Randomised study of EndoRings™-assisted vs. standard colonoscopy for detection of polyps in at risk individuals with Lynch Syndrome.

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Declaration

I *Rohin Dhar* hereby declare that the research reported in this dissertation is based on my original, independent work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I also confirm that this work has not been reported or published prior to registration for the above-mentioned degree.

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Abstract

Introduction: Lynch syndrome (LS) is an autosomal dominant condition and is the most common cause of inherited colorectal cancer (CRC), contributing to approximately 3%-5% of newly diagnosed cases of colorectal malignancy. LS affected individuals bear 18% – 53% lifetime risk for development of CRC. The only therapeutic approach to prevent development of CRC among individuals with LS is periodic colonoscopic screening for detection and removal of adenomas and polyps, which are the precursors for cancer. Despite being the current gold standard, and accounting for all other variables (such as experience of the physician), conventional colonoscopy has been known to sometimes miss detecting adenomas/polyps, specifically those present in the folds of the colonic mucosa and on the inner luminal wall of the colonic flexures. EndoRings™ assisted colonoscopy has therefore been developed to improve colonoscopy outcomes in terms of enhancing adenoma detection rates (ADR)/polyp detection rates (PDR) and involves flexible silicone rings mechanically stretching the colonic folds and thus enhancing total colon visualisation.

Objectives: The present study aims to primarily investigate the efficacy of EndoRings™ assisted colonoscopy compared to traditional colonoscopy in terms of ADR/PDR in a known cohort of individuals with LS in a South African setting.

Methods: The study was conducted as a cross-sectional randomised controlled trial. Individuals from the Northern Cape province of South Africa with LS were enrolled into the study during our Annual Northern Cape Colonoscopy Outreach trip for the year 2015. A total of 54 individuals (per-protocol) were included in the study and randomised blindly using computer randomisation into a control arm undergoing standard colonoscopy (n=27) and a study arm undergoing EndoRings™-assisted colonoscopy (n=27). Number of polyps detected (the primary outcome) along with a set of secondary outcomes was recorded in real time on data sheets for each individual and statically analysed using IPython.

Results: The female to male ratio in the EndoRings™ group was 19:8 versus 15:12 in the standard colonoscopy group (P = 0.40) whereas the mean age of patients was 43.98±15.27 years and 44.26±14.67 years (P = 0.05) respectively. The average number of polyps detected in the EndoRings™ group was 1.4 versus 0.9 in the non-EndoRings™ group (P = 0.60).

Conclusion: The present study outcomes observed comparable ADR/PDR in EndoRings™ assisted versus standard colonoscopy with no statistically significant difference. This result may be due to the study's limitations (small sample size) and design. Though no statistically significant conclusions could be reached, EndoRings™ assisted colonoscopy was perceived as being helpful in terms of increasing total colonic visualisation and allowing better scope stabilisation during interventions. Comparable intubation times, withdrawal times, total procedure times and similar complication rates were observed in both study arms. Although this study demonstrated non-inferiority of EndoRings™ compared to standard colonoscopy, further studies with a larger sample size in an easily accessible population over a longer study period are recommended.

Acknowledgements, Contributions & Conflict of Interest

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I would also like to thank and acknowledge First Medical for suppling the EndoRings™ devices, and for paying the cost of my trip to the Northern Cape to conduct this study.

Conflict of interest statement: I would like to declare that neither I, nor my supervisor or any person involved with this research had/has any stake, be it financial or otherwise in First Medical. Although First Medical provided the EndoRings™ and the funds to support me during the trip to the Northern Cape, they and/or none of their representatives had any involvement at any stage in the conduction of this trail. The trial design, methodologies, analysis, findings as well as the final manuscript is the result of the author's own work with no influence from First Medical or its representatives.

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List of Abbreviations

ACF	aberrant crypt foci
ADR	adenoma detection rate
APC	adenomatous polyposis coli
ASGE	American Society for Gastrointestinal Endoscopy
BRAF	B - RAF proto-oncogene
CI	confidence interval
CIMP	CpG island hypermethylation phenotype
CIN	chromosomal instability
CRC	colorectal cancer
CpG	5'—C—phosphate—G—3' sequence
DCC	deleted in colon cancer
DNA	deoxyribonucleic acid
EPCAM	epithelial cell adhesion molecule
FAP	familial adenomatous polyposis
FUSE	Full spectrum endoscopy
GSH	Groote Schuur Hospital
HNPCC	hereditary non-polyposis colorectal cancer
HREC	Human Research Ethics Committee
HPP	hyperplastic polyp
IHC	immunohistochemistry
kRAS	kRAS proto-oncogene
LOH	loss of heterozygosity
LS	Lynch syndrome

MAP	MUTYH – associated polyposis
MLH1	mut - L homolog 1
MLH3	mut - L homolog 3
MMR	mismatch repair
MSI	microsatellite instability
MSI-H	high frequency microsatellite instability
MSI-L	low frequency microsatellite instability
MSH2	mut - S homolog 2
MSH6	mut - S homolog 6
MutS – alpha	mutator – S – alpha
MutS – beta	mutator – S – beta
MutL – alpha	mutator – L – alpha
MutL – beta	mutator – L – beta
MutL – gamma	mutator – L – gamma
NCCN	National Comprehensive Cancer Network
p53	tumour protein 53
PCR	polymerase chain reaction
PDR	polyp detection rate
PMS1	post meiotic segregation 1
PMS2	post meiotic segregation 2
RCT	randomised control trial
SD	standard deviation
SMAD2	mothers against decapentaplegic homolog 2
SMAD4	mothers against decapentaplegic homolog 4
SSA	sessile serrated adenoma
TSA	traditional serrated adenoma
UCT	University of Cape Town

Chapter 1: Introduction and Literature review.

1.1 Search methods.

For this literature review and for the entirety of this thesis a relevant literature search was performed using PubMed, PubMed Central, Medical Subject Headings (MeSH), and UpToDate databases. All abstracts and full texts yielding from the search terms were used, provided they were in English. Some South African articles were obtained directly from the author concerned. Internal citations and references within the initial search articles were also sourced.

1.2 Colorectal cancer.

1.2.1 Incidence and epidemiology.

Colorectal cancer (CRC) is a major cause of morbidity and mortality [1]. Globally it accounts for approximately 10% of all cancers [2,3]. Worldwide CRC is the 3rd most common cancer in men and 2nd most common in women, with 1.4 million new cases and almost 694,000 deaths estimated to have occurred in 2012 [4]. According to GLOBOCAN, the reported incidence in western countries, or countries following a western lifestyle is significantly higher than in most African and South Asian countries [5]. However, trends in CRC incidence and mortality are reversing. In economically developed nations, incidence and mortality are currently on a decline, whilst in economically underdeveloped nations an uptrend is emerging [9]. Another worrying development is an increase in the incidence of young (<40 years) CRC. This phenomenon is occurring globally but is much more noticeable in the Middle Eastern and South and East Asian countries [9].

Risk factors for developing CRC can broadly be classified as environmental or inherited. Advanced age, lower socio-economic status, modifiable behaviors such as lack of exercise, smoking, poor diet, obesity, and a lack of a national screening program are all important risk factors [6-8].

In 2013 (latest data available), the South African National Cancer Registry reported the number of new cases of CRC diagnosed in South Africa as 1906 for men and 1542 for women. This translates to 5.3% and 4.2% of all cancers at age standardized incidence of 11.47/100000 and 6.23/100000 respectively [10].

1.2.2 Classification.

CRC present in three distinct patterns, sporadic, familial or hereditary. Sporadic CRC is the most common (60-70%) and occurs in individuals with no prior family history. Aetiologically these tumors are thought to be caused by modifiable environmental, dietary, behavioral risk factors as well as non-modifiable factors such as advanced age (>50 years) and sex [11].

Familial CRC, the 2nd most common (25%) is the least well understood and is not associated with any identifiable gene. Individuals have a positive family history, but the pattern is not entirely consistent with the hereditary syndromes. First degree family members of affected individuals are at two to three times greater risk than normal of developing CRC. The risk also increases if multiple first-degree relatives are affected or if the index case was less than 50 years of age at diagnosis [12]. Familial CRC type X is a good example that has all the clinical criteria of Lynch syndrome (LS) but as yet no identifiable germline mutation [13].

Hereditary CRC (10%) has two main tumor variants that are distinguished by the propensity to develop a vast number of adenomatous polyps or not. The polyp forming variants include familial adenomatous polyposis (FAP) and its variants (attenuated FAP, MYH-associated FAP), MUTYH-associated polyposis (MAP) and various hamartomatous polyposis syndromes such as Peutz-Jeghers, juvenile polyposis and Cowden syndrome [14]. LS is the second variant, in which the affected individuals do not develop polyposis and only a few to no polyps may be identified. These hereditary conditions incur a very high risk of developing CRC, with almost 90-100% developing cancer in FAP and 50-70% in individuals with LS during their lifetime.

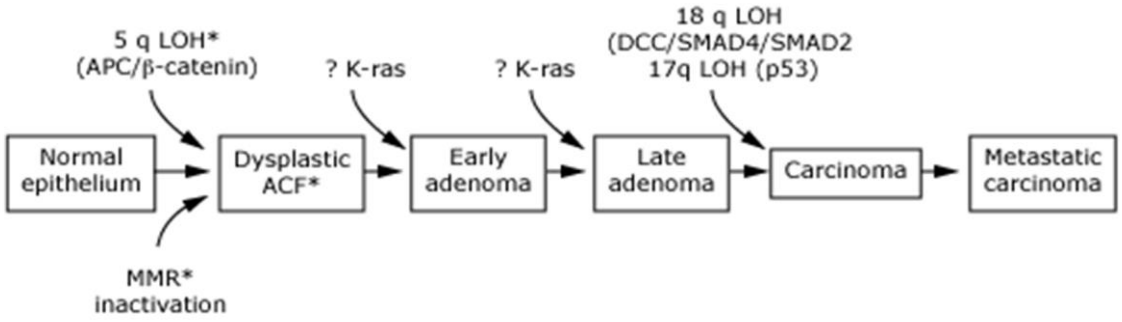
1.2.3 Genetics.

Our understanding of the molecular events involved in the development of CRC is greater than for other solid tumors. Germline mutations are involved in the development of the inherited syndromes, while a stepwise accumulation of somatic mutations is responsible for most sporadic cases resulting in a normal cell becoming malignant. Mostly CRCs develop

from mutations, suppression, deletions, overexpression, altered deoxyribonucleic acid (DNA) methylation, gene rearrangements, amplifications and deletions of a limited number of genes. The genes usually implicated are the adenomatous polyposis coli (APC), kRAS oncogene (kRAS), tumor protein 53 (p53), mismatch repair genes (MMR) MSH2 and MLH1 and more recently the deleted in colon cancer (DCC), SMAD4, and SMAD2 genes [15]. Development of CRC has been attributed to the breakdown of signaling pathways that are otherwise responsible for normal regulation of cellular function. These pathways include the following:

The adenoma-carcinoma sequence pathway: Normally, intestinal cells are continuously lost and replaced by apoptosis and exfoliation into the intestinal lumen. New cells originate at the intestinal crypt base and progress towards the lumen at which time proliferation of cells ceases and they differentiate into their final form [16]. Most CRCs rise from adenomas (adenomatous polyps) in the colon that over time, due to stepwise disruption of the normal mechanisms, lead to dysplasia and ultimately carcinoma formation as illustrated in Figure 1 below.

Figure 1. A genetic model for colorectal tumourigenesis [17]



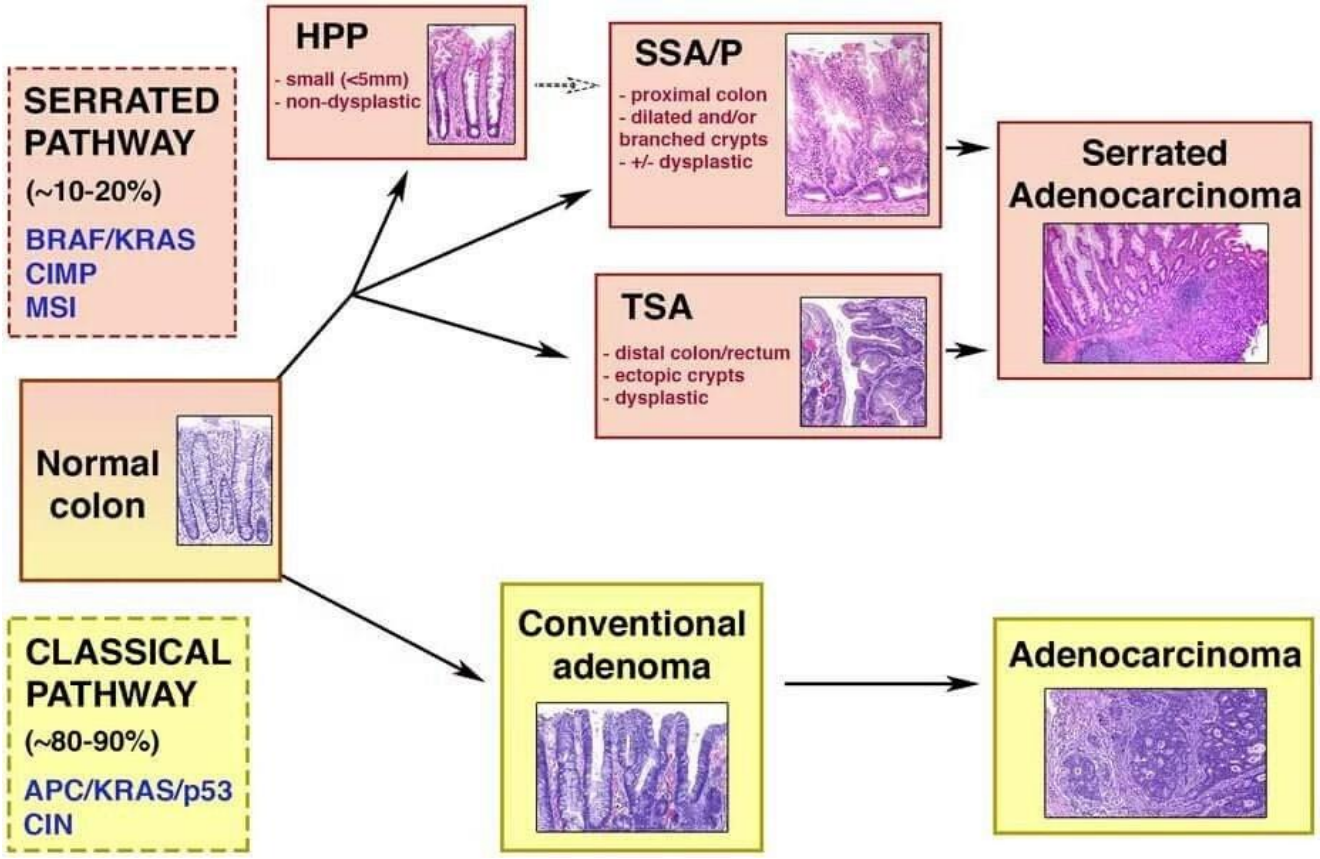
LOH: loss of heterozygosity; DCC: deleted in colon cancer gene; APC: adenomatous polyposis coli gene; ACF: aberrant crypt foci; MMR: DNA mismatch repair enzyme [17].

In 1990, Fearon and Vogelstein provided molecular data to supplement the above concept. According to the Vogelstein model, germline or somatic mutations are necessary for carcinoma to develop, and it is the accumulation rather than the sequence of multiple genetic mutations that ultimately determines the biological behaviour of the tumour [16]. Germline mutations are responsible for inherited syndromes such as FAP and LS. Sporadic cancers generally occur due to a stepwise accumulation of multiple somatic mutations. Mutations in the APC gene occurs early in the tumorigenesis of both inherited and sporadic colorectal

cancers, while p53 tumour suppressor gene mutations generally occur later in the process [17].

Serrated polyp pathway: Although most CRCs are thought to develop through the adenoma-carcinoma sequence, recent evidence suggests the existence of an alternate pathway in which serrated polyps replace the traditional adenoma as the precursor lesion (Figure 2.) [18].

Figure 2. Serrated pathway versus classical pathway in tumourigenesis [19].



HPP: hyperplastic polyps, SSA: sessile serrated adenoma, TSA: traditional serrated adenoma [19].

Molecular tumourigenesis pathways: Currently three known molecular pathways leading to tumour formation exist. These are the chromosomal instability (CIN) pathway, the mutator- phenotype/DNA mismatch repair pathway and the hypermethylation phenotype hyperplastic/serrated polyp pathway [20]. Tumour formation via the CIN (APC) pathway results from "gain of function" mutations. This occurs due to activation of growth promoting oncogenes or secondary to diminished activity of tumour suppressor genes or apoptotic

pathways [16]. CIN tumours may be inherited (FAP) or sporadic, and are characterized by chromosomal abnormalities such as deletions, insertions, and loss of heterozygosity [16].

Inherited CRC such as LS characteristically progress through the mutator phenotype/mismatch repair pathway. This involves the dysfunction of DNA MMR enzymes, due to germline mutations in one of several different MMR genes, most commonly MLH1 or MSH2. Suppression of MMR genes result in accumulation of DNA errors in the genome. The accumulation of these abnormalities in short sequences of nucleotide bases may be repeated dozens to hundreds of times within the genome and are known as microsatellites. Therefore, tumours can phenotypically have high levels of microsatellite instability (MSI-H) or low levels of microsatellite instability (MSI-L). It should be noted that MSI -H occurs in approximately 15% of sporadic colorectal cancers as well, though in most of these cases the gene 'silencing' is not due to a MMR mutation but due to epigenetic reasons such as hypermethylation of the gene promoter for the MMR enzyme [21].

Lastly the hypermethylation phenotype (CIMP+) pathway involves epigenetic alterations such as DNA hypomethylation and loss of imprinting, as well as DNA hypermethylation resulting in silencing of certain genes [21-24]. CIMP+ colorectal tumours have a particularly high frequency of methylation of CpG islands. CpG islands consist of a cytosine [C] and a guanine [G] base linked by a phosphodiester bond. This defect results in hypermethylation of the promoter region of MMR enzymes especially MLH1 ultimately resulting in silencing of gene expression [21,25]. MSI-H, CIMP+ colorectal cancers present almost entirely with BRAF gene mutations and do not carry mutations in kRAS [26-28]. Lynch-related CRCs present only with kRAS and not BRAF mutations [26,27]. Tumours with BRAF mutations tend to have a worse prognosis than that which is typically associated with MSI-H tumours [29,30].

1.3 Lynch Syndrome.

LS is an autosomal dominant condition characterised by germline mutation in one of the MMR genes (MLH1, MSH2, MSH6, PMS2) or the EPCAM gene. It is also commonly referred to as hereditary non-polyposis colorectal cancer (HNPCC) but there is a subtle difference between the two. While LS is characterised by gene mutations, HNPCC is defined clinically usually by family history and fulfilment of the Amsterdam criteria. LS is associated with an increased susceptibility to developing CRC cancer and extracolonic malignancies of

which endometrial cancer is most prevalent. LS accounts for approximately 3% of newly diagnosed CRCs and 3% of endometrial cancers [31]. It is estimated that 1 in 279 of the population carry mutations in DNA MMR genes [32]. In the Northern Cape region of South Africa, the incidence of colorectal cancer is relatively low at around 3-4/100000 [33]. Here 10.5% of colorectal cancers were found to harbour a MMR gene mutation. This is approximately three times the international rate in high incidence areas [34].

1.3.1 Genetics.

The following MMR genes are involved [31]:

- MLH1, on chromosome 3p22.2, mutated in 37%.
- MSH2, on chromosome 2p21-16, mutated in 41%.
- MSH6, on chromosome 2p16.3, mutated in 13%.
- PMS2, on chromosome 7p22.1, mutated in 9%.

In normal DNA replication the role of the MMR system is to preserve genomic integrity by correcting base substitution and small insertion-deletion mismatches. This is achieved through coordinated function of different gene products. Two heterodimer protein complexes, MutS-alpha and MutS-beta are responsible for the recognition of base pair mismatches. MutS-alpha is a MSH2 and MSH6 heterodimer where as MutS-beta is a heterodimer of MSH2/MSH3 [35]. Three heterodimer pairs termed MutL-alpha, MutL-beta, and MutL-gamma are responsible for the repair component of the MMR system. MutL-alpha is a heterodimer of MLH1 and PMS2, MutL-beta is a heterodimer of MLH1 and PMS1, whereas MutL-gamma is a MLH1/MLH3 heterodimer.

For a defective MMR system to exist both alleles of any one of the MMR genes must be inactivated. Even though on a clinical level the syndrome is autosomal dominant, on a molecular level, germline mutations are recessive as evidenced by two hits being necessary to disable the gene. Generally, one allele of a MMR gene has a germline mutation and the second gene is inactivated somatically by mutation, loss of heterozygosity or epigenetic silencing by protein hypermethylation. Once both alleles are inactive, mutation rate increases leading to genomic instability due to the failure of the MMR system to repair the DNA mismatches. DNA mismatches generally occur in areas of repetitive nucleotide sequences

known as microsatellites. Expansion or contraction of these microsatellite regions is known as microsatellite instability (MSI) and is characteristic of what happens when there is loss of mismatch repair in Lynch-associated cancers. Failure of mismatch repair affects not only the DNA MMR genes but also genes that control cell growth and apoptotic processes. Accumulation of mutations in these genes is thought to be the driving force behind carcinogenesis [36,37]. Finally, deletions in the 3' terminal codon of the EPCAM gene, leads to inactivation of the MSH2 gene found neighbouring the EPCAM gene. Mosaic inactivation of the MSH2 gene in cells that express EPCAM 3' end deletion results in tumours that are different from typical germline MSH2 mutations or deletions but phenotypically are similar to LS [38].

1.3.2 Clinical features.

Colonic manifestations: Most individuals with LS are asymptomatic until they present with features of CRC. The lifetime (70 year) risk of CRC in patients with LS ranges from 10% to 47% and to a degree is influenced by the sex of the patient and the mutated MMR gene (Table 1) [39-44]. In general, CRC resulting from LS presents at a younger age than sporadic CRC (45 years to 60 years versus 69 years) [39,40,42-44].

Table 1. Lifetime cancer risk related to Lynch genotypes [39-44]

Cancer site	MLH1		MSH2		MSH6		PMS2	
	Men	Women	Men	Women	Men	Women	Men	Women
Any Lynch cancer	59%	80%	71%	75%	31%	71%	-	-
Colorectal	34 to 47%	36 to 45%	37 to 47%	33 to 37%	14 to 22%	10 to 26%	19 to 20%	11 to 15%
Endometrial	NA	18 to 54%	NA	21 to 51%	NA	16 to 49%	NA	12 to 24%
Ovarian	NA	11 to 20%	NA	15%	NA	1%	NA	-
Urinary tract	1.2%	3%	8%	10%	0.7%		-	
Gastric	20%	8%	2%	9%	-		-	
Small bowel	0.4%*		1.1%*		-		-	

Biliary/pancreatic	1.9%*	0.02%*	-	-
Brain tumours (gliomas)	1.7%*	2.5%*	-	-

NA: not applicable. * Not reported separately by sex.

Synchronous (at the same time) and metachronous (at another time) tumours occur more frequently in patients with LS and approximately 7% of patients have more than one tumour at the time of their presentation [45]. It has been shown that development of a metachronous CRC in a patient who underwent segmental resection of their 1st cancer occurred in 16% at 10 years, 41% at 20 years and 62% at 30 years. In those patients where the primary tumour was rectal, 14% developed metachronous tumours at 10 years, 47% at 20 years and 69% at 30 years [46,47].

Lynch related CRC shows a predilection for the right side of the colon. These tumours tend to develop from larger, flatter, more proximal adenomas/polyps and exhibit higher rates of high-grade dysplasia and/or villous histology than sporadic adenomas/polyps. The adenoma-carcinoma sequence is also greatly accelerated in LS versus sporadic CRC (35 months versus 10-15 years). The overall 10-year survival for Lynch related CRC is high at approximately 91% [48].

Extracolonic manifestations: LS is associated with the development of extracolonic tumours of which endometrial cancer is the commonest. Risk depends on the MMR gene mutated and is illustrated above (Table 1) [39-44]. Other common cancers include cancers of the ovary, stomach, small bowel, hepatobiliary system, renal pelvis and ureter, brain (glioma, Turcot syndrome), sebaceous neoplasms (Muir-Torre syndrome). Cancer of the pancreas, prostate, breast and cervix have also been reported but are rare [42,49-57].

Genotype phenotype correlation: With regards to the four MMR genes, PMS2 mutations have the lowest penetration (Table 1). Due to the inherent cancer risk associated with any of these mutated genes, the screening and treatment of at-risk individuals is not modified to the gene involved and remains the same regardless of the gene mutated. The risk of CRC and endometrial cancer is approximately the same in MLH1 and MSH2 mutations, however the overall cancer risk is greater for those with a MSH2 gene mutation, especially where

urothelial cancers and sebaceous tumours are concerned (Table 1) [40,42,58]. With regards to EPCAM mutations, risk of progression to cancer is similar to those with MSH2 mutations. The risk of endometrial cancer is lower when compared to those with MSH2 mutations unless the base pair deletion extends close to the promoter region of MSH2[59,60]. MLH1/MSH2 mutations have higher penetrance when compared to MSH6/PMS2. MSH6/PMS2 mutations exhibit an attenuated cancer phenotype and a later age at which cancer is first diagnosed [38-40].

Histological features of Lynch-associated CRC include mucinous, signet ring or medullary histologic type. They are poorly differentiated and have a brisk lymphocytic infiltrate or are rimmed by a Crohn-like, germinal centre-producing lymphoid reaction [61,62].

1.3.3 Identification of individuals at risk for Lynch Syndrome.

LS is largely under-recognized [63]. Historically the primary tool to identify individuals was family history of CRC and other cancers. These family history-based strategies have been shown to have poor sensitivity and specificity for identifying patients with LS. After recognising that Lynch-associated CRCs exhibit MSI, tumour testing has become an important adjunct in identification of individuals with LS and their families.

Clinical criteria: The Amsterdam I criteria were proposed in 1990 to help identify families likely to be mutation carriers for LS. However, the Amsterdam I criteria was found to be too rigid and in 1998 was expanded to include the extracolonic cancers associated with LS. This expansion resulted in the present-day Amsterdam II criteria with a sensitivity and specificity of 22% and 98% respectively [64,65].

For the Amsterdam II criteria to be fulfilled each of the following must be met:

- 3 or more relatives with an associated cancer (CRC, or cancer of the endometrium, small intestine, ureter or renal pelvis).
- 2 or more successive generations affected.
- 1 or more relatives diagnosed before the age of 50 years.
- 1 should be a first-degree relative of the other two.
- FAP should be excluded in cases of colorectal carcinoma.
- Tumours should be verified by pathologic examination [66].

In 1997 (with a revision in 2004) a new set of recommendations called the Bethesda guidelines were published by the National Cancer Institute for the purpose of identifying individuals who should undergo genetic testing for LS related tumours.

The revised Bethesda Guidelines are as follows [65,67,68]:

- CRC diagnosed in a patient who is less than 50 years old.
- Presence of synchronous or metachronous CRC or other LS-associated tumours, regardless of age.
- CRC with MSI-H histology diagnosed in a patient less than 60 years old.
- CRC diagnosed in one or more first-degree relatives with a LS-associated tumour, with one of the cancers being diagnosed at less than 50 years of age.
- CRC diagnosed in two or more first-degree or second-degree relatives with LS-associated tumours, regardless of age.

The revised guidelines have been found to be more sensitive (82%) and specific (77%) than the Amsterdam II criteria in identifying individuals and families at risk of developing LS [67].

Prediction models: Several computational clinical prediction models have been developed as an adjunct to clinical criteria and clinical suspicion to better estimate the risk of a MMR gene mutation and identify individuals and families who would benefit from genetic testing. Three well known risk prediction models are the MMRpredict, MMRpro and PREMM models [69-72]. The MMRpredict model has been validated in a study involving 725 consecutive patients with CRC whose MMR status was available, the sensitivity and specificity of the model is 94% and 91%, respectively [73]. The MMRpro model estimates the chance of germline mutations in MLH1, MSH2, MSH6 genes. MMRpro also serves to estimate future risk in unaffected individuals or mutation carriers and untested persons. The MMRpro model, in validation studies has been shown to be more discerning than the Bethesda criteria [74]. Of the three models, the PREMM has the highest sensitivity but lowest specificity (90% and 67%, respectively) [63] and when followed by genetic testing seems to be cost effective in individuals between ages of 25-35 years in whom the risk estimate exceeds 5% [75].

Tumour-based strategies: Some experts advocate universal testing of all CRCs while others employ a selective strategy of tumour testing reserved for high risk individuals [63,76-80]. Evidence suggests that universal testing has a slightly greater sensitivity for identifying

individuals with LS when compared to other strategies, including the Bethesda criteria, or other selective tumour testing strategy. However, the diagnostic yield from universal testing and selective testing is comparable and in fact selective approaches resulted in far fewer cases needing tumour testing and germline testing without a significant decrease in diagnostic yield or strain on resources [31,81,82].

MSI testing: Due to loss of DNA MMR genes, tumours in LS demonstrate MSI. MSI testing is done using polymerase chain reaction (PCR) to amplify DNA sequences containing nucleotide repeats. If more than 30% of the markers in a panel show expansion or contraction of these sequences in a tumour when compared with normal mucosa from the same patient, the tumour is said to be MSI-H. the sensitivity and specificity of MSI testing are 85% and 90% respectively [61]. However, MSH6 associated cancers can sometimes be missed on MSI testing as MSH6 mononucleotide markers are not included in all MSI panels [59].

Immunohistochemistry: Immunohistochemistry (IHC) can be used to detect loss of staining of MMR protein that occurs in LS with a sensitivity and specificity of 83% and 89% respectively. It is inexpensive, easily accessible and can be performed on small biopsies with the added benefit of being able to determine the exact gene which may be the problem [83,84]. In addition, IHC can also be used on endometrial tumour samples to detect LS, though the evidence is lacking with regards to other Lynch associated tumours and abnormal IHC results in these should be evaluated along with family and personal history before diagnosing Lynch syndrome. IHC (and MSI) can also be used in testing of large (>1cm) adenomas/polyps if no CRCs are available for testing in a family, but it must be kept in mind that the absence of MSI in these cases does not rule out LS and further testing is needed if enough suspicion exists [85,86].

1.3.4 Diagnosis of Lynch Syndrome.

LS should be suspected in individuals who exhibit the following:

- synchronous or metachronous CRC.
- CRC prior to 50 years of age.
- multiple LS associated cancers.
- familial clustering of LS associated cancers.

Before a definitive diagnosis is made a pathogenic germline mutation in the MMR gene or

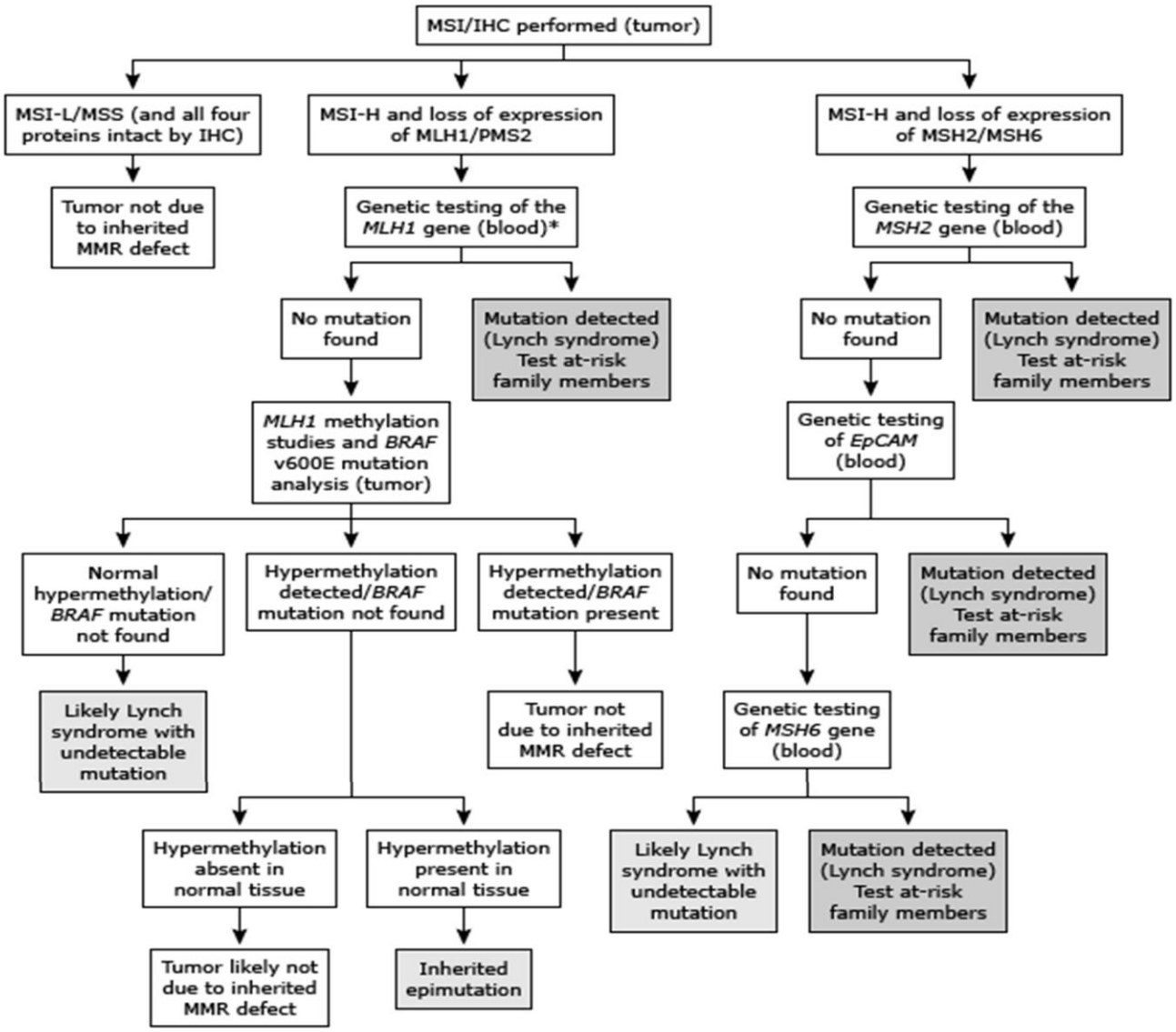
EPCAM gene is required. Sequential genetic evaluation of patients suspected to have LS begins with tumour testing. Germline testing on all patients is not viable due to the exorbitant expense [57,87].

Genetic evaluation should be considered in the following individuals:

- All newly diagnosed patients with CRC (alternatively, those diagnosed prior to age 70 years).
- Endometrial cancer prior to age 60 years.
- First-degree relative of those with known MMR/EPCAM gene mutation.
- Individuals with a CRC with a >5 % chance of a MMR gene mutation by prediction models.
- Family cancer history meeting Amsterdam I or II criteria or revised Bethesda guidelines.

Tumour evaluation: Genetic evaluation begins with MSI and/or IHC testing. If no MSI is found and all four MMR proteins are intact on IHC, this effectively rules out most cases of LS. If evidence of MSI-H or loss of expression of a MMR protein is noted further evaluation is based on the MSI/IHC results and outlined in the algorithm below.

Figure 3. Tumour evaluation algorithm [88].



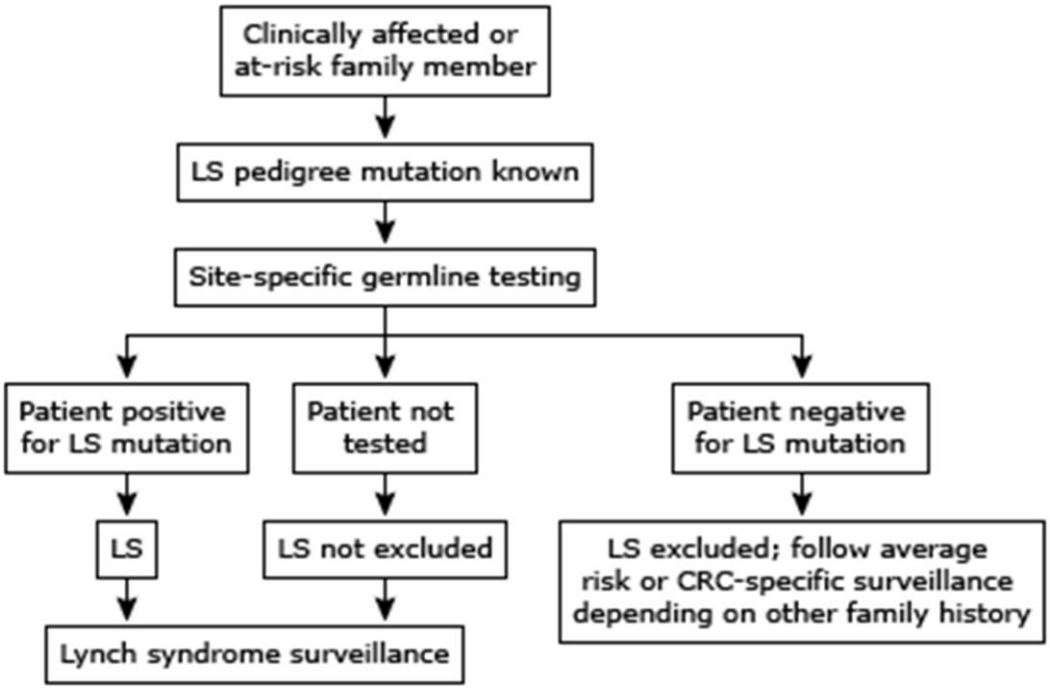
MSI: microsatellite instability; IHC: immunohistochemistry; MSI-L: low MSI; MSS: microsatellite stable; MSI-H: high MSI; MMR: mismatch repair [88].

Germline testing: Germline testing is required to establish the definitive diagnosis of LS. Comprehensive germline mutation testing involves gene sequencing and deletion/duplication analyses Germline testing should be offered to the following individuals:

- Patients with microsatellite unstable tumours by MSI/IHC testing.
- If tumour testing is not feasible and if the clinical suspicion of LS is strong (e.g., individual meets revised Bethesda criteria).
- If a patient meets the Amsterdam criteria, some experts recommend germline testing without prior tumour testing.

Once a pathogenic MMR/EPCAM mutation is found the diagnosis of LS is established. At-risk relatives should then be referred for genetic counselling and site-specific testing for the mutation to determine the pedigree. Screening should be started in at risk individuals from the ages of 20 to 25 years or at 10 years before the earliest onset of cancer in the family.

Figure 4. Germline testing algorithm [88].



LS: Lynch syndrome; CRC: colorectal cancer.

1.3.5 Cancer screening and surveillance of Lynch Syndrome.

Guidelines for cancer screening for individuals with LS are largely based on expert opinion and limited observational data [89,90]. The United States Multi-Society Task Force on Colorectal Cancer and the American College of Gastroenterology recommend the guidelines discussed below.

All patients with a definitive diagnosis of LS should undergo screening for associated cancers. Individuals at risk for LS with indeterminate genetic results and those who have not undergone genetic evaluation should also be considered for screening depending on their personal and family history and evidence of MSI on tumour testing.

Colorectal cancer: Colonoscopic surveillance in individuals with LS should begin at age 20 to 25 years (at one or two-year intervals), or two to five years before the earliest age of CRC diagnosis in the family. The exception to this is families with MSH6 and PMS2 mutations which are known to have a lower CRC risk and a later age at CRC diagnosis. Colonoscopic screening in these individuals with an attenuated phenotype can begin at age 25 to 30 or two to five years prior to the earliest CRC in the family, repeated every one to two years [57,87]. Colonoscopy has been shown to significantly decrease cancer related mortality in individuals with LS. Additionally, colonoscopic surveillance of at-risk family members have been associated with a gain of 14 quality-adjusted life years per screened individual versus no screening [95]. A fifteen-year prospective study published in 2000 studied 22 families with LS and compared cancer incidence and mortality between 133 regularly screened at-risk members and 119 members who had refused screening. Participants who underwent colonoscopic screening at approximately 3-year intervals had a lower CRC incidence (6% versus 16%) and overall mortality (8% versus 22%) as compared with the unscreened group [93]. With regards to interval timing of surveillance colonoscopy, there have been no randomized trials done to determine the optimal interval between colonoscopes. Observational studies, and prospective cohort studies, however, have found that annual colonoscopy currently is appropriate [92,94,96,97].

Endometrial and ovarian cancer: Women with LS, starting at 30 to 35 years of age or three to five years before the earliest age of diagnosis of these cancers in the family should undergo annual pelvic examination with endometrial biopsies taken to look for endometrial cancer as well as have transvaginal ultrasound done to detect ovarian cancer [57,87]. Prophylactic hysterectomy and bilateral salpingo-oophorectomy at the end of childbearing or around age 40 years for mutation carriers is also recommended. Those who do not consent to surgery need to continue screening annually.

Other Cancers: Screening for gastric cancer in LS is controversial and not recommended [91]. Lifetime risk of small bowel cancer in LS is small (0.4% to 12%) and routine screening currently is not recommended as it is not cost effective [63,98]. However, the National Comprehensive Cancer Network (NCCN) guidelines controversially do suggest that wireless capsule endoscopy be considered at two- to three-year intervals. Regarding urinary tract cancers, the recommendations are for annual urine analysis starting from age 30-35 years [57,87,91,99]. Annual skin examinations to detect sebaceous tumours and cutaneous

keratoacanthomas associated with Muir-Torre syndrome are recommended [100]. As per current guidelines screening for pancreatic cancers should be reserved for individuals with a MMR mutation and a first degree relative with pancreatic cancer. Routine screening is not recommended [87,101].

1.3.6 Management of Cancer.

The ideal surgical management of an individual with LS who is found to have CRC or endoscopically unresectable adenoma is total abdominal colectomy with ileorectal anastomosis followed by annual endoscopic surveillance of the remaining rectum. Segmental colectomy with annual postoperative surveillance should be reserved for patients who are not candidates for total colectomy [87]. Individuals who undergo segmental colectomy are at greater risk of a subsequent adenoma/polyp or carcinoma as compared to those who have had a total colectomy [47,102]. Women should be offered prophylactic hysterectomy and bilateral salpingo-oophorectomy at the time of colectomy. The role of chemotherapy in Lynch associated CRC is currently controversial. Most are MSI-H and are often poorly differentiated. The effect of chemotherapy on these cancers is currently unknown. Fluorouracil seems to have little effect on MSI-H tumours whereas in some studies irinotecan seemed to have increased efficacy against MSI-H tumours. Ultimately more studies need to be conducted before recommendations can be given [103].

1.4 Lynch syndrome in South Africa

Historic LS data in South Africa is unfortunately lacking. The index case of LS in South Africa dates back to 1985 when LS was first described in South Africa. The index case involved a 30-year-old male who developed CRC with a family pedigree spanning over four generations. Most of the available data in South Africa pertaining to LS comes from around 50 satellite families consisting of approximately 2000 individuals located in the Northern Cape Province of South Africa. This cohort of individuals has been identified over the years through genetic testing of at-risk family members[104].

1.4.1 Colorectal cancer screening and surveillance in South Africa.

At the time of writing no formal CRC screening or surveillance program exists in South Africa. The South African experience with regards to LS is enhanced by the Annual Northern Cape Colonoscopy Outreach program. This mobile service provides annual genetic testing

and surveillance colonoscopy in small district hospitals and clinics in the Northern Cape and makes quality surveillance accessible to an at-risk group who may otherwise find annual screening an economic and logistical challenge. At risk individual and their family members upon reaching the age of 18 years undergo genetic counselling and site-specific testing to determine if a MMR mutation is present. Patients identified as having an MMR gene mutation undergo surveillance colonoscopy every 2 years starting at age 18 years, then annually from 30 years old [105]. In line with international evidence, mobile surveillance colonoscopy has been shown to have improved outcomes overall, and in CRC-related survival rates in individuals carrying a single MMR gene mutation [106]. One of the main challenges the mobile surveillance program faces is that with each year compliance with surveillance colonoscopy is decreasing with a 2007 study showing fewer than 25% adhering to all their recommended screening appointments [107]. Major factors identified for noncompliance were financial constraints (18%), logistical difficulties (16.4%), the unpleasant experience of bowel preparation (16.4%) and pain (4.9%) as a reason for nonattendance.

1.4.2 Role of EndoRings™ in surveillance colonoscopy for Lynch Syndrome.

Colonoscopy is currently the gold standard for detection and removal of colorectal adenomas/polyps that if left unchecked develop into CRC [108,110]. Despite being the best available and most sensitive method for adenoma/polyp detection, colonoscopy is not without its limitations. The most serious limitation being that precancerous lesions can easily be missed during standard colonoscopy due to patient factors, examiner factors, poor bowel preparation, flat lesions and hidden lesions (in haustral folds and colonic flexures) (Table 2) [109,110]. Older studies place adenoma/polyp miss rates by standard colonoscopy to be approximately 20-25%. With newer studies, while evaluating technologies that improve colonic visualisation, miss rates using standard colonoscopy approach 40%. This has serious implications regarding morbidity and mortality of at-risk individuals. Standard colonoscopes (140 and 170-degree scopes) only allow visualisation of approximately 90% of the colon [108].

Table 2. Factors influencing the adenoma detection rate (ADR) [109].

	ADR
Examiner-dependent factors	
Endoscopist's expertise	Higher
Withdrawal time >6 min	Higher
Coecum intubation rate >90%	Higher
Patient-dependent factors	
Age >40	Higher
Male gender	Higher
Optimal bowel preparation	Higher
Aspirin intake	Higher
Presence of fundic gland polyps	Higher
Vegetable food fibers	Lower
Procedure-dependent factors	
Second investigator	Higher
Back-to-back colonoscopy	Higher
Water immersion	Higher
Water exchange	Higher
Staining procedures	Higher
Endoscopic devices	
Transparent cap	Conflicting results
Endocuff	Higher in 2 RCT's
Endorings	Higher in one RCT
Technical improvements	
FICE	No effect
NBI	No effect
i-Scan	No effect
G-Eye	Higher
FUSE	Higher
Retroflexion	No effect

In an attempt to increase ADR/PDR multiple new technologies have been developed to improve colonic visualisation in the hopes of positively influencing the above while maintaining the capabilities that standard colonoscopy offers. These technologies range from less practical and more expensive inventions such as Full Spectrum Endoscopy (FUSE), Third eye technologies, virtual chemo-endoscopy to more practical and relatively more affordable devices such as EndoCuff™, cap assisted colonoscopy and the subject of this study - the EndoRings™ [108-110].

The EndoRings™ is a silicone-rubber device that fits onto the distal end of a colonoscope. It boasts flexible circular 'petals' (rings) that allow easy intubation to the caecum but upon withdrawal stretches out the haustral folds of the colon to allow for better visualisation of the mucosal surface. Additionally, the rings provide a stabilising anchor that prevents slippage

of the scope and keeps it centred in the lumen when preforming interventions such as snaring or taking biopsies [108-110].

There is a lack of abundant data available with regards to the actual benefit that these devices provide but from the evidence we have it is generally suggested that techniques like FUSE, Third Eye and retroflection colonoscopy are promising but involve a significant financial investment to be feasible [108,109]. Staining techniques and water infusion technologies might be useful in select patients but are too time consuming to be used for screening purposes [109]. Cap assisted colonoscopy results are equivocal with some studies showing improvement and some showing no difference in ADR/PDR. EndoCuff™ assisted colonoscopy again is promising but not all randomised trials show superiority of EndoCuff™ to standard colonoscopy [109]. With regards to EndoRings™ only one major randomized control trial has been completed, the CLEVER trial, which reported a statistically significant reduction in missed adenomas with EndoRings™ (14%) versus standard colonoscopy (48%). However, it should be noted that the CLEVER trial was funded by EndoAid Limited, the company that produces the EndoRings™ device [108-110]. It is clear that more studies are needed evaluating the absolute efficacy of these novel devices before major changes to current practice can be implemented, especially in the South African setting. Ultimately the major factors that influence improved ADR/PDR and are more cost effective to enact are the expertise of the endoscopist, optimal bowel-cleansing, correct withdrawal times, meeting quality markers for screening colonoscopy as set by ASGE and patient compliance with screening intervals [109].

1.5 Chapter 1 References.

2. Parkin DM, Whelan SL, Ferlay J, et al. Cancer incidence in five continents, Vol VIII [IARC Scientific Publication No. 155]. Lyon: International Agency for Research on Cancer; 2002.

3. World Cancer Research Fund / American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington DC: American Institute for Cancer Research. 2007.

4. Boyle P, Langman J S. ABC of colorectal cancer: Epidemiology. BMJ 2000;321(7264):805–808. [https://doi.org/10.1136/bmj.321.7264.805] [PMID: 11009523]

5. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65(2):87-108. [https://doi.org/10.3322/caac.21262] [PMID: 25651787]

6. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90. [https://doi.org/10.3322/caac.20107] [PMID: 21296855]

7. Doubeni CA, Laiyemo AO, Major JM, et al. Socioeconomic status and the risk of colorectal cancer: an analysis of more than a half million adults in the National Institutes of Health-AARP Diet and Health Study. Cancer 2012;118(14):3636-3644. [https://doi.org/10.1002/cncr.26677] [PMID: 22898918]

8. Doubeni CA, Major JM, Laiyemo AO, et al. Contribution of behavioral risk factors and obesity to socioeconomic differences in colorectal cancer incidence. J Natl Cancer Inst 2012;104(18):1353-1362. [https://doi.org/10.1093/jnci/djs346] [PMID: 22952311]

9. Klabunde CN, Cronin KA, Breen N, et al. Trends in colorectal cancer test use among vulnerable populations in the United States. Cancer Epidemiol Biomarkers Prev 2011;20(8):1611-1621. [https://doi.org/10.1158/1055-9965.EPI-11-0220] [PMID: 21653643]

10. Marley A, Hongmei N. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet. 2016;7(3):105–114. Published online 2016 Sep 30. [PMID: 27766137]

11. South African National Cancer Registry. Cancer in South Africa 2013 Full Report. South Africa. National Cancer Registry, 2013. http://www.nicd.ac.za/centres/national-cancer-registry/ (accessed 15 December 2017)

12. Watson AJ CP. Colon cancer: a civilization disorder. Dig Dis. 2011;29(2):222-228. [https://doi.org/10.1159/000323926] [PMID: 21734388]

13. Arvelo F, Sojo F, Cotte C. Biology of colorectal cancer. Ecancermedicalscience 2015;9:520. [https://doi.org/10.3332/ecancer.2015.520] [PMID: 25932044]

14. Lindor NM. Familial colorectal cancer type X: the other half of hereditary nonpolyposis colon cancer syndrome. Surg Oncol Clin N Am. 2009;18(4):637-645. [https://doi.org/10.1016/j.soc.2009.07.003] [PMID: 19793571]

15. Wirtzfeld DA, Petrelli NJ, Rodriguez-Bigas MA. Hamartomatous polyposis syndromes: molecular genetics, neoplastic risk, and surveillance recommendations. Ann Surg Oncol 2001;8(4):319-327. [https://doi.org/10.1007/s10434-001-0319-7] [PMID: 11352305]

16. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol. 2011;6:479-507. [https://doi.org/10.1146/annurev-pathol-011110-130235] [PMID: 21090969]

17. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61(5):759-767. [https://doi.org/10.1016/0092-8674(90)90186-I] [PMID: 2188735]

18. Lynch JP, Hoops TC. The genetic pathogenesis of colorectal cancer. Hematol Oncol Clin North Am 2002;16(4):775-810. [https://doi.org/10.1016/S0889-8588(02)00029-1] [PMID: 12418049]

19. Patai AV, Molnár B, Tulassay Z, et al. Serrated pathway: alternative route to colorectal cancer. World J Gastroenterol. 2013;19(5):607-615. [https://doi.org/10.3748/wjg.v19.i5.607] [PMID: 23431044]

20. Guarinos C, Sanchez-Fortun C, Rodriguez-Soler M, et al. Serrated polyposis syndrome: Molecular, pathological and clinical aspects. World J Gastroenterol 2012;18(20):2452-2461. [https://doi.org/10.3748/wjg.v18.i20.2452] [PMID: 22654442]

21. Noffsinger AE. Serrated polyps and colorectal cancer: new pathway to malignancy. Annu Rev Pathol. 2009;4:343-364. [https://doi.org/10.1146/annurev.pathol.4.110807.092317] [PMID: 19400693]

22. Weisenberger DJ, Siegmund KD, Campan M, 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):787-793. [https://doi.org/10.1038/ng1834] [PMID: 16804544]

23. Das PM, Singal R. DNA methylation and cancer. J Clin Oncol. 2004;22(22):4632-4642.[https://doi.org/10.1200/JCO.2004.07.151] [PMID: 15542813]

24. Shen L, Kondo Y, Rosner GL, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. J Natl Cancer Inst. 2005;97(18):1330-1338. [https://doi.org/10.1093/jnci/dji275] [PMID: 16174854]

25. van Engeland M, Derks S, Smits KM, et al. Colorectal cancer epigenetics: complex simplicity. J Clin Oncol. 2011;29(10):1382-1391. [https://doi.org/10.1200/JCO.2010.28.2319] [PMID: 21220596]

26. Goel A, Nagasaka T, Arnold CN, et al. The CpG island methylator phenotype and chromosomal instability are inversely correlated in sporadic colorectal cancer. Gastroenterology. 2007;132(1):127-138. [https://doi.org/10.1053/j.gastro.2006.09.018] [PMID: 17087942]

27. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature. 2002;418(6901):934. [https://doi.org/10.1038/418934a] [PMID: 12198537]

28. Domingo E, Niessen RC, Oliveira C, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. Oncogene. 2005;24(24):3995-3998. [https://doi.org/10.1038/sj.onc.1208569] [PMID: 15782118]

29. Samowitz WS, Albertsen H, Sweeney C, et al. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst. 2006;98(23):1731-1738. [https://doi.org/10.1093/jnci/djj468] [PMID: 17148775]

30. French AJ, Sargent DJ, Burgart LJ, et al. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clin Cancer Res. 2008;14(11):3408-3415. [https://doi.org/10.1158/1078-0432.CCR-07-1489] [PMID: 18519771]

31. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009;58(1):90-96. [https://doi.org/10.1136/gut.2008.155473] [PMID: 18832519]

32. Moreira L, Balaguer F, Lindor N, et al. Identification of Lynch syndrome among patients with colorectal cancer. JAMA. 2012;308(15):1555-1565. [https://doi.gov/10.1001/jama.2012.13088] [PMID: 23073952]

33. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2017;26(3):404-412. [https://doi.org/10.1158/1055-9965.EPI-16-0693] [PMID: 27799157]

34. Wentink M, Räkers M, Stupart D, et al. Incidence and histological features of colorectal cancer in the Northern Cape province, South Africa. S Afr J Surg. 2010;48(4):109-113. [PMID: 21542398]

35. Vergouwe F, Boutall A, Stupart D, et al. Mismatch repair deficiency in colorectal cancer patients in a low-incidence area. S Afr J Surg. 2013;51(1):16-21. [https://doi.org/10.7196/SAJS.1314] [PMID: 23472647]

36. Koessler T, Oestergaard MZ, Song H, et al. Common variants in mismatch repair genes and risk of colorectal cancer. Gut. 2008;57(8):1097-1101. [https://doi.org/10.1136/gut.2007.137265] [PMID: 18364438]

37. Tannergård P, Lipford JR, Kolodner R, et al. Mutation screening in the hMLH1 gene in Swedish hereditary nonpolyposis colon cancer families. Cancer Res 1995;55(24):6092-6096. [PMID: 8521398]

38. Kakar S, Burgart LJ, Thibodeau SN, et al. Frequency of loss of hMLH1 expression in colorectal carcinoma increases with advancing age. Cancer. 2003;97(6):1421-1427. [https://doi.org/10.1002/cncr.11206] [PMID: 12627505]

39. Kempers MJ, Kuiper RP, Ockeloen CW, et al. Risk of colorectal and endometrial cancers in EPCAM deletion-positive Lynch syndrome: a cohort study. Lancet Oncol. 2011;12(1):49-55. [https://doi.org/10.1016/S1470-2045(10)70265-5] [PMID: 21145788]

40. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology. 2008;135(2):419-428. [https://doi.org/10.1053/j.gastro.2008.04.026] [PMID: 18602922]

41. Bonadona V, Bonaïti B, Olschwang S, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA. 2011;305(22):2304-2310. [https://doi.org/10.1001/jama.2011.743] [PMID: 21642682]

42. Moller P, SeppäläT, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut. 2017;66(3):464-472. [https://doi.org/10.1136/gutjnl-2015-309675] [PMID: 26657901]

43. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. Hum Mutat. 2013;34(3):490-497. [https://doi.org/10.1002/humu.22262] [PMID: 23255516]

44. Baglietto L, Lindor NM, Dowty JG, et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst. 2010;102(3):193-201. [https://doi.org/10.1093/jnci/djp473] [PMID: 20028993]

45. ten Broeke SW, Brohet RM, Tops CM, et al. Lynch syndrome caused by germline PMS2 mutations: delineating the cancer risk. J Clin Oncol. 2015;33(4):319-325. [https://doi.org/10.1200/JCO.2014.57.8088] [PMID: 25512458]

46. Win AK, Buchanan DD, Rosty C, et al. Role of tumour molecular and pathology features to estimate colorectal cancer risk for first-degree relatives. Gut. 2015;64(1):101-110. [https://doi.org/10.1136/gutjnl-2013-306567] [PMID: 24615377]

47. Parry S, Win AK, Parry B, et al. Metachronous colorectal cancer risk for mismatch repair gene mutation carriers: the advantage of more extensive colon surgery. Gut. 2011;60(7):950-957. [https://doi.org/10.1136/gut.2010.228056] [PMID: 21193451]

48. Win AK, Parry S, Parry B, et al. Risk of metachronous colon cancer following surgery for rectal cancer in mismatch repair gene mutation carriers. Ann Surg Oncol. 2013;20(6):1829-1836.[https://doi.org/10.1245/s10434-012-2858-5] [PMID: 23358792]

49. Moller P, SeppäläT, Bernstein I, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective Lynch syndrome database. Gut. 2017;66(9):1657-1664. [https://doi.org/10.1136/gutjnl-2016-311403] [PMID: 27261338]

50. Win AK, Young JP, Lindor NM, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol. 2012;30(9):958-964. [https://doi.org/10.1200/JCO.2011.39.5590] [PMID: 22331944]

51. Win AK, Lindor NM, Young JP, et al. Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. J Natl Cancer Inst. 2012;104(18):1363-1372. [https://doi.org/10.1093/jnci/djs351] [PMID 22933731]

52. Win AK, Lindor NM, Winship I, et al. Risks of colorectal and other cancers after endometrial cancer for women with Lynch syndrome. J Natl Cancer Inst. 2013;105(4):274-279. [https://doi.org/10.1093/jnci/djs525] [PMID: 23385444]

53. Kastrinos F, Mukherjee B, Tayob N, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA. 2009;302(16):1790-1795. [https://doi.org/10.1001/jama.2009.1529] [PMID: 19861671]

54. Raymond VM, Mukherjee B, Wang F, et al. Elevated risk of prostate cancer among men with Lynch syndrome. J Clin Oncol. 2013;31(14):1713-1718. [https://doi.org/10.1200/JCO.2012.44.1238] [PMID: 23530095]

55. Win AK, Lindor NM, Jenkins MA. Risk of breast cancer in Lynch syndrome: a systematic review. Breast Cancer Res. 2013;15(2): R27. [https://doi.org/10.1186/bcr3405] [PMID: 23510156]

56. Ryan S, Jenkins MA, Win AK. Risk of prostate cancer in Lynch syndrome: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2014;23(3):437-449. [https://doi.org/10.1158/1055-9965.EPI-13-1165] [PMID: 24425144]

57. Antill YC, Dowty JG, Win AK, et al. Lynch syndrome and cervical cancer. Int J Cancer. 2015;137(11):2757-2761. [https://doi.org/10.1002/ijc.29641] [PMID: 26077226]

58. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. Gastroenterology. 2014;147(2):502-526. [https://doi.org/10.1053/j.gastro.2014.04.001] [PMID: 25043945]

59. Watson P, Vasen HF, Mecklin JP, et al. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer. 2008;123(2):444-449. [https://doi.org/10.1002/ijc.23508] [PMID: 18398828]

60. Clendenning M, Senter L, Hampel H, et al. A frame-shift mutation of PMS2 is a widespread cause of Lynch syndrome. J Med Genet. 2008;45(6):340-345. [https://doi.org/10.1136/jmg.2007.056150] [PMID: 18178629]

61. Ligtenberg MJ, Kuiper RP, Geurts van Kessel A, Hoogerbrugge N. EPCAM deletion carriers constitute a unique subgroup of Lynch syndrome patients. Fam Cancer. 2013;12(2):169-174. [https://doi.org/10.1007/s10689-012-9591-x] [PMID:23264089]

62. Jenkins MA, Hayashi S, O'Shea AM, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007;133(1):48-56. [https://doi.org/10.1053/j.gastro.2007.04.044] [PMID: 17631130]

63. Bessa X, Alenda C, Paya A,Álvarez C, et al. Validation microsatellite path score in a population-based cohort of patients with colorectal cancer. J Clin Oncol. 2011;29(25):3374-3380. [https://doi.org/10.1200/JCO.2010.34.3947] [PMID: 21788563]

64. Hampel H, de la Chapelle A. The search for unaffected individuals with Lynch syndrome: do the ends justify the means? Cancer Prev Res (Phila). 2011;4(1):1-5. [https://doi.org/10.1158/1940-6207.CAPR-10-0345] [PMID: 21205737]

65. American Gastroenterological Association. American Gastroenterological Association medical position statement: hereditary colorectal cancer and genetic testing. Gastroenterology. 2001;121(1):195-197. [https://doi.org/10.1053/gast.2001.25580] [PMID: 11438508]

66. Bellizzi AM, Frankel WL. Colorectal cancer due to deficiency in DNA mismatch repair function: a review. Adv Anat Pathol. 2009;16(6):405–417. [https://doi.org/10.1097/PAP.0b013e3181bb6bdc] [PMID: 1985113]

67. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. 1999;116 (6):1453–1456. [https://doi.org/10.1016/S0016-5085(99)70510-x] [PMID: 10348829]

68. Piñol V, Castells A, Andreu M, et al. Accuracy of Revised Bethesda Guidelines, Microsatellite Instability, and Immunohistochemistry for the Identification of Patients With Hereditary Nonpolyposis Colorectal Cancer. JAMA. 2005;293(16): 1986–1994. [https://doi.org/10.1001/jama.293.16.1986] [PMID: 15855432]

69. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261–268. [https://doi.org/10.1093/jnci/djh034] [PMID: 14970275]

70. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med. 2006;354(26):2751-2763. [https://doi.org/10.1056/NEJMoa053493] [PMID: 16807412]

71. Balmaña J, Stockwell DH, Steyerberg EW, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. JAMA. 2006;296(12):1469-1478. [https://doi.org/10.1001/jama.296.12.1469] [PMID: 17003395]

72. Wijnen JT, Vasen HF, Khan PM, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med. 1998;339(8):511-518. [https://doi.org/10.1056/NEJM199808203390804] [PMID: 9709044]

73. Kastrinos F, Ojha RP, Leenen C, et al. Comparison of Prediction Models for Lynch Syndrome Among Individuals With Colorectal Cancer. J Natl Cancer Inst. 2015;108(2) [https://doi.org/10.1093/jnci/djv308] [PMID: 26582061]

74. Green RC, Parfrey PS, Woods MO, Younghusband HB. Prediction of Lynch syndrome in consecutive patients with colorectal cancer. J Natl Cancer Inst. 2009;101(5):331-340. [https://doi.org/10.1093/jnci/djn499] [PMID: 19244167]

75. Chen S, Wang W, Lee S, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA. 2006;296(12):1479-1487. [https://doi.org/10.1001/jama.296.12.1479] [PMID: 17003396]

76. Dinh TA, Rosner BI, Atwood JC, et al. Health benefits and cost-effectiveness of primary genetic screening for Lynch syndrome in the general population. Cancer Prev Res (Phila). 2011;4(1):9-22. [https://doi.org/10.1158/1940-6207.CAPR-10-0262] [PMID: 21088223]

77. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005;352(18):1851-1860. [https://doi.org/10.1056/NEJMoa043146] [PMID: 15872200]

78. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001;69(4):780-790. [https://doi.org/10.1086/323658] [PMID: 11524701]

79. Percesepe A, Borghi F, Menigatti M, et al. Molecular screening for hereditary nonpolyposis colorectal cancer: a prospective, population-based study. J Clin Oncol. 2001;19(19):3944-3950. [https://doi.org/10.1200/JCO.2001.19.19.3944] [PMID: 11579115]

80. Ravnik-Glavac M, Potocnik U, Glavac D. Incidence of germline hMLH1 and hMSH2 mutations (HNPCC patients) among newly diagnosed colorectal cancers in a Slovenian population. J Med Genet. 2000;37(7):533-536. [https://doi.org/10.1136/jmg.37.7.533] [PMID: 10970186]

81. Julié C, Trésallet C, Brouquet A, et al. Identification in daily practice of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. Am J Gastroenterol. 2008;103(11):2825-2835. [https://doi.org/10.1111/j.1572-0241.2008.02084.x] [PMID: 18759827]

82. Mvundura M, Grosse SD, Hampel H, Palomaki GE. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. Genet Med. 2010;12(2):93-104. [https://doi.org/10.1097/GIM.0b013e3181cd666c] [PMID: 20084010]

83. Ladabaum U, Wang G, Terdiman J, et al. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. Ann Intern Med. 2011;155(2):69-79. [https://doi.org/10.7326/0003-4819-155-2-201107190-00002] [PMID: 21768580]

84. Weissman SM, Bellcross C, Bittner CC, et al. Genetic counseling considerations in the evaluation of families for Lynch syndrome--a review. J Genet Couns. 2011;20(1):5-19. [https://doi.org/10.1007/s10897-010-9325-x] [PMID: 20931355]

85. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn. 2008;10(4):293-300. [https://doi.org/10.2353/jmoldx.2008.080031] [PMID: 18556767]

86. Yurgelun MB, Goel A, Hornick JL, et al. Microsatellite instability and DNA mismatch repair protein deficiency in Lynch syndrome colorectal polyps. Cancer Prev Res (Phila). 2012;5(4):574-582. [https://doi.org/10.1158/1940-6207.CAPR-11-0519] [PMID: 22262812]

87. Walsh MD, Buchanan DD, Pearson SA, et al. Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry. Mod Pathol. 2012;25(5):722-730. [https://doi.org/10.1038/modpathol.2011.209] [PMID 22322191]

88. Syngal S, Brand RE, Church JM, et al. ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol. 2015;110(2):223-262. [https://doi.org/10.1038/ajg.2014.435] [PMID: 25645574]

89. Goodenberger M, Lindor NM. Lynch syndrome and MYH-associated polyposis: review and testing strategy. J Clin Gastroenterol 2011;45(6):488-500. [https://doi.org/10.1097/MCG.0b013e318206489c] [PMID: 21325953]

90. Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA. 1997;277(11):915-919. [PMID 9062331]

91. Dunlop MG, British Society for Gastroenterology, Association of Coloproctology for Great Britain and Ireland. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut. 2002;51 Suppl 5: V21. [https://doi.org/10.1136/gut.51.suppl_5.v21] [PMID: 12221036]

92. Vasen HF, Blanco I, Aktan-Collan K et al. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut. 2013;62(6):812-823. [https://doi.org/10.1136/gutjnl-2012-304356] [PMID: 23408351]

93. Engel C, Rahner N, Schulmann K, et al. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol. 2010;8(2):174-182. [https://doi.org/10.1016/j.cgh.2009.10.003] [PMID: 19835992]

94. Järvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology. 2000;118(5):829-834. [https://doi.org/10.1016/s0016-5085(00)70168-5] [PMID: 10784581]

95. Mecklin JP, Aarnio M, LääräE, et al. Development of colorectal tumors in colonoscopic surveillance in Lynch syndrome. Gastroenterology. 2007;133(4):1093-1098. [https://doi.org/10.1053/j.gastro.2007.08.019] [PMID: 17919485]

96. Syngal S, Weeks JC, Schrag D, et al. Benefits of colonoscopic surveillance and prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer

mutations. Ann Intern Med. 1998;129(10):787-796. [https://doi.org/10.7326/0003-4819-129-10-199811150-00007] [PMID: 9841584]

97. Vasen HF, Abdirahman M, Brohet R, et al. One to 2-year surveillance intervals reduce risk of colorectal cancer in families with Lynch syndrome. Gastroenterology. 2010;138(7):2300-2306. [https://doi.org/10.1053/j.gastro.2010.02.053] [PMID: 20206180]

98. de Vos tot Nederveen Cappel WH, Nagengast FM, Griffioen G, et al. Surveillance for hereditary nonpolyposis colorectal cancer: a long-term study on 114 families. Dis Colon Rectum. 2002;45(12):1588-1594. [https://doi.org/10.1007/s10350-004-7244-3] [PMID: 12473880]

99. ten Kate GL, Kleibeuker JH, Nagengast FM, et al. Is surveillance of the small bowel indicated for Lynch syndrome families? Gut. 2007;56(9):1198-1201. [https://doi.org/10.1136/gut.2006.118299] [PMID: 17409122]

100. Myrhøj T, Andersen MB, Bernstein I. Screening for urinary tract cancer with urine cytology in Lynch syndrome and familial colorectal cancer. Fam Cancer 2008;7(4):303-307. [https://doi.org/10.1007/s10689-008-9193-9] [PMID: 18389386]

101. South CD, Hampel H, Comeras I, et al. The frequency of Muir-Torre syndrome among Lynch syndrome families. J Natl Cancer Inst. 2008;100(4):277-281. [https://doi.org/10.1093/jnci/djm291] [PMID: 18270343]

102. Canto MI, Harinck F, Hruban RH, et al. International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. Gut. 2013;62(3):339-347. [https://doi.org/10.1136/gutjnl-2012-303108] [PMID: 23135763]

103. Kalady MF, McGannon E, Vogel JD, et al. Risk of colorectal adenoma and carcinoma after colectomy for colorectal cancer in patients meeting Amsterdam criteria. Ann Surg. 2010;252(3):507-511. [https://doi.org/10.1097/SLA.0b013e3181f20bd2] [PMID: 20739851]

104. Vasen HFA, Möslein G, Alonso A, et al. Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). J Med Genet. 2007;44(6):353–362. [https://doi.org/10.1136/jmg.2007.048991] [PMID: 17327285]

105. Goldblatt J, Madden MV, Boshoff PJ, et al. Hereditary non-polyposis colorectal cancer in a Namaqualand-Kindred. S Afr Med J. 1990;77(1):42-44. [PMID: 2294615]

106. Anderson DW, Goldberg PA, Algar U, et al. Mobile colonoscopic surveillance provides quality care for hereditary nonpolyposis colorectal carcinoma families in South Africa. Colorectal Dis. 2007;9(6):509-514. [https://doi.org/10.1111/j.1463-1318.2006.01172.x] [PMID: 17477847]

107. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis. 2009;11(2):126-130. [https://doi.org/10.1111/j.1463-1318.2008.01702.x] [PMID: 19143775]

108. Bruwer Z, Futter M, Ramesar R. A mobile colonoscopic unit for lynch syndrome: Trends in surveillance uptake and patient experiences of screening in a developing country. J Genet Couns. 2013;22(1):125-137. [https://doi.org/10.1007/s10897-012-9523-9] [PMID: 23299947]

109. Dik VK, Gralnek IM, Segol O, et al. Multicenter, randomized, tandem evaluation of EndoRings colonoscopy – results of the CLEVER study. Endoscopy 2015;47(12):1151-1158. [https://doi.org/10.1055/s-0034-1392421] [PMID: 26220283]

110. Floer M, Meister T. Endoscopic Improvement of the Adenoma Detection Rate during Colonoscopy – Where Do We Stand in 2015? Digestion 2016;93(3):202–213. [https://doi.org/10.1159/000442464] [PMID: 26986225]

111. Jain D, Sandhu N, Singhal S. New Developments in Mechanical Enhancement of Colonoscopy: Cuffs, Caps and Rings. Digestion 2016;93(3):234–247. [https://doi.org/10.1159/000445108] [PMID: 27119347]

Chapter 2: Publication ready manuscript.

2.1 Introduction.

Lynch syndrome (LS) is an autosomal dominant disorder characterised by specific germline mutations in one of the four DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) or the EPCAM gene [1]. Historically known as hereditary non-polyposis colorectal cancer (HNPCC) (which is defined clinically, usually by satisfaction of the Amsterdam I or II criteria, in the absence of a known genetic mutation) it is the most common of the inherited syndromes that significantly increase the life time risk of developing colorectal cancer (CRC) as well as cancers of multiple other organs (endometrium, stomach, ovaries etc.) from 5% in the general population to 18%-53% depending on the gene affected [2]. Progression to CRC in these individuals is accelerated (1-2 years) and cancers tend to develop at a younger age when compared to non-hereditary CRC. Additionally, individuals who do go on to develop CRC are at significant risk of synchronous tumours or of developing metachronous tumours after their index surgery, depending on the type of surgery performed (15% at 10 years, 40% at 20 years, 60% at 30 years) [3]. Even though the name HNPCC may suggest an absence of adenomas/polyps, individuals with LS can certainly develop a few adenomas/polyps. These are usually fast growing, flat and more prevalent in the right colon displaying higher grades of dysplasia and villous histology [4].

Estimates suggest that 1 in 300 individuals carry mutations in their DNA MMR genes. Though not all carriers progress to cancer, LS still accounts for approximately 3%-5% of all newly diagnosed CRCs yearly. Currently LS is still under recognised. Multiple clinical criteria (Amsterdam I & II, Bethesda), prediction models (MMRpredict, MMRpro) as well as tumour-based strategies have been developed as adjuncts to clinical suspicion to better identify individual and families who would benefit genetic testing [5-10]. Current international consensus guidelines recommend that high-risk individuals with LS undergo screening colonoscopy every 1-2 years beginning at 20-25 years of age or 2-5 years prior to earliest age of CRC in the family (whichever occurs 1st), repeated ever 1-2 years. Several screening recommendations for extracolonic Lynch associated cancers also exist [11-14].

CRC in South Africa is the 4th most common cancer among both men and women. Crude incidence rate is 6.5-8.5/100000 yearly [15]. The Northern Cape is the largest and most sparsely populated province in South Africa with a predominantly Afrikaans speaking population. In the Northern Cape CRC incidence is relatively lower at 3-4/100000, however

it has been shown that 10.5% of these cancers have a MMR gene mutation, translating to three times the normal international rate in high incidence areas [16,17]. The majority of the LS data from South Africa comes from 50 satellite families (2000 individuals) from the Northern Cape.

At the time of writing no national CRC/LS screening and surveillance program exists in South Africa. However, the South African experience with regards to LS is enhanced by the Annual Northern Cape Colonoscopy Outreach Program. In the remote areas of Northern Cape, the Colorectal Unit of the University of Cape Town and Groote Schuur Hospital has provided an annual (August/September) outreach colonoscopy service for mutation positive individuals for the past 21 years. Each annual trip spans a period of one week (preceded by a preparatory and informative trip) and involves visits to 4-5 district towns by specialists, nurses, registrars, technicians from the GSH colorectal department and over the past few years gynaecological and breast services as well [16,18,19]. It has been shown that individuals who comply with the outreach service benefit from overall and CRC related survival extending their lifespan by up to 20 quality adjusted life years [19]. Outreach colonoscopy has been shown to provide a safe, cost-effective and comparable service to impatient colonoscopy in this setting [20]. However, the outreach service does face several challenges. These include challenges with patient adherence to the service (25% obtained 100% adherence) and poor compliance with bowl preparation prior to colonoscopy, resulting in a steady decline in attendance over the years [1]. Major factors influencing this decline are mostly economical and logistical in nature. Pain and discomfort are other major deterrent factors. Any traumatic experiences likely result in further non-compliance of not only the individuals involved but also other family members. Therefore, it is essential to have experts providing the service to make the experience as smooth as possible for the patients. Having experts also limits the number of major complications (e.g. perforation) which is of paramount importance as the nearest metropolitan hospital is usually hours away from the towns where the service visits.

Colonoscopy as we know is a colon inspection and intervention technique involving a fibre optic instrument being inserted through the anus for colonic visualisation and/or intervention. Standard colonoscopy is currently the gold standard for detection and removal of colorectal adenomas and polyps, the precursor lesions of most CRCs [19,21]. Despite being the most sensitive surveillance method for prevention of future CRCs, colonoscopy is not without its

limitations, particularly in terms of visualisation of the entire colon [22]. Known causes of missed precancerous lesions include inadequate bowel preparation, difficulties in detection of flat, small (1mm -5mm) lesions and lesions on the proximal side of haustral folds and the internal curves of colonic flexures [23,24]. Several studies have reported the adenoma/polyp miss rates of standard colonoscopy to be approximately 20–25%, with newer studies citing miss rates approaching 40% [25,26]. This has created a need for the development of novel technologies/techniques to improve visualisation of the colon especially behind colonic folds and flexures.

Several technologies such as cap-assisted colonoscopy, virtual chromo endoscopy, Third Eye colonoscopy, Full Spectrum Endoscopy (FUSE) colonoscopy, EndoCuff™ and EndoRings™ (Fig 4.) exist to try improving adenoma detection rate (ADR)/polyp detection rate (PDR), however their true efficacy remains to be established conclusively [24,27]. Arguably some positive evidence for the use of cuff devices such as EndoCuff™ (2 RCTs) and EndoRings™ (1 RCT) with regards to increased ADR/PDR in at risk individuals exists, however too few studies currently exist to draw any definitive conclusions as to their benefit. EndoRings™ is a silicone-rubber device that is fitted onto the distal end of the colonoscope. The alleged improvement in detection of adenomas/polyps offered by the EndoRings™ is provided by three circular rows of flexible silicone rubber rings that engage and mechanically straighten the colonic folds during withdrawal (Fig 4.) [1]. The EndoRings™ additionally improves visualization of the total colonic surface area by keeping the distal tip of the colonoscope centred in the colonic lumen and stabilizing the scope tip where interventions are needed. Colonoscopy with the EndoRings™ does not interfere with the normal washing, suctioning, and therapeutic capabilities of the colonoscope and does not block parts of the camera view as is the case with some of the other devices [1].

The utility of EndoRings™ in terms of increased ADR/PDR has not been studied in the South African setting. The primary objective of this study therefore aims to determine the efficacy of EndoRings™ assisted colonoscopy in terms of ADR/PDR when compared with standard colonoscopy in individuals with Lynch syndrome. The primary endpoint of this study measures the number of adenomas/polyps detected within both study arms and provides a comparison of the two. Secondary end points include intubation time, withdrawal time, total procedure time, complications and adequacy of bowel preparation in both arms.

2.2 Methodology.

The study was designed as a cross sectional randomized controlled trial after obtaining ethical approval from the Research Ethics Committee of the University of Cape Town (HREC number 536/2015). A total of 57 individuals from the Northern Cape province of South Africa with LS awaiting scheduled surveillance colonoscopies were enrolled into the study over a one-week period during the Annual Northern Cape Colonoscopy Outreach trip for the year 2015. Individuals satisfying the inclusion criteria (n = 54) were enrolled and informed consent (in local language of preference) was obtained from each individual for the colonoscopic procedure as well as for the study (see appendix for consent form). Those not meeting the inclusion criteria and those who refused to provide consent were excluded (n = 3). All study participants were well informed about the study details, the risks, benefits and alternatives and had an extended opportunity to ask any questions. Identity of participants was kept anonymous throughout and all data was recorded on numbered data sheets without any personal identifiers (see appendix). Study participants were randomised blindly using computer randomisation to a control arm undergoing standard colonoscopy and a study arm undergoing EndoRings™ assisted colonoscopy, each arm numbering 27 participants.

All colonoscopies were performed by experienced senior colorectal and gastrointestinal consultants to maintain standards. Bowel preparation was started 24 hours prior to the procedure using MOVIPREP™ according to the standard bowel preparation protocol provided by the manufacturer. A stopwatch was used to measure caecum intubation time. A photograph of the appendix orifice was used to confirm complete colonoscopy. The time stamp on the photograph was used as the reference for the start of the withdrawal time. At the end of the procedure, a photograph of the distal rectum and anus was taken with a retroflexed colonoscope. The time stamp on this photograph served as the end of procedure time. Time spent removing polyps and cleaning the colon was documented and subtracted from the total withdrawal time (caecum to rectum) to determine net withdrawal time. Pethidine and Midazolam were administered for analgesia and sedation. All relevant data, including patient demographics, primary outcome (number of adenomas/polyps detected) and secondary outcomes (procedure times and bowel preparation and complications) were recorded on data sheets during the procedure in real-time by a registered nurse or surgical trainee so as not to interrupt the consultants during the procedure.

Inclusion and exclusion criteria.

Inclusion criteria:

- Known LS MMR gene mutation carriers undergoing scheduled surveillance colonoscopy during the Annual Northern Cape Colonoscopy Outreach trip.
- Individuals who provided informed consent for the procedure.

Exclusion criteria:

- Individuals under the age of 18 years.
- Individuals with a history of inflammatory bowel disease, known colonic stricture and history of other polyposis syndromes (FAP, MAP).
- Refusal to provide informed consent.

Statistical analysis.

A power calculation (R, 64 bit, version 3.1.3) was carried out using a z-test to compare the means of the number of polyps expected in the two arms (with a significance level of 5% and assuming that the variances were known). The distribution for the mean number of polyps was normal, using large sample theory (central limit theorem). However, the results should be treated with caution especially at the smaller sample sizes. To calculate the sample size required for a study power of 80%, six hypothetical contexts were considered (see Appendix E for all six scenarios). Using hypothetical context 5 (Fig 5. below) for our best estimates (based on current studies), it was determined that a total of 80 individuals (40 in each arm) were needed to have an 80% chance of detecting a significant difference in the means between the two arms at a 5% significance level, based on a coefficient of variation of 100%,and assuming a true mean of 0.2 polys in Arm 1 (10 polyps/50 colons), and a mean of 0.4 in Arm 2 (20 polyps/50 colons). The distribution of the number of polyps per colon was heavily right-skewed, as a large proportion of 0 polyps were expected.

Data analysis was performed using an IPython kernel in a Jupyter notebook together with the numerical python, scientific python, statsmodels, and scikit.bootstrap libraries. Data management was performed using the pandas library and plotting was based on the matplotlib library. Descriptive statistics used mean and median as point estimates and standard deviation, range, and 95% confidence intervals as measure of dispersion. The assumptions for the use of parametric tests were done using the Shapiro-Wilk test and

examination for statistical outliers. Student's t-test and analysis of variance we used to compare numerical variables if these assumptions were met. The Mann-Whitney-Wilcoxon and Kruskal-Wallis tests were used as non-parametric alternatives. Univariate linear regression was used to compare numerical variables. Proportions we compared using the $\chi 2$ for independence. A confidence levels of 95% was used throughout. Unless otherwise indicated, a two-tail test hypothesis was used with an alpha-value of 0.05 as discriminator for rejection of the null-hypothesis.

2.3 Results.

Demographics.

Out of a total of 57, data for 54 colonoscopies was available for analysis. Three individuals were excluded for protocol violations. All enrolled individuals agreed to participate in the study. The use of EndoRings™ during colonoscopy was randomly assigned by computer randomisation, with 27 individuals in each study arm. Colonoscopies were performed on 34 (63%) females and 20 (37%) males. The male cohort was slightly older, with a mean age and standard deviation (SD) of 46.3±18.1 (range: 22 to 82) years versus a mean age and SD of 42.4±12.7 (range: 20 to 64) years for the female cohort (P = 0.55) as shown in Fig 1. below.

The mean age of all participants who underwent EndoRings™ assisted colonoscopy was 43.98±15.27 years (range: 20 to 57, 95% CI 33.9 to 42.8 years) compared to a mean age of 44.26±14.67 years (range 22 to 82, 95% CI 43.6 to 55.2 years) for those who underwent standard colonoscopy (P = 0.05).The female to male ratio in the EndoRings™ group was 19:8 versus 15:12 in the standard colonoscopy group (P = 0.40).

In both arms, out of 27 patients, 24 patients (88.9%) had previously had a colonoscopy and 25 patients (92.6%) successfully completed their colonoscopy. Out of the 27 patients, 10 patients (37.04%) in the standard colonoscopy arm and 4 patients (14.8%) in the EndoRings™ arm had had prior surgery (segmental resections (11) and subtotal colectomy (3) with ileorectal anastomosis. 4 colonoscopies (2 in each arm) could not be carried out to completion due to colonic narrowing.

Bowel preparation and procedure time.

Using the Harefield Cleansing Scale, bowel preparation during colonoscopy was reported as

excellent in 48.1% (n = 13) of cases in both arms, good in 33.3% (n = 9) and 44.4% (n= 12), and fair in 18.5% (n = 5) and 7.4% (n = 2) of cases in the standard colonoscopy versus EndoRings™ arms respectively. The variations in the adequacy of bowel preparation in both arms did not influence the completion of the procedures (P = 0.49) or the number of polyps detected (P= 0.24). We found the total time taken to complete the colonoscopy was somewhat influenced by the adequacy of the bowel preparation. Excellent preparation - mean time to completion 22.4 minutes (95% CI, 19.5 to 26.8 minutes), good preparation - mean time 31.2 minutes (95% CI, 25.1 to 38 minutes) and fair preparation - mean time 24.1 minutes (95% CI, 21 to 26.4 minutes) as shown in Fig 2, though this was not clinically or statistically significant.

As illustrated in Table 1, no significant differences were found with regards to time taken to reach the caecum (95% CI 12.3 to 18.9 minutes in EndoRings™ arm versus 95% CI 11.5 to 17.9 minutes standard colonoscopy arm, P =0.84), the withdrawal time (95% CI 8.0 to 11.2 minutes in EndoRings™ arm versus 95% CI 8.4 to 12.6 minutes in standard colonoscopy arm) (P = 0.84) as well as the total procedure time in both arms. Please refer to Appendix A for supplementary data.

Adenoma/Polyp detection rate.

Table 2 highlights the number of adenomas/polyps per person found as a percentage in both arms. The average number of adenomas/polyps detected in the EndoRings™ assisted colonoscopy arm was 1.4 versus 0.9 in the standard colonoscopy group (p = 0.60) as shown in Fig 3. Similarly, Table 3 displays the location of adenomas/polyps found in the different regions of the colon as well as the number of times adenomas/polyps were found in those areas. Furthermore, in both arms the size of adenomas/polyps detected were less than 1 cm in size and no large adenomas/polyps were reported in any individual. No complication occurred in the EndoRings™ assisted colonoscopy arm. Two individuals reported limited rectal bleeding immediately after their colonoscopy in the standard colonoscopy group (P = 0.54). There were no device related issues such as dislodgement or impaction noted with the use of the EndoRings™ device, and no difference in the discomfort experienced during the procedures by the participants between the two arms.

Subgroup analysis (exclusion of individuals with previous colon resections).

Excluding individuals who had undergone previous colon resection yielded 23 individuals (n=27-4) in the EndoRings™ assisted colonoscopy arm (6 males/17 females) and 17

individuals (n=27-10) in the standard colonoscopy arm (8 males/9 females). The total number of adenomas/polyps found in the former group was 20 (mean 0.86, SD 1.42) versus 6 (mean 0.35, SD 0.76) in the standard colonoscopy group (see Table 4 below). Analysis of this data, using the above-mentioned tests yielded a p-value of 0.32, which again is not statistically significant.

2.4 Discussion.

Currently standard colonoscopy is the gold standard when it comes to detection and removal of premalignant lesions in the colon and rectum [1]. Despite being the best available technique, standard colonoscopy does have some serious limitations of which the most significant being the inability to adequately visualise the entirety of the colorectal mucosal lining [23]. Missed lesions hidden in the proximal aspects of haustral folds and flexures can have significant consequences in terms of morbidity and mortality for the individuals concerned [24]. In an attempt to improve visualisation of the colon and in effect increase detection rates of sinister lesions multiple novel techniques and devices have been proposed. The EndoRings™, a small silicone-rubber device which attaches to the distal end of the colonoscope is one such novel device and it has been proposed that EndoRings™ assisted colonoscopy increases total colonic visualisation, particularly in the folds and flexures as well as significantly reduces the adenoma/polyp miss rates associated with standard colonoscopy [23]. Therefore, the purpose of this randomised cross-sectional controlled trial was to investigate the efficacy in terms of increased ADR/PDR of the EndoRings™ device in the South African setting. In this study at risk individuals with LS presenting for their annual screening colonoscopy via the Northern Cape Outreach Colonoscopy program were divided into a control group undergoing standard colonoscopy and a test group undergoing EndoRings™ assisted colonoscopy. During the course of this particular study we were unable to demonstrate a statistically significant difference in ADR/PDR between the standard colonoscopy and EndoRings™ assisted colonoscopy groups (P = 0.60). The total number of lesions detected with standard colonoscopy were 24 (mean 0.9) as compared to 40 (mean 1.4) with EndoRings™ assisted colonoscopy. Furthermore, subgroup analysis excluding all those individuals who had previously undergone colonic resection of any extent also failed to yield any statistically significant findings (P=0.34) between the two groups. These findings are not in line with those reported by the CLEVER trial, the only other RCT comparing standard colonoscopy to EndoRings™ assisted colonoscopy to date. The CLEVER trial reported a significant increase in ADR/PDR during their study [23]. At the time of designing this study

we had anticipated a much larger cohort of LS affected individuals from the Northern Cape to present themselves for their annual screening colonoscopy than the number that actually attended their screening. This reduced sample size as well as constraints imposed on investigator time for the purpose of this study and logistical issues faced by the mobile surveillance team, contributed significantly to this study being under powered and may therefore be the reason why a statistically significant difference between the two arms could not be demonstrated (type 2 error). It should be noted that even though the CLEVER trial reported a significant difference in total detection rates in their trial, they too were unable to demonstrate a statistically significant difference in missed advanced adenoma/polyp rates (size > 10 mm, high grade dysplasia, villous architecture) [23].

In line with available data, we did not observe any statistically significant difference in the total procedure time, time to caecum and withdrawal time between the two arms. In an attempt to maintain uniformity time taken to carry out interventions (e.g. polypectomies) was not considered for analysis in either arm. Overall bowel preparation was found to be excellent in 26, good in 21 and fair in 7 individuals according to the Harefield Cleansing Scale and did not have any impact on ADR/PDR in this study. Completion of colonoscopy could not be achieved in 4 individuals (2 in each arm). All these individuals had previous abdominal/colonic surgery causing some degree of colonic narrowing limiting the progression of the scope. Two individuals reported experiencing complications post colonoscopy (minor bleeding), however these complications were found in the standard colonoscopy arm alone whereas no complications were reported in the EndoRings™ assisted colonoscopy arm. As expected, lesions were more frequently encountered in the proximal colon in both study arms (n = 9 standard colonoscopy, n = 13 EndoRings™ assisted colonoscopy) but once again out of line with the CLEVER trial there was no clinically significant difference between the two arms. According to the manufacturer, EndoRings™ improve total colonic visualisation by not only straightening out the colonic folds by also stabilises the scope tip within the lumen. The device does not interfere with washing, suctioning and the therapeutic capabilities of the scope and does not block any part of the camera view [28]. Anecdotal evidence from our endoscopists supports this claim as we found that the use of the EndoRings™ did in fact mechanically straightened the colonic folds and stabilise the scope within the colonic lumen without compromising vision or ability to perform interventions. Another advantage of the EndoRings™ device is that it readily fits most available colonoscopes and is therefore relatively inexpensive when compared to other

novel devices (e.g. FUSE). This may be of some consideration for cash strapped departments wishing to adopt the technology.

As eluded to above our study had some limitation. In addition to the small sample size to achieve power, the study may have benefited from back to back comparison of ADR/PDR using standard colonoscopy followed by EndoRings™ assisted colonoscopy on each individual patient rather than splitting the patients into two arms each undergoing one colonoscopy. However, this would have been an impossibility for us as the mobile unit moves through five towns in the week that it operates, attempting to provide a desperately needed service to a dwindling and already apprehensive population. The time and resources required in subjecting an individual to two procedures one after the other in small district hospitals, many without proper surgical services should a complication arise was simply not acceptable.

Although our data is insufficient and statistically not significant given the small sample size, the improved efficacy of EndoRings™-assisted colonoscopy may be implied if total numbers are taken into consideration. Overall more adenomas/polyps were found in the test arm of the study as compared to the control arm with no overall increase in procedure time or change in procedure experience. Some may even argue that operator experience with the EndoRings™ is superior to standard colonoscopy but whether this translates into a clinically significant, practice changing paradigm remains to be determined.

2.5 Conclusion.

In conclusion, this randomized cross-sectional study failed to demonstrate a statistically significant advantage in terms of ADR/PDR between EndoRings™ assisted colonoscopy and standard colonoscopy in patients with LS. Although there was a trend noticed favoring EndoRings™ assisted colonoscopy, no statistically significant conclusions could be reached, likely due to study limitations. EndoRings™ assisted colonoscopy was perceived as being advantageous in terms of improved total colonic visualisation and aiding in making interventions easier. The findings of comparable intubation times, withdrawal times, total procedure times and similar complication rates as standard colonoscopy are all in favour of EndoRings™ assisted colonoscopy. However, before any changes to clinical practice and/or surveillance programs are made these advantages need to be considered carefully as adopting new technologies places addition financial and logistical burden on already resource poor

systems, especially in our setting. However, having said that if future larger trials conducted in a more easily accessible population group (general population etc.) over a longer period prove EndoRings™ to be superior in detecting premalignant lesions then serious consideration to practice change would be validated if aiming to halt progression to cancer.

2.6 Tables and Figures.

Fig 1. Description of patients based on gender.

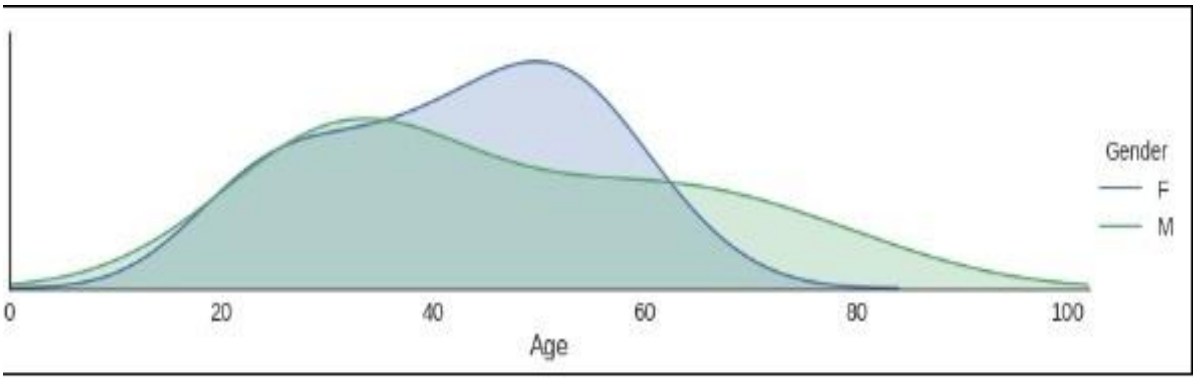


Fig 2. Procedure time based on adequacy of bowel preparation.

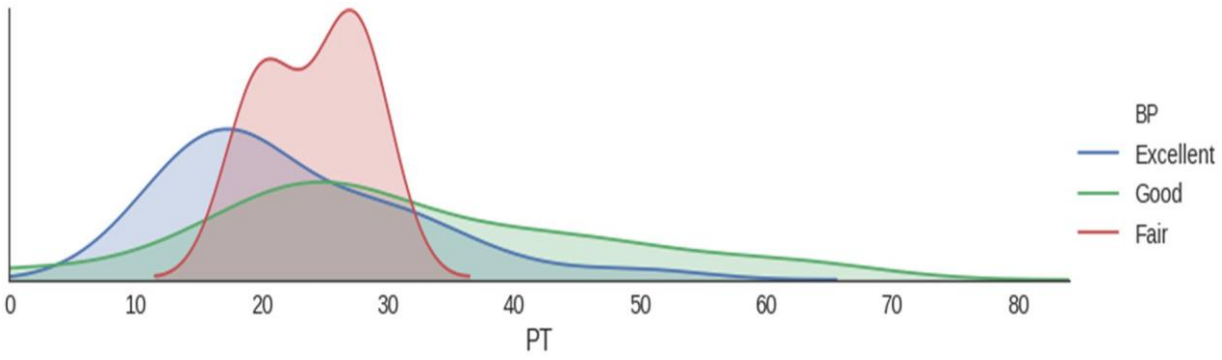


Fig 3. Number of polyps found in EndoRings™ arm VS standard colonoscopy arm.

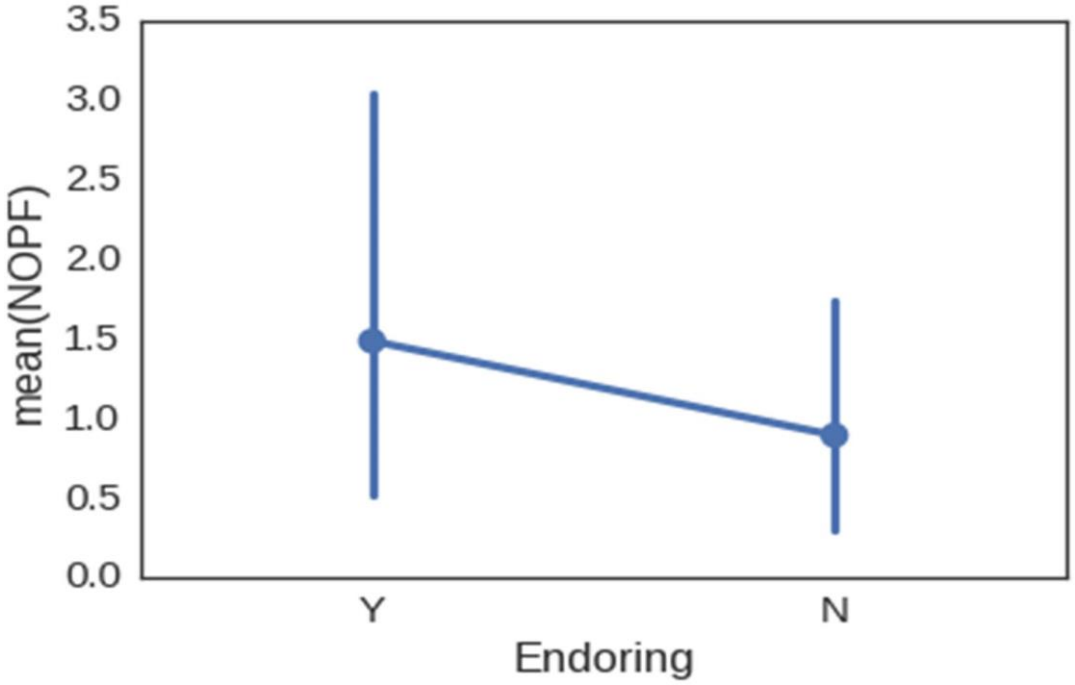


Fig 4. EndoRing™ on the tip of a colonoscope and in the colon [23].

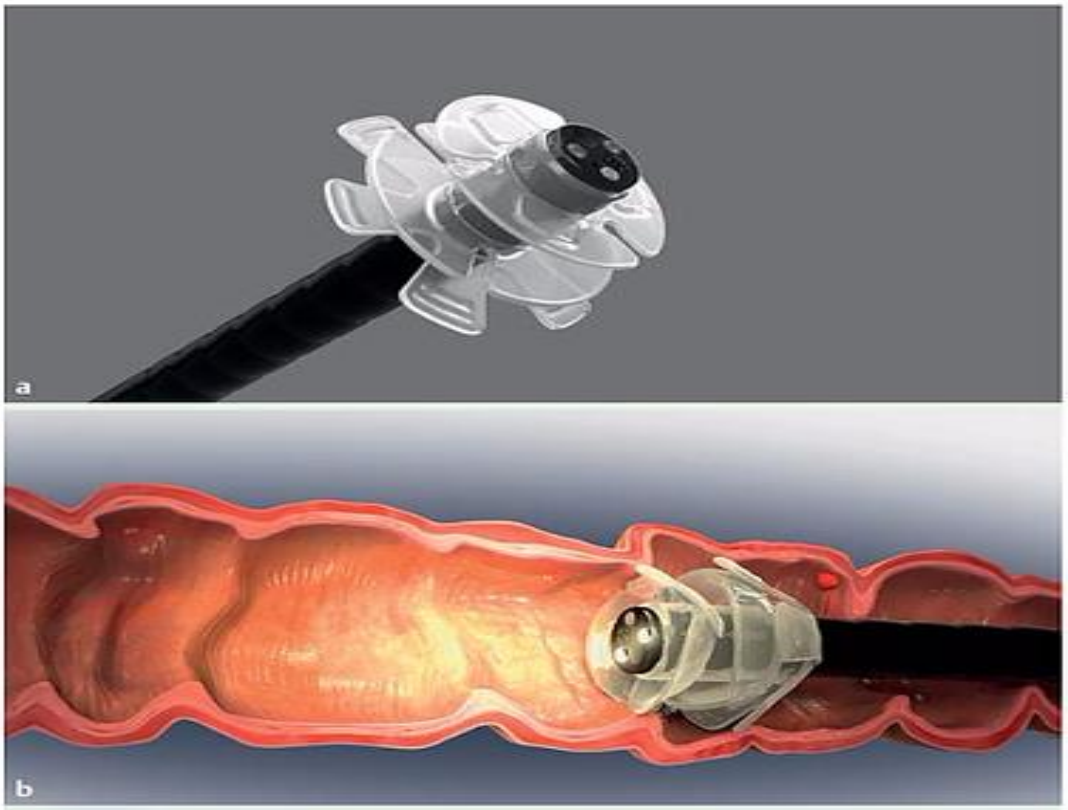
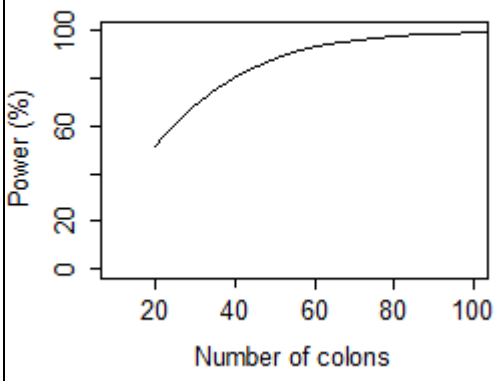


Fig. The EndoRings device. a Illustration of the device attached to the distal end of a colonoscope. b Illustration showing how the device mechanically straightens the colonic folds during withdrawal of the colonoscope.

Fig 5. Hypothetical Context 5 for power calculation.

Context 5



Power	Sample size
50	<20
60	25
70	31
80	39
90	53

Table 1: Procedure time in standard colonoscopy vs EndoRings™.

	Standard Colonoscopy	EndoRings™
N=27 each	(Mean ± SD)	(Mean ± SD)
Time taken to reach caecum	14.66 ± 8.35	14.42 ± 8.45
Time taken to withdraw	9.6 ± 4.72	9.46 ± 4.65
Total procedure time	26.25 ± 12.34	25.9 ± 12.5

Table 2: Number of polyps in standard colonoscopy vs EndoRings™.

	Standard Colonoscopy		EndoRings™	
Number of Adenomas/Polyps	(%)	Number of People	(%)	Number of People
0	66.67	18	62.96	17
1	18.52	5	7.4	2
2	3.7	1	14.8	4
3	3.7	1	3.7	1
4	3.7	1	3.7	1
5	0	0	3.7	1
10	3.7	1	0	0
18	0	0	3.7	1
Total	100	27	100	27

Table 3: Number of polyps at different locations in standard colonoscopy vs EndoRings.

	Standard Colonoscopy	EndoRings™
Location of Adenomas/Polyps	Number of times adenoma/polyp found in this location	Number of times adenoma/polyp found in this location
Caecum	4	5
Ascending colon	1	3
Transverse colon	4	5
Descending colon	1	1
Sigmoid colon	2	4
Rectum	2	2

Table 4: Subgroup analysis (exclusion of individuals with previous colon resections).

	No. of individuals in EndoRings™ assisted colonoscopy group (excluding those who had previous colectomies). N= 23 (27-4)		No. of individuals in standard colonoscopy group (excluding those who had previous colectomies). N= 17 (27-10)	
Number of Adenomas/Polyps	Male	Female	Male	Female
0	4	11	5	8
1	1	1	1	2
2	0	3	0	0
3	0	1	1	0
4	0	1	0	0
5	1	0	0	0
Total Adenomas/Polyps	6	14	4	27

2.7 Chapter 2 References

1. Bruwer Z, Futter M, Ramesar R. A mobile colonoscopic unit for lynch syndrome: Trends in surveillance uptake and patient experiences of screening in a developing country. J Genet Couns. 2013;22(1):125-137. [https://doi.org/10.1007/s10897-012-9523-9] [PMID: 23299947]

2. Shawki S, Kalady MF. Recent advances in understanding Lynch syndrome. F1000Research 2016;5(0):2889. [https://doi.org/10.12688/f1000research.9654.1] [PMID: 28105321]

3. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. Gastroenterology. 2014;147(2):502-526. [https://doi.org/10.1053/j.gastro.2014.04.001] [PMID: 25043945]

4. Järvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology. 2000;118(5):829-834. [https://doi.org/10.1016/s0016-5085(00)70168-5] [PMID: 10784581]

5. Bessa X, Alenda C, Paya A,Álvarez C, et al. Validation microsatellite path score in a population-based cohort of patients with colorectal cancer. J Clin Oncol. 2011;29(25):3374-3380. [https://doi.org/10.1200/JCO.2010.34.3947] [PMID: 21788563]

6. American Gastroenterological Association. American Gastroenterological Association medical position statement: hereditary colorectal cancer and genetic testing. Gastroenterology. 2001;121(1):195-197. [https://doi.org/10.1053/gast.2001.25580] [PMID: 11438508]

7. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. 1999;116 (6):1453–1456. [https://doi.org/10.1016/S0016-5085(99)70510-x] [PMID: 10348829]

8. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary

nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261–268. [https://doi.org/10.1093/jnci/djh034] [PMID: 14970275]

9. Ligtenberg MJ, Kuiper RP, Geurts van Kessel A, Hoogerbrugge N. EPCAM deletion carriers constitute a unique subgroup of Lynch syndrome patients. Fam Cancer. 2013;12(2):169-174. [https://doi.org/10.1007/s10689-012-9591-x] [PMID: 23264089]

10. Ladabaum U, Wang G, Terdiman J, et al. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. Ann Intern Med. 2011;155(2):69-79. [https://doi.org/10.7326/0003-4819-155-2-201107190-00002] [PMID: 21768580]

11. Antill YC, Dowty JG, Win AK, et al. Lynch syndrome and cervical cancer. Int J Cancer. 2015;137(11):2757-2761. [https://doi.org/10.1002/ijc.29641] [PMID: 26077226]

12. Walsh MD, Buchanan DD, Pearson SA, et al. Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry. Mod Pathol. 2012;25(5):722-730. [https://doi.org/10.1038/modpathol.2011.209] [PMID 22322191]

13. Dunlop MG, British Society for Gastroenterology, Association of Coloproctology for Great Britain and Ireland. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut. 2002;51 Suppl 5: V21. [https://doi.org/10.1136/gut.51.suppl_5.v21] [PMID: 12221036]

14. Myrhøj T, Andersen MB, Bernstein I. Screening for urinary tract cancer with urine cytology in Lynch syndrome and familial colorectal cancer. Fam Cancer 2008;7(4):303-307. [https://doi.org/10.1007/s10689-008-9193-9] [PMID: 18389386]

15. Marley A, Hongmei N. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet. 2016;7(3):105–114. Published online 2016 Sep 30. [PMID: 27766137]

16. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and Penetrance of Major Genes and

Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2017;26(3):404-412. [https://doi.org/10.1158/1055-9965.EPI-16-0693] [PMID: 27799157]

17. Wentink M, Räkers M, Stupart D, et al. Incidence and histological features of colorectal cancer in the Northern Cape province, South Africa. S Afr J Surg. 2010;48(4):109-113. [PMID: 21542398]

18. Goldblatt J, Madden MV, Boshoff PJ, et al. Hereditary non-polyposis colorectal cancer in a Namaqualand-Kindred. S Afr Med J. 1990;77(1):42-44. [PMID: 2294615]

19. Anderson DW, Goldberg PA, Algar U, et al. Mobile colonoscopic surveillance provides quality care for hereditary nonpolyposis colorectal carcinoma families in South Africa. Colorectal Dis. 2007;9(6):509-514. [https://doi.org/10.1111/j.1463-1318.2006.01172.x] [PMID: 17477847]

20. Klabunde CN, Cronin KA, Breen N, et al. Trends in colorectal cancer test use among vulnerable populations in the United States. Cancer Epidemiol Biomarkers Prev 2011;20(8):1611-1621. [https://doi.org/10.1158/1055-9965.EPI-11-0220] [PMID: 21653643]

21. Mecklin JP, Aarnio M, LääräE, et al. Development of colorectal tumors in colonoscopic surveillance in Lynch syndrome. Gastroenterology. 2007;133(4):1093-1098. [https://doi.org/10.1053/j.gastro.2007.08.019] [PMID: 17919485]

22. Jain D, Sandhu N, Singhal S. New Developments in Mechanical Enhancement of Colonoscopy: Cuffs, Caps and Rings. Digestion 2016;93(3):234–247. [https://doi.org/10.1159/000445108] [PMID: 27119347]

23. Dik VK, Gralnek IM, Segol O, et al. Multicenter, randomized, tandem evaluation of EndoRings colonoscopy – results of the CLEVER study. Endoscopy 2015;47(12):1151- 1158. [https://doi.org/10.1055/s-0034-1392421] [PMID: 26220283]

24. Floer M, Meister T. Endoscopic Improvement of the Adenoma Detection Rate during Colonoscopy – Where Do We Stand in 2015? Digestion 2016;93(3):202–213. [https://doi.org/10.1159/000442464] [PMID: 26986225]

25. Ahn SB, Han DS, Bae JH, et al. The Miss Rate for Colorectal Adenoma Determined by Quality-Adjusted, Back-to-Back Colonoscopies. Gut Liver 2012;6(1):64-70. [https://doi.org/10.5009/gnl.2012.6.1.64] [PMID: 22375173]

26. Leufkens AM, Van Oijen MGH, Vleggaar FP, Siersema PD. Factors influencing the miss rate of polyps in a back-to-back colonoscopy study. Endoscopy 2012; 44:470-475. [https://doi.org/10.1055/s-0031-1291666] [PMID: 22441756]

27. Chin M, Karnes W, Jamal MM, et al. Use of the Endocuff during routine colonoscopy examination improves adenoma detection: A meta-analysis. World J Gastroenterol 2016;22(43): 9642-9649. [https://doi.org/10.3748/wjg.v22.i43.9642] [PMID: 27920485]

28. Mamula P, Tierney W. M, Banerjee S, et al. Devices to improve colon polyp detection. Gastrointestinal Endoscopy 2011;73(6), 1092-1097. [https://doi.org/10.1016/j.gie.2011.01.062]

Appendix

Appendix A: Supplementary data.

Fig 1. Time (min.) to Caecum: EndoRings™ assisted colonoscopy VS standard colonoscopy.

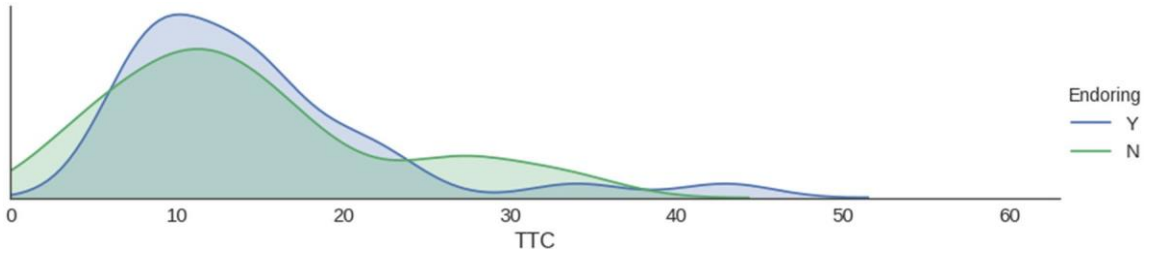


Fig 2. Time to Caecum probability plot for EndoRings™ arm.

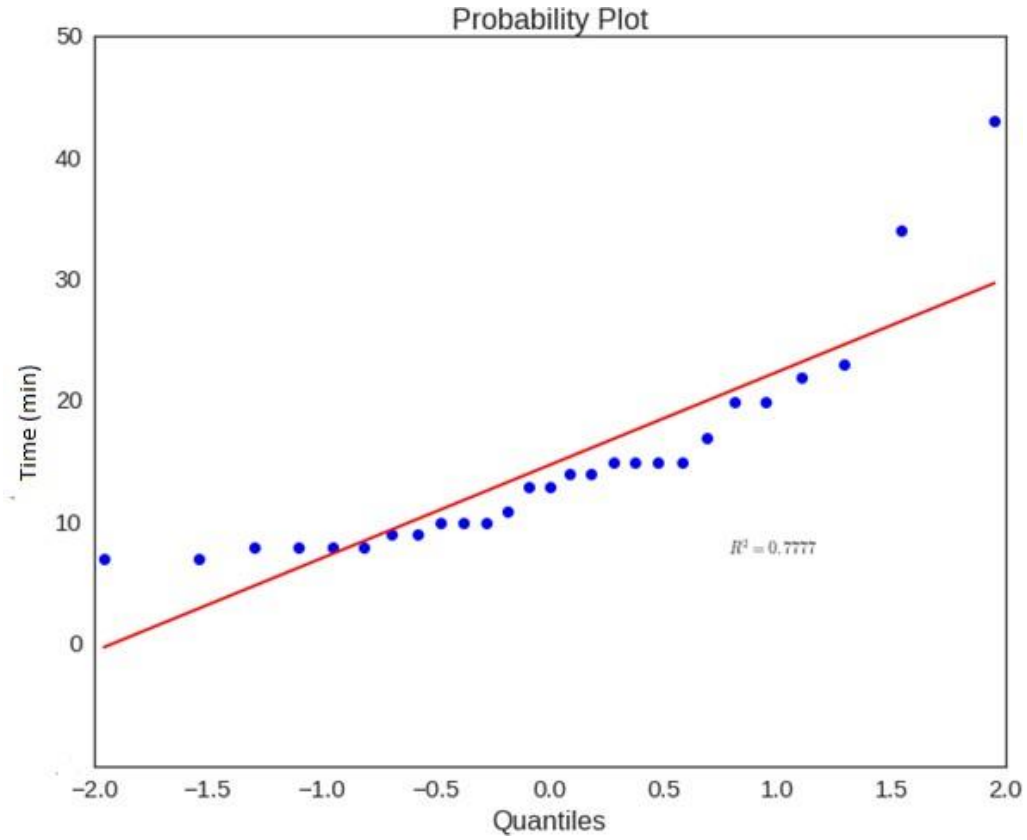


Fig 3. Time to caecum: Probability plot for standard colonoscopy arm.

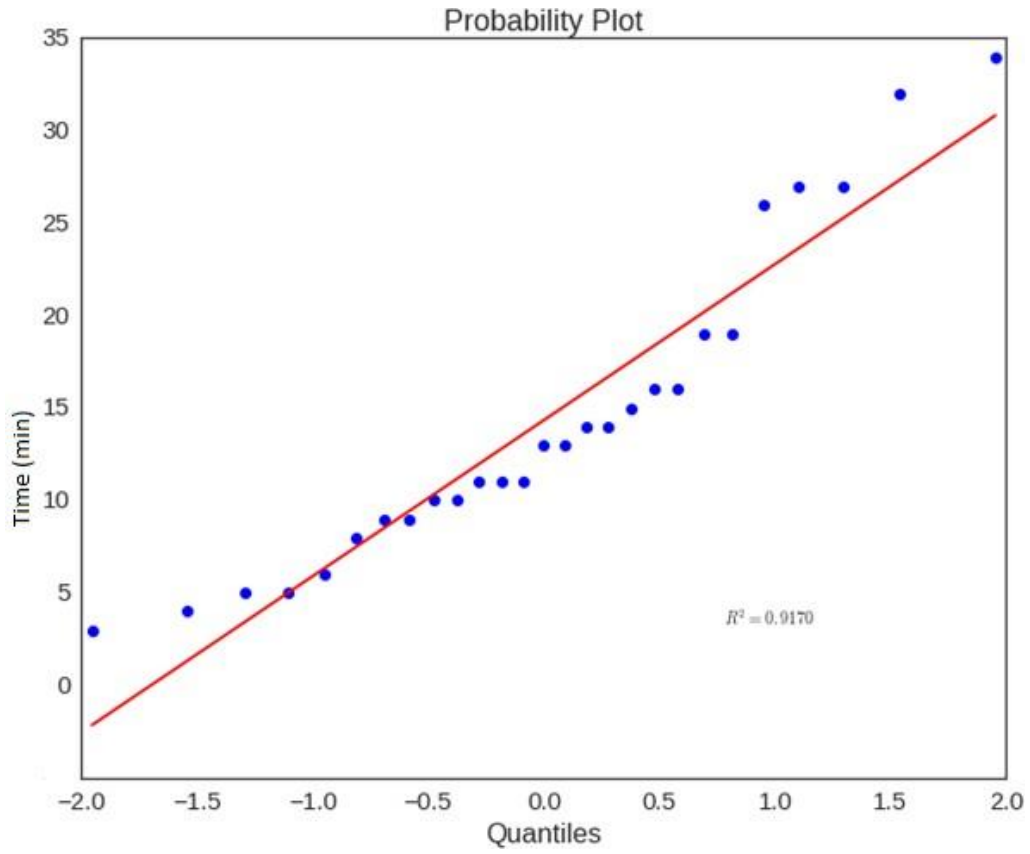


Fig 4. Withdrawal time (minutes): EndoRings™ assisted colonoscopy VS standard colonoscopy.

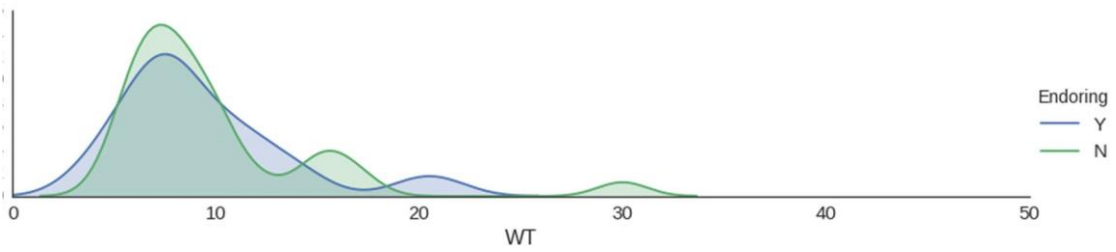


Fig 5. Withdrawal time: Probability plot for EndoRings™ arm.

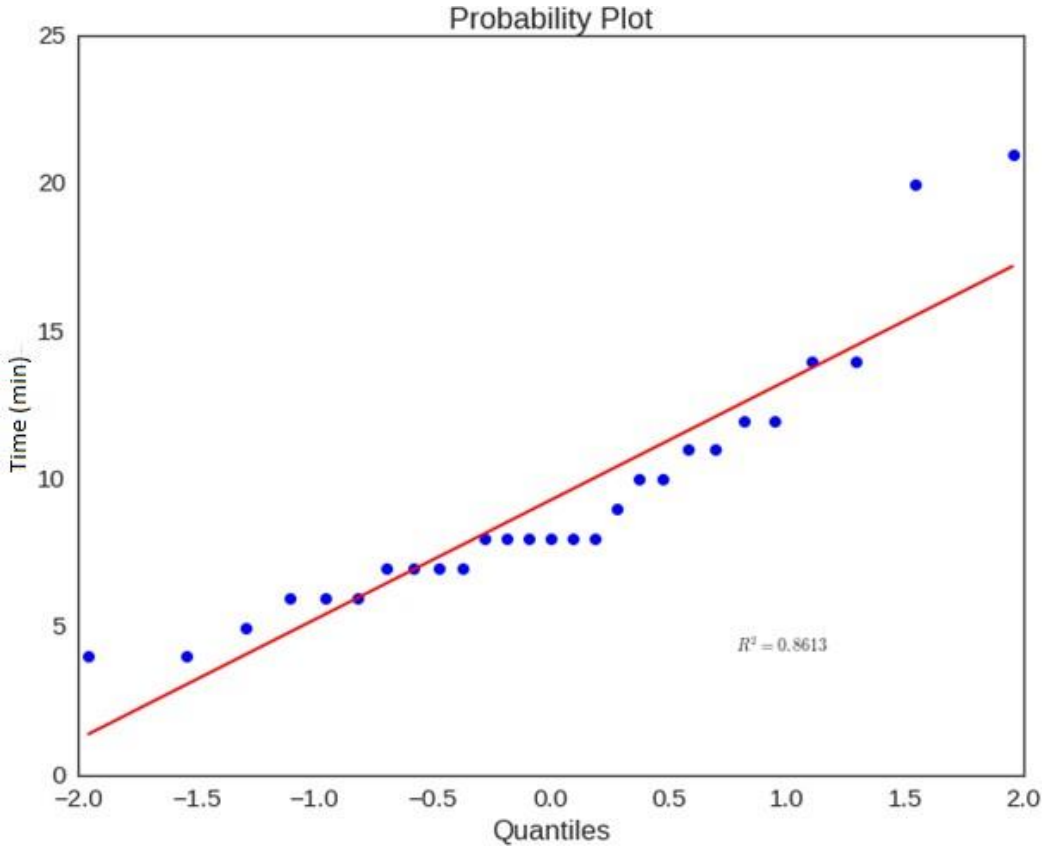
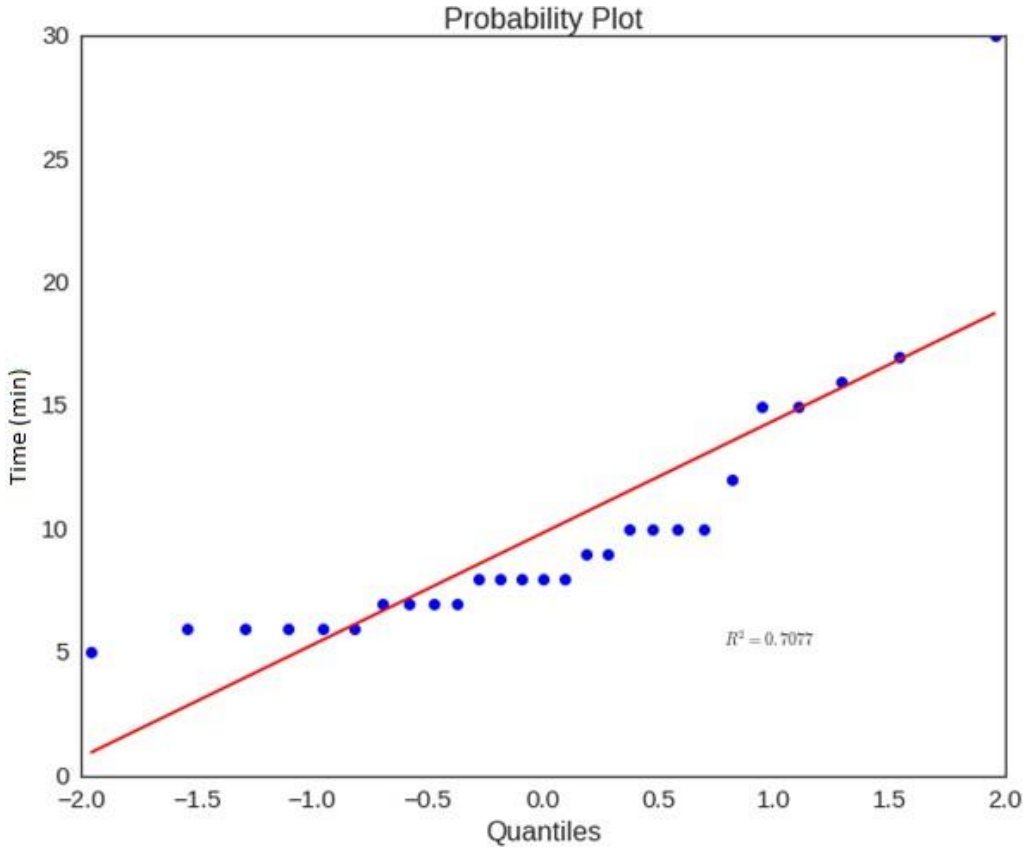


Fig 6. Withdrawal time: Probability plot for standard colonoscopy.



Appendix B: Ethics approval:

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: nosi.tsama@uct.ac
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 July 2015

HREC REF: 536/2015

Prof P Goldberg
General Surgery
Colorectal Unit
Old Main Building

Dear Prof Goldberg

PROJECT TITLE: A RANDOMIZED STUDY OF ENDORINGS™ ASSISTED VS STANDARD COLONOSCOPY FOR SURVEILLANCE OF AT RISK INDIVIDUALS WITH LYNCH SYNDROME (MMed-candidate- Dr R Dhar)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th July 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr R Dhar will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

Appendix C: Consent form (English):

Consent Form to participate in medical research

A randomized study of Endorings™ assisted vs standard colonoscopy for surveillance of at-risk individuals with Lynch Syndrome

Dr. R Dhar
Supervisor
Prof. PA Goldberg
Department of Colorectal Surgery
Groote Schuur Hospital

Contact for research: Dr. R Dhar (Tel: +27 848866894; e-mail: rohindhar83@gmail.com)
Contact for UCT Human Research Ethics Committee (Tel: +27 21 406 6346)

I,_____hereby agree to participate in the research project evaluating the use of Endorings™-assisted vs. Standard colonoscopy for detection of polyps in at risk individuals with Lynch Syndrome. The study will take place during the first week of September 2015, and I will be required to undergo one colonoscopy. The risks and benefits have been explained to me by Dr. R Dhar and Sr. Ursula Algar which I understand and have been given the opportunity to ask questions.

I understand that my participation in this study is entirely voluntary.

I understand there will not be any financial compensation involved for participation in this research.

I agree to the use of my medical records which might include a physical examination. This will remain confidential but may be used for presentations and articles (on an anonymous basis).

_____ _____
Patient Doctor

_____ _____
Witness Translator

_____
Date

Consent form: Afrikaans.

Toestemming tot deelname aan mediese navorsing

Vergelyking tussen Endorings™-geassisteerde kolonoskopie en konvensionele kolonoskopie in Lynch Sindroom pasiënte

Dr. R Dhar
Toesighouer
Prof. PA Goldberg
Departement van Kolorektale Chirurgie
Groote Schuur Hospitaal

Kontak persoon: Dr. R Dhar (Tel: +27 848866894; e-pos: rohindhar83@gmail.com)
Kontak persoon, Menslike Novorsings Etiek Komitee (Tel: +27 21 406 6346)

Ek,_____gee hiermee toestemming tot deelname aan hierdie studie wat die gebruik van Endorings™ geassisteerde kolonoskopie met konvensionele kolonoskopie vergelyk. Die doel van beide Endorings™ geassisteerde- en konvensionele kolonoskopie is die identifikasie van kolon poliepe in individue met Lynch Sindroom. Hierdie studie vind plaas gedurende die eerste week van September 2015 en sluit een kolonoskopie ondersoek in. Die voordele asook die risiko's verbonde aan hieride studie is deur Dr. R Dhar en Sr. Ursula Algar aan my verduidelik. Ek verstaan die risiko's en voordele en was die geleentheid gebied om vrae te stel.

Ek besef dat my deelname aan hierdie studie vrywillig is en dat daar geen finansiële kompensasie betrokke is nie.

Ek stem saam met die gebruik van my mediese rekords wat kan insluit 'n fisiese ondersoek. Alle inligting sal as konfidensieel geag word. Dit kan ook in voordragte en artikels gebruik word, steeds op n anonieme basis.

_____ _____
Pasiënt Dokter

_____ _____
Getuie Vertaler

_____
Datum

Appendix D: Data sheet:

Data collection sheet Number:

Endorings™ Study

Field 1

Patient data:

Age_____

Gender: O Female O Male

Previous operations of the abdomen: _____

First colonoscopy in his/her life: O Yes O No

Field 2

O use of Endorings™

O without Endorings™

Field 3

Bowel preparation:

O Excellent (>90% of mucosa seen, mostly liquid colonic contents, minimal suctioning needed for adequate visualization)

O Good (>90% of mucosa seen, mostly liquid colonic contents, significant suctioning needed for adequate visualization)

O Fair (>90% of mucosa seen, mixture of liquid and semisolid colonic contents, which could be suctioned and/or washed)

O Inadequate (<90% of mucosa seen, mixture of semisolid and solid colonic contents, which could not be suctioned or washed)

Field 4

O Complete colonoscopy

- O with terminal ileum intubation
- O without terminal ileum intubation

O Partial colonoscopy (until____cm from anus)

Field 5

O No polyps detected during procedure

O Polyps detected during procedure

Location/ size / number of polyps

Cecum

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Ascending colon

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Hepatic flexure (right)

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Transvers colon

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Splenic flexure (left)

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Descending colon

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Sigmoid colon

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Rectum

O Polyp Size <1cm, Number O Polyp size >1cm, Number _

Field 6

Caecum intubation time (min) _____

Procedure time (min): ____

Withdrawal time (min) (stopwatch is paused for procedures like: suction, biopsy, polypectomy): ___

Sedation necessity: Type (mg)____ Analgesia: Type (mg) _____

Field 7

Complications

O none

O yes O perforation, O mucosa-laceration, O bleeding, O loss of Endorings™

Appendix E: Power Calculation Scenarios

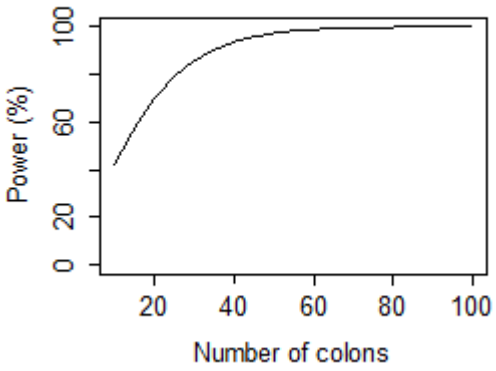
Power Analysis Summary

Objective	Perform a power calculation (sampling unit = colon) to compare the average number of polyps per colon in arms one and two. Inputs: • Arm 1 – 10 polyps in 50 colons, 15 -20 polyps in 50 colons (50% - 100% increase) • To get a sense of variability – about 90% of colons have 0 polyps, of the remaining 10%, about 80% have 1 polyp, 10% have 2…
Approach	Perform a power calculation based on a z-test • Data is highly non-normal, therefore rely on approximate normality of mean (large sample theory) • Based on inputs above, try to get a sense of reasonable inputs for variability The data is highly right-skewed (will have a large variance), so we cannot expect much power in a comparison of means.
Software	R (64 bit, version 3.1.3)
Report version	v1.0
Data file	NA
Data modifications	NA
Accompanying files	None

Six hypothetical contexts were considered

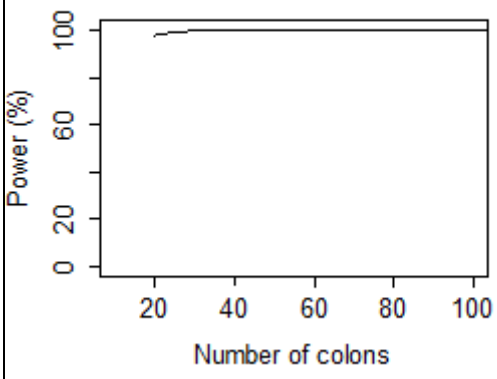
	Number of polyps per 50 colons		Coefficient of variation (ratio of standard deviation to mean) for number of polyps per colon
	Arm 1	Arm2	
1	10	15	50%
2	10	15	100%
3	10	15	200%
4	10	20	50%
5	10	20	100%
6	10	20	200%

Context 1



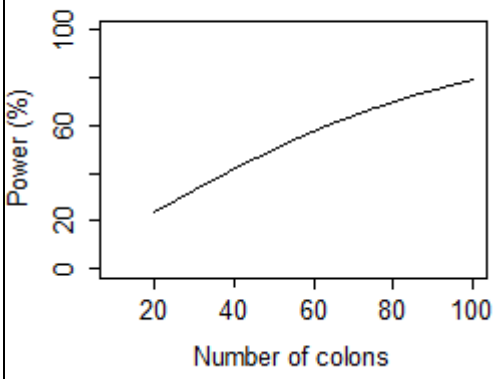
	Power	Sample size
1	50	<20
2	60	<20
3	70	20
4	80	26
5	90	34

Context 4



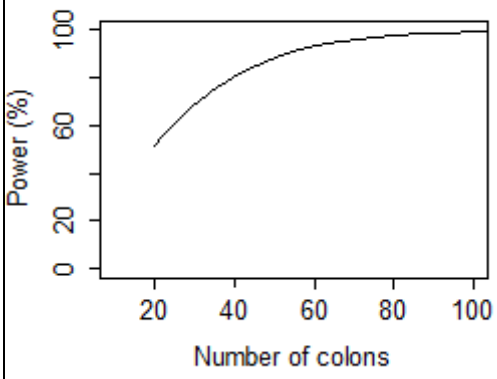
	Power	Sample size
1	50	<20
2	60	<20
3	70	<20
4	80	<20
5	90	<20

Context 2



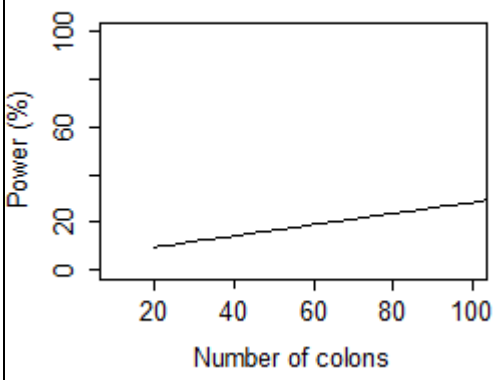
	Power	Sample size
1	50	50
2	60	64
3	70	80
4	80	102
5	90	137

Context 5



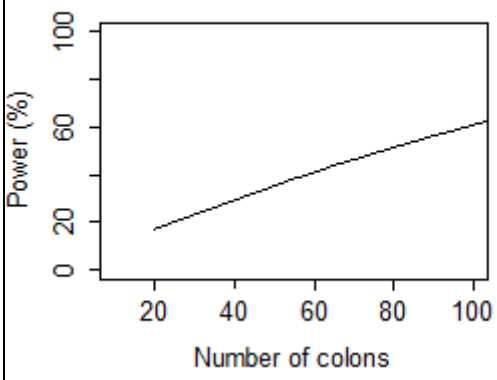
	Power	Sample size
1	50	<20
2	60	25
3	70	31
4	80	39
5	90	53

Context 3



	Power	Sample size
1	50	200
2	60	>200

Context 6



	Power	Sample size
1	50	77
2	60	98
3	70	123
4	80	157
5	90	>200

Appendix F: Instruction to Authors

International Journal of Colorectal Disease

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.

- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Figures

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.
- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.
- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.
- Figures should be submitted separately from the text, if possible.
- When preparing your figures, size figures to fit in the column width.

Informed consent

The following statement should be included:

Informed consent: “Informed consent was obtained from all individual participants included in the study.”

If identifying information about participants is available in the article, the following statement should be included:

“Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.”