-14.
62. De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The Molecular Hallmarks of the Serrated Pathway in Colorectal Cancer. Cancers. 2019;11(7):1017.
63. Yamagishi H, Kuroda H, Imai Y, Hiraishi H. Molecular pathogenesis of sporadic colorectal cancers. Chinese Journal of Cancer. 2016;35(1).
64. Parmar S, Easwaran H. Genetic and epigenetic dependencies in colorectal cancer development. Gastroenterol Rep (Oxf). 2022;10:goac035.
65. Bardhan K, Liu K. Epigenetics and colorectal cancer pathogenesis. Cancers (Basel). 2013;5(2):676-713.
66. Muzny DM, Bainbridge MN, Chang K, Dinh HH, Drummond JA, Fowler G, et al. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(7407):330-7.
67. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nature medicine. 2015;21(11):1350-6.
68. Singh MP, Rai S, Pandey A, Singh NK, Srivastava S. Molecular subtypes of colorectal cancer: An emerging therapeutic opportunity for personalized medicine. Genes Dis. 2021;8(2):133-45.
69. Sawayama H, Miyamoto Y, Ogawa K, Yoshida N, Baba H. Investigation of colorectal cancer in accordance with consensus molecular subtype classification. Ann Gastroenterol Surg. 2020;4(5):528-39.
70. Jass JR. Molecular heterogeneity of colorectal cancer: Implications for cancer control. Surg Oncol. 2007;16 Suppl 1:S7-9.
71. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007;50(1):113-30.
72. Porter RJ, Arends MJ, Churchhouse AMD, Din S. Inflammatory Bowel Disease-Associated Colorectal Cancer: Translational Risks from Mechanisms to Medicines. J Crohns Colitis. 2021;15(12):2131-41.
73. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61(5):759-67.
74. Baran B, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference Between Left-Sided and Right-Sided Colorectal Cancer: A Focused Review of Literature. Gastroenterology Res. 2018;11(4):264-73.
75. Kasi A, Handa S, Bhatti S, Umar S, Bansal A, Sun W. Molecular Pathogenesis and Classification of Colorectal Carcinoma. Curr Colorectal Cancer Rep. 2020;16(5):97-106.

76. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer. 2009;9(7):489-99.
77. Vogelstein B, Kinzler KW. The Path to Cancer — Three Strikes and You're Out. New England Journal of Medicine. 2015;373(20):1895-8.
78. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic Alterations during Colorectal-Tumor Development. New England Journal of Medicine. 1988;319(9):525-32.
79. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene. 2017;36(11):1461-73.
80. Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol. 2016;99:141-9.
81. Bettington M, Walker N, Clouston A, Brown I, Leggett B, Whitehall V. The serrated pathway to colorectal carcinoma: current concepts and challenges. Histopathology. 2013;62(3):367-86.
82. Murcia O, Juárez M, Hernández-Illán E, Egoavil C, Giner-Calabuig M, Rodríguez-Soler M, et al. Serrated colorectal cancer: Molecular classification, prognosis, and response to chemotherapy. World Journal of Gastroenterology. 2016;22(13):3516.
83. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005;65(14):6063-9.
84. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nature Reviews Genetics. 2006;7(8):606-19.
85. Danielsen SA, Eide PW, Nesbakken A, Guren T, Leithe E, Lothe RA. Portrait of the PI3K/AKT pathway in colorectal cancer. Biochim Biophys Acta. 2015;1855(1):104-21.
86. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. Cancers (Basel). 2017;9(5).
87. Jung B, Staudacher JJ, Beauchamp D. Transforming Growth Factor β Superfamily Signaling in Development of Colorectal Cancer. Gastroenterology. 2017;152(1):36-52.
88. Itatani Y, Kawada K, Sakai Y. Transforming Growth Factor-β Signaling Pathway in Colorectal Cancer and Its Tumor Microenvironment. Int J Mol Sci. 2019;20(23).
89. Liebl MC, Hofmann TG. The Role of p53 Signaling in Colorectal Cancer. Cancers (Basel). 2021;13(9).
90. Farooqi AA, de la Roche M, Djamgoz MBA, Siddik ZH. Overview of the oncogenic signaling pathways in colorectal cancer: Mechanistic insights. Semin Cancer Biol. 2019;58:65-79.
91. Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. 1990;50(23):7717-22.
92. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. The British journal of surgery. 2002;89(7):845-60.
93. Shia J. The diversity of tumours with microsatellite instability: molecular mechanisms and impact upon microsatellite instability testing and mismatch repair protein immunohistochemistry. Histopathology. 2021;78(4):485-97.
94. Seeman NC. DNA in a material world. Nature. 2003;421(6921):427-31.
95. Bateman AC. DNA mismatch repair proteins: scientific update and practical guide. Journal of Clinical Pathology. 2021;74(4):264-8.
96. Cerretelli G, Ager A, Arends MJ, Frayling IM. Molecular pathology of Lynch syndrome. J Pathol. 2020;250(5):518-31.
97. East JE, Atkin WS, Bateman AC, Clark SK, Dolwani S, Ket SN, et al. British Society of Gastroenterology position statement on serrated polyps in the colon and rectum. Gut. 2017;66(7):1181-96.
98. Toyota M, Ho C, Ahuja N, Jair KW, Li Q, Ohe-Toyota M, et al. Identification of differentially methylated sequences in colorectal cancer by methylated CpG island amplification. Cancer Res. 1999;59(10):2307-12.

99. Lee MS, Menter DG, Kopetz S. Right Versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes. Journal of the National Comprehensive Cancer Network : JNCCN. 2017;15(3):411-9.
100. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann Intern Med. 1990;113(10):779-88.
101. Iacopetta B. Are there two sides to colorectal cancer? International journal of cancer Journal international du cancer. 2002;101(5):403-8.
102. Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? - a systematic review. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2015;41(3):300-8.
103. Menigatti M, Truninger K, Gebbers JO, Marbet U, Marra G, Schär P. Normal colorectal mucosa exhibits sex- and segment-specific susceptibility to DNA methylation at the hMLH1 and MGMT promoters. Oncogene. 2009;28(6):899-909.
104. Nawa T, Kato J, Kawamoto H, Okada H, Yamamoto H, Kohno H, et al. Differences between right- and left-sided colon cancer in patient characteristics, cancer morphology and histology. J Gastroenterol Hepatol. 2008;23(3):418-23.
105. Gualco G, Reissenweber N, Cliché I, Bacchi CE. Flat elevated lesions of the colon and rectum: a spectrum of neoplastic and nonneoplastic entities. Ann Diagn Pathol. 2006;10(6):333-8.
106. Okamoto M, Kawabe T, Yamaji Y, Kato J, Ikenoue T, Togo G, et al. Flat-type early colorectal cancer preferentially develops in right-sided colon in older patients. Diseases of the colon and rectum. 2005;48(1):101-7.
107. Benedix F, Kube R, Meyer F, Schmidt U, Gastinger I, Lippert H. Comparison of 17,641 patients with right- and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. Diseases of the colon and rectum. 2010;53(1):57-64.
108. Passardi A, Canale M, Valgiusti M, Ulivi P. Immune Checkpoints as a Target for Colorectal Cancer Treatment. Int J Mol Sci. 2017;18(6).
109. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467-80.
110. Vasen HF, Tomlinson I, Castells A. Clinical management of hereditary colorectal cancer syndromes. Nature reviews Gastroenterology & hepatology. 2015;12(2):88-97.
111. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. The New England journal of medicine. 2003;348(10):919-32.
112. Sinicrope FA. Lynch Syndrome-Associated Colorectal Cancer. The New England journal of medicine. 2018;379(8):764-73.
113. Tiwari AK, Roy HK, Lynch HT. Lynch syndrome in the 21st century: clinical perspectives. Qjm. 2016;109(3):151-8.
114. Miller CA, Barnes AJ, Fuemmeler BF, Thomson MD. Colorectal cancer lifetime risk accuracy and behavior change intentions before and after risk assessment. Cancer Causes & Control. 2021;32(4):423-8.
115. Emilsson L, Holme Ø, Bretthauer M, Cook NR, Buring JE, Løberg M, et al. Systematic review with meta-analysis: the comparative effectiveness of aspirin vs. screening for colorectal cancer prevention. Alimentary Pharmacology & Therapeutics. 2017;45(2):193-204.
116. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology. 2005;129(3):837-45.
117. Longacre TA, Fenoglio-Preiser CM. Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. The American journal of surgical pathology. 1990;14(6):524-37.
118. Baretton GB, Aust DE. Sessile Serrated Adenomas: Possible Precursors of So-called Interval Colorectal Cancers? Endoskopie Heute. 2009;22(1):17-21.

119. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182-8.
120. Nazemalhosseini Mojarad E, Kuppen PJ, Aghdaei HA, Zali MR. The CpG island methylator phenotype (CIMP) in colorectal cancer. Gastroenterol Hepatol Bed Bench. 2013;6(3):120-8.
121. Satorres C, Garcia-Campos M, Bustamante-Balen M. Molecular Features of the Serrated Pathway to Colorectal Cancer: Current Knowledge and Future Directions. Gut Liver. 2021;15(1):31-43.
122. Mezzapesa M, Losurdo G, Celiberto F, Rizzi S, D'Amati A, Piscitelli D, et al. Serrated Colorectal Lesions: An Up-to-Date Review from Histological Pattern to Molecular Pathogenesis. International Journal of Molecular Sciences. 2022;23(8):4461.
123. Snover DC. Update on the serrated pathway to colorectal carcinoma. Human pathology. 2011;42(1):1-10.
124. Carr NJ, Mahajan H, Tan KL, Hawkins NJ, Ward RL. Serrated and non-serrated polyps of the colorectum: their prevalence in an unselected case series and correlation of BRAF mutation analysis with the diagnosis of sessile serrated adenoma. Journal of Clinical Pathology. 2009;62(6):516-8.
125. Fu X, Li L, Peng Y. Wnt signalling pathway in the serrated neoplastic pathway of the colorectum: possible roles and epigenetic regulatory mechanisms. Journal of Clinical Pathology. 2012;65(8):675-9.
126. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature. 2002;418(6901):934.
127. Bettington M, Walker N, Rosty C, Brown I, Clouston A, McKeone D, et al. Clinicopathological and molecular features of sessile serrated adenomas with dysplasia or carcinoma. Gut. 2017;66(1):97-106.
128. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet. 2006;38(7):787-93.
129. Pai RK, Bettington M, Srivastava A, Rosty C. An update on the morphology and molecular pathology of serrated colorectal polyps and associated carcinomas. Modern Pathology. 2019;32(10):1390-415.
130. O'Brien MJMDMPH, Yang SMD, Mack CBS, Xu HMD, Huang CSMD, Mulcahy EMD, et al. Comparison of Microsatellite Instability, CpG Island Methylation Phenotype, BRAF and KRAS Status in Serrated Polyps and Traditional Adenomas Indicates Separate Pathways to Distinct Colorectal Carcinoma End Points. American Journal of Surgical Pathology. 2006;30(12):1491-501.
131. Yang SMD, Farraye FAMDMSC, Mack CBS, Posnik OMD, O'Brien MJMDMPH. BRAF and KRAS Mutations in Hyperplastic Polyps and Serrated Adenomas of the Colorectum: Relationship to Histology and CpG Island Methylation Status. American Journal of Surgical Pathology. 2004;28(11):1452-9.
132. Kim JH. Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. World Journal of Gastroenterology. 2014;20(15):4230.
133. Murakami T, Akazawa Y, Yatagai N, Hiromoto T, Sasahara N, Saito T, et al. Molecular characterization of sessile serrated adenoma/polyps with dysplasia/carcinoma based on immunohistochemistry, next-generation sequencing, and microsatellite instability testing: a case series study. Diagnostic pathology. 2018;13(1).
134. Lee JA, Park HE, Yoo S-Y, Jeong S, Cho N-Y, Kang GH, et al. CpG Island Methylation in Sessile Serrated Adenoma/Polyp of the Colorectum: Implications for Differential Diagnosis of Molecularly High-Risk Lesions among Non-dysplastic Sessile Serrated Adenomas/Polyps. Journal of Pathology and Translational Medicine. 2019;53(4):225-35.
135. Bettington M, Brown I, Rosty C, Walker N, Liu C, Croese J, et al. Sessile Serrated Adenomas in Young Patients may have Limited Risk of Malignant Progression. J Clin Gastroenterol. 2019;53(3):e113-e6.

136. Hashimoto T, Yamashita S, Yoshida H, Taniguchi H, Ushijima T, Yamada T, et al. WNT Pathway Gene Mutations Are Associated With the Presence of Dysplasia in Colorectal Sessile Serrated Adenoma/Polyps. The American journal of surgical pathology. 2017;41(9):1188-97.
137. Bond CE, McKeone DM, Kalimutho M, Bettington ML, Pearson SA, Dumenil TD, et al. RNF43 and ZNRF3 are commonly altered in serrated pathway colorectal tumorigenesis. Oncotarget. 2016;7(43):70589-600.
138. Koinuma K, Yamashita Y, Liu W, Hatanaka H, Kurashina K, Wada T, et al. Epigenetic silencing of AXIN2 in colorectal carcinoma with microsatellite instability. Oncogene. 2006;25(1):139-46.
139. Bettington ML, Walker NI, Rosty C, Brown IS, Clouston AD, McKeone DM, et al. A clinicopathological and molecular analysis of 200 traditional serrated adenomas. Mod Pathol. 2015;28(3):414-27.
140. Tsai J-H, Liau J-Y, Lin Y-L, Lin L-I, Cheng Y-C, Cheng M-L, et al. Traditional serrated adenoma has two pathways of neoplastic progression that are distinct from the sessile serrated pathway of colorectal carcinogenesis. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2014;27(10):1375-85.
141. Sekine S, Yamashita S, Tanabe T, Hashimoto T, Yoshida H, Taniguchi H, et al. Frequent PTPRK-RSPO3 fusions and RNF43 mutations in colorectal traditional serrated adenoma. J Pathol. 2016;239(2):133-8.
142. Sekine S, Ogawa R, Hashimoto T, Motohiro K, Yoshida H, Taniguchi H, et al. Comprehensive characterization of RSPO fusions in colorectal traditional serrated adenomas. Histopathology. 2017;71(4):601-9.
143. Tsai JH, Liau JY, Yuan CT, Lin YL, Tseng LH, Cheng ML, et al. RNF43 Is an Early and Specific Mutated Gene in the Serrated Pathway, With Increased Frequency in Traditional Serrated Adenoma and Its Associated Malignancy. The American journal of surgical pathology. 2016;40(10):1352-9.
144. Sohier P, Sanson R, Leduc M, Audebourg A, Broussard C, Salnot V, et al. Proteome analysis of formalin-fixed paraffin-embedded colorectal adenomas reveals the heterogeneous nature of traditional serrated adenomas compared to other colorectal adenomas. J Pathol. 2020;250(3):251-61.
145. Kim JH, Kang GH. Evolving pathologic concepts of serrated lesions of the colorectum. J Pathol Transl Med. 2020;54(4):276-89.
146. Phipps AI, Limburg PJ, Baron JA, Burnett-Hartman AN, Weisenberger DJ, Laird PW, et al. Association Between Molecular Subtypes of Colorectal Cancer and Patient Survival. Gastroenterology. 2015;148(1):77-87.e2.
147. Phipps AI, Alwers E, Harrison T, Banbury B, Brenner H, Campbell PT, et al. Association Between Molecular Subtypes of Colorectal Tumors and Patient Survival, Based on Pooled Analysis of 7 International Studies. Gastroenterology. 2020;158(8):2158-68.e4.
148. Kim JH, Bae JM, Cho NY, Kang GH. Distinct features between MLH1-methylated and unmethylated colorectal carcinomas with the CpG island methylator phenotype: implications in the serrated neoplasia pathway. Oncotarget. 2016;7(12):14095-111.
149. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. 2013;24(6):1207-22.
150. Bailie L, Loughrey MB, Coleman HG. Lifestyle Risk Factors for Serrated Colorectal Polyps: A Systematic Review and Meta-analysis. Gastroenterology. 2017;152(1):92-104.
151. Williams GT, Arthur JF, Bussey HJR, Morson BC. Metaplastic polyps and polyposis of the colorectum. Histopathology. 1980;4(2):155-70.
152. Torlakovic E, Snover DC. Serrated adenomatous polyposis in humans. Gastroenterology. 1996;110(3):748-55.
153. Bosman FTC, F. ; Hruban, R. H. ; Theise, N. D. WHO classification of tumours of the digestive system 2010 No.Ed. 4 pp.417 pp. ref.3728. Switzerland: World Health Organization; 2010.
154. Rouphael C, Macaron C, Burke CA. Serrated Polyposis Syndrome: an Update and Review of the Literature. Current Treatment Options in Gastroenterology. 2021;19(2):253-65.

155. Ijspeert JEG, Bevan R, Senore C, Kaminski MF, Kuipers EJ, Mroz A, et al. Detection rate of serrated polyps and serrated polyposis syndrome in colorectal cancer screening cohorts: a European overview. Gut. 2017;66(7):1225-32.
156. Rivero-Sanchez L, Lopez-Ceron M, Carballal S, Moreira L, Bessa X, Serradesanferm A, et al. Reassessment colonoscopy to diagnose serrated polyposis syndrome in a colorectal cancer screening population. Endoscopy. 2017;49(1):44-53.
157. Pohl J, Schneider A, Vogell H, Mayer G, Kaiser G, Ell C. Pancolonic chromoendoscopy with indigo carmine versus standard colonoscopy for detection of neoplastic lesions: a randomised two-centre trial. Gut. 2011;60(4):485-90.
158. Kokko A, Laiho P, Lehtonen R, Korja S, Carvajal-Carmona LG, Jarvinen H, et al. EPHB2 germline variants in patients with colorectal cancer or hyperplastic polyposis. BMC Cancer. 2006;6:145.
159. Quintana I, Mejías-Luque R, Terradas M, Navarro M, Piñol V, Mur P, et al. Evidence suggests that germline RNF43 mutations are a rare cause of serrated polyposis. Gut. 2018;67(12):2230-2.
160. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG). Gut. 2020;69(3):411-44.
161. Weisenberger DJ, Levine AJ, Long TI, Buchanan DD, Walters R, Clendenning M, et al. Association of the Colorectal CpG Island Methylator Phenotype with Molecular Features, Risk Factors, and Family History. Cancer Epidemiology Biomarkers & Prevention. 2015;24(3):512-9.
162. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, et al. Serrated lesions of the colorectum: review and recommendations from an expert panel. The American journal of gastroenterology. 2012;107(9):1315-29; quiz 4, 30.
163. Anderson. Risk Factors for Sessile Serrated Adenomas. Journal of Clinical Gastroenterology. 2011;45(8):694.
164. Rustagi T, Anderson J. Sessile Serrated Adenomas in the Proximal Colon are Likely to be Flat, Large and Occur in Smokers. American Journal of Gastroenterology. 2012;107:S814-S5.
165. Walker RG, Landmann JK, Hewett DG, Worthley DL, Buttenshaw RL, Knight N, et al. Hyperplastic polyposis syndrome is associated with cigarette smoking, which may be a modifiable risk factor. The American journal of gastroenterology. 2010;105(7):1642-7.
166. Davenport JR, Su T, Zhao Z, Coleman HG, Smalley WE, Ness RM, et al. Modifiable lifestyle factors associated with risk of sessile serrated polyps, conventional adenomas and hyperplastic polyps. Gut. 2018;67(3):456-65.
167. Haque TR, Bradshaw PT, Crockett SD. Risk Factors for Serrated Polyps of the Colorectum. Digestive Diseases and Sciences. 2014;59(12):2874-89.
168. Liang PS, Chen T-Y, Giovannucci E. Cigarette smoking and colorectal cancer incidence and mortality: Systematic review and meta-analysis. International Journal of Cancer. 2009;124(10):2406-15.
169. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, et al. Cigarette Smoking and Colorectal Cancer Risk by Molecularly Defined Subtypes. JNCI: Journal of the National Cancer Institute. 2010;102(14):1012-22.
170. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. JAMA : the journal of the American Medical Association. 2004;291(5):585-90.
171. Wallace K, Grau MV, Ahnen D, Snover DC, Robertson DJ, Mahnke D, et al. The association of lifestyle and dietary factors with the risk for serrated polyps of the colorectum. Cancer Epidemiol Biomarkers Prev. 2009;18(8):2310-7.
172. Anderson JCMD, Rangasamy PMD, Rustagi TMD, Myers MMD, Sanders MMD, Vaziri HMD, et al. Risk Factors for Sessile Serrated Adenomas. Journal of Clinical Gastroenterology. 2011;45(8):694-9.

173. Song M, Chan AT, Sun J. Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer. Gastroenterology. 2020;158(2):322-40.
174. Yu JH, Chen YY, Fu XS, Zhou X, Peng Y, Shi L, et al. Invasive Fusobacterium nucleatum may play a role in the carcinogenesis of proximal colon cancer through the serrated neoplasia pathway. International Journal of Cancer. 2016;139(6):1318-26.
175. Ito M, Kanno S, Nosho K, Sukawa Y, Mitsuhashi K, Kurihara H, et al. Association ofFusobacterium nucleatumwith clinical and molecular features in colorectal serrated pathway. International Journal of Cancer. 2015;137(6):1258-68.
176. Parian AM, Lazarev MG. Serrated Colorectal Lesions in Patients With Inflammatory Bowel Disease. Gastroenterology & hepatology. 2018;14(1):19-25.
177. Brcic I, Dawson H, Gröchenig HP, Högenauer C, Kashofer K. Serrated Lesions in Inflammatory Bowel Disease: Genotype-Phenotype Correlation. International Journal of Surgical Pathology. 2021;29(1):46-53.
178. Ko HM, Harpaz N, McBride RB, Cui M, Ye F, Zhang D, et al. Serrated colorectal polyps in inflammatory bowel disease. Mod Pathol. 2015;28(12):1584-93.
179. East JE, Vleugels JL, Roelandt P, Bhandari P, Bisschops R, Dekker E, et al. Advanced endoscopic imaging: European Society of Gastrointestinal Endoscopy (ESGE) Technology Review. Endoscopy. 2016;48(11):1029-45.
180. Tada M, Katoh S, Kohli Y, Kawai K. On the dye spraying method in colonofiberscopy. Endoscopy. 1977;8(2):70-4.
181. Machida H, Sano Y, Hamamoto Y, Muto M, Kozu T, Tajiri H, et al. Narrow-Band Imaging in the Diagnosis of Colorectal Mucosal Lesions: A Pilot Study. Endoscopy. 2004;36(12):1094-8.
182. JE IJ, Bastiaansen BA, van Leerdam ME, Meijer GA, van Eeden S, Sanduleanu S, et al. Development and validation of the WASP classification system for optical diagnosis of adenomas, hyperplastic polyps and sessile serrated adenomas/polyps. Gut. 2016;65(6):963-70.
183. Hazewinkel Y, López-Cerón M, East JE, Rastogi A, Pellisé M, Nakajima T, et al. Endoscopic features of sessile serrated adenomas: validation by international experts using high-resolution white-light endoscopy and narrow-band imaging. Gastrointest Endosc. 2013;77(6):916-24.
184. Sano Y, Ikematsu H, Fu KI, Emura F, Katagiri A, Horimatsu T, et al. Meshed capillary vessels by use of narrow-band imaging for differential diagnosis of small colorectal polyps. Gastrointest Endosc. 2009;69(2):278-83.
185. Tadepalli US, Feihel D, Miller KM, Itzkowitz SH, Freedman JS, Kornacki S, et al. A morphologic analysis of sessile serrated polyps observed during routine colonoscopy (with video). Gastrointest Endosc. 2011;74(6):1360-8.
186. Nakao Y, Saito S, Ohya T, Aihara H, Arihiro S, Kato T, et al. Endoscopic features of colorectal serrated lesions using image-enhanced endoscopy with pathological analysis. Eur J Gastroenterol Hepatol. 2013;25(8):981-8.
187. Yamada M, Sakamoto T, Otake Y, Nakajima T, Kuchiba A, Taniguchi H, et al. Investigating endoscopic features of sessile serrated adenomas/polyps by using narrow-band imaging with optical magnification. Gastrointest Endosc. 2015;82(1):108-17.
188. Yamashina T, Uedo N. Diagnosis of sessile serrated adenoma/polyp with magnifying narrow band imaging. Journal of Gastroenterology & Hepatology. 2012;27 Supplement(4):48-9.
189. Kimura T, Yamamoto E, Yamano HO, Suzuki H, Kamimae S, Nojima M, et al. A novel pit pattern identifies the precursor of colorectal cancer derived from sessile serrated adenoma. The American journal of gastroenterology. 2012;107(3):460-9.
190. Tate DJ, Jayanna M, Awadie H, Desomer L, Lee R, Heitman SJ, et al. A standardized imaging protocol for the endoscopic prediction of dysplasia within sessile serrated polyps (with video). Gastrointest Endosc. 2018;87(1):222-31.e2.
191. Sano W, Fujimori T, Ichikawa K, Sunakawa H, Utsumi T, Iwatate M, et al. Clinical and endoscopic evaluations of sessile serrated adenoma/polyps with cytological dysplasia. J Gastroenterol Hepatol. 2018;33(8):1454-60.

192. Burgess NG, Pellise M, Nanda KS, Hourigan LF, Zanati SA, Brown GJ, et al. Clinical and endoscopic predictors of cytological dysplasia or cancer in a prospective multicentre study of large sessile serrated adenomas/polyps. Gut. 2016;65(3):437-46.
193. Murakami T, Sakamoto N, Nagahara A. Endoscopic diagnosis of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. World journal of gastroenterology : WJG. 2018;24(29):3250-9.
194. Tanaka Y, Yamano HO, Yamamoto E, Matushita HO, Aoki H, Yoshikawa K, et al. Endoscopic and molecular characterization of colorectal sessile serrated adenoma/polyps with cytologic dysplasia. Gastrointest Endosc. 2017;86(6):1131-8.e4.
195. Sano Y, Saito Y, Fu K-I, Matsuda T, Uraoka T, Kobayashi N, et al. Efficacy of magnifying chromoendoscopy for the differential diagnosis of colorectal lesions. Digestive Endoscopy. 2005;17(2):105-16.
196. Sano W, Hirata D, Teramoto A, Iwatate M, Hattori S, Fujita M, et al. Serrated polyps of the colon and rectum: Remove or not? World journal of gastroenterology. 2020;26(19):2276-85.
197. East JE, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps & serrated adenomas of the colon: Classification, molecular genetics, natural history, and clinical management. Gastroenterology clinics of North America. 2008;37(1):25-+.
198. Huang CS, Farraye FA, Yang S, O'Brien MJ. The clinical significance of serrated polyps. The American journal of gastroenterology. 2011;106(2):229-40; quiz 41.
199. Leonard DF, Dozois EJ, Smyrk TC, Suwanthanma W, Baron TH, Cima RR, et al. Endoscopic and surgical management of serrated colonic polyps. British Journal of Surgery. 2011;98(12):1685-94.
200. Gupta V, East JE. Optimal Endoscopic Treatment and Surveillance of Serrated Polyps. Gut Liver. 2020;14(4):423-9.
201. Hassan C, Quintero E, Dumonceau JM, Regula J, Brandão C, Chaussade S, et al. Post-polypectomy colonoscopy surveillance: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2013;45(10):842-51.
202. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR, et al. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. Gastroenterology. 2012;143(3):844-57.
203. Rex DK, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, et al. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. Gastroenterology. 1997;112(1):24-8.
204. Anderson R, Burr NE, Valori R. Causes of Post-Colonoscopy Colorectal Cancers Based on World Endoscopy Organization System of Analysis. Gastroenterology. 2020;158(5):1287-99.e2.
205. Bisschops R, East JE, Hassan C, Hazewinkel Y, Kamiński MF, Neumann H, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline – Update 2019. Endoscopy. 2019;51(12):1155-79.
206. Hassan C, Spadaccini M, Iannone A, Maselli R, Jovani M, Chandrasekar VT, et al. Performance of artificial intelligence in colonoscopy for adenoma and polyp detection: a systematic review and meta-analysis. Gastrointest Endosc. 2021;93(1):77-85.e6.
207. Repici A, Badalamenti M, Maselli R, Correale L, Radaelli F, Rondonotti E, et al. Efficacy of Real-Time Computer-Aided Detection of Colorectal Neoplasia in a Randomized Trial. Gastroenterology. 2020;159(2):512-20.e7.
208. Wang P, Liu X, Berzin TM, Glissen Brown JR, Liu P, Zhou C, et al. Effect of a deep-learning computer-aided detection system on adenoma detection during colonoscopy (CADe-DB trial): a double-blind randomised study. Lancet Gastroenterol Hepatol. 2020;5(4):343-51.
209. Liu WN, Zhang YY, Bian XQ, Wang LJ, Yang Q, Zhang XD, et al. Study on detection rate of polyps and adenomas in artificial-intelligence-aided colonoscopy. Saudi J Gastroenterol. 2020;26(1):13-9.
210. Su JR, Li Z, Shao XJ, Ji CR, Ji R, Zhou RC, et al. Impact of a real-time automatic quality control system on colorectal polyp and adenoma detection: a prospective randomized controlled study (with videos). Gastrointest Endosc. 2020;91(2):415-24.e4.

211. Wang P, Berzin TM, Glissen Brown JR, Bharadwaj S, Becq A, Xiao X, et al. Real-time automatic detection system increases colonoscopic polyp and adenoma detection rates: a prospective randomised controlled study. Gut. 2019;68(10):1813-9.
212. Sanduleanu S, le Clercq CMC, Dekker E, Meijer GA, Rabeneck L, Rutter MD, et al. Definition and taxonomy of interval colorectal cancers: a proposal for standardising nomenclature. Gut. 2015;64(8):1257.
213. Heigh RI, Yab TC, Taylor WR, Hussain FT, Smyrk TC, Mahoney DW, et al. Detection of colorectal serrated polyps by stool DNA testing: comparison with fecal immunochemical testing for occult blood (FIT). PLoS One. 2014;9(1):e85659.
214. Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T, Shiratori Y. A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. Gastroenterology. 2005;129(2):422-8.
215. Park DI, Ryu S, Kim YH, Lee SH, Lee CK, Eun CS, et al. Comparison of guaiac-based and quantitative immunochemical fecal occult blood testing in a population at average risk undergoing colorectal cancer screening. The American journal of gastroenterology. 2010;105(9):2017-25.
216. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. New England Journal of Medicine. 2014;370(14):1287-97.
217. Zorzi M, Senore C, Da Re F, Barca A, Bonelli LA, Cannizzaro R, et al. Detection rate and predictive factors of sessile serrated polyps in an organised colorectal cancer screening programme with immunochemical faecal occult blood test: the EQuIPE study (Evaluating Quality Indicators of the Performance of Endoscopy). Gut. 2017;66(7):1233-40.
218. Lash RH, Genta RM, Schuler CM. Sessile serrated adenomas: prevalence of dysplasia and carcinoma in 2139 patients. Journal of Clinical Pathology. 2010;63(8):681-6.
219. Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, et al. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. Gastroenterology. 2006;131(5):1400-7.
220. Higuchi T, Sugihara K, Jass JR. Demographic and pathological characteristics of serrated polyps of colorectum. Histopathology. 2005;47(1):32-40.
221. Payne SR, Church TR, Wandell M, Rosch T, Osborn N, Snover D, et al. Endoscopic detection of proximal serrated lesions and pathologic identification of sessile serrated adenomas/polyps vary on the basis of center. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(7):1119-26.
222. Hetzel JT, Huang CS, Coukos JA, Omstead K, Cerda SR, Yang S, et al. Variation in the detection of serrated polyps in an average risk colorectal cancer screening cohort. The American journal of gastroenterology. 2010;105(12):2656-64.
223. Kahi CJ, Li XC, Eckert GJ, Rex DK. High colonoscopic prevalence of proximal colon serrated polyps in average-risk men and women. Gastrointestinal Endoscopy. 2012;75(3):515-20.
224. East JE, Vieth M, Rex DK. Serrated lesions in colorectal cancer screening: detection, resection, pathology and surveillance. Gut. 2015;64(6):991-1000.
225. de Wijkerslooth TR, Stoop EM, Bossuyt PM, Tytgat KM, Dees J, Mathus-Vliegen EM, et al. Differences in proximal serrated polyp detection among endoscopists are associated with variability in withdrawal time. Gastrointest Endosc. 2013;77(4):617-23.
226. Abdeljawad K, Vemulapalli KC, Kahi CJ, Cummings OW, Snover DC, Rex DK. Sessile serrated polyp prevalence determined by a colonoscopist with a high lesion detection rate and an experienced pathologist. Gastrointest Endosc. 2015;81(3):517-24.
227. Alvarez C, Andreu M, Castells A, Quintero E, Bujanda L, Cubiella J, et al. Relationship of colonoscopy-detected serrated polyps with synchronous advanced neoplasia in average-risk individuals. Gastrointestinal Endoscopy. 2013;78(2):333-+.

228. Pullens B, Dekker E, Ellis AJ, Guy R, Madronal K, Chetty R, et al. Impact of the consideration of serrated polyps to the interval of colonoscopic surveillance in the NHS Bowel Cancer Screening Programme. Colorectal Dis. 2014.
229. Anderson JC, Butterly LF, Goodrich M, Robinson CM, Weiss JE. Differences in Detection Rates of Adenomas and Serrated Polyps in Screening Versus Surveillance Colonoscopies, Based on the New Hampshire Colonoscopy Registry. Clinical Gastroenterology and Hepatology. 2013;11(10):1308-12.
230. Lee CK, Kim YW, Shim J-J, Jang JY. Prevalence of Proximal Serrated Polyps and Conventional Adenomas in an Asymptomatic Average-Risk Screening Population. Gut Liver. 2013;7(5):524-31.
231. Leung WK, Tang V, Lui PCW. Detection rates of proximal or large serrated polyps in Chinese patients undergoing screening colonoscopy. Journal of Digestive Diseases. 2012;13(9):466-71.
232. Min YW, Lee JH, Lee SH, Park DI, Han DS, Rhee PL, et al. Prevalence of proximal colon serrated polyps in a population at average risk undergoing screening colonoscopy: a multicenter study. Clin Res Hepatol Gastroenterol. 2012;36(6):604-8.
233. Hazewinkel Y, de Wijkerslooth TR, Stoop EM, Bossuyt PM, Biermann K, van de Vijver MJ, et al. Prevalence of serrated polyps and association with synchronous advanced neoplasia in screening colonoscopy. Endoscopy. 2013.
234. Buda A, De Bona M, Dotti I, Piselli P, Zabeo E, Barbazza R, et al. Prevalence of different subtypes of serrated polyps and risk of synchronous advanced colorectal neoplasia in average-risk population undergoing first-time colonoscopy. Clin Transl Gastroenterol. 2012;3(1):e6.
235. Subramanian V, Mannath J, Hawkey CJ, Ragunath K. High definition colonoscopy vs. standard video endoscopy for the detection of colonic polyps: a meta-analysis. Endoscopy. 2011;43(6):499-505.
236. East JE, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. Gastroenterology clinics of North America. 2008;37(1):25-46, v.
237. Erichsen R, Baron JA, Hamilton-Dutoit SJ, Snover DC, Torlakovic EE, Pedersen L, et al. Increased Risk of Colorectal Cancer Development Among Patients With Serrated Polyps. Gastroenterology. 2016;150(4):895-902 e5.
238. Hazewinkel Y, de Wijkerslooth TR, Stoop EM, Bossuyt PM, Biermann K, van de Vijver MJ, et al. Prevalence of serrated polyps and association with synchronous advanced neoplasia in screening colonoscopy. Endoscopy. 2014;46(3):219-24.
239. Hiraoka S, Kato J, Fujiki S, Kaji E, Morikawa T, Murakami T, et al. The presence of large serrated polyps increases risk for colorectal cancer. Gastroenterology. 2010;139(5):1503-10, 10.e1-3.
240. Kahi CJ, Li X, Eckert GJ, Rex DK. High colonoscopic prevalence of proximal colon serrated polyps in average-risk men and women. Gastrointest Endosc. 2012;75(3):515-20.
241. Li DMD, Jin CP, McCulloch CP, Kakar SMD, Berger BMMD, Imperiale TFMD, et al. Association of Large Serrated Polyps With Synchronous Advanced Colorectal Neoplasia. American Journal of Gastroenterology. 2009;104(3):695-702.
242. Gao Q, Tsoi KK, Hirai HW, Wong MC, Chan FK, Wu JC, et al. Serrated polyps and the risk of synchronous colorectal advanced neoplasia: a systematic review and meta-analysis. The American journal of gastroenterology. 2015;110(4):501-9; quiz 10.
243. Lee LH, Iacucci M, Fort Gasia M, Ghosh S, Panaccione R, Urbanski S. Prevalence and Anatomic Distribution of Serrated and Adenomatous Lesions in Patients with Inflammatory Bowel Disease. Canadian Journal of Gastroenterology and Hepatology. 2017;2017:1-8.
244. Biswas S, Ellis AJ, Guy R, Savage H, Madronal K, East JE. High prevalence of hyperplastic polyposis syndrome (serrated polyposis) in the NHS bowel cancer screening programme. Gut. 2013;62(3):475-.
245. Moreira L, Pellise M, Carballal S, Bessa X, Ocana T, Serradesanferm A, et al. High prevalence of serrated polyposis syndrome in FIT-based colorectal cancer screening programmes. Gut. 2013;62(3):476-7.

246. Atkin WS, Edwards R, Kralj-Hans I, Wooldrage K, Hart AR, Northover JM, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. Lancet. 2010;375(9726):1624-33.
247. Schoen RE. Interrupting the adenoma-carcinoma sequence: screening for adenomas and cancer, now and in the future. Seminars in gastrointestinal disease. 2000;11(4):219-28.
248. Kahi CJ, Vemulapalli KC, Snover DC, Abdel Jawad KH, Cummings OW, Rex DK. Findings in the distal colorectum are not associated with proximal advanced serrated lesions. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2015;13(2):345-51.
249. Fidler JL, Johnson CD, MacCarty RL, Welch TJ, Hara AK, Harmsen WS. Detection of flat lesions in the colon with CT colonography. Abdom Imaging. 2002;27(3):292-300.
250. Gluecker TM, Fletcher JG, Welch TJ, MacCarty RL, Harmsen WS, Harrington JR, et al. Characterization of lesions missed on interpretation of CT colonography using a 2D search method. AJR Am J Roentgenol. 2004;182(4):881-9.
251. Kim DH, Matkowskyj KA, Lubner MG, Hinshaw JL, Munoz Del Rio A, Pooler BD, et al. Serrated Polyps at CT Colonography: Prevalence and Characteristics of the Serrated Polyp Spectrum. Radiology. 2016;280(2):455-63.
252. Ijspeert JEG, Tutein Nolthenius CJ, Kuipers EJ, van Leerdam ME, Nio CY, Thomeer MGJ, et al. CT-Colonography vs. Colonoscopy for Detection of High-Risk Sessile Serrated Polyps. The American journal of gastroenterology. 2016;111(4):516-22.
253. González-Suárez B, Pagés M, Araujo IK, Romero C, Rodríguez De Miguel C, Ayuso JR, et al. Colon capsule endoscopy versus CT colonography in FIT-positive colorectal cancer screening subjects: a prospective randomised trial—the VICOCA study. BMC Medicine. 2020;18(1).
254. Meester RGS, Van Herk MMAGC, Lansdorp-Vogelaar I, Ladabaum U. Prevalence and Clinical Features of Sessile Serrated Polyps: A Systematic Review. Gastroenterology. 2020;159(1):105-18.e25.
255. Kahi CJ, Hewett DG, Norton DL, Eckert GJ, Rex DK. Prevalence and Variable Detection of Proximal Colon Serrated Polyps During Screening Colonoscopy. Clinical Gastroenterology and Hepatology. 2011;9(1):42-6.
256. Carballal S, Rodríguez-Alcalde D, Moreira L, Hernández L, Rodríguez L, Rodríguez-Moranta F, et al. Colorectal cancer risk factors in patients with serrated polyposis syndrome: a large multicentre study. Gut. 2016;65(11):1829-37.
257. Boparai KS, Mathus-Vliegen EMH, Koornstra JJ, Nagengast FM, Van Leerdam M, Van Noesel CJM, et al. Increased colorectal cancer risk during follow-up in patients with hyperplastic polyposis syndrome: a multicentre cohort study. Gut. 2010;59(8):1094-100.
258. Kaminski MF, Regula J, Kraszewska E, Polkowski M, Wojciechowska U, Didkowska J, et al. Quality indicators for colonoscopy and the risk of interval cancer. New England Journal of Medicine. 2010;362(19):1795-803.
259. Kaminski MF, Wieszczy P, Rupinski M, Wojciechowska U, Didkowska J, Kraszewska E, et al. Increased Rate of Adenoma Detection Associates With Reduced Risk of Colorectal Cancer and Death. Gastroenterology. 2017;153(1):98-105.
260. Hassan C, Repici A. Defeating Cancer by Boosting the Adenoma Detection Rate: The Circle of Life. Gastroenterology. 2017;153(1):8-10.
261. Desai M, Anderson JC, Kaminski M, Thoguluva Chandrasekar V, Fathallah J, Hassan C, et al. Sessile serrated lesion detection rates during average risk screening colonoscopy: A systematic review and meta-analysis of the published literature. Endoscopy International Open. 2021;09(04):E610-E20.
262. Keswani RN, Crockett SD, Calderwood AH. AGA Clinical Practice Update on Strategies to Improve Quality of Screening and Surveillance Colonoscopy: Expert Review. Gastroenterology. 2021;161(2):701-11.

263. van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. The American journal of gastroenterology. 2006;101(2):343-50.
264. Bressler B, Paszat LF, Chen Z, Rothwell DM, Vinden C, Rabeneck L. Rates of New or Missed Colorectal Cancers After Colonoscopy and Their Risk Factors: A Population-Based Analysis. Gastroenterology. 2007;132(1):96-102.
265. Sanduleanu S, Masclee AM, Meijer GA. Interval cancers after colonoscopy-insights and recommendations. Nature reviews Gastroenterology & hepatology. 2012;9(9):550-4.
266. Corley DA, Jensen CD, Marks AR, Zhao WK, Lee JK, Doubeni CA, et al. Adenoma Detection Rate and Risk of Colorectal Cancer and Death. New England Journal of Medicine. 2014;370(14):1298-306.
267. Rabeneck L, Paszat LF, Saskin R. Endoscopist Specialty Is Associated With Incident Colorectal Cancer After a Negative Colonoscopy. Clinical Gastroenterology and Hepatology. 2010;8(3):275-9.
268. le Clercq C, Rondagh E, Riedl R, Bosman FT, Beets GL, Lameeteman WI, et al. Interval Colorectal Cancers Frequently Have Subtle Macroscopic Appearance: A 10 Year-Experience in an Academic Center. Gastroenterology. 2011;140(5):S112-S3.
269. Singh S, Singh PP, Murad MH, Singh H, Samadder JN. Prevalence, Risk Factors, and Outcomes of Interval Colorectal Cancers: A Systematic Review and Meta-Analysis. Official journal of the American College of Gastroenterology | ACG. 2014;109(9):1375-89.
270. Rex DK, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty BM, et al. Quality Indicators for Colonoscopy. Official journal of the American College of Gastroenterology | ACG. 2015;110(1):72-90.
271. Lee TJW, Rutter MD, Blanks RG, Moss SM, Goddard AF, Chilton A, et al. Colonoscopy quality measures: experience from the NHS Bowel Cancer Screening Programme. Gut. 2012;61(7):1050.
272. le Clercq CMC, Bouwens MWE, Rondagh EJA, Bakker CM, Keulen ETP, de Ridder RJ, et al. Postcolonoscopy colorectal cancers are preventable: a population-based study. Gut. 2013.
273. Lakoff J, Paszat LF, Saskin R, Rabeneck L. Risk of Developing Proximal Versus Distal Colorectal Cancer After a Negative Colonoscopy: A Population-Based Study. Clinical Gastroenterology and Hepatology. 2008;6(10):1117-21.
274. Obrien M, Winawer S, Zauber A, Bushey M, Sternberg S, Gottlieb L, et al. Flat adenomas in the National Polyp Study: Is there increased risk for high-grade dysplasia initially or during surveillance? Clinical Gastroenterology and Hepatology. 2004;2(10):905-11.
275. Brenner H, Chang-Claude J, Seiler CM, Hoffmeister M. Interval cancers after negative colonoscopy: population-based case-control study. Gut. 2012;61(11):1576-82.
276. Robertson DJ, Greenberg ER, Beach M, Sandler RS, Ahnen D, Haile RW, et al. Colorectal cancer in patients under close colonoscopic surveillance. Gastroenterology. 2005;129(1):34-41.
277. Lee T, Rees C, Blanks R, Moss S, Nickerson C, Wright K, et al. Colonoscopic factors associated with adenoma detection in a national colorectal cancer screening program. Endoscopy. 2014;46(03):203-11.
278. Hurlstone DP, Cross SS, Adam I, Shorthouse AJ, Brown S, Sanders DS, et al. A prospective clinicopathological and endoscopic evaluation of flat and depressed colorectal lesions in the United Kingdom. The American journal of gastroenterology. 2003;98(11):2543-9.
279. Soetikno RM. Prevalence of Nonpolypoid (Flat and Depressed) Colorectal Neoplasms in Asymptomatic and Symptomatic Adults. JAMA : the journal of the American Medical Association. 2008;299(9):1027.
280. McGill SK, Kaltenbach T, Friedland S, Soetikno R. The learning curve for detection of non-polypoid (flat and depressed) colorectal neoplasms. Gut. 2015;64(1):184-5.
281. Coe SG, Crook JE, Diehl NN, Wallace MB. An endoscopic quality improvement program improves detection of colorectal adenomas. The American journal of gastroenterology. 2013;108(2):219-26; quiz 27.

282. Kaltenbach T, McGill SK, Kalidindi V, Friedland S, Soetikno R. Proficiency in the Diagnosis of Nonpolypoid Colorectal Neoplasm Yields High Adenoma Detection Rates. Digestive Diseases and Sciences. 2012;57(3):764-70.
283. Barclay RL, Vicari JJ, Greenlaw RL. Effect of a time-dependent colonoscopic withdrawal protocol on adenoma detection during screening colonoscopy. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(10):1091-8.
284. Butterly L, Robinson CM, Anderson JC, Weiss JE, Goodrich M, Onega TL, et al. Serrated and adenomatous polyp detection increases with longer withdrawal time: results from the New Hampshire Colonoscopy Registry. The American journal of gastroenterology. 2014;109(3):417-26.
285. Brown SR, Baraza W. Chromoscopy versus conventional endoscopy for the detection of polyps in the colon and rectum. The Cochrane database of systematic reviews. 2010(10):Cd006439.
286. Kahi CJ, Anderson JC, Waxman I, Kessler WR, Imperiale TF, Li X, et al. High-definition chromocolonoscopy vs. high-definition white light colonoscopy for average-risk colorectal cancer screening. The American journal of gastroenterology. 2010;105(6):1301-7.
287. Kahi C, Hewett D, Norton D, Rex D. Prevalence and Variable Detection of Proximal Colon Serrated Polyps during Screening Colonoscopy. American Journal of Gastroenterology. 2010;105:S559-S.
288. Crockett SD, Nagtegaal ID. Terminology, Molecular Features, Epidemiology, and Management of Serrated Colorectal Neoplasia. Gastroenterology. 2019;157(4):949-66 e4.
289. Kahi CJ, Hewett DG, Norton DL, Eckert GJ, Rex DK. Prevalence and variable detection of proximal colon serrated polyps during screening colonoscopy. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2011;9(1):42-6.
290. Zorzi M, Senore C, Da Re F, Barca A, Bonelli LA, Cannizzaro R, et al. Detection rate and predictive factors of sessile serrated polyps in an organised colorectal cancer screening programme with immunochemical faecal occult blood test: the EQuIPE study (Evaluating Quality Indicators of the Performance of Endoscopy). Gut. 2017;66(7):1233-40.
291. Burgess NG, Tutticci NJ, Pellise M, Bourke MJ. Sessile serrated adenomas/polyps with cytologic dysplasia: a triple threat for interval cancer. Gastrointestinal Endoscopy.80(2):307-10.
292. Rau TT, Agaimy A, Gehoff A, Geppert C, Jung K, Knobloch K, et al. Defined morphological criteria allow reliable diagnosis of colorectal serrated polyps and predict polyp genetics. Virchows Archiv : an international journal of pathology. 2014;464(6):663-72.
293. Wong NA, Hunt LP, Novelli MR, Shepherd NA, Warren BF. Observer agreement in the diagnosis of serrated polyps of the large bowel. Histopathology.55(1):63-6.
294. Ensari A, Bilezikci B, Carneiro F, Dogusoy GB, Driessen A, Dursun A, et al. Serrated polyps of the colon: how reproducible is their classification? Virchows Archiv. 2012;461(5):495-504.
295. Baldin RKS, Júnior RAA, Azevedo M, Sebastião APM, Montemor M, Tullio LF, et al. Interobserver variability in histological diagnosis of serrated colorectal polyps. Journal of Coloproctology. 2015;35(04):193-7.
296. Turner JK, Williams GT, Morgan M, Wright M, Dolwani S. Interobserver agreement in the reporting of colorectal polyp pathology among bowel cancer screening pathologists in Wales. Histopathology.62(6):916-24.
297. Vennelaganti S, Cuatrecasas M, Vennalaganti P, Kennedy KF, Srinivasan S, Patil DT, et al. Interobserver Agreement Among Pathologists in the Differentiation of Sessile Serrated From Hyperplastic Polyps. Gastroenterology. 2021;160(1):452-4.e1.
298. Foss FA, Milkins S, McGregor AH. Inter-observer variability in the histological assessment of colorectal polyps detected through the NHS Bowel Cancer Screening Programme. Histopathology. 2012;61(1):47-52.

299. Van Putten PG, Hol L, Van Dekken H, Han Van Krieken J, Van Ballegooijen M, Kuipers EJ, et al. Inter-observer variation in the histological diagnosis of polyps in colorectal cancer screening. Histopathology. 2011;58(6):974-81.
300. Kumbhari V, Behary J, Hui JM. Prevalence of adenomas and sessile serrated adenomas in Chinese compared with Caucasians. J Gastroenterol Hepatol. 2013;28(4):608-12.
301. Sonnenberg A, Turner KO, Genta RM. Ethnic variations in the occurrence of colonic neoplasms. United European Gastroenterol J. 2017;5(3):424-31.
302. Li D, Liu L, Fevrier HB, Alexeeff SE, Doherty AR, Raju M, et al. Increased Risk of Colorectal Cancer in Individuals With a History of Serrated Polyps. Gastroenterology. 2020;159(2):502-11.e2.
303. Leggett B, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology. 2010;138(6):2088-100.
304. Farris AB, Srivastava A, Misdraji J, Lauwers GY, Mino-Kenudson M. Sessile serrated polyp/adenoma: Challenging discrimination from other colonic polyps. Laboratory Investigation. 2006;86:107A-8A.
305. Chetty R, Bateman AC, Torlakovic E, Wang LM, Gill P, Al-Badri A, et al. A pathologist's survey on the reporting of sessile serrated adenomas/polyps. J Clin Pathol. 2014.
306. Crockett SD, Snover DC, Ahnen DJ, Baron JA. Sessile serrated adenomas: an evidence-based guide to management. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2015;13(1):11-26.e1.
307. Ma MX, Bourke MJ. Sessile Serrated Adenomas: How to Detect, Characterize and Resect. Gut Liver. 2017;11(6):747-60.
308. Brenner H, Tao S. Superior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy. Eur J Cancer. 2013;49(14):3049-54.
309. Franco DL, Leighton JA, Gurudu SR. Approach to Incomplete Colonoscopy: New Techniques and Technologies. Gastroenterology & hepatology. 2017;13(8):476-83.
310. Gill P, Rafferty H, Munday D, Bailey A, Wang LM, East JE, et al. Proximal colon cancer and serrated adenomas - hunting the missing 10%. Clinical Medicine. 2013;13(6):557-61.
311. Bouwens MW, van Herwaarden YJ, Winkens B, Rondagh EJ, de Ridder R, Riedl RG, et al. Endoscopic characterization of sessile serrated adenomas/polyps with and without dysplasia. Endoscopy. 2014;46(3):225-35.
312. Buchner AM. The Role of Chromoendoscopy in Evaluating Colorectal Dysplasia. Gastroenterology & hepatology. 2017;13(6):336-47.
313. Singh R. White light endoscopy, narrow band imaging and chromoendoscopy with magnification in diagnosing colorectal neoplasia. World Journal of Gastrointestinal Endoscopy. 2009;1(1):45.
314. Nagata K, Endo S, Honda T, Yasuda T, Hirayama M, Takahashi S, et al. Accuracy of CT Colonography for Detection of Polypoid and Nonpolypoid Neoplasia by Gastroenterologists and Radiologists: A Nationwide Multicenter Study in Japan. The American journal of gastroenterology. 2017;112(1):163-71.
315. Liang J, Kalady MF, Appau K, Church J. Serrated polyp detection rate during screening colonoscopy. Colorectal Disease. 2012;14(11):1323-7.
316. Min YW, Lee JH, Lee SH, Park DI, Han DS, Rhee PL, et al. Prevalence of proximal colon serrated polyps in a population at average risk undergoing screening colonoscopy: A multicenter study. Clinics and Research in Hepatology and Gastroenterology. 2012;36(6):604-8.
317. Rondagh E, Masclee A, Bouwens M, Winkens B, Riedl R, De Bruïne A, et al. Endoscopic red flags for the detection of high-risk serrated polyps: an observational study. Endoscopy. 2011;43(12):1052-8.
318. Hewett DG, Rex DK. Miss rate of right-sided colon examination during colonoscopy defined by retroflexion: an observational study. Gastrointest Endosc. 2011;74(2):246-52.

319. Anderson JC, Butterly LF, Robinson CM, Goodrich M, Weiss JE. Impact of fair bowel preparation quality on adenoma and serrated polyp detection: data from the New Hampshire Colonoscopy Registry by using a standardized preparation-quality rating. Gastrointestinal Endoscopy. 2014;80(3):463-70.
320. Clark BT, Laine L. High-quality Bowel Preparation Is Required for Detection of Sessile Serrated Polyps. Clinical Gastroenterology and Hepatology. 2016;14(8):1155-62.
321. Sehgal A, Aggarwal S, Mandaliya R, Loughney T, Mattar MC. Improving sessile serrated adenoma detection rates with high definition colonoscopy: A retrospective study. World Journal of Gastrointestinal Endoscopy. 2022;14(4):226-35.
322. Roelandt P, Demedts I, Willekens H, Bessissow T, Braeye L, Coremans G, et al. Impact of endoscopy system, high definition, and virtual chromoendoscopy in daily routine colonoscopy: a randomized trial. Endoscopy. 2019;51(03):237-43.
323. Rex DK, Clodfelter R, Rahmani F, Fatima H, James-Stevenson TN, Tang JC, et al. Narrow-band imaging versus white light for the detection of proximal colon serrated lesions: a randomized, controlled trial. Gastrointest Endosc. 2016;83(1):166-71.
324. Hazewinkel Y, Tytgat KM, van Leerdam ME, Koornstra JJ, Bastiaansen BA, van Eeden S, et al. Narrow-band imaging for the detection of polyps in patients with serrated polyposis syndrome: a multicenter, randomized, back-to-back trial. Gastrointest Endosc. 2015;81(3):531-8.
325. Ket SN, Bird-Lieberman E, East JE. Electronic Imaging to Enhance Lesion Detection at Colonoscopy. Gastrointestinal Endoscopy Clinics of North America. 2015;25(2):227-42.
326. Jaramillo E, Watanabe M, Slezak P, Rubio C. Flat neoplastic lesions of the colon and rectum detected by high-resolution video endoscopy and chromoscopy. Gastrointestinal Endoscopy.42(2):114-22.
327. Pal P, Singh AP, Kanuri ND, Banerjee R. Electronic chromo-endoscopy: technical details and a clinical perspective. Translational Gastroenterology and Hepatology. 2022;7:6-.
328. Rutter MD, Saunders BP, Schofield G, Forbes A, Price AB, Talbot IC. Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. Gut. 2004;53(2):256-60.
329. Laine L, Kaltenbach T, Barkun A, McQuaid KR, Subramanian V, Soetikno R. SCENIC international consensus statement on surveillance and management of dysplasia in inflammatory bowel disease. Gastrointest Endosc. 2015;81(3):489-501.e26.
330. Subramanian V, Mannath J, Ragunath K, Hawkey CJ. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. Aliment Pharmacol Ther. 2011;33(3):304-12.
331. Soetikno R, Subramanian V, Kaltenbach T, Rouse RV, Sanduleanu S, Suzuki N, et al. The detection of nonpolypoid (flat and depressed) colorectal neoplasms in patients with inflammatory bowel disease. Gastroenterology. 2013;144(7):1349-52, 52.e1-6.
332. Ignjatovic A, East JE, Subramanian V, Suzuki N, Guenther T, Palmer N, et al. Narrow band imaging for detection of dysplasia in colitis: a randomized controlled trial. The American journal of gastroenterology. 2012;107(6):885-90.
333. Dekker E, van den Broek FJ, Reitsma JB, Hardwick JC, Offerhaus GJ, van Deventer SJ, et al. Narrow-band imaging compared with conventional colonoscopy for the detection of dysplasia in patients with longstanding ulcerative colitis. Endoscopy. 2007;39(3):216-21.
334. van den Broek FJ, Fockens P, van Eeden S, Reitsma JB, Hardwick JC, Stokkers PC, et al. Endoscopic tri-modal imaging for surveillance in ulcerative colitis: randomised comparison of high-resolution endoscopy and autofluorescence imaging for neoplasia detection; and evaluation of narrow-band imaging for classification of lesions. Gut. 2008;57(8):1083-9.
335. Subramanian V, Mannath J, Ragunath K, Hawkey CJ. Meta-analysis: the diagnostic yield of chromoendoscopy for detecting dysplasia in patients with colonic inflammatory bowel disease. Alimentary Pharmacology & Therapeutics. 2011;33(3):304-12.

336. Leong RW, Butcher RO, Picco MF. Implementation of image-enhanced endoscopy into solo and group practices for dysplasia detection in Crohn's disease and ulcerative colitis. Gastrointest Endosc Clin N Am. 2014;24(3):419-25.
337. Rahmi G, Lecomte T, Malka D, Maniere T, Le Rhun M, Guimbaud R, et al. Impact of chromoscopy on adenoma detection in patients with Lynch syndrome: a prospective, multicenter, blinded, tandem colonoscopy study. The American journal of gastroenterology. 2015;110(2):288-98.
338. Lecomte T, Cellier C, Meatchi T, Barbier JP, Cugnenc PH, Jian R, et al. Chromoendoscopic colonoscopy for detecting preneoplastic lesions in hereditary nonpolyposis colorectal cancer syndrome. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2005;3(9):897-902.
339. Haanstra JF, Dekker E, Cats A, Nagengast FM, Hardwick JC, Vanhoutvin SA, et al. Effect of chromoendoscopy in the proximal colon on colorectal neoplasia detection in Lynch syndrome: a multicenter randomized controlled trial. Gastrointest Endosc. 2019;90(4):624-32.
340. Rivero-Sánchez L, Arnau-Collell C, Herrero J, Remedios D, Cubiella J, García-Cougil M, et al. White-Light Endoscopy Is Adequate for Lynch Syndrome Surveillance in a Randomized and Noninferiority Study. Gastroenterology. 2020;158(4):895-904.e1.
341. López-Vicente J, Rodríguez-Alcalde D, Hernández L, Riu Pons F, Vega P, Herrero Rivas JM, et al. Panchromoendoscopy Increases Detection of Polyps in Patients With Serrated Polyposis Syndrome. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2019;17(10):2016-23.e6.
342. Boparai KS, van den Broek FJ, van Eeden S, Fockens P, Dekker E. Increased polyp detection using narrow band imaging compared with high resolution endoscopy in patients with hyperplastic polyposis syndrome. Endoscopy.43(8):676-82.
343. Brown SR, Baraza W, Din S, Riley S. Chromoscopy versus conventional endoscopy for the detection of polyps in the colon and rectum. The Cochrane database of systematic reviews. 2016;4:CD006439.
344. Chung SJ, Kim D, Song JH, Kang HY, Chung GE, Choi J, et al. Comparison of detection and miss rates of narrow band imaging, flexible spectral imaging chromoendoscopy and white light at screening colonoscopy: a randomised controlled back-to-back study. Gut. 2014;63(5):785-91.
345. Hong SN, Choe WH, Lee JH, Kim SI, Kim JH, Lee TY, et al. Prospective, randomized, back-to-back trial evaluating the usefulness of i-SCAN in screening colonoscopy. Gastrointest Endosc. 2012;75(5):1011-21.e2.
346. Bowman EA, Pfau PR, Mitra A, Reichelderfer M, Gopal DV, Hall BS, et al. High Definition Colonoscopy Combined with i-SCAN Imaging Technology Is Superior in the Detection of Adenomas and Advanced Lesions Compared to High Definition Colonoscopy Alone. Diagn Ther Endosc. 2015;2015:167406.
347. Min M, Deng P, Zhang W, Sun X, Liu Y, Nong B. Comparison of linked color imaging and white-light colonoscopy for detection of colorectal polyps: a multicenter, randomized, crossover trial. Gastrointest Endosc. 2017;86(4):724-30.
348. Paggi S, Mogavero G, Amato A, Rondonotti E, Andrealli A, Imperiali G, et al. Linked color imaging reduces the miss rate of neoplastic lesions in the right colon: a randomized tandem colonoscopy study. Endoscopy. 2018;50(4):396-402.
349. Ikematsu H, Sakamoto T, Togashi K, Yoshida N, Hisabe T, Kiriyama S, et al. Detectability of colorectal neoplastic lesions using a novel endoscopic system with blue laser imaging: a multicenter randomized controlled trial. Gastrointest Endosc. 2017;86(2):386-94.
350. Shukla R, Salem M, Hou JK. Use and barriers to chromoendoscopy for dysplasia surveillance in inflammatory bowel disease. World Journal of Gastrointestinal Endoscopy. 2017;9(8):359.
351. Lebwohl B, Kastrinos F, Glick M, Rosenbaum AJ, Wang T, Neugut AI. The impact of suboptimal bowel preparation on adenoma miss rates and the factors associated with early repeat colonoscopy. Gastrointest Endosc. 2011;73(6):1207-14.

352. Gurudu SR, Ramirez FC, Harrison ME, Leighton JA, Crowell MD. Increased adenoma detection rate with system-wide implementation of a split-dose preparation for colonoscopy. Gastrointest Endosc. 2012;76(3):603-8.e1.
353. Nutalapati V, Desai M, Thoguluva-Chandrasekar VS, Olyaee M, Rastogi A. Effect of dynamic position changes on adenoma detection rate during colonoscope withdrawal: systematic review and meta-analysis. Endosc Int Open. 2020;8(12):E1842-e9.
354. East JE, Bassett P, Arebi N, Thomas-Gibson S, Guenther T, Saunders BP. Dynamic patient position changes during colonoscope withdrawal increase adenoma detection: a randomized, crossover trial. Gastrointest Endosc. 2011;73(3):456-63.
355. Lee SW, Chang JH, Ji JS, Maeong IH, Cheung DY, Kim JS, et al. Effect of Dynamic Position Changes on Adenoma Detection During Colonoscope Withdrawal: A Randomized Controlled Multicenter Trial. The American journal of gastroenterology. 2016;111(1):63-9.
356. Corte C, Dahlenburg L, Selby W, Griffin S, Byrne C, Chua T, et al. Hyoscine butylbromide administered at the cecum increases polyp detection: a randomized double-blind placebo-controlled trial. Endoscopy. 2012;44(10):917-22.
357. Rondonotti E, Zolk O, Amato A, Paggi S, Baccarin A, Spinzi G, et al. The impact of hyoscine-N-butylbromide on adenoma detection during colonoscopy: meta-analysis of randomized, controlled studies. Gastrointest Endosc. 2014;80(6):1103-12.e2.
358. Ashraf I, Ashraf S, Siddique S, Nguyen DL, Choudhary A, Bechtold ML. Hyoscine for polyp detection during colonoscopy: A meta-analysis and systematic review. World J Gastrointest Endosc. 2014;6(11):549-54.
359. Leung FW, Amato A, Ell C, Friedland S, Harker JO, Hsieh YH, et al. Water-aided colonoscopy: a systematic review. Gastrointest Endosc. 2012;76(3):657-66.
360. Yen AW, Leung JW, Leung FW. A new method for screening and surveillance colonoscopy: Combined water-exchange and cap-assisted colonoscopy. Journal of interventional gastroenterology. 2012;2(3):114-9.
361. Waldmann E, Britto-Arias M, Gessl I, Heinze G, Salzl P, Sallinger D, et al. Endoscopists with low adenoma detection rates benefit from high-definition endoscopy. Surgical endoscopy. 2015;29(2):466-73.
362. Banks MR, Haidry R, Butt MA, Whitley L, Stein J, Langmead L, et al. High resolution colonoscopy in a bowel cancer screening program improves polyp detection. World journal of gastroenterology : WJG. 2011;17(38):4308-13.
363. Dinesen L, Chua TJ, Kaffes AJ. Meta-analysis of narrow-band imaging versus conventional colonoscopy for adenoma detection. Gastrointest Endosc. 2012;75(3):604-11.
364. Pasha SF, Leighton JA, Das A, Harrison ME, Gurudu SR, Ramirez FC, et al. Comparison of the yield and miss rate of narrow band imaging and white light endoscopy in patients undergoing screening or surveillance colonoscopy: a meta-analysis. The American journal of gastroenterology. 2012;107(3):363-70; quiz 71.
365. Nagorni A, Bjelakovic G, Petrovic B. Narrow band imaging versus conventional white light colonoscopy for the detection of colorectal polyps. The Cochrane database of systematic reviews. 2012;1:Cd008361.
366. Sabbagh LC, Reveiz L, Aponte D, de Aguiar S. Narrow-band imaging does not improve detection of colorectal polyps when compared to conventional colonoscopy: a randomized controlled trial and meta-analysis of published studies. BMC Gastroenterol. 2011;11:100.
367. Zhao ZY, Guan YG, Li BR, Shan YQ, Yan FH, Gao YJ, et al. Detection and miss rates of autofluorescence imaging of adenomatous and polypoid lesions during colonoscopy: a systematic review and meta-analysis. Endosc Int Open. 2015;3(3):E226-35.
368. Takeuchi Y, Sawaya M, Oka S, Tamai N, Kawamura T, Uraoka T, et al. Efficacy of autofluorescence imaging for flat neoplasm detection: a multicenter randomized controlled trial (A-FLAT trial). Gastrointest Endosc. 2019;89(3):460-9.

369. de Wijkerslooth TR, Stoop EM, Bossuyt PM, Mathus-Vliegen EM, Dees J, Tytgat KM, et al. Adenoma detection with cap-assisted colonoscopy versus regular colonoscopy: a randomised controlled trial. Gut. 2012;61(10):1426-34.
370. Patil R, Ona MA, Ofori E, Reddy M. Endocuff-Assisted Colonoscopy-A Novel Accessory in Improving Adenoma Detection Rate: A Review of the Literature. Clinical endoscopy. 2016;49(6):533-8.
371. Ngu WS, Bevan R, Tsiamoulos ZP, Bassett P, Hoare Z, Rutter MD, et al. Improved adenoma detection with Endocuff Vision: the ADENOMA randomised controlled trial. Gut. 2019;68(2):280-8.
372. Facciorusso A, Triantafyllou K, Murad MH, Prokop LJ, Tziatzios G, Muscatiello N, et al. Compared Abilities of Endoscopic Techniques to Increase Colon Adenoma Detection Rates: A Network Meta-analysis. Clinical Gastroenterology and Hepatology. 2019;17(12):2439-54.e25.
373. Adler A, Wegscheider K, Lieberman D, Aminalai A, Aschenbeck J, Drossel R, et al. Factors determining the quality of screening colonoscopy: a prospective study on adenoma detection rates, from 12,134 examinations (Berlin colonoscopy project 3, BECOP-3). Gut. 2013;62(2):236-41.
374. Jorgensen OD, Kronborg O, Fenger C. A randomised study of screening for colorectal cancer using faecal occult blood testing: results after 13 years and seven biennial screening rounds. Gut. 2002;50(1):29-32.
375. Singh H, Nugent Z, Demers AA, Kliewer EV, Mahmud SM, Bernstein CN. The reduction in colorectal cancer mortality after colonoscopy varies by site of the cancer. Gastroenterology. 2010;139(4):1128-37.
376. Burnett-Hartman AN, Newcomb PA, Phipps AI, Passarelli MN, Grady WM, Upton MP, et al. Colorectal Endoscopy, Advanced Adenomas, and Sessile Serrated Polyps: Implications for Proximal Colon Cancer. American Journal of Gastroenterology. 2012;107(8):1213-9.
377. Schreiner MA, Weiss DG, Lieberman DA. Proximal and large hyperplastic and nondysplastic serrated polyps detected by colonoscopy are associated with neoplasia. Gastroenterology. 2010;139(5):1497-502.
378. Stoffel EM, Turgeon DK, Stockwell DH, Normolle DP, Tuck MK, Marcon NE, et al. Chromoendoscopy detects more adenomas than colonoscopy using intensive inspection without dye spraying. Cancer prevention research (Philadelphia, Pa). 2008;1(7):507-13.
379. Le Rhun M, Coron E, Parlier D, Nguyen JM, Canard JM, Alamdari A, et al. High resolution colonoscopy with chromoscopy versus standard colonoscopy for the detection of colonic neoplasia: a randomized study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2006;4(3):349-54.
380. van den Broek FJ, Fockens P, Van Eeden S, Kara MA, Hardwick JC, Reitsma JB, et al. Clinical evaluation of endoscopic trimodal imaging for the detection and differentiation of colonic polyps. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(3):288-95.
381. Dolwani S, Ragunath K. Quality criteria for a good screening colonoscopy. Current Colorectal Cancer Reports. 2010;6(1):38-43.
382. Rutter M, Chilton A. Quality Assurance Guidelines for Colonoscopy. NHS BCSP Publication. 2011;6:24.
383. Picco MF, Pasha S, Leighton JA, Bruining D, Loftus EV, Jr., Thomas CS, et al. Procedure time and the determination of polypoid abnormalities with experience: implementation of a chromoendoscopy program for surveillance colonoscopy for ulcerative colitis. Inflammatory Bowel Diseases.19(9):1913-20.
384. Ijspeert JEG, de Wit K, van der Vlugt M, Bastiaansen BAJ, Fockens P, Dekker E. Prevalence, distribution and risk of sessile serrated adenomas/polyps at a center with a high adenoma detection rate and experienced pathologists. Endoscopy. 2016;48(08):740-6.
385. JEG IJ, Bevan R, Senore C, Kaminski MF, Kuipers EJ, Mroz A, et al. Detection rate of serrated polyps and serrated polyposis syndrome in colorectal cancer screening cohorts: a European overview. Gut. 2017;66(7):1225-32.

386. Singh M, Sacatos M, Laine L. Impact of Changeover to Newer Endoscopic Systems on Quality and Efficiency of Screening and Surveillance Colonoscopy: Equipment or Endoscopist. J Clin Gastroenterol. 2018;52(10):891-5.
387. Sawyer EJ, Cerar A, Hanby AM, Gorman P, Arends M, Talbot IC, et al. Molecular characteristics of serrated adenomas of the colorectum. Gut. 2002;51(2):200-6.
388. Torlakovic EMD, Skovlund EPD, Snover DCMD, Torlakovic GMD, Nesland JMMDPD. Morphologic Reappraisal of Serrated Colorectal Polyps. American Journal of Surgical Pathology. 2003;27(1):65-81.
389. Bettington M, Walker N, Rosty C, Brown I, Clouston A, Wockner L, et al. Critical appraisal of the diagnosis of the sessile serrated adenoma. The American journal of surgical pathology. 2014;38(2):158-66.
390. Lane N, Kaplan H, Pascal RR. Minute adenomatous and hyperplastic polyps of the colon: divergent patterns of epithelial growth with specific associated mesenchymal changes. Contrasting roles in the pathogenesis of carcinoma. Gastroenterology. 1971;60(4):537-51.
391. Bateman AC, Shepherd NA. UK guidance for the pathological reporting of serrated lesions of the colorectum. Journal of Clinical Pathology. 2015;68(8):585-91.
392. Snover DC AD, Burt RW, Odze RD. Serrated polyps of the colon and rectum and serrated polyposis. In: Bosman FT, Carneiro F, Hruban RH, et al, editors. WHO classification of tumours of the digestive system. Lyon, France: IARC; 2010. p. 160–5. 2010.
393. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, et al. Serrated lesions of the colorectum: Review and recommendations from an expert panel. American Journal of Gastroenterology. 2012;107(9):1315-29.
394. Bastos Junior CDS, Pannain VLN, Caroli-Bottino A. Morphological Characteristics, Classifications and Difficulties in the Use of Diagnostic Criteria for Serrated Lesions of the Large Intestine. Journal of Coloproctology. 2021;41(04):430-7.
395. Schlemper RJ, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, et al. The Vienna classification of gastrointestinal epithelial neoplasia. Gut. 2000;47(2):251.
396. Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. Biometrics. 1977;33(1):159.
397. Jaravaza DR, Rigby JM. Hyperplastic polyp or sessile serrated lesion? The contribution of serial sections to reclassification. Diagnostic pathology. 2020;15(1).
398. Bettington M, Walker N, Rosty C, Brown I, Clouston A, McKeone D, et al. Serrated tubulovillous adenoma of the large intestine. Histopathology. 2016;68(4):578-87.
399. Hashimoto T, Tanaka Y, Ogawa R, Mori T, Yoshida H, Taniguchi H, et al. Superficially serrated adenoma: a proposal for a novel subtype of colorectal serrated lesion. Mod Pathol. 2018;31(10):1588-98.
400. Mizuguchi Y, Sakamoto T, Hashimoto T, Tsukamoto S, Iwasa S, Saito Y, et al. Identification of a novel PRR15L-RSPO2 fusion transcript in a sigmoid colon cancer derived from superficially serrated adenoma. Virchows Archiv : an international journal of pathology. 2019;475(5):659-63.
401. Kim KM, Lee EJ, Ha S, Kang SY, Jang KT, Park CK, et al. Molecular features of colorectal hyperplastic polyps and sessile serrated adenoma/polyps from Korea. American Journal of Surgical Pathology. 2011;35(9):1274-86.
402. Liu C, Walker NI, Leggett BA, Whitehall VL, Bettington ML, Rosty C. Sessile serrated adenomas with dysplasia: morphological patterns and correlations with MLH1 immunohistochemistry. Modern Pathology. 2017;30(12):1728-38.
403. Farris AB, Misdraji J, Srivastava A, Muzikansky A, Deshpande V, Lauwers GY, et al. Sessile serrated adenoma: challenging discrimination from other serrated colonic polyps. The American journal of surgical pathology. 2008;32(1):30-5.
404. Glatz K, Pritt B, Glatz D, Hartmann A, O'Brien MJ, Blaszyk H. A multinational, internet-based assessment of observer variability in the diagnosis of serrated colorectal polyps. Am J Clin Pathol. 2007;127(6):938-45.

405. Khalid O, Radaideh S, Cummings OW, O'Brien MJ, Goldblum JR, Rex DK. Reinterpretation of histology of proximal colon polyps called hyperplastic in 2001. World journal of gastroenterology : WJG. 2009;15(30):3767-70.
406. Bustamante-Balén M, Bernet L, Cano R, Morell L, López A. Assessing the reproducibility of the microscopic diagnosis of sessile serrated adenoma of the colon. Rev Esp Enferm Dig. 2009;101(4):258-64.
407. Gunia S, Berg T, Gradhand E, Becker S. Knowledge of the anatomical polyp location might bias the pathological classification of histologically equivocal colorectal serrated polyps - a consensus study performed by pathology trainees. Pathol Res Pract. 2011;207(2):116-20.
408. Farris ABMD, Misdraji JMD, Srivastava AMD, Muzikansky AMA, Deshpande VMD, Lauwers GYMD, et al. Sessile Serrated Adenoma: Challenging Discrimination From Other Serrated Colonic Polyps. American Journal of Surgical Pathology. 2008;32(1):30-5.
409. Gill MD, Bramble MG, Rees CJ, Bradburn M, Lee TJ, Mills SJ. Comparison of Screen Detected and Interval Colorectal Cancers in the Bowel Cancer Screening Programme: Experience from the North East of England. Gut. 2012;61:A50-A.
410. Rosty C, Hewett DG, Brown IS, Leggett BA, Whitehall VL. Serrated polyps of the large intestine: current understanding of diagnosis, pathogenesis, and clinical management. Journal of gastroenterology. 2013;48(3):287-302.
411. Anderson JC, Lisovsky M, Greene MA, Hagen C, Srivastava A. Factors Associated With Classification of Hyperplastic Polyps as Sessile Serrated Adenomas/Polyps on Morphologic Review. J Clin Gastroenterol. 2018;52(6):524-9.
412. Tao EW, Wang YF, Zou TH, Cui Y, Chen YX, Gao QY. Relationship between serrated polyps and synchronous and metachronous advanced neoplasia: A retrospective study. Journal of Digestive Diseases. 2020;21(10):558-65.
413. Nasir-Moin M, Suriawinata AA, Ren B, Liu X, Robertson DJ, Bagchi S, et al. Evaluation of an Artificial Intelligence–Augmented Digital System for Histologic Classification of Colorectal Polyps. JAMA Network Open. 2021;4(11):e2135271.
414. Nguyen LH, Goel A, Chung DC. Pathways of Colorectal Carcinogenesis. Gastroenterology. 2020;158(2):291-302.
415. Tornillo L, Lehmann FS, Garofoli A, Paradiso V, Ng CKY, Piscuoglio S. The Genomic Landscape of Serrated Lesion of the Colorectum: Similarities and Differences With Tubular and Tubulovillous Adenomas. Front Oncol. 2021;11:668466.
416. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458(7239):719-24.
417. Diaz-Gay M, Alexandrov LB. Unraveling the genomic landscape of colorectal cancer through mutational signatures. Adv Cancer Res. 2021;151:385-424.
418. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-21.
419. Pfeifer GP. Environmental exposures and mutational patterns of cancer genomes. Genome Med. 2010;2(8):54.
420. Peña-Diaz J, Bregenhorn S, Ghodgaonkar M, Follonier C, Artola-Borán M, Castor D, et al. Noncanonical mismatch repair as a source of genomic instability in human cells. Mol Cell. 2012;47(5):669-80.
421. Georgeson P, Pope BJ, Rosty C, Clendenning M, Mahmood K, Joo JE, et al. Evaluating the utility of tumour mutational signatures for identifying hereditary colorectal cancer and polyposis syndrome carriers. Gut. 2021;70(11):2138-49.
422. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Research. 2018;47(D1):D941-D7.
423. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed. 2013;98(6):236-8.
424. Qin D. Next-generation sequencing and its clinical application. Cancer Biol Med. 2019;16(1):4-10.

425. Gargis AS, Kalman L, Bick DP, da Silva C, Dimmock DP, Funke BH, et al. Good laboratory practice for clinical next-generation sequencing informatics pipelines. Nat Biotechnol. 2015;33(7):689-93.
426. Goncearenco A, Rager SL, Li M, Sang Q-X, Rogozin IB, Panchenko AR. Exploring background mutational processes to decipher cancer genetic heterogeneity. Nucleic Acids Research. 2017;45(W1):W514-W22.
427. Do H, Dobrovic A. Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization. Clin Chem. 2015;61(1):64-71.
428. Arreaza G, Qiu P, Pang L, Albright A, Hong L, Marton M, et al. Pre-Analytical Considerations for Successful Next-Generation Sequencing (NGS): Challenges and Opportunities for Formalin-Fixed and Paraffin-Embedded Tumor Tissue (FFPE) Samples. International Journal of Molecular Sciences. 2016;17(9):1579.
429. Nagahashi M, Shimada Y, Ichikawa H, Nakagawa S, Sato N, Kaneko K, et al. Formalin-fixed paraffin-embedded sample conditions for deep next generation sequencing. J Surg Res. 2017;220:125-32.
430. Guo Q, Lakatos E, Bakir IA, Curtius K, Graham TA, Mustonen V. The mutational signatures of formalin fixation on the human genome. Nature Communications. 2022;13(1).
431. Ahlquist DA, Sargent DJ, Loprinzi CL, Levin TR, Rex DK, Ahnen DJ, et al. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. Ann Intern Med. 2008;149(7):441-50, w81.
432. Anderson JC, Hisey W, Mackenzie TA, Robinson CM, Srivastava A, Meester RGS, et al. Clinically significant serrated polyp detection rates and risk for postcolonoscopy colorectal cancer: data from the New Hampshire Colonoscopy Registry. Gastrointest Endosc. 2022;96(2):310-7.
433. Qiao Y, Zhao L, Luo C, Luo Y, Wu Y, Li S, et al. Multi-modality artificial intelligence in digital pathology. Briefings in Bioinformatics. 2022;23(6).

Appendices

Appendix A: Published Paper and author contribution

Feasibility and economic assessment of chromocolonoscopy for detection of proximal serrated neoplasia within a population-based colorectal cancer screening programme (CONSCOP): an open-label, randomised controlled non-inferiority trial.

Lancet Gastroenterol Hepatol 2019; 4: 364-75.

R Ramaraj*, C Hurt*, A Farr, M Morgan, N Williams, CJ Philips, GT Williams, G Gardner, C Porter, J Sampson, S Hillier, H Heard, S Dolwani

*Joint first authors

Author contribution:

RR- clinical research fellow, conceptual design of the trial including responsibility for application for approval through IRAS, design of CRF, participant information sheet, training material, writing trial protocol, data collection design and methodology, data collection and curation, management of pathology meetings and data, investigation, training, and site initiation, database and data validation, formal analysis, writing-original draft.

CH-statistician, conceptual design of the trial, writing trial protocol, methodology, statistical analysis and data curation, data management from trial, writing- original draft and review.

AF, CJP- Health Economic Analysis

MM, NW, GTW- Pathology Expert Review

GG- Trial Management- site initiation and overall trial management

CP- Data collation from CRF

JS, HH, SH- Conceptual design of the trial and funding

SD- chief investigator for CONSCOP, conceptual design of the trial, data curation, funding acquisition, training and site initiation, formal analysis, resources, supervision, writing-original draft, review and editing

Feasibility and economic assessment of chromocolonoscopy for detection of proximal serrated neoplasia within a population-based colorectal cancer screening programme (CONSCOP): an open-label, randomised controlled non-inferiority trial

Chris Hurt*, Rajeswari Ramaraj*, Angela Farr, Meleri Morgan, Namor Williams, Ceri J Philips, Geraint T Williams, Georgina Gardner, Catherine Porter, Julian Sampson, Sharon Hillier, Hayley Heard, Sunil Dalwani, on behalf of the CONSCOP Clinical Research Consortium†

Appendix B: CONSCOP Protocol V2.0

CONSCOP - Feasibility of reduction in right sided bowel cancer through CONtrast enhanced colonoSCOPy

A feasibility randomised controlled trial (RCT) of contrast enhanced vs non-enhanced colonoscopy in index bowel cancer screening to reduce bowel cancer mortality

Clinical Trial Protocol

Version: 2.0

Date: 24th January 2014

ClinicalTrials.gov Number: NCT01972451

Funder: National Institute for Social Care and Health Research (NISCHR)

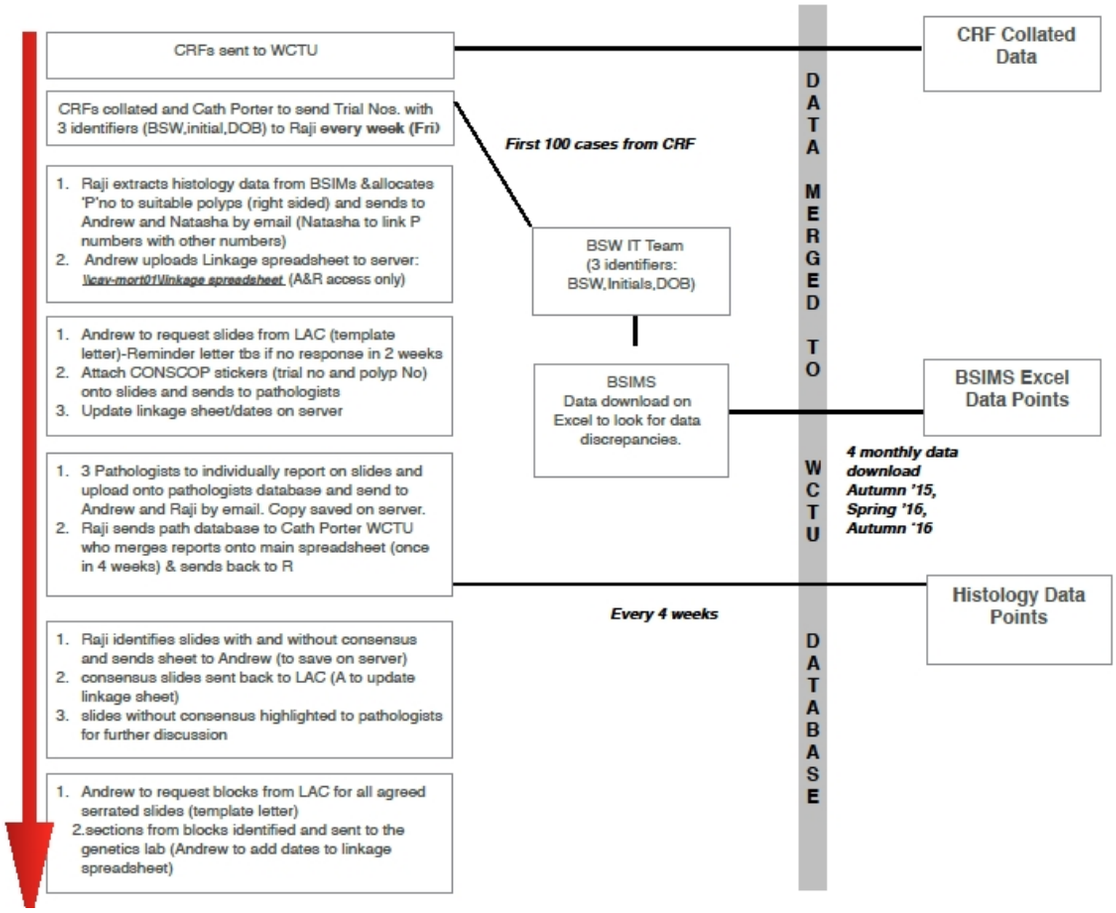
Funder No: RfPPB–1021

Name of Sponsor: Cardiff & Vale University Health Board

Sponsor No: 13/DMD/5786

Authorised by:			
Name:	Sunil Dolwani	Role:	Chief Investigator
Signature:		Date:	26/2/14
Name:	Gareth Griffiths	Role:	Director, WCTU
Signature:		Date:	26/2/14

Appendix C: Data Flow Chart



Appendix D: List of training day documents

LIST OF DOCUMENTS REQUIRED FOR CONSCOP WORKSHOPS

No	Document	Issuer
1	Protocol	Sunil / Raji
2	Patient information sheet	Sunil / Raji
3	List of activity required by Screening Colonoscopist	Sunil / Raji
4	List of activity required by SSP	Sunil / Raji
5	Agenda	Helena
6	Communication sheets	Sunil / Raji
7	CRF forms	Sunil / Raji
8	Contact Details	Sunil / Raji
9	FAQ's	Sunil / Raji
10	File for papers to include blank paper for writing	Helena

Appendix E: CONSCOP randomisation specification

Randomisation of participants (total time 2-3 minutes)

Checklist:

1. Assessment completed on the phone (allocate list as usual, make a note on the list and CRF of potential eligible patients)

2. Website address for randomization –(to be provided)

3. Tel number for randomization in case of problems with website (Mon to Fri 9am to 5pm)–

4. Details to be entered:

Access will be through a 'log in' screen where the recruiting site is selected from a drop down list (eg. Select wrexham maelor hospital) and the associated PIN code is also entered. Each site should have a 3 digit 'site code' associated for use in generating a site number:

5. The system will then check that the PIN and centre match before moving to a 'randomisation' screen where the following data is entered:

    1. The initials of the clinician performing the randomisation (3 characters)
    2. Bowel screening wales Number (starts with BSW followed by the 9 numbers).
    3. Date of colonoscopy appointment.
    4. Time of colonoscopy appointment (AM/PM)

6. The user should then press 'OK' when finished

7. The system should ask the user to verify a summary of the above data by pressing 'verified'

8. The system will then perform simple 1:1 randomisation (site/date/time needs to be entered accurately so that no more than 2 enhanced dye participants are not on the same list)

9. The system will then display the input data together with the output data which is :

- Randomisation outcome:
    - Arm A: Colonoscopy without enhanced dye
    - Arm B: Colonoscopy with enhanced dye
- CONSCOP trial number (a unique identifier made up of the site number then 3 digits allocated in consecutive order starting at 001)
- Date of randomisation

10. The user should be asked to "log out" or "randomise another patient". If they select the latter they should go back to the "randomisation" screen otherwise go to the "log in" screen.

Appendix F: Participant Information Sheet

<PRINT ON HOSPITAL OR BSW HEADED PAPER>

Feasibility of reduction in right sided bowel cancer through CONtrast enhanced colonoSCOPy

Patient Information Sheet

We would like to invite you to take part in a research study. Please take time to read the following information carefully and to decide whether or not you wish to take part. Talk to others about the study if you wish. Ask us if anything is unclear or if you would like more information.

Why have I been invited?

You provided a stool sample as part of the NHS Bowel Cancer Screening Programme (Bowel Screening Wales). Your test result shows that blood has been found in your bowel movement. It does not mean that you have cancer. The result may have been caused by bleeding from polyps (small growths) or other conditions such as haemorrhoids (piles).

You have recently spoken to a Specialist Screening Practitioner and have agreed to have a colonoscopy to investigate the cause of the result.

We are inviting every person in Wales who is to have a colonoscopy as part of the NHS Bowel Cancer Screening Programme to take part in this research study.

What is the purpose of this study?

The Bowel Cancer Screening Programme was implemented to reduce the risk of deaths from bowel cancer by identifying them early when treatment is more effective. Regular bowel screening has been shown to reduce the risk of dying from bowel cancer by 15%. Polyps may be seen during colonoscopy and are usually removed as they could grow and cause problems later. Sometimes polyps develop into cancer. Polyps and cancer can occur anywhere in the bowel and they are usually detected at colonoscopy but there is a small percentage of people who develop cancers despite having colonoscopies as a part of the screening programme. These cancers are usually in the right side of the upper large bowel. There is some evidence to suggest that these cancers could be caused by a certain type of flat polyp called a serrated polyp. Small studies have suggested that these polyps may have a faster rate of growth into cancer than the usual polyps (adenomas). These serrated polyps are typically flat and quite difficult to detect.

A colonoscopy is a way of looking at the lining of the large bowel (colon) to see whether there is any disease present. The test allows us to look at your bowel and take small samples (biopsies) to analyse in the laboratory if necessary. The instrument used is called a colonoscope and is a

Appendix G: Frequently asked questions for Patients

Most of the information relating to the study is in the patient information sheet that is sent out in the pack (this is to keep handy)

FAQs:

1. What is a flat polyp and why is it important?

Polyps (growth) and cancer can occur anywhere in the bowel and they are usually detected at colonoscopy but there is a small percentage of people who develop cancers despite having colonoscopies as a part of the screening programme. These cancers are usually in the right side of the upper large bowel. There is some evidence to suggest that a certain type of flat polyp called a serrated polyp could cause these cancers. Small studies have suggested that these polyps may have a faster rate of growth into cancer than the usual polyps (adenomas). These serrated polyps are typically flat and quite difficult to detect.

2. Why do we think the use of blue dye (Indigo Carmine) will help?

During a colonoscopy we may find polyps. Sometimes polyps develop into cancer. It may be easier to see the surface pattern and size of a polyp if it is sprayed with blue dye. It may also help to detect very subtle or flat polyps.

3. What is blue dye and will it harm me (any complications)?

The Blue dye that is used to spray the colon is called Indigo Carmine. It is very safe and is a food colouring agent and is already routinely used in camera tests. It does not cause any permanent stains in the bowel and just pools over the surface to allow better visualisation of the bowel and highlight any abnormalities i.e. Polyps etc. Unless you have a known allergy to this dye specifically then there is no need to worry about safety.

4. How much longer will the procedure take if using blue dye?

The doctor who does your colonoscopy test will be spraying the right side of your bowel (upper part of your bowel) with the dye using a spraying catheter through the camera scope. It should not take more than a few minutes extra to spray the bowel. Then the doctor will look at the inside of your bowels carefully and remove any polyps if the need arises.

5. Will I see blue dye in my stools after the procedure?

Appendix H: Protocol for Dye Dilution

PROTOCOL FOR DYE DILUTION:

1. Mix 3.5 ampoules (each ampoule is 10 ml therefore 35 ml of the dye) of indigo carmine in 140 ml of water (total 175ml) to be used per patient.

2. If more dye needs to be used for the second patient then to mix another 3.5 ampoules in 140ml of water or make it up together for the list of both dye cases as 7 amoules (70 ml dye) with 280 ml water = 350 ml of diluted dye solution.

3. Remember the tubing has some fluid to prime it and it may be useful to prime initially with water

4. Technical tip – irrigate with water through the pump as normal if necessary during insertion. Swap bottles during withdrawal to diluted dye bottle if dye spray group patient

5. Dye spray only from the caecum to the splenic flexure on withdrawal

6. Please let the Endoscopy nurse running the list know the number of cases to make up dye or prior to the list as soon as consent confirmed with the participant in order to avoid wastage of dye

* Please confirm points 4 and 5 with the screening colonoscopist

Note:

[1] Each Ampoule of indigo carmine contains 10ml of dye, which needs 40ml water dilution. Hence for 3.5 ampoules, this will be 35ml of dye mixed in 140 ml (40*3=120ml+20ml) of water. Total: 35+140ml=175ml

Appendix I: Laminated sheet for SSP information

Feasibility of reduction in right-sided bowel cancer through CONtrast enhanced colonoSCOPy

Telephonic interview	After phone call	3 days before each list
1. Usual assessment 2. Checks eligibility for study 3. Gives brief info (2 min laminated sheet) 4. No consent at this point 5. Print but consent and PIS and send along with BCP pack as usual	Allocate to list and date as usual (make a note on list re: eligible patients)	1. Checks complete list 2. For those whom have had PIS etc sent-note BSW no. in file 3. Randomiser (internet/ phone)-enter BSW no/ date/time-get random trial no and trial arm (2 min) 4. Enter trial no/BSW no. on labels provided (1 sticker in front of notes, the other on CRF form) 5. Only a maximum of 2 patients will have dye spray-not more

Randomisation checklist (2 minutes)

1. Assessment completed on the phone (allocate list as usual, make a note on the list and Case Report Form (CRF) of potential eligible patients)
2. Website address for randomisation (to be provided during site initiation)
3. Tel number for randomisation in case of problems with website (Mon to Fri 9am to 5pm)– **02920 64 5500**
4. Details to be entered:
   - The initials of the clinician performing the randomisation (3 characters)
   - Bowel screening wales Number (starts with BSW followed by the 9 numbers)
   - Date of colonoscopy appointment
   - Time of colonoscopy appointment (AM/PM)
5. A CONSCOP trial number along with details of which Trial Arm will be displayed
6. Enter CONSCOP No, BSW No and date of procedure onto provided sticker/label (stick in front of notes and CRF)

IMPORTANT: Enter Site/Date/Time accurately as randomiser will ensure not more than 2 enhanced dye Arm is on one list depending on data provided

***NB – If for some reason the date and list needs to be changed – please keep the same randomisation group as original and discuss with Wales Cancer Trials Unit (WCTU) – Tel no – (to be provided)**

Appendix J: List of activity required by Screening Practitioners

CONSCOP

LIST OF ACTIVITY REQUIRED BY SSPS

1. During and after telephonic interview
2. During and immediately after Procedure
3. When Histology report received

1. During and after the telephonic interview :

Telephonic interview

1. Usual assessment
2. Checks eligibility for study
3. Gives brief info (2 min laminated sheet)
4. No consent at this point
5. Print out consent and PIS and send along with GCP pack as usual

After phone call

Allocated to list and date as usual (make a note of BSW No. on list and file re: eligible patients)

3 days before each list

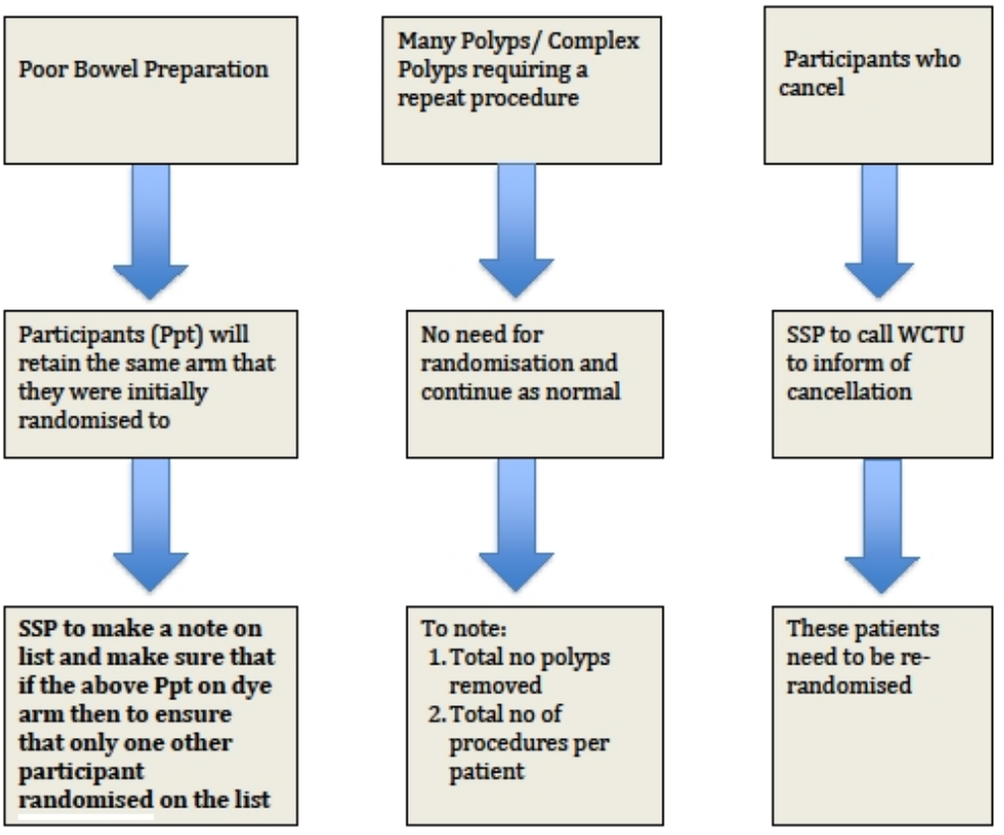
1. Checks complete list
2. For those whom have had PIS etc sent-note BSW no. in file
3. Randomiser (internet/ phone)-enter BSW no/date/ time-get random trial no and trial arm (2 min)
4. Enter trial no/BSW no. on labels provided (1 sticker in front of notes, the other on CRF form)
5. Only a maximum of 2 patients will have dye spray – not more

- See separate leaflet on randomisation
- Please make sure that whilst interviewing patients that an accurate smoking history is elicited i.e. current or ex smoker
- When entering details onto the randomisation site accurately enter date and time of list (am/pm) as this will ensure that no more than two enhanced dye trial arm is on any list

Appendix K: Early Repeat Procedures- scenarios

INFORMATION ON EARLY REPEAT COLONOSCOPY

Early Repeat Colonoscopy's can occur in 3 settings:



Appendix L: Trial Specific Case Report Form Page 1

Initials	DOB	Trial No.
	D D M M Y Y Y Y	

WCTU
WALES CANCER TRIALS UNIT
UNED YMCHWIL CANSER CYMRU

CONSCOP Case Report Form
Page 1 of 3

CONSCOP

Inclusion Criteria

Please refer to Section 6 of the ELCID Protocol for further details on the eligibility criteria *which must be confirmed prior to randomisation of participants.* ***All*** *of the following questions must be answered yes.*

Does the patient meet ALL of the following inclusion criteria? *(enter number in the box)*

1. Tested positive on FOBT in the screening program and eligible and appropriate for an index screening colonoscopy ☐ 1=yes, 2=no
2. Given written informed consent ☐ 1=yes, 2=no

Exclusion Criteria

All *of the following questions must be answered no.*

Does the patient meet ANY of the following exclusion criteria? *(enter number in the box)*

3. Not fit for colonoscopy on the screening program or undergoing alternative investigation such as CT pneumocolon or minimal prep CT scan as their index procedure instead ☐ 1=yes, 2=no
4. Undergone previous colorectal surgery ☐ 1=yes, 2=no
5. Known allergy to a food colouring agent . ☐ 1=yes, 2=no
6. Previous inclusion in the trial ☐ 1=yes, 2=no

List Information

7. BSW number B S W
8. Date of appointment D D M M Y Y Y Y
9. Time of list ☐ 1=A.M. 2=P.M
10. Position on list ☐ 1=1st 2=2nd 3=3rd 4=4th
11. Randomisation arm ☐ 1= standard 2= Enhanced dye

Demographics

12. Current Smoking status ☐ 1=Smoker 2=Ex-smoker 3=Never smoker

If smoker or ex-smoker:
- 12a. Approximate number of cigarettes a day ☐☐☐
- 12b. Approximate number of years ☐☐☐

13. Family history of bowel cancer? ☐ 1=yes, 2=no
14. Family history of bowel polyps? ☐ 1=yes, 2=no

Completed by: PRINT NAME SIGNATURE Date: D D M M Y Y Y Y

MACRO entry by: SIGNATURE Date: D D M M Y Y Y Y

Return to: CONSCOP, Wales Cancer Trials Unit, School of Medicine, Cardiff University, 6th Floor, Neuadd Meirionnydd, Heath Park, Cardiff, CF14 4YS

Version 1.0 June 2014

Appendix M: Trial Specific Case Report Form Page 2 and 3

Initials	DOB	Trial No.
	D D M M Y Y Y Y	

WCTU
WALES CANCER TRIALS UNIT
UNED YMCHWIL CANSER CYMRU

CONSCOP Case Report Form
Page 2 of 3

CONSCOP

The colonoscopy procedure

15. Was bowel preparation adequate or inadequate? ☐
1=Adequate
2=Inadequate for both standard and enhanced dye
3=Patient randomised to enhanced dye but bowel preparation only adequate for standard colonoscopy

If 15=c then please continue with procedure without using dye and go to question 18

If 15=b, please give date and time of scheduled repeat procedure:

16. Date of appointment D D M M Y Y Y Y

17. Time of list ☐ 1=A.M. 2=P.M

Do not complete anything else on this form but complete another of these forms after the rescheduled procedure. Do not re-randomise the patient, keep the patient in the originally allocated trial arm.

18. Endoscopist assessment of procedural difficulty: ☐ 1=Easy 2=Average 3=Difficult 4=Unable to complete

19. Were any of the following procedures necessary? ☐ 1=yes, 2=no

If yes, please answer all the questions below:

a. Use of patient position change ☐ 1=yes, 2=no
b. Adequate insufflation ☐ 1=yes, 2=no
c. Repetitive examination of colonic segment ☐ 1=yes, 2=no
d. Examination of flexures and proximal sides of folds ☐ 1=yes, 2=no
e. Use of torque to flatten folds ☐ 1=yes, 2=no
f. Suctioning of liquid ☐ 1=yes, 2=no
g. Use of mucolytic over polyp ☐ 1=yes, 2=no

20. Previous abdominal/pelvic surgery ☐ 1=yes, 2=no

21. Presence of diverticular disease ☐ 1=yes, 2=no

22. Were any polyps found? ☐ 1=yes, 2=no

22a. If yes, were all polyps removed at this colonoscopy ? ☐ 1=yes, 2=no

22b. If no to 22a, is further colonoscopy scheduled for removal of remaining polyps? ☐ 1=yes, 2=no

22c. If no to 22a, reason ☐

Please check for SAEs at 7 and 30 days after colonoscopy

Completed by: PRINT NAME SIGNATURE Date: D D M M Y Y Y Y

MACRO entry by: SIGNATURE Date: D D M M Y Y Y Y

Return to: CONSCOP, Wales Cancer Trials Unit, School of Medicine, Cardiff University, 6th Floor, Neuadd Meirionnydd, Heath Park, Cardiff, CF14 4YS

Version 1.0 June 2014

Initials	DOB	Trial No.
	D D M M Y Y Y Y	

WCTU
WALES CANCER TRIALS UNIT
UNED YMCHWIL CANSER CYMRU

CONSCOP Case Report Form
Page 3 of 3

CONSCOP

Resource utilisation data (*Llandough only*)

23. Was enhance dye given? ☐ 1=yes, 2=no

If yes, please answer all the questions below:

a. Amount of dye used (number of vials) ☐ 0-5 expected

b. Volume of dilutant (ml) ☐☐☐ 0-250 expected

c. Method of dye dispersion ☐ 1=Spray catheter 2=Pump

24. Were any of the following resources used: ☐ 1=yes, 2=no

If yes, please answer all the questions below:

a. ABC probes	☐ 1=yes, 2=no			
b. Coagrasper	☐ 1=yes, 2=no			
c. Injectors	☐ 1=yes, 2=no	If yes, number	☐	1-2 expected
d. Number of clips	☐ 1=yes, 2=no	If yes, number	☐	1-2 expected
e. Number of snares	☐ 1=yes, 2=no	If yes, number	☐☐	1-20 expected
f. Number of specimen pots	☐ 1=yes, 2=no	If yes, number	☐	1-3 expected
g. Other resource use	☐ 1=yes, 2=no			

If 24g. is yes, please specify below with amounts

	number ☐☐
	number ☐☐
	number ☐☐

Appendix N: CONSCOP Cost Study – What are the likely costs associated with the introduction of enhanced colonoscopy techniques?

Aim

To assess the additional resources needed for successful implementation of enhanced colonoscopy techniques.

Approach

The costs associated with the colonoscopy procedures within the trial were assessed in two parts, the additional costs to the NHS of providing new resources required to implement the new enhanced dye technique and those resources used during routine practice. The costs were assessed from the perspective of the UK NHS. The analysis was conducted on procedures with a complete set of original data across all resource use variables to assess the extent of cost variation between the groups and also on all available cases (intention to treat analysis) with mean imputation for sites that did not collect data on equipment and instruments related to the dye technique and used during a colonoscopy.

Costing Methods - Implementation cost of chromocolonoscopy

All screening units in the Bowel Screening Wales programme were invited to join the trial – those who were interested and had the resources and experience to carry out the trial were recruited and trained in the use of the trial enhanced dye techniques. Additional resources in the form of staff time to train in the new enhanced procedure are listed in Table 1 below.

Table 1. Staff training required for chromocolonoscopy

Staff requiring training	**Training needs**	**Training time**	**Unit cost** Source of costs: PSSRU 2016[1]
Training for BSW Screening Colonoscopist	To carry out the colonoscopic procedure using the contrast dye technique.	1 hour	Hospital based doctors: Consultant surgical £137 Per working hour
Training for BSW SSP	BSW SSPs need to be trained to provide adequate information to the patients,	2 hours	Hospital based nurses - band 6: £44 Per working hour

	correct data collection, reporting of Adverse events (AE and SAEs)		
Screening unit staff	Trained in preparation of the dye solution	1 hour	Hospital based nurses - band 6: £44 Per working hour
Training provided by			
Consultant grade colonoscopist already expert in the enhanced dye technique.	All the above staff trained in one teaching session	2 hours	Hospital based doctors: Consultant surgical £137 Per working hour
PSSRU - Personal Social Services Research Unit			

Also included in the implementation cost is the cost of the contrast dye and equipment necessary to disperse the dye during the colonoscopy procedure. Table 2 outlines the resources required and accompanying unit costs. The technique of spraying the colon with contrast dye can be done in two different ways, with a pump which is widely used to cleanse a colon with water during a standard colonoscopy but is adapted using bungs and tubing to allow the spraying of dye onto the colon walls or alternatively a spray catheter. This data was collected by one site only during the study (n=110 procedures). The mean cost of dye and accompanying equipment calculated from the data collected at this site is applied to the remaining chromocolonoscopy procedures performed at sites that did not collect data.

Table 2. Consumables required for the chromocolonoscopy		
Consumable	**Use**	**Unit cost** **Source of costs: Trial team (April 2017)**

1% indigo carmine dye	Each Ampoule of indigo carmine contains 10ml of dye, which needs 40ml distilled water for dilution. The standard dye dose per procedure is 175ml which requires 3.5 10ml dye ampoules.	Cost per 10ml ampoule is £7.74 which is purchased in packs of 10 - £77.40 (inc VAT). Cost per standard dye dose per procedure = £27.09.
Spray Catheter	Specifically used to spray the dye onto the colon walls	£199.99 (inc VAT) for 5 £40 per spray catheter
Bung	BioShield Biopsy Valves with Irrigation Line 30mm (Box of 50) 22 boxes/year	£129.78 / £2.5956 per valve (inc VAT)
	BioShield Biopsy Valves with Irrigation Line Pentax Scope (Box of 50) 1 box/year	£129.78 / £2.5956 per valve (inc VAT)
Tubing costs	Torrent Irrigation Tubing (Box of 25) 20 boxes/year	£314.40 / £12.576 per tube (inc VAT) 1 tube is used per list = 2 patients Cost = £6.288 per patient
Note: When adapting the pump for dye spraying the bungs are changed per patient while the tubing can be reused. A patient list contains 1 to 2 patients, therefore 2 bungs and 1 tube is used per patient list.		

Calculation of Implementation costs

The implementation costs to the NHS consist of specialist equipment to perform the chromocolonoscopy procedure and the required training to carry it out. The chromocolonoscopy technique is only performed during the first index colonoscopy and not during subsequent repeat procedures. Table 3 shows the mean training cost per procedure is £4.94 and the mean equipment cost per procedure is £47.99. In total the implementation cost per procedure is £52.93. The spray

catheter was used in only 30% of procedures with a cost of £40 per colonoscopy. This compares to the technique of adapting existing pumps with tubing and a valve which added £8.88 to the cost of the colonoscopy and was the preferred method of clinicians in 70% or 77 procedures out of the 110 performed. At the site elected to collect resource use related to the enhanced dye technique 143 patients were randomised to the intervention index procedure. Data was collected for 110 procedures and 8 procedures did not use the enhanced dye technique.

Table 3. Calculation of Implementation cost of chromocolonoscopy		
Training costs	**per screening unit performing 110* procedures**	**Cost**
Colonoscopist - consultant	I hour of training	£137
SSP – Band 6 nurse	2 hours of training	£88
Enoscopy nurse – band 6	1 hour of training	£44
Expert colonoscopist trained in the new enhanced technique	2 hours to train the above	£274
Total training cost per screening unit		**£543**
Training cost per patient procedure	**£543/110 procedures**	**£4.94**
Consumable cost		
Contrast dye (with <175ml samples corrected = 175ml)		£29.77 (s.d £5.52) Min £27.09 max £54.18

Bungs and tubing used with pumps	77 (70%) procedures	£684.04
Spray catheter	33 (30%) procedures	£1,320.00
	Mean cost of Instruments used for dye dispersal	£2,004.04/110=£18.22
Total dye/consumable cost per procedure	**Dye plus dispersal instruments per procedure**	**£47.99 (s.d £14.77)** **Min £35.97,** **max £86.44**
TOTAL IMPLEMENTATION COST PER PROCEDURE	**Training plus dye/dispersal instruments**	**£52.93 (s.d £14.77)** **Min £40.91,** **max £91.38**
*Note: data on the resources used to perform the enhanced dye technique was collected at one site during the index colonoscopy – 228 patients were randomised with 110 to the standard colonoscopy group and 118 to the enhanced dye colonoscopy group, 8 of which did not receive the dye thus leaving 110 in the enhanced colonoscopy group.		

Costing Methods - Resource use during colonoscopy procedure

Resource use data regarding staff time performing the procedure and medications administered during a procedure were collected from all participating screening sites. Resources classified as consumables were only collected at one site during index colonoscopies. At this site 183 procedures (20% of 904 total colonoscopies) documented the use of instruments as listed in the CRF during the colonoscopy - 91 standard arm and 92 chromocolonoscopy arm. As this is a feasibility study the mean consumable cost for each arm of the trial was calculated and used as a proxy cost for all procedures performed at sites which did not collect data related to consumables. This allows an

overall resource use comparative cost to be calculated for all patient procedures in the available cases analysis.

Staff

The NHS staff required to perform the colonoscopy and associated unit cost are listed in Table 4. The cost per working hour for NHS staff is taken from the PSSRU 2016[1] and a cost per minute calculated (cost per minute is rounded to the nearest whole number). The cost per minute for each staff member involved in the procedure is multiplied by the duration variable (measured in minutes) to produce a total staff cost per patient procedure.

Table 4. RESOURCE USE COSTS: Staff			
		Source of costs: PSSRU 2016[1]	
Staff time	Cost of staff performing procedure:	Staff group	Unit cost
(Duration variable)	1.Colonoscopist	Hospital based doctors: Consultant surgical	Per working hour £137 (£2.28 per minute)
	2.Endoscopy Nurse and 3.Specialist Screening Practitioner - SSP	Hospital based nurses: Staff band 6	Per working hour = £44 (£0.73 per minute)

Consumables

Data recording the instruments used during a procedure specifically listed in the CRF was collected on patients receiving a colonoscopy at one treatment centre and are listed in Table 5. The unit costs for any consumables used are supplied by the trial team. A total consumables cost per patient procedure is calculated based on the total number of instruments required during the colonoscopy. For the purposes of calculating an overall resource use mean cost per patient procedure, the mean

cost from the sample data is used to impute a cost for procedures at sites that did not collect data – standard arm (n=91) mean cost = £66.65 (sd 84.39) and chromocolonoscopy arm (n=92) = £78.57 (sd 79.72) – a non-significant difference of £11.92 (p=.327). The cost data reported in the results tables are based on the sample data only as imputation artificially reduces variation producing a false significant difference in cost.

The cost per arm of 'other' consumable instruments is evaluated separately from the instruments considered to be integral to the procedure. The mean cost of other consumables for standard arm (n=91) is £7.08 compared to £8.34 for chromocolonoscopy arm (n=92), producing a non-significant difference of £1.26 (p=0.579). No further analysis is undertaken with this data as it is unknown if every questionnaire respondent answered the question fully as the data gathered is rather patchy and some instruments which would be expected to be reported were not. This factor maybe be perceived as a limitation to the study.

Table 5. RESOURCE USE COSTS: Consumables		
		Source of costs: Trial team (April 2017)
Instrument costs	Notes	**Current Unit cost per item (£)**
A P C Probes		£86.70
Coagrasper		£147.25
Injectors		£22.90
Clips		£40.80
Snares		£19.99
Specimen pots		£0.19

Medication

Costing medication used during the colonoscopy is based on the number of ampules used during the procedure based on the total quantity reported in either milligrams or micrograms. If total quantity of

medication reported included the half measure of an ampule, the cost of the whole ampule was applied. For example, if a total of 3mg of midazolam was reported, indicating the administration of one and a half 2mg/ml, 5-ml ampules during the procedure, two ampules are included in the costs. Aside from the standard medications administered during the procedure Buscopan is the only other medication reported. Table 6 below shows the medications reported and associated unit cost. A cost per patient is calculated based on all medication received during the procedure. Bowel preparation laxative medication was also calculated per procedure.

Table 6. RESOURCE USE COSTS: Medication	
Drug	**BNF[2] Unit cost (May 2017)**
Benzodiazepine	
Sedation - Midazolam	Injection, midazolam (as hydrochloride) 2 mg/mL, 5-mL amp = 65p;
Opioid	
Analgesia - Pethidine	Injection, pethidine hydrochloride 50 mg/mL, , 2-mL amp = 47p;
Analgesia – Fentanyl	Injection, fentanyl (as citrate) 50 micrograms/mL, net price 2-mL amp = 45p,
Reversal drugs	
Naloxone	Naloxone use was not reported
Flumazenil	Injection, flumazenil 100 micrograms/mL, net price 5-mL amp = £13.50
Other medication – Buscopan	Injection, hyoscine butylbromide 20 mg/mL, net price 1-mL amp = 29p
Bowel prep costs	**Source: Trial team April 2017**
Kleenprep	£10.83

Moviprep	£8.01
Senna and citrameg	£6.59
Picolax (two sachets per patient)	£3.39

Missing data

As consumables resource data relating to surgical instruments, equipment and contrast dye solution was only collected at one screening site for the initial index colonoscopy procedure, the mean cost per study arm from this sample is applied to the remaining sites that did not collect data. Where data is missing for staff time performing the procedure and type of bowel preparation medication prescribed, the mean cost per study arm is applied. Missing duration data includes 6 procedures from the enhanced dye group and 3 procedures belonging to the standard group. If data was not entered noting medication administered during the procedure it is assumed that medication was not prescribed and a zero cost applied. Five procedures are excluded from the analysis as no data was entered across any of the resource use categories.

Data Analysis

Available case analysis was conducted for procedures where the majority of resource use data has been collected and where mean imputation had been used to substitute missing data. A descriptive analysis reporting mean cost and standard deviation together with the minimum and the maximum resource use costs are reported. Independent samples T-Test is used to evaluate differences in resource use costs between the two trial arms and 95% confidence intervals show the lower and upper parameters of the cost difference between the standard and enhanced procedure.

References

1. **Curtis, L. Unit Costs of Health and Social Care 2016, Personal Social Services Research Unit (PSSRU), University of Kent, Canterbury**

2. Joint Formulary Committee. British National Formulary (online) London: BMJ Group and Pharmaceutical Press https://bnf.nice.org.uk/drug/

March 2014

GeneRead™ DNA FFPE Handbook

For purification of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissues for reliable next-generation sequencing analysis

Making improvements in life possible®

QIAGEN®

Sample & Assay Technologies

Appendix P: Datasheet for DNA extraction from FFPE samples

Group	Polyp No	Matched adenoma	Trial No	Male/female	Smoking	Size of the Polyp	Interval from slide scraped to extraction	Area in mm2	How many slides?	Slide um	DNA Quantification ng/ul	Volume (ul)	Box Label	Total Volume	Yield for library prep	WES at WGP
1	55	N/A	123063	M	ExSmoker	8	2	56	5	10	7.63	20	5D	152.6	Y	N
1	85	N/A	123087	F	ExSmoker	9	2	28	5	10	2.37	20	5E	47.4	Y	N
1	103	N/A	123094	F	Never Smoked	3	1	24	5	10	2.64	20	7E	52.8	Y	N
1	168	N/A	100001	F	Never Smoked	7	1	30	5	10	5.39	20	9B	107.8	Y	N
1	169	N/A	100001	F	Never Smoked	5	2	35	5	10	7.54	20	10C	150.8	Y	N
1	216	N/A	100009	M	ExSmoker	16	1	30	5	10	0.2	20	9C	4	N	N
1	217	N/A	100009	M	ExSmoker	18	1	35	5	10	0.89	20	9D	17.8	Y	N
1	236	N/A	123155	M	ExSmoker	5	3	24	5	10	0.344	20	9E	6.88	N	N
1	374	N/A	123190	M	ExSmoker	3	2	12.3	5	10	6.17	20	2B	123.4	Y	N
1	505	N/A	100052	M	ExSmoker	3	1	24	5	10	2.01	20	9A	40.2	Y	N
1	506	N/A	100052	M	ExSmoker	3	2	25	5	10	1.89	20	2C	37.8	Y	N
2	71	Y	53008	M	Never Smoked	4	1	9	5	10	2.55	20	8A	51	Y	N
2	72	Adenoma	53008	M	Never Smoked	8	1	20	5	10	9.35	20	8B	187	Y	N
2	206	N-too small	94016	M	ExSmoker	7	1	9	5	10	0.317	20	8C	6.34	N	N
2	393	Y	123201	M	Never Smoked	5	2	16	5	10	15.4	20	2D	308	Y	Y
2	654	N-too small	123166	M	Current smoker	4	2	20.3	5	10	12.7	20	2E	254	Y	Y
3	17	N	123014	F	Current smoker	10	2	160	5	10	32.4	20	8D	648	Y	Y
3	128	Y	53031	F	Never Smoked	4	3	14	5	10	3.04	20	5B	60.8	Y	N
3	128.1	Adenoma	53031	F	Never Smoked	3	3	20	5	10	4.21	20	5A	84.2	Y	N
3	151	N	123014	F	Current smoker	13	1	72	5	10	44	20	7A	880	Y	Y
3	214	N	53072	M	Current smoker	10	1	50	5	10	19.1	20	6B	382	Y	N
3	255	Y	123170	M	ExSmoker	5	2	16	5	10	0.278	20	10A	5.56	N	N
3	399	Y	123210	M	Current smoker	6	2	30	5	10	11.4	20	10B	228	Y	Y
3	400	Adenoma	123210	M	Current smoker	1	2	54	5	10	29.2	20	7D	584	Y	Y
3	447	Y	29032	M	ExSmoker	3	1	48	5	10	>60	20	6E	1200	Y	Y
3	449	Adenoma	29032	M	ExSmoker	8	2	20	5	10	8.74	20	7B	174.8	Y	N
3	450	Y	29032	M	ExSmoker	5	2	25	5	10	16.1	20	7C	322	Y	Y
3	624	Y	29042	F	ExSmoker	4	1	21	5	10	19.2	20	6D	384	Y	N
3	625	Adenoma	29042	F	ExSmoker	4	1	12	5	10	30.9	20	6C	618	Y	Y
3	673	Adenoma	337903	M	ExSmoker	5	2	25	5	10	1.74	20	4E	34.8	Y	N
3	677	Y	337903	M	ExSmoker	3	3	16	5	10	1.3	20	5C	26	Y	N
3	708	N	53072	M	Current smoker	5	0	98	5	10	41.4	20	2A	828	Y	Y
3	715	Adenoma	123170	M	ExSmoker	2	1	91	5	10	>60	20	6A	1200	Y	Y
4	172	N/A	123120	F	ExSmoker	5	1	77	5	10	>60	20	3E	1200	Y	Y

June 2015

GeneRead™ DNAseq Targeted Panels V2 Handbook

For targeted enrichment prior to next-generation sequencing

(96-well plate compatible, all-bead purification)

QIAGEN®

Sample & Assay Technologies

BMJ PUBLISHING GROUP LTD. LICENSE
TERMS AND CONDITIONS

Feb 18, 2024

This Agreement between Dr. Rajeswari Ramaraj ("You") and BMJ Publishing Group Ltd. ("BMJ Publishing Group Ltd.") consists of your license details and the terms and conditions provided by BMJ Publishing Group Ltd. and Copyright Clearance Center.

License Number	5732010707111
License date	Feb 18, 2024
Licensed Content Publisher	BMJ Publishing Group Ltd.
Licensed Content Publication	Gut
Licensed Content Title	Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002)
Licensed Content Author	Stuart R Cairns,John H Scholefield,Robert J Steele,Malcolm G Dunlop,Huw J W Thomas,Gareth D Evans,Jayne A Eaden,Matthew D Rutter,Wendy P Atkin,Brian P Saunders,Anneke Lucassen,Paul Jenkins,Peter D Fairclough,Christopher R J Woodhouse,developed on behalf of The British Society of Gastroenterology, and the Association of Coloproctology for Great Britain and Ireland
Licensed Content Date	May 1, 2010
Licensed Content Volume	59
Licensed Content Issue	5
Type of Use	Dissertation/Thesis
Requestor type	Individual

BMJ PUBLISHING GROUP LTD. LICENSE
TERMS AND CONDITIONS

Feb 18, 2024

This Agreement between Dr. Rajeswari Ramaraj ("You") and BMJ Publishing Group Ltd. ("BMJ Publishing Group Ltd.") consists of your license details and the terms and conditions provided by BMJ Publishing Group Ltd. and Copyright Clearance Center.

License Number	5731110445969
License date	Feb 16, 2024
Licensed Content Publisher	BMJ Publishing Group Ltd.
Licensed Content Publication	Gut
Licensed Content Title	British Society of Gastroenterology position statement on serrated polyps in the colon and rectum
Licensed Content Author	James E East,Wendy S Atkin,Adrian C Bateman,Susan K Clark,Sunil Dolwani,Shara N Ket,Simon J Leedham,Perminder S Phull,Matt D Rutter,Neil A Shepherd,Ian Tomlinson,Colin J Rees
Licensed Content Date	Jul 1, 2017
Licensed Content Volume	66
Licensed Content Issue	7
Type of Use	Dissertation/Thesis
Requestor type	Individual
Format	Print and electronic
Portion	Figure/table/extract

ELSEVIER LICENSE
TERMS AND CONDITIONS

Feb 18, 2024

This Agreement between Dr. Rajeswari Ramaraj ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5731090841097
License date	Feb 16, 2024
Licensed Content Publisher	Elsevier
Licensed Content Publication	Modern Pathology
Licensed Content Title	An update on the morphology and molecular pathology of serrated colorectal polyps and associated carcinomas
Licensed Content Author	Rish K. Pai,Mark Bettington,Amitabh Srivastava,Christophe Rosty
Licensed Content Date	Oct 1, 2019
Licensed Content Volume	32
Licensed Content Issue	10
Licensed Content Pages	26
Start Page	1390
End Page	1415
Type of Use	reuse in a thesis/dissertation

BMJ PUBLISHING GROUP LTD. LICENSE
TERMS AND CONDITIONS

Feb 18, 2024

This Agreement between Dr. Rajeswari Ramaraj ("You") and BMJ Publishing Group Ltd. ("BMJ Publishing Group Ltd.") consists of your license details and the terms and conditions provided by BMJ Publishing Group Ltd. and Copyright Clearance Center.

License Number	5732100368384
License date	Feb 18, 2024
Licensed Content Publisher	BMJ Publishing Group Ltd.
Licensed Content Publication	Gut
Licensed Content Title	Development and validation of the WASP classification system for optical diagnosis of adenomas, hyperplastic polyps and sessile serrated adenomas/polyps
Licensed Content Author	Joep E G IJspeert,Barbara A J Bastiaansen,Monique E van Leerdam,Gerrit A Meijer,Susanne van Eeden,Silvia Sanduleanu,Erik J Schoon,Tanya M Bisseling,Manon CW Spaander,Niels van Lelyveld,Marloes Bargeman,Junfeng Wang,Evelien Dekker,Dutch Workgroup serrAted polypS & Polyposis (WASP)
Licensed Content Date	Jun 1, 2016
Licensed Content Volume	65
Licensed Content Issue	6
Type of Use	Dissertation/Thesis
Requestor type	Individual

ELSEVIER LICENSE
TERMS AND CONDITIONS

Feb 18, 2024

This Agreement between Dr. Rajeswari Ramaraj ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5731100860261
License date	Feb 16, 2024
Licensed Content Publisher	Elsevier
Licensed Content Publication	Gastroenterology
Licensed Content Title	Increased Risk of Colorectal Cancer Development Among Patients With Serrated Polyps
Licensed Content Author	Rune Erichsen,John A. Baron,Stephen J. Hamilton-Dutoit,Dale C. Snover,Emina Emilia Torlakovic,Lars Pedersen,Trine Frøslev,Mogens Vyberg,Stanley R. Hamilton,Henrik Toft Sørensen
Licensed Content Date	Apr 1, 2016
Licensed Content Volume	150
Licensed Content Issue	4
Licensed Content Pages	13
Start Page	895
End Page	902.e5
Type of Use	reuse in a thesis/dissertation