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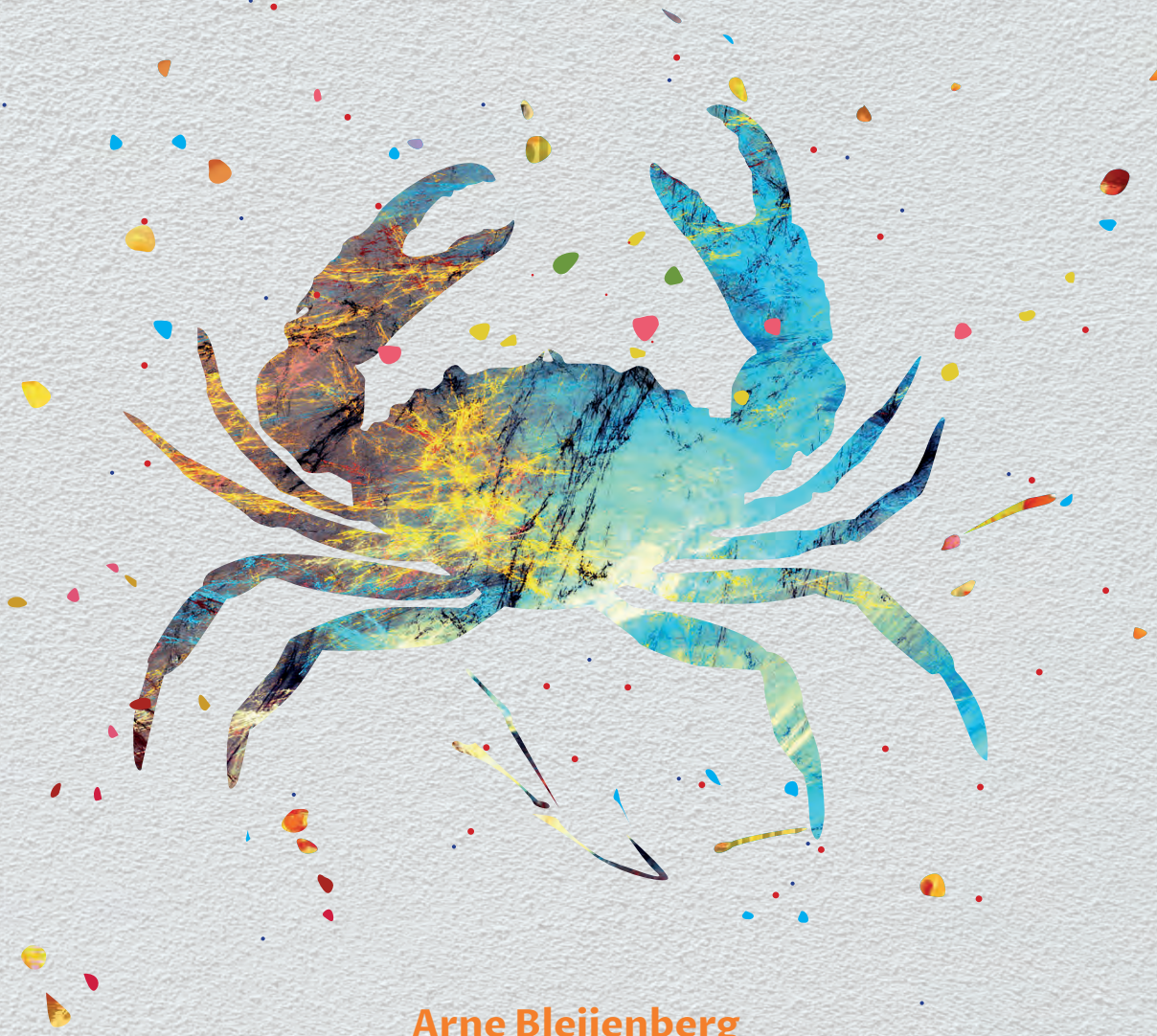
THE SERRATED NEOPLASIA PATHWAY TO COLORECTAL CANCER

Molecular biology and clinical management



Arne Bleijenberg

The serrated neoplasia pathway to colorectal cancer

Molecular biology and clinical management

Arne Bleijenberg

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The serrated neoplasia pathway to colorectal cancer
Molecular biology and clinical management

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op woensdag 28 Oktober 2020, te 11.00 uur

door

Arne Gerardus Cornelis Bleijenberg
geboren te Warnsveld

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1

General introduction and outline of this thesis

GENERAL INTRODUCTION

Colorectal cancer (CRC) is one of the most common types of cancer worldwide, and despite ongoing improvement in early diagnosis and treatment, it remains the second leading cause of cancer-related death in western countries.[1] This has made CRC prevention a top priority nationally and internationally, as is illustrated by the countless community screening programs that are being implemented throughout the world.[2, 3] CRC incidence can be effectively reduced by endoscopic removal of precursor lesions, more commonly known as polyps.

Serrated polyps

Because adenomas were long considered the only precursor lesion to CRC[4], endoscopists were until recently trained to detect and resect adenomas only. In contrast, serrated polyps, a different type of precursor lesion, have long been considered harmless, and as such they received little attention and were rarely removed during colonoscopy.

However, nowadays serrated polyps are held responsible for 15-30% of all CRCs. This has caused a paradigm shift in CRC prevention over the past years.[5-8] CRC prevention no longer consists of detection and resection of adenomas only. Instead, endoscopists are now also tasked to detect and resect serrated polyps.

Problematically however, this recent discovery of the malignant potential of serrated polyps means that our knowledge and awareness about these polyps grossly lags behind compared to our understanding of adenomatous polyps. This is not only true from a clinical perspective, but also for our understanding of the molecular alterations in the so-called serrated neoplasia pathway.

Clinically, the detection of serrated polyps is suboptimal, and inferior to adenoma detection.[9, 10] Large inter-endoscopist variation in detection rates of serrated polyps suggests that serrated polyps are often missed during colonoscopy.[9, 10] This is likely a result of both the indistinct and subtle appearance of serrated polyps[11] as well as the lower awareness among endoscopists about their malignant potential. Of concern, this higher miss-rate actually results in CRC development, as is illustrated by the overrepresentation of the serrated neoplasia pathway in so-called postcolonoscopy CRCs (PCCRCs).[5, 12, 13] Such PCCRCs are CRCs arising *after* a colonoscopy but before the due-date of a surveillance procedure, and are believed to arise frequently from missed pre-malignant polyps.[14]

It is not only the clinical knowledge and awareness of serrated polyps that is lagging behind compared to adenomas. The molecular alterations in the progression from a serrated polyp to CRC are poorly described as well. Better understanding of these molecular events could guide identification of new (faecal) biomarkers for early detection of serrated polyps, and could potentially provide novel treatment targets for serrated-derived CRC. The latter is especially important considering that serrated polyps give rise to a group of CRC with the poorest prognosis of all CRC subtypes, namely *BRAF*-mutant, microsatellite stable (MSS) CRC.[15-19]

The studies in **PART I** of this thesis have aimed to increase both our clinical and molecular knowledge of serrated polyps, as well as our ability to educate endoscopists in detecting serrated polyps.

Serrated polyposis syndrome

One specific group of patients most clearly experiences the malignant potential of serrated polyps: those with serrated polyposis syndrome (SPS). If serrated polyps occur in unusually high numbers, patients might fulfil the diagnostic criteria for this syndrome (SPS).[20] SPS is associated with a high risk of CRC. If left untreated, CRC prevalence rates of 15-70% have been reported.[21-26] Although previously considered rare, recent studies have demonstrated that up to 1:111 average risk individuals undergoing colonoscopy after a positive faecal immunochemical test (FIT) might fulfil the diagnostic criteria of SPS, making it (by far) the most common polyposis syndrome currently known.[27, 28]

However, just like for sporadic serrated polyps, our understanding of SPS is limited and most international guidelines for treatment and surveillance rely on low-quality, retrospective evidence to support recommendations. In order to prevent CRC, SPS patients are currently put under very stringent surveillance regimens, such as annual colonoscopy.[29] This leads to substantial colonoscopy burden for both patients as well as their physicians, even though the benefit of such very intensive surveillance is questionable.

The studies in **PART II** of this thesis aimed to increase our understanding of SPS, and to decrease colonoscopy burden for SPS patients without increasing their CRC risk.

OUTLINE OF THIS THESIS

Part I – Serrated polyps

Chapter 2 describes a study in which we aimed to increase the awareness of serrated polyps and their malignant potential among endoscopists. We trained these endoscopists in face-to-face sessions, and compared their subsequent proximal serrated polyp detection rate (PSPDR) to a random group of untrained endoscopists.

In **Chapter 3** we focus on a different problem regarding management of serrated polyps. Because of the relative lack of supporting evidence, international guidelines give conflicting recommendations regarding the post-polypectomy surveillance after serrated polyp resection. Specifically, some guidelines advocate risk stratification based on serrated polyp subtype, while other guidelines recommend a one-size-fits all approach to all serrated polyp subtypes. In this **chapter 3** we therefore aimed to address differences between two widely used post-polypectomy surveillance guidelines: the guideline of the United States Multisociety Taskforce on CRC (US-MSTF), and the guideline of the European Society for Gastrointestinal Endoscopy (ESGE).

Lastly, in **Chapter 4** we have aimed to identify key molecular driver events in the malignant progression of serrated polyps with an early focus of dysplasia or invasive cancer. Using microdissection techniques and whole exome sequencing, we isolated

and sequenced both DNA from dysplastic/cancerous foci as well as from adjacent non-progressed serrated polyp tissue. The mutational landscapes of the progressed and non-progressed components were compared, which allowed us to identify several recurrent oncogenic mutations that might play a significant role in the earliest steps in malignant progression in the serrated neoplasia pathway.

Part II – Serrated polyposis syndrome

Part II of this thesis contains several chapters focusing on patients with an unusually high number of serrated polyps, as part of the serrated polyposis syndrome (SPS).

To prevent cancer development, SPS patients are advised to undergo life-long endoscopic surveillance. However, the optimal interval between surveillance colonoscopies is uncertain because of an almost complete lack of prospective cohort studies. Therefore, in **chapter 5** we have aimed to develop and prospectively assess a personalised surveillance protocol that could bring down colonoscopy burden without increasing a patient's risk of developing cancer. Because SPS manifests almost entirely in the colon, patients who previously underwent extensive colonic surgery might not be at increased risk of cancer anymore. Therefore, in **chapter 6** we describe the cancer risk of SPS patients with a history of (sub)total colectomy. We hypothesized that such patients could benefit from even less frequent endoscopic surveillance.

In **chapter 7** we describe the characteristics of a group of individuals that previously fulfilled the diagnostic SPS criteria, but at present do not fulfil these criteria anymore due to substantial changes in the diagnostic guideline in the summer of 2019.

Finally, in **chapter 8**, we have exploited the fact that the Amsterdam UMC has maintained a prospective database of SPS patients since 2007. Based on these long-term follow-up data, we were able to describe how this syndrome behaves during long-term endoscopic surveillance. Also, we describe the risks and benefits associated with such long-term surveillance.

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2

Substantial and sustained improvement of serrated polyp detection after a simple educational intervention – Results from a prospective controlled trial

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ABSTRACT

Objective

Serrated polyps (SPs) are an important cause of postcolonoscopy colorectal cancers (PCCRs), which is likely the result of suboptimal SP detection during colonoscopy. We assessed the long-term effect of a simple educational intervention focusing on optimising SP detection.

Design

An educational intervention, consisting of two 45-minute training sessions (held three years apart) on serrated polyp detection, was given to endoscopists from 9 Dutch hospitals. Hundred randomly selected and untrained endoscopists from other hospitals were selected as control group. Our primary outcome measure was the proximal SP detection rate (PSPDR) in trained vs. untrained endoscopists who participated in our FIT-based population screening program.

Results

Seventeen trained and 100 untrained endoscopists were included, who performed 11,305 and 51,039 colonoscopies, respectively. At baseline, PSPDR was equal between the groups (9.3% vs. 9.3%). After training, the PSPDR of trained endoscopists gradually increased to 15.6% in 2018. This was significantly higher than the PSPDR of untrained endoscopists, which remained stable around 10% (p=.018). All below-average (i.e., PSPDR ≤6%) endoscopists at baseline improved their PSPDR after training session one, as did 57% of endoscopists with average PSPDR (6-12%) at baseline. The second training session further improved the PSPDR in 44% of endoscopists with average PSPDR after the first training.

Conclusion

A simple educational intervention was associated with substantial long-term improvement of PSPDR in a prospective controlled trial within FIT-based population screening. Widespread implementation of such interventions might be an easy way to improve SP detection, which may ultimately result in fewer PCCRCs; clinicaltrials.gov,
trial-ID NCT03902899.

INTRODUCTION

Colorectal cancer (CRC) is a leading causes of cancer-related mortality world-wide[1, 2], and arises from premalignant polyps.[3] CRC incidence can be reduced dramatically by removal of these polyps.[4] Adenomatous polyps have long been considered the only precursor lesion for CRC, and therefore CRC prevention has traditionally focused on detection and removal of adenomas. However, recent evidence shows that up to one-third of CRCs arise through serrated polyps (SP).[5-7] This has caused a paradigm shift in CRC prevention; endoscopists should nowadays also detect and resect these SPs.[8, 9]

However, due to the relative recent discovery of their malignant potential, the clinical relevance and appearance of SPs might be less well-known among endoscopists. In addition, detection of SPs is more difficult due to their flat shape, pale colour and vague borders. Furthermore, mucus is often attached to the polyp, thereby hiding it from the endoscopist's eye.[9, 10] Two studies reported proximal SP detection rate (PSPDR) ranging from 1-2% in low-detecting endoscopists up to 18% in high-detecting endoscopists[11, 12], suggesting that low-detectors frequently miss SPs.

Poor detection therefore seems to be one of the main challenges in the current management of SPs. This is illustrated by the disproportionally high contribution of SPs to the development of postcolonoscopy CRC (PCCRC): both molecular characteristics as well as the preferential right-sided location of PCCRCs suggest that PCCRCs disproportionally often arise from missed or incompletely resected SPs.[13-16]

Analogous to the risk reduction of PCCRCs at increased adenoma detection rate (ADR)[17, 18], it therefore seems possible that an increase in SP detection will result in a decline in PCCRCs. Educational interventions aiming to improve adenoma detection have resulted in an increased ADR[19-21], but not in increased SP detection rates.[22] Hence, dedicated interventions specifically aiming to improve SP detection seem warranted. To our knowledge, the effect of such educational interventions has not been studied.

We performed a study to evaluate whether a simple and easily applicable educational intervention consisting of two 45-minute training sessions (approximately 3 years apart) on optimizing SP detection, could lead to a long-term increase in SP detection in a large prospective controlled trial nested within the Dutch FIT-based CRC screening program.

METHODS

Study design

In this prospective trial we assessed the effect of a simple educational intervention on the detection of SPs in the Dutch nationwide CRC screening program. All residents of the Netherlands aged 55 to 75 years old are invited 2-yearly to perform a faecal immunochemical test (FIT). All individuals with a positive test result are invited for follow up by colonoscopy. Each year, all residents from the same birth year are invited, thereby assuring comparable invitees throughout the country.

All endoscopists from 9 Dutch hospitals spread throughout the Netherlands, performing colonoscopies in FIT-positives in the Dutch national screening program, were invited to receive our educational intervention and were thus allocated to the intervention-arm. This intervention consisted of two 45-minute training sessions; one in 2014 and another one 3 years later (for details see below). The selection of these 9 centres was pragmatic, and mainly based on scattered location throughout the Netherlands. Hundred randomly selected endoscopists employed in other hospitals throughout the Netherlands were selected as controls.

Our study fell beyond the Dutch legislation regarding Medical Research Involving Human Subjects Act (WMO) since patients were not exposed to any intervention, and because their privacy was guaranteed by anonymization of the database. Formal ethical approval was therefore not deemed necessary by the institutional review board (IRB) of the Academic Medical Centre in Amsterdam. This study was in accordance with the declaration of Helsinki[23] as well as the General Data Protection Regulation (GDPR)[24], and was registered on the publicly accessible clinicaltrials.gov; trial-ID NCT03902899. Patients were not involved in the design and conduct of this study.

Inclusion criteria

Endoscopists were eligible for inclusion if they were accredited to perform colonoscopies within the Dutch FIT-based screening program. This meant all included endoscopists were subjected to strict quality monitoring and auditing throughout the study duration, which have been described in detail previously.[25] Briefly, among several other quality criteria, each endoscopist had to perform ≥200 colonoscopies per year, ≥50 polypectomies per year, and achieve a cecum intubation rate of ≥95%, withdrawal time of ≥6 minutes in ≥90% of colonoscopies, adenoma detection rate of ≥30%, removal of ≥90% of detected polyps and retrieval for pathologic examination of ≥90% of resected polyps in screening colonoscopies.[25] All screening colonoscopies performed by the included endoscopists were analysed to calculate our primary and secondary endpoints.

Exclusion criteria

Endoscopists allocated to the intervention arm who did not attend both training sessions were excluded for primary analysis. We performed a sensitivity analysis in which endoscopists that attended only one training session were included in the intervention group. Endoscopists who did not perform screening colonoscopies before the first training session were excluded, since no baseline detection rates of these endoscopists could be established.

Educational intervention

The educational intervention we implemented consisted of two training sessions. All endoscopists in the intervention arm received these two training sessions; one in 2014 and a second session in 2017 (2 hospitals received the latter in October and November 2016,

respectively). Training sessions were given by one of three research fellows specialized in serrated polyps (JIJ, AB and YH).

Training session one and two were almost identical. Both consisted of an oral presentation of about 45 minutes. The training sessions primarily focused on making endoscopists aware of the importance of diagnosing, detecting and resecting SPs. Statistics about the contribution of SPs to sporadic and postcolonoscopy CRC were presented, and typical features of the different SP subtypes (hyperplastic polyps (HPs), sessile serrated lesions (SSLs) and traditional serrated adenomas (TSAs)) were highlighted. Optical diagnosis of colorectal polyps using the WASP-classification was discussed.[26] The slides of these presentations are available as supplementary material.

Data collection – Dutch CRC screening program

Data from the national CRC screening program were used for our analyses. All colonoscopies in FIT-positives are routinely registered in this centralized database, which is coordinated by the governmental National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu (RIVM)). This database includes detailed information of every colonoscopy performed in the population screening program in the Netherlands, such as the endoscopist performing the endoscopy, cecal intubation, withdrawal time, polyp detection and resection. Histologic data was extracted from PALGA, the nationwide network and registry of histopathology and cytopathology in The Netherlands.[27]

For the purpose of this study we extracted from this database all colonoscopies that were performed between January 2014 and September 2018 by the included endoscopists.

All identifying variables were removed by employees of the RIVM before the data was made available to us in order to comply with privacy legislation of the European General Data Protection Regulation act (GDPR).[24] As such, no personal information about endoscopists (e.g. age, gender, years of experience) could be included in the provided data.

Outcome parameters

The primary outcome measure was the difference in proximal serrated polyp detection rate (PSPDR) in colonoscopies performed by trained vs. untrained endoscopists based on all colonoscopies performed by trained endoscopists after they received their first training, and all colonoscopies performed by the untrained endoscopists in the control arm. PSPDR was defined as the percentage of patients in whom at least one SP proximal to the descending colon was removed.[12] The difference was expressed as absolute percentage and as odds-ratio for the detection of a proximal SP by control group vs. the intervention group.

Secondary outcome measures included the detection rates of subtypes of SPs (i.e. HP, SSL, and TSA) and (advanced) adenomas.

Another secondary outcome measure was the effect of our intervention on individual endoscopists. For this purpose, baseline detection rates were measured using all

colonoscopies prior to the first training session in 2014. The endoscopists were divided into three groups based on their baseline PSPDR: below-average (PSPDR ≤6%), average (PSPDR 6-12%) and above-average (PSPDR ≥12%). The cut-off PSPDR values for these three groups were chosen arbitrarily based on baseline PSPDR. Baseline detection rates were compared with those after the first and the second training respectively, for each individual endoscopist. The effect was assessed by quantifying how many endoscopists improved, remained stable or declined in PSPDR over time, with the three baseline strata as benchmark (i.e. below-average, average and above-average).

Statistical analyses

Detection rates of trained and untrained endoscopists were compared using mixed effects logistic regression analysis. Training was analysed as a fixed effect into the model. To adjust for the fact that our data was clustered on endoscopist-level and for the fact that colonoscopies were performed in different types of centres (i.e. academic hospital, general hospital, private clinic), we added random intercepts in the model for the endoscopist and the type of centre.

Differences in colonoscopy and patient characteristics were assessed using Mann-Whitney U tests (nonparametric numerical variables) and chi-square tests (discrete variables). All analyses were performed using Statistical Package for Social Sciences (SPSS) version 24 (IBM, Somers, New York, USA) and RStudio[28] with *lme4* package[29], with the function of *glmer*. Figures were produced using GraphPad Prism (version 7.03, GraphPad Software, La Jolla California USA).

Since at the time of initiation of our study in 2014 too little evidence was available about baseline PSPDR in a FIT-based cohort, and because we were unable to make a reliable estimation about the number of colonoscopies per endoscopist, it was difficult to reliably produce the required parameters for sample size calculations. Instead, we therefore aimed to include as many endoscopists as possible in the intervention arm by selecting a relatively large number of hospitals for our intervention. The large number of control endoscopists was chosen pragmatically.

RESULTS

Baseline characteristics

A total of 34 endoscopists from 9 centres were potentially eligible for inclusion in the intervention arm. Eleven were excluded due to absence during one of the training sessions, while six were excluded because they did not yet perform colonoscopies for the population screening program at the start of the study and thus no baseline PSPDR could be measured. Thus, 17 endoscopists attended both training sessions and were included in the intervention arm. In addition, 100 randomly selected untrained endoscopists were included as controls (Table 1). Three trained endoscopists (18%) were employed in an academic hospital, 10 (59%) in a general hospital and 4 (23.5%) worked mainly in a private clinic. In the control group, 3 (3%) of the untrained endoscopists worked

in an academic centre, while 91 (91%) and 6 (6%) were employed by general and private clinics, respectively (Table 1).

Endoscopists in the intervention arm performed a total of 928 baseline colonoscopies (i.e., colonoscopies in 2014 prior to the first training session), while endoscopists in the control arm performed 6,069 baseline colonoscopies (i.e., colonoscopies in 2014; Table 1). Age and gender distribution of patients were similar between the intervention and control arm, but median inspection time of colonoscopy was longer (16 minutes vs. 13 minutes, p<.001) for colonoscopies performed by endoscopists in the intervention arm. Detection rates at baseline did not differ across all different polyp subtypes (Table 2).

After these baseline colonoscopies, trained and untrained endoscopists performed 10,377 and 44,967 post-training colonoscopies, respectively (Table 1). Age and gender distribution of patients were similar, but colonoscopies performed by trained endoscopists had a longer median withdrawal time (17 minutes vs. 13 minutes, p<.001).

Primary outcome measure: PSPDR before and after training

After receiving their first training, trained endoscopists showed an increase in PSPDR from 9.3% at baseline to 12.5%, 13.0%, 15.1% and 15.6% in 2015, 2016, 2017 and 2018 respectively (Table 3 & figure 1A). In contrast, no clear improvement was seen in untrained endoscopists (Figure 1A). When stratified per year of follow-up, the difference between trained and untrained endoscopists was non-significant in 2015 (p=.08) and 2016 (p=.14), but statistically significant in 2017 (p=.003) and 2018 (p=.04). The average PSPDR of trained endoscopists was 35% higher than the PSPDR of untrained endoscopists (13.8% vs. 10.2%, p=.018).

PSPDR was not associated with the type of centre in which endoscopists worked: compared to academic hospitals, SPs were as frequently detected in general hospitals (p=.48) and in private hospitals (p=.45).

When stratifying for SP subtypes, trained endoscopists were more likely to detect both SSLs (9.7% vs. 7.7%, OR 1.30 [95%CI 1.02-1.66], p=.034) as well as HPs (22% vs. 18%, OR 1.30 [95%CI 1.02-1.66], p=.035, Table 3).

Sensitivity analyses

Because of the overrepresentation of academic centres among the group of trained endoscopists, we compared the post-training PSPDR of trained vs. untrained endoscopists after exclusion of all colonoscopies performed in academic centres. This yielded similar results to our primary analyses, with an average post-training PSPDR for trained endoscopists of 14.3% vs. 10.0% for untrained endoscopists (p=.004).

Furthermore, we performed sensitivity analyses in which we included the subgroup of endoscopists (n=11) that participated in only one of the training sessions (supplementary table 1). Based on the 3,464 colonoscopies that these endoscopists performed after baseline, they showed a slight increase in PSPDR compared to baseline (10.6% at baseline

Table 1. Characteristics endoscopists and colonoscopies

Percentages ≥10% are rounded to the nearest integer. INCLUDED ENDOSCOPISTS (n=117)				
	Overall (n=117)	Intervention arm (n=17)	Control arm (n=100)	
Type of centre of endoscopist, n (%)				
Academic hospital	6 (5.1%)	3 (18%)	3 (3.0%)	
General hospital	101 (86%)	10 (59%)	91 (91%)	
Private clinic	10 (8.5%)	4 (23.5%)	6 (6.0%)	
Total colonoscopies per endoscopist, median (IQR)	498 (288-724)	640 (517 – 823)	445 (274-677)	
PRE-TRAINING COLONOSCOPIES* (n=6,997) Characteristics of scoped patients				
	Overall (n=6,997)	Intervention arm (n=928)	Control arm (n=6,069)	P-value
Age, mean (SD)	71 (SD 5.1)	72 (SD 4.7)	71 (SD 5.1)	<.001
Male gender, n (%)	4,119 (59%)	563 (61%)	3,556 (59%)	.24
Type of centre, n (%)				<.001
Academic hospital	940 (13%)	226 (24%)	714 (12%)	
General hospital	5,357 (77%)	574 (62%)	4,783 (79%)	
Private clinic	700 (10%)	128 (14%)	572 (9.4%)	
Quality parameters, n (%)				
Cecal intubation, n (%)	6,642 (95%)	882 (95%)	5,760 (95%)	1.00
Inspection time, median (IQR)	14 (9-21)	16 (10-26)	13 (9-20)	<.001
Inspection time ≥6 minutes	6,541 (94%)	912 (98%)	5,629 (93%)	<.001
BBPS ≥2 in all segments	6,727 (96%)	896 (96%)	5,831 (96%)	0.94

Table 1. (continued)

POST-TRAINING COLONOSCOPIES* (n=55,344) Characteristics of scoped patients				
	Overall (n=55,344)	Intervention arm (n=10,377)	Control arm (n=44,967)	P-value
Age, mean (SD)	66 (SD 5.3)	66 (SD 5.1)	66 (SD 5.4)	.005
Male gender, n (%)	32,587 (59%)	6,053 (58%)	26,534 (59%)	.21
Type of centre, n (%)				<.001
Academic hospital	3,516 (6.4%)	2,057 (20%)	1,459 (3.2%)	
General hospital	42,705 (79%)	5,993 (58%)	37,712 (84%)	
Private clinic	8,123 (15%)	2,327 (22%)	5,796 (13%)	
Year of colonoscopy, n (%)				
2014	616 (1.1%)	616 (5.9%)*	Not applicable*	
2015	13,107 (24%)	2,687 (26%)	10,420 (23%)	
2016	15,216 (27.5%)	2,921 (28%)	12,295 (27%)	
2017	16,437 (30%)	2,700 (26%)	13,737 (30.5%)	
2018	9,968 (18%)	1,453 (14%)	8,515 (19%)	<.001
Quality parameters, n (%)				
Cecal intubation, n (%)	53,844 (97%)	10,061 (97%)	43,783 (97%)	.02
Inspection time, median (IQR)	14 (10-21)	17 (12-26)	13 (9-20)	<.001
Inspection time ≥6 minutes	53,973 (97.5%)	10,276 (99%)	43,697 (97%)	<.001
BBPS ≥2 in all segments	52,896 (96%)	9,818 (95%)	43,078 (96%)	<.001

* Pre-training refers to baseline colonoscopies performed in 2014 prior to the first training session (trained endoscopists), or all colonoscopies in 2014 (untrained endoscopists). Post-training refers to all colonoscopies after the first training (trained endoscopists), or all colonoscopies from 2015 onwards (untrained endoscopists).

Abbreviations: IQR, interquartile range; SD, standard deviation; BBPS, Boston bowel preparation score

Table 2. Baseline detection rates based on 928 colonoscopies by trained endoscopists and 6,069 colonoscopies by untrained endoscopists* Percentages ≥10% are rounded to the nearest integer.

	Endoscopists in intervention arm (n=17)	Endoscopists in control arm (n=100)	P**
≥1 Proximal SP (=PSPDR), % (95%CI)	9.3% (7.4-11%)	9.3% (8.6-10%)	.48
≥1 SP	23% (20-26%)	24% (23-25%)	.87
≥1 HP	20% (17-22%)	20% (19-21%)	.61
≥1 SSL	5.9% (4.4-7.4%)	5.4% (4.9-6.0%)	.72
≥1 TSA	1.1% (0.4-1.7%)	1.4% (1.1-1.8%)	.49
≥1 Adenoma (=ADR), % (95%CI)	66% (63-70%)	66% (65-68%)	.99
≥1 Advanced adenoma	41% (38-44%)	45% (44-47%)	.13

* Based on colonoscopies in 2014 prior to the first training session (trained endoscopists), or *all* colonoscopies in 2014 (untrained endoscopists)

** P-values are based on mixed effects logistic regression analyses, with fixed effects for training and adjusted with random intercepts for type of centre and endoscopist

Abbreviations: SP, serrated polyp; PSPDR, proximal serrated polyp detection rate; CI, confidence interval; HP, hyperplastic polyp; SSL, sessile serrated lesion; TSA, traditional serrated adenoma; ADR, adenoma detection rate

vs. 12.8%), but this was not significantly different compared to untrained endoscopists (OR 1.37, 95%CI 0.91-2.06, p=.13).

Effect of first and second training session on trained endoscopists

Based on baseline detection rates, we arbitrarily defined cut-off values to stratify endoscopists into below-average PSPDR (≤6%), average PSPDR (6-12%) and above-average PSPDR (≥12%) (Figure 2&3).

Endoscopists with below-average PSPDR at baseline (n=5)

All five endoscopists with below-average PSPDR at baseline improved after the first training session. After the second training session, one endoscopist showed additional improvement and fell in the range of above-average, while one fell back to the below-average range after training session two. The other three remained in the range of average PSPDR after training session two.

Endoscopists with average PSPDR at baseline (n=7)

Of the seven endoscopists with average PSPDR at baseline, four improved to above-average PSPDR after training session one. The second training session resulted in improvement of the remaining three, who also moved to above average PSPDR. In other words, all endoscopists with average baseline PSPDR had above-average PSPDR after the second training session.

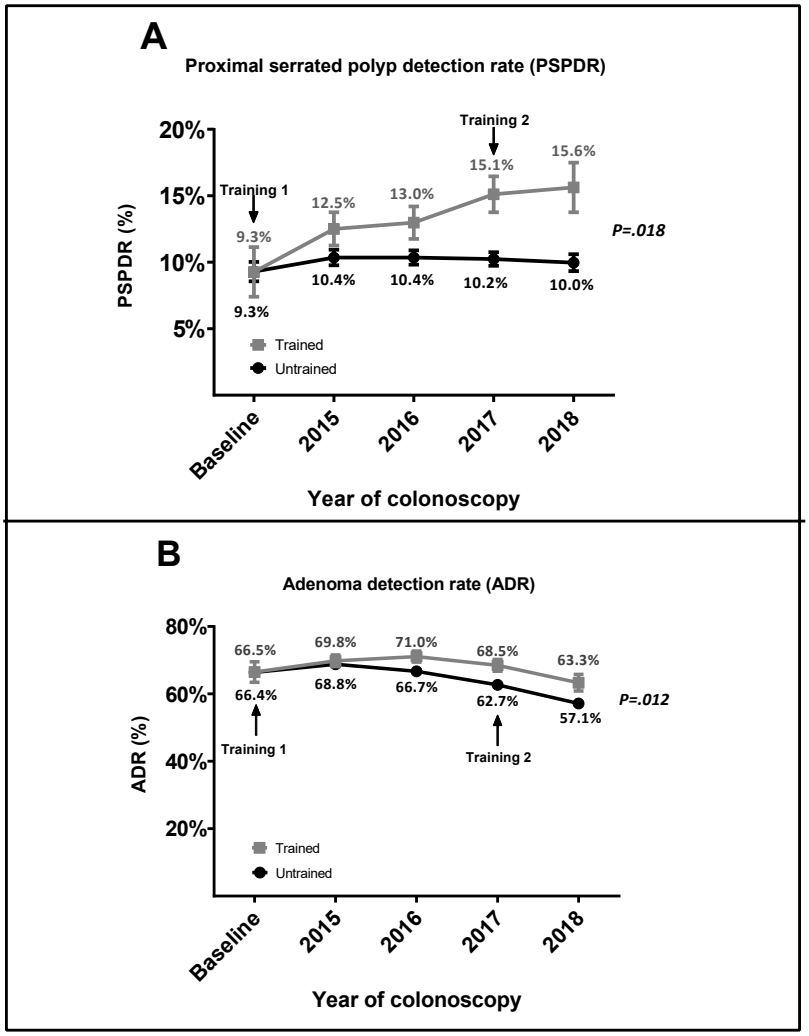

Figure 1. A: PSPDR before and after training; B: ADR before and after training. *Abbreviations: PSPDR, proximal serrated polyp detection rate. ADR, adenoma detection rate.*

Endoscopists with above-average PSPDR at baseline (n=5)

Of the five endoscopists with an above-average PSPDR at baseline, four remained in the above-average range after training session one, while one fell back to average PSPDR. After training session two, one additional endoscopists fell back to average PSPDR. In other words, three of the five endoscopists with above-average PSPDR at baseline also had above-average PSPDR after the second training session, while two fell back to average PSPDR.

Table 3. Post-training detection rates based on colonoscopies performed by trained vs. untrained endoscopists. Percentages ≥10% are rounded to the nearest integer.

	2015		2016		2017
	Trained endoscopists (n=2,687)	By untrained endoscopists (n=10,420)	Trained endoscopists (n=2,921)	Untrained endoscopists (n=12,295)	Trained endoscopists (n=2,700)
≥1 Proximal SP (=PSPDR), %	12.5%	10%	13%	10%	15%
≥1 SP	28%	25%	28%	25%	27%
≥1 HP	23%	20%	21.5%	19%	20%
≥1 SSL	8.9%	7.5%	10%	7.8%	11%
≥1 TSA	0.7%	1.0%	0.9%	1.0%	0.7%
≥1 Adenoma (=ADR), %	70%	69%	71%	67%	68.5%
≥1 Advanced adenoma	49%	50%	47%	44%	41%

* P-values are based on mixed effects logistic regression analyses, with fixed effects for training and adjusted with random intercepts

Abbreviations: SP, serrated polyp; PSPDR, proximal serrated polyp detection rate; HP, hyperplastic polyp; SSL, sessile serrated lesion

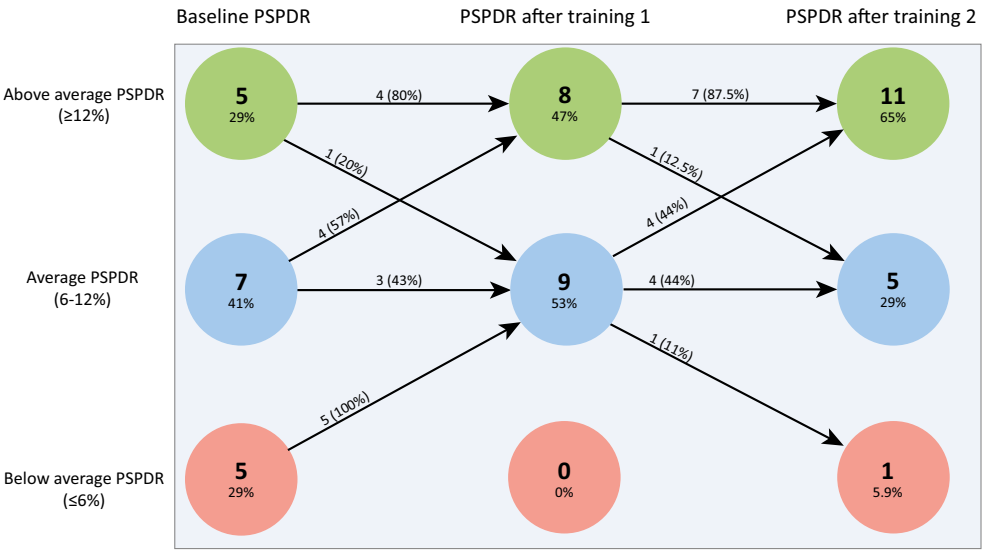

Figure 2. Effect of training sessions 1&2 per endoscopist, stratified according to their PSPDR at baseline, after the first training and after the second training. For example, of the 7 endoscopists with average PSPDR at baseline, 4 (57%) moved up to 'above average PSPDR' after training session 1, while 3 remained in the 'average PSPDR' group. *Abbreviations: PSPDR, proximal serrated polyp detection rate.*

	2018		Overall post-training			
Untrained endoscopists (n=13,737)	Trained endoscopists (n=1,453)	Untrained endoscopists (n=8,515)	Trained endoscopists (n=10,377)	Untrained endoscopists (n=44,967)	OR (95%CI)	P*
10%	16%	10%	14%	10%	1.49 (1.07-2.07)	**.018**
23%	2.7%	21%	28%	24%	1.27 (1.04-2.55)	**.021**
17%	21.5%	16%	22%	18%	1.30 (1.02-1.65)	**.035**
7.9%	9.3%	7.4%	9.7%	7.7%	1.30 (1.02-1.66)	**.034**
0.9%	0.6%	0.6%	0.7%	0.9%	0.89 (0.62-1.30)	.58
63%	63%	57%	68.5%	64%	1.20 (1.04-1.38)	**.012**
39%	37%	36%	45%	42%	1.08 (0.95-1.22)	.23

s for type of centre and endoscopist

n; TSA, traditional serrated adenoma; ADR, adenoma detection rate

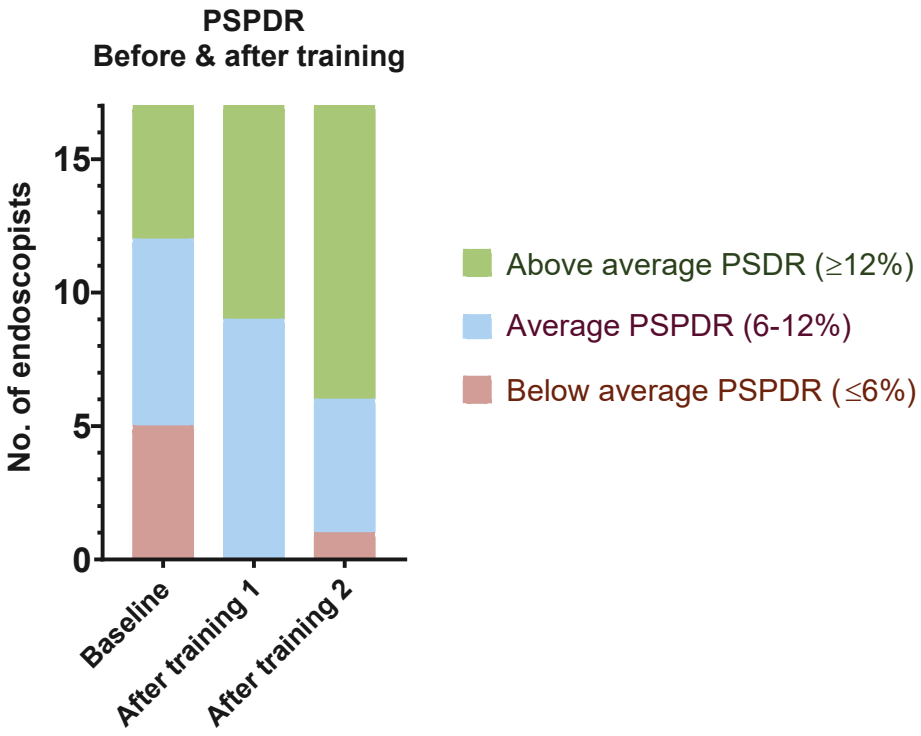

Figure 3. PSPDR before & after training. *Abbreviations: PSPDR, proximal serrated polyp detection rate.*

Secondary outcome measure: ADR before and after training

The ADR of trained and untrained endoscopists did not differ at baseline (66.5% vs. 66.4%, respectively). Overall, trained endoscopist had an average post-training ADR of 68.5%, while untrained endoscopists had an average ADR of 64.1%, corresponding to a relative difference of 6.9%, and an OR of 1.2 (95%CI 1.04-1.38, p=.012) for the detection of one or more adenomas (Table 3 & Figure 1B). During follow-up, the ADR of both trained and untrained endoscopists initially increased before decreasing in 2017 and 2018 to below baseline detection rates: the ADR of trained endoscopists slightly increased to 69.8% and 71.0% in 2015 and 2016, and then dropped to 68.5% and 63.3% in 2017 and 2018, respectively. Comparably, the ADR of untrained endoscopists slightly increased to 68.8% in 2015, before falling to 66.7%, 62.7% and 57.1% in 2016, 2017 and 2018, respectively (Table 3 & Figure 1B).

DISCUSSION

In this large prospective trial, we demonstrate that a simple educational intervention was associated with substantial and sustained increase in PSPDR during colonoscopy within a homogeneous FIT-positive screening population. Considering that these lesions are held responsible for a disproportionately high number of interval carcinomas[8, 16, 30, 31], our intervention might ultimately lead to a significant reduction in PCCRCs within FIT-based screening-programs. In addition, we demonstrate that the most prominent effect was achieved in endoscopists that had a below-average PSPDR at baseline (Figure 2), suggesting this intervention should be mainly targeted at the low-performers.

Interpreting our findings, lack of *awareness* about SPs might be one of the most important contributors to the highly variable (and often suboptimal) SP detection as described in previous studies.[11, 12] After all, the content of our training sessions mostly focused on *why* SPs should be detected and resected, rather than how this should be done. This might be due to the fact that the contribution of SPs to CRC has only gradually come into focus over the past two decades[5, 9, 32-35]. Many endoscopists were trained in an era in which adenomas were considered the sole precursors to CRC, or were trained *by* endoscopists that were trained in this pre-SP era. Indeed, a recent large multi-centre study by Crockett and colleagues demonstrated that endoscopists with fewer years of practice detected significantly more SPs than their older colleagues.[36]

Apart from the increased SP detection, we saw an interesting time-trend in the ADR: after initially increasing, both groups showed a decreasing ADR in 2017 and 2018 (Figure 1B). This trend was in line with an overall slow decrease in ADR in our national screening program. This can be attributed to three factors related to the implementation of the Dutch biennial FIT-based screening program from 2014 onwards: an increase of the iFOBT cut-off from 88ng/mL to 275ng/mL in 2014. First, the initial increase in ADR was most likely a result of the increase of the cut-off of the iFOBT from 88ng/mL to 275ng/mL [37] The subsequent decrease in ADR can be attributed to the decreasing age

of invitees from 2015 onwards, which was in turn a result of the phased invitation of screenees from 2014-2019 (supplementary figure 1). Lastly, the screening program consists of biennial FIT. As a result, the proportion of participants in their 2nd or 3rd test-round grew from 0% in 2014 and 2015, to 30% in 2016 and 53% in 2017. [37-40] In line with literature[41-44], the 2nd/3rd-round screenees had a lower incidence of CRC, advanced adenomas and non-advanced adenomas, which reflects the lower positive-predictive value of FIT after multiple test-rounds. Regardless of this overall trend, endoscopists that received our SP detection training seemed to have a slightly higher ADR than endoscopists that did not receive our training (p=.012). Albeit statistically significant, the clinical relevance of this minor difference in ADR is questionable. The reason for this difference in ADR between intervention and control group is unclear, but could potentially relate to the longer withdrawal time in the intervention group. Alternatively, more thorough mucosal inspection in search of SPs in the intervention group might have also resulted in higher detection of adenomas.

To interpret our results, it should be questioned whether these background developments in the population screening program might have also affected our study outcomes. This does not seem to be the case because these developments have equally affected the intervention and control arm of our study.

Our study has several strengths. First of all, this is the first study focusing on educational interventions to improve SP detection. Such targeted interventions are needed, as Racho and colleagues showed that training focused on improving ADR detection had no effect whatsoever on SP detection, despite its long-lasting beneficial effect on ADR.[22] Second, we had the opportunity to perform our study within the Dutch FIT-based screening program. This allowed us to include a large number of endoscopists and colonoscopies. Our cohort of 17 intervention and 100 control endoscopists can be considered large compared to for example the well-known EQUIP-trial which assessed the effectivity of a training which aimed to improve ADR, in which 8 and 7 endoscopists were randomized into the intervention and control arm, respectively.[19] Equally important, nesting our study in the Dutch screening program provided us with a prospectively collected database of a homogeneous group of patients with high-quality colonoscopies performed by accredited endoscopists. Quality indicators (i.e. bowel preparation, cecum intubation and inspection time) were each met in over 95% of colonoscopies (Table 1). Third, we included almost five years of prospective follow-up data, which allowed us to assess the long-term impact of our educational intervention. Last, the histopathologic assessment, especially the SP sub classification, can be considered high-quality as well since all pathologists received online training in order to optimize correct SP diagnosis. This online training module was shown to be very effective.[45]

Nevertheless, several alternative explanations for the improved PSPDR have to be considered as well. First of all, trained endoscopists were aware of the fact that they participated in this study, while the untrained endoscopists were not. As such, the Hawthorne effect, which describes an individual's change in behaviour due to awareness of being observed[46], might have positively influenced the PSPDR of trained,

but not of untrained endoscopists. However, we believe this effect has not played a major role in our study. First, the awareness of being monitored among trained endoscopists was kept to an absolute minimum in order to minimize interference of the Hawthorne effect. The background monitoring of PSPDR was only communicated verbally during both training sessions. Aside from these brief occasions in 2014 and 2017, no communication existed between the researchers and the endoscopists, and the endoscopists were not reminded in any other way about our ongoing study. It therefore seems likely that our ongoing study was not on top of the mind of participating endoscopists during their daily work in the 5 years of follow-up. Moreover, *all* Dutch endoscopists are aware that they are being monitored as part of standard care, since they continuously have to meet several minimal quality requirements (e.g., ADR ≥30%).[25] Since endoscopists face penalties when they fail to reach such quality parameters (e.g. withdrawal of accreditation), our monitoring was probably perceived as far less urgent and thus received less attention from endoscopists than the ongoing quality assurance parameters they were confronted with in daily practice. Nevertheless, it seems advisable that future similar studies make the control arm aware of the fact that they are monitored too, in order to reduce any potential influence of the Hawthorne effect.

Furthermore, our study was not randomized, and therefore we cannot rule out some degree of selection bias. Baseline differences between the trained and untrained endoscopists might have played a role in the increased detection rates we observed in the trained group. First of all, trained endoscopists more often operated in academic centres (12% vs. 24%, p<.001). However, we accounted for this by including type of centre in our multivariable model. In addition, our regression analyses did not show any association between type of centre and PSPDR, neither in trained or untrained endoscopists. Moreover, we ran separate sensitivity analyses in which we excluded colonoscopies in academic centres, which yielded similar results. A second baseline difference concerns the mucosal inspection time (16 minutes vs. 13 minutes, p<.001), suggesting trained endoscopists already took slightly more time for mucosal inspection than untrained endoscopists before their first training. However, although a prolonged withdrawal time has been linked to superior ADR[47-51], both groups exceeded withdrawal times of 10 minutes. A study by Lee and colleagues demonstrated that the additional benefit of withdrawal times beyond 10 minutes is questionable, and minimal at best.[51] We therefore consider it unlikely that this baseline difference represents a clinically relevant bias.

We cannot rule out that these or other baseline differences might have influenced our results. Most importantly, however, our baseline comparison of detection rates shows that across all polyp subtypes, detection rates of trained and untrained endoscopists were virtually identical (Table 2). This strongly suggests that trained and untrained endoscopists were indeed 'equal' at baseline.

Several other limitations have to be acknowledged as well. We chose to include endoscopists that attended both training sessions only, which meant several had to be excluded due to absence during either training session. Since attendance was voluntary,

this might have led to selection of more devoted or motivated endoscopists. Considering that the effect of our training strongly relies on endoscopists' motivation to pay extra attention to serrated lesions, this selection might have caused an overestimation of our outcome parameters. However, this potential selection bias seems less plausible taking into account our sensitivity analysis, in which endoscopists that only participated in one of the two training sessions also showed an increase in PSPDR from 10.6% to 12.8%, although this was not significantly different from the untrained group (p=.13), possibly due to the small subgroup sample size (3,464 colonoscopies in 11 endoscopists).

Another limitation was the scarcity of endoscopist characteristics we could include in our analysis. It would have been very informative to assess whether characteristics like age, years of experience or gender might predict the effect of our intervention. Unfortunately such parameters could not be provided by the RIVM because their database did not contain this information. Finally, our secondary analyses to assess which endoscopists benefitted most from our interventions required us to split up our intervention group in three subgroups. The resulting subgroups are quite small (i.e. 5, 7 and 5 endoscopists in the below average, average and above average groups, respectively). These subgroup analyses should therefore be interpreted with caution and warrant validation in future larger studies.

Our results would ideally be interpreted in the light of established, evidence-based targets for PSPDR to optimize PCCRC prevention, similar to the studies that were used to guide ADR targets. For example, Kaminski and colleagues demonstrated that an ADR of at least 20% was associated with a much lower risk of PCCRC[17], while Corley and colleagues later demonstrated that every 1% increase in ADR was associated with a 3% decrease in PCCRC incidence.[18] Such data has been invaluable in determining clinically meaningful ADR targets. Unfortunately, no such data has been published on SP detection rates, and therefore it is much more difficult to determine clinically meaningful PSPDR targets at this moment. We do know, however, that PCCRCs disproportionately often bear typical molecular and clinical characteristics of the serrated pathway, and therefore it is generally believed that missing SPs is an important factor in PCCRC development.[5, 8, 16, 52] This suggests that improvement of SP detection would result in a decline in PCCRC incidence. Indeed, recently the British Society for Gastroenterology even suggested that improvement of SP detection might have a greater impact on PCCRC incidence than improvement of ADR.[8] In this light, and in the absence of established thresholds, it seems safe to assume that any improvement in PSPDR might be clinically relevant. Future studies will hopefully help to establish evidence-based PSPDR thresholds to further guide the implementation and targeting of quality improvement interventions such as ours.

In conclusion, our results show that improvement of SP detection can be achieved relatively easily by a simple educational intervention, which consists of two 45-minute training sessions which are held 3 years apart. Giving SPs a more prominent place in current endoscopy programs could potentially lead to improved CRC prevention. Endoscopists with a low PSPDR at baseline benefitted most from our intervention, which

supports introduction of continuous PSPDR monitoring in order to identify and target low-performers and provide additional training to them.

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3

Implications of different surveillance guidelines after serrated polyp resection in United States of America and Europe

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ABSTRACT

Introduction

Because individuals with serrated polyps (SP) and adenomas are at increased risk of developing new polyps and colorectal cancer (CRC), surveillance after resection is justified. After adenoma resection most international guidelines are consistent but recommendations for surveillance after SP resection vary. The United States Multi-Society Taskforce on CRC (US-MSTF) base surveillance intervals on SP subtype (traditional serrated adenoma, sessile serrated polyp, hyperplastic polyps), while the European Society on Gastrointestinal Endoscopy (ESGE) guidelines do not take SP subtype into account. We evaluated the implications of this difference in a primary colonoscopy screening cohort.

Methods

We included participants from a large colonoscopy screening trial. In a post-hoc simulation assuming full protocol adherence, we determined the surveillance interval for each subject based on their polyp burden, using the most recent US-MSTF and the ESGE guidelines.

Results

We included 5,323 participants, of whom 1,228 had one or more SP. In 5,202 participants (98%, Cohen's Kappa 0.90) and in 1,106 of those with SP (90%, Cohen's Kappa 0.80), both guidelines recommended identical surveillance intervals. Recommendations for a 3-year surveillance interval were identical between the two guidelines. All 122 subjects with discordant recommendations would receive a follow-up colonoscopy after 10 years based on ESGE and after 5 years based on US-MSTF.

Conclusion

Despite their different criteria used to determine surveillance after SP resection, most individuals are recommended identical colonoscopy surveillance intervals following the ESGE or US-MSTF guideline. This suggests that surveillance recommendations do not need to consider the serrated polyp subtype.

INTRODUCTION

Serrated polyps (SP) are estimated to be the precursor of 15-30% of colorectal cancers (CRC).[1],{Jass, 2007 #56}[2] Among the three subtypes of SP - traditional serrated adenomas (TSAs), hyperplastic polyps (HPs) and sessile serrated lesions (SSLs)[3] - only TSAs and SSLs are thought to be precursors of CRC.[4, 5] Because TSAs are rare, SSL are probably the predominant serrated precursor to CRC.[4, 6] Some observational studies found an increased risk of metachronous CRC and advanced adenomas (polyps ≥10mm, with villous histology or high-grade dysplasia) in individuals with SSL or TSA, especially when SPs occurred together with adenomas.[7-12] In a large case-control study, the odds ratios of being diagnosed with CRC within 10 years were 3.07 and 4.84 after SSL and TSA resection, respectively, compared to individuals without polyps.[7] In other studies individuals with SSLs had a similar risk of metachronous advanced neoplasia compared to individuals with non-advanced adenomas.[8, 9] In two studies the risk of developing metachronous polyps or CRC in individuals with only HPs has hardly been studied. In a prospective chemoprevention trial, the presence of HPs at baseline was not associated with the risk of developing metachronous adenomas.[13] Another retrospective study did not show an increased risk of metachronous CRC after HP removal.[7] Hence, available (albeit limited) evidence suggests a higher risk of adenomas or CRC in individuals with SSLs and TSAs compared to individuals with only HPs at baseline. These results imply that some SP subtypes (i.e. SSLs and TSAs) might require more stringent surveillance than other SP subtypes (i.e. HPs).

Histopathologic subtyping of SPs into SSLs, HPs and TSAs is, however, difficult, as reflected by high inter-rater variation between pathologists. While TSAs might be less often confused with SSLs or HPs due to their characteristic eosinophilic cytoplasm and the presence of ectopic crypt foci [14], SSLs are often classified as HPs and vice versa.[15-18] Hence, SP sub-classification does not seem reliable in clinical practice. As a result, establishing and utilizing surveillance recommendations based on SP subtype is complicated. Nevertheless, the United States Multi-Society Task Force on CRC (US-MSTF) guideline recommends different surveillance intervals for subjects with SSLs, TSAs and HPs[19]. In contrast, the European Society on Gastrointestinal Endoscopy (ESGE) post-polypectomy surveillance guideline is not based on SP subtype, but only on polyp size and presence of dysplasia (table 1).[20] To this end, we evaluated if a surveillance strategy based on histopathologic subtypes of SPs entails different recommendations than a strategy without taking SP subtypes into account.

METHODS

Study design

This is a post-hoc analysis of prospectively collected data from the Norwegian part of the Nordic-European Initiative on Colorectal Cancer (NordICC), a large colonoscopy screening trial. We utilized the Norwegian NordICC database to simulate surveillance

Table 1. US-MSTF and ESGE post-polypectomy surveillance guideline

Surveillance interval	US-MSTF guideline*	ESGE Guideline
3 years	· ≥3 tubular adenomas · ≥1 tubular adenoma ≥10mm · ≥1 (tubulo)villous adenoma · ≥1 adenoma with HGD · ≥1 SSL ≥10mm · ≥1 HP ≥10mm* · ≥1 SSL with dysplasia · ≥1 TSA	· ≥3 tubular adenomas · ≥1 tubular adenoma ≥10mm · ≥1 (tubulo)villous adenoma · ≥1 adenoma with HGD · ≥1 SP ≥ 10mm · ≥1 SP with dysplasia
5 years	· ≥1 SSL <10mm w/o dysplasia	
10 years (c.q. return to regular screening)	· No polyps · 1 or 2 small (<10mm) tubular adenomas† · Only HPs <1cm*	· No polyps · 1-2 small (<10mm) · tubular adenomas · SP <10mm

* The recommendation for large HPs is ambiguous in the US-MSTF guideline. The decision to consider HP≥10mm as high-risk, and HP <10mm as low-risk was therefore based on previous literature and expert opinion. See supplementary table 1 for alternative management of large HP in sensitivity analyses.

† The US-MSTF guideline recommends surveillance after 5-10 years after resection of 1 or 2 non-advanced adenomas. For the purpose of the current study, we assigned subjects with 1-2 non-advanced adenomas a 10 year surveillance interval.

US-MST, United states multi-society taskforce on colorectal cancer; ESGE, European Society of Gastrointestinal Endoscopy; SSL, Sessile Serrated Lesion; HP, Hyperplastic Polyp; TSA, Traditional Serrated Adenoma; SP, Serrated Polyp.

intervals in two scenarios: one scenario in which SP subtype is taken into account and one scenario in which all SP subtypes are treated identically. The different scenarios of this post-hoc analysis are discussed in detail below.

NordICC is a large randomized colonoscopy screening trial, as described in detail elsewhere.[21] In brief, a population-based sample of men and women aged 55-64 years without prior diagnosis of CRC from Norway, Poland, Sweden and the Netherlands were randomized to be invited to either once-only screening colonoscopy, or no screening (standard care in the trial areas during the study period). All endoscopists had performed at least 300 colonoscopies and a minimum of 200 colonoscopies per year before contributing to the trial. Using standard equipment, all lesions detected at screening were removed whenever possible. Intubation of the cecum was registered. Bowel preparation was scored by the endoscopist as either "good", "moderate but adequate for detecting polyps >5mm", "poor in segments" or "generally poor". Additional interventions to ensure high-quality colonoscopy are extensively discussed elsewhere.[21, 22] All colonoscopies were performed between 2011 and 2014.

Post-hoc analysis

For the current study, we included participant and polyp data (including size, morphology and histopathology of resected polyps) from the NordICC trial database.[22] Only Norwegian participants were included in the current study because the quality of SP classification was deemed especially high in this arm of the NordICC. All SPs in Norway were diagnosed by a dedicated group of three pathologists with ≥10 years of experience. These three pathologists received a dedicated training in SP classification at the beginning of the NordICC trial. Because such homogeneity and quality of SP classification could not be guaranteed in the other participating countries, we chose to only include Norwegian individuals. We excluded subjects who had CRC detected at screening and those who fulfilled the diagnostic criteria for serrated polyposis syndrome [23], because different surveillance recommendations apply to these individuals.

For the current study we assigned each individual two separate 'virtual' surveillance intervals based on their poly burden: one interval calculated using the US-MSTF guideline, and one interval calculated using the ESGE guideline (Table 1).[20, 24] These virtual surveillance intervals were only used in our database and analyses, and were independent from the interval that was actually chosen by the endoscopist, since endoscopists might not always adhere to surveillance guidelines. We thus simulated a scenario with complete protocol adherence. The two guidelines are concordant on surveillance intervals after adenoma removal, but provide different recommendations following SP removal (table 1).

Surveillance guidelines

US-MSTF surveillance guideline

The US-MSTF guideline stratifies individuals into low, intermediate or high-risk categories by histopathological subclasses. Although this guideline explicitly classifies TSAs, SSLs ≥10mm and SSLs with dysplasia as high-risk, no recommendations are provided for HPs ≥10mm. For the current study, we considered HPs ≥10mm as high-risk SPs, and HPs <10mm as low-risk SPs. We also performed a sensitivity analysis where all HPs were considered low-risk lesions, regardless of size.

According to the guideline, individuals with one or more high-risk SPs, advanced adenomas (≥10mm, high-grade dysplasia or ≥25% villous histology) or ≥3 non-advanced adenomas are recommended surveillance after 3 years and individuals with one or more non-dysplastic SSL <10mm after 5 years. Lastly individuals with any number of HPs <10mm or no polyps are recommended screening colonoscopy after 10 years (table 1). The US-MSTF guideline recommends a surveillance interval of "5-10 years" for patients with 1-2 non-advanced adenomas. Because our aim is to study differences in surveillance after SP resection, we assigned all patients with 1-2 low-risk adenomas a 10-year surveillance interval for the current study.

ESGE surveillance guideline

In the ESGE guideline, all SPs ≥10mm in diameter and those containing dysplasia are considered high-risk and are recommended surveillance colonoscopy after 3 years. All other SPs are considered low-risk, irrespective of histopathological sub-classification, and are recommended to return to CRC screening programs or re-colonoscopy after 10 years. Identical to the US-MSTF guideline, advanced adenomas or ≥3 non-advanced adenomas result in a 3-year surveillance interval, whereas individuals with 1 or 2 non-advanced adenomas are recommended to return to CRC screening or re-colonoscopy after 10 years.

Histopathologic re-evaluation

Initially, all resected polyps in the study sample were assessed by a dedicated group of pathologists. For serrated polyps, the criteria by Aust *et al.* and Rex et al. were applied.[4, 25] SPs were sub-classified into HP, SSL (with or without dysplasia) and TSA. Based on these diagnostic criteria, SSLs were differentiated from HPs by the presence of one or more SSL-like crypts: distorted, dilated and/or horizontally branched crypts.[4, 25] Endoscopic and histologic examples of these subclasses are presented in Figure 1. To ensure robust histologic diagnosis for this post-hoc analysis, a random sample of 488 polyps was re-evaluated by an independent expert pathologist (DK), who was blinded for both the original histological assessment, and for location and size of the polyp. During this post-hoc revision, differentiation between HP and SSL was similarly based on the presence of SSL-like crypts. Of note, at the time of this post-hoc revision, up-to-date diagnostic criteria dictated that ≥2 SSL-like crypts were required for SSL-diagnosis instead of ≥1 SSL-like crypt.[23]

In case of disagreement between the initial and revised diagnosis, a second expert pathologist was consulted (EML), and a final diagnosis was based on consensus between these two pathologists. A priori, an agreement of at least 80% between the initial and revised diagnostic sub-classification of SPs was considered adequate for the current study.

Study outcomes and statistical analyses

The primary outcome was the agreement in surveillance recommendation between the US-MSTF and ESGE guideline, expressed as percentage agreement and tested using Cohen's kappa statistic (κ). Kappa values between 0.60 and 0.80 were regarded as moderate agreement and values over 0.80 as strong agreement.[26] Agreement was calculated for all individuals as well as for individuals with at least one SP. We used logistic regression to test if individuals with SP had a higher risk of co-occurring adenomas compared to patients without SP, and presented the results as relative risk (RR) with 95% confidence intervals (CI). Mean and standard deviations (SD) were reported for normally distributed variables; skewed variables were described using median and inter-quartile range (IQR). We analysed our data using Statistical Package for Social Sciences (SPSS), version 24.0.

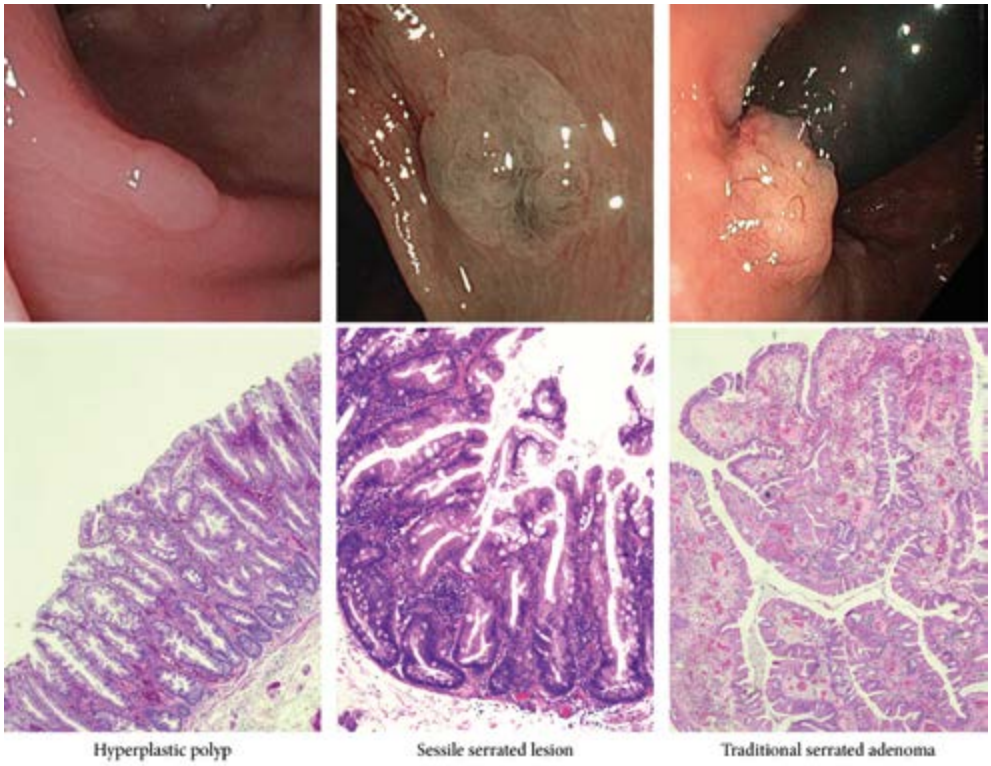

Figure 1. Histologic and endoscopic examples of a hyperplastic polyp (with typical narrow, serrated crypts), sessile serrated lesion (with typical widened serrated crypts with L- or anchor-shaped crypt bases) and a traditional serrated adenoma (with typical ectopic crypt formation, eosinophilic cytoplasm and jigsaw-shaped serrations).

RESULTS

Baseline characteristics

All screening participants

In the Norwegian part of the NordICC trial, 5,354 individuals were screened between 2011 and 2014. Bowel preparation was characterized as moderate or good in 91% of the colonoscopies; the cecal intubation rate was 97%. Thirty-one subjects were excluded; 21 due to screening-detected CRC and 10 because of serrated polyposis syndrome (Figure 2 & Table 2). The mean age of the 5,323 screening participants was 60 years (SD 2.9), and 2,563 were male (48%). In total, 4,804 lesions were resected, of which 2,187 (46%) were SPs and 2,315 (48%) were adenomas. There were 1,402 participants (26%) with at least one adenoma, and 332 participants (6.2%) had at least one advanced adenoma. One or more SPs were found in 1,228 subjects (23%)

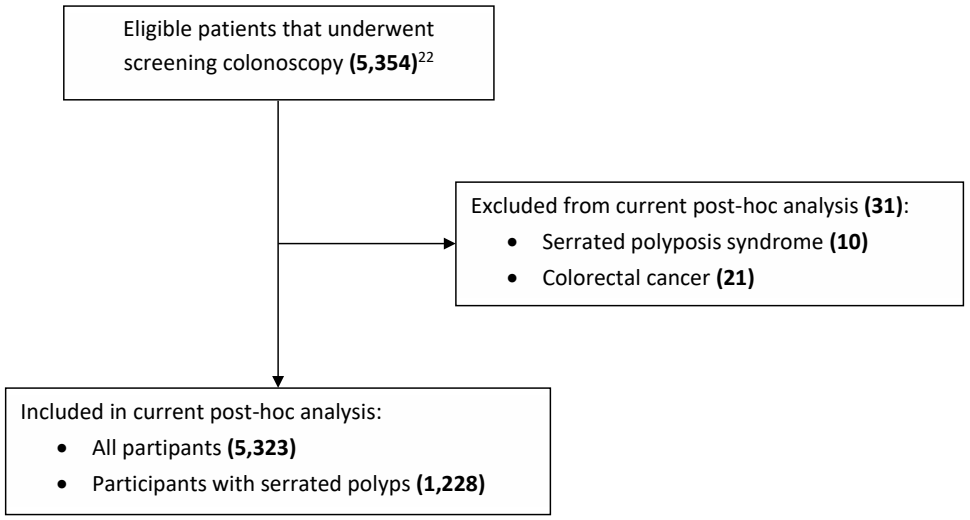

Figure 2. Study flow-chart

Participants with serrated polyps

The mean age of the 1,228 subjects with SPs was 60 years (SD 2.9), and 703 (57%) were male. In total, 3,222 polyps were resected from subjects with SPs, of which 2,187 (68%) were SP and 825 (26%) were adenomas. Of the subjects with SPs, 1,081 participants had at least one HP (88%), 284 had at least one SSL (23%) and four had at least one TSA (0.3%). Furthermore, 458 (37%) had at least one adenoma, of whom 125 (10%) had at least one advanced adenoma (Table 2).

Histopathologic re-evaluation

Of the 488 re-evaluated tissue samples, 57 were excluded because the initial assessor classified them as multiple polyps in one test tube, no diagnostic material, normal mucosa, unspecified SP or polyps of other diagnosis than SP or adenoma. In the revised 431 polyps (338 SPs and 93 adenomas), there was agreement between the initial and revised diagnosis in 364 polyps (84%). The initial and revised diagnosis of the 338 SPs showed agreement in 272 SPs (80%). Of the 228 initially diagnosed HPs, 32 were re-diagnosed as SSL (14%). Of the 108 initially diagnosed SSLs, 22 were re-diagnosed as HP (20%). One of the two initially diagnosed TSAs was re-diagnosed as SSL (50%). Last, 92 out of 93 adenomas showed agreement between initial and revised diagnosis (99%).

Table 2. baseline characteristics & polyp info

	All participants	Participants with at least 1 SP
Cohort size, n (%)	5,323 (100%)	1,228 (100%)
Age, mean (SD)	60 (2.9)	60 (2.9)
Male gender, n (%)	2,563 (48%)	703 (57%)
Resected polyps		
Total resected polyps, n (%)	4,804 (100%)	3,222 (100%)
Adenomas, n (%)	2315 (48%)	825 (26%)
Advanced adenomas*	417 (8.7%)	153 (4.7%)
Serrated polyps, n (%)	2,187 (46%)	2,187 (68%)
HP	1,776 (37%)	1,776 (55%)
SSL	407 (8.5%)	407 (13%)
TSA	4 (0.8%)	4 (0.1%)
SP ≥10mm	281 (5.8%)	281 (8.7%)
SP containing dysplasia	32 (0.7%)	32 (1%)
Other histology†, n (%)	302 (6.3%)	210 (6.5%)
Polyp detection rates		
At least 1 polyp	2,173 (41%)	1,228 (100%)
At least 1 SP, n (%)	1,228 (23%)	1,228 (100%)
At least 1 proximal SP§	537 (10%)	537 (44%)
At least 1 SP ≥10mm	220 (4.1%)	220 (18%)
At least 1 HP, n (%)	1081 (20%)	1,081 (88%)
At least 1 HP ≥10mm	115 (2.2%)	115 (9.4%)
At least 1 SSL, n (%)	284 (5.3%)	284 (23%)
At least 1 SSL ≥10mm	122 (2.3%)	122 (9.9%)
At least 1 SSL with dysplasia	27 (0.5%)	27 (2.2%)
At least 1 TSA, n (%)	4 (0.08%)	4 (0.3%)
At least 1 adenoma, n (%)	1402 (26%)	458 (37%)
At least 1 advanced adenoma	332 (6.2%)	125 (10%)

* Advanced adenoma: ≥10mm, and/or villous histology, and/or high-grade dysplasia

† Other histology includes: granulomatous, hamartomatous and inflammatory polyps; polypous lymphoid hyperplasia or neuro-endocrine tumours.

§Proximal to the splenic flexure

SD, standard deviation; HP, hyperplastic polyp; SSL, sessile serrated lesion; TSA, traditional serrated adenoma; SP, serrated polyp;

Guideline agreement

All screening participants

Of the 5,323 screened participants, 5,201 (98%) received the same interval recommendation by both guidelines (κ=0.90, Figure 3 & 4). All 642 subjects classified as high-risk using the US-MSTF were also classified as high-risk using the ESGE guideline (Table 3). In our sensitivity analyses in which large HP were treated as low-risk polyps according to the US-MSTF guideline, agreement was 96% between the two guidelines (supplementary table 1).

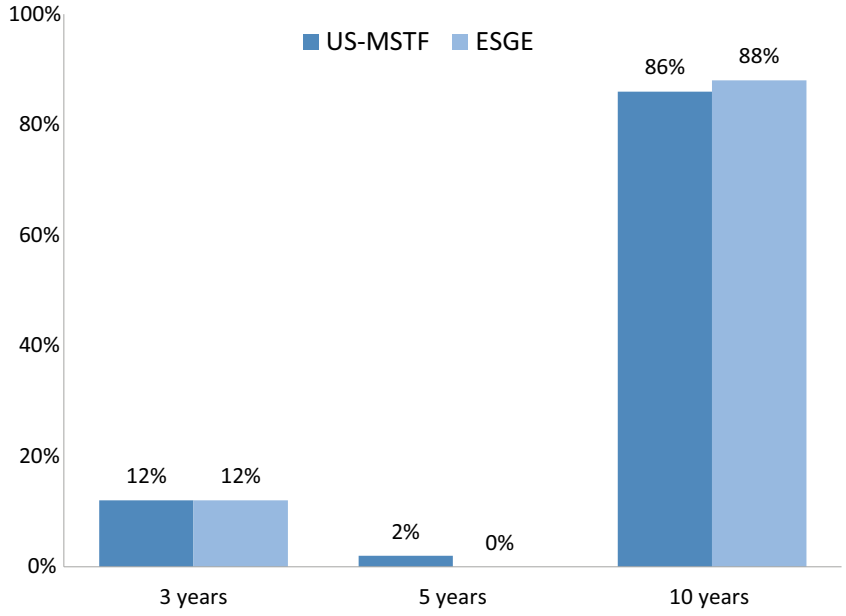

Figure 3. Distribution of surveillance intervals using the US-MSTF and ESGE guidelines, respectively

Table 3. Surveillance intervals according to US-MSTF and ESGE for all screened participants and for subjects with one or more serrated polyps

All participants (5,323)			
		ESGE surveillance strategy	
		3 years	10 years
US-MSTF surveillance strategy	3 years	642 (12%)	0
	5 years	0	122 (2%)
	10 years	0	4,559 (86%)
Subjects with serrated polyps (1,228)			
US-MSTF surveillance strategy	3 years	371 (30%)	0
	5 years	0	122 (10%)
	10 years	0	735 (60%)

US-MSTF: United Stated Multi-Society Taskforce on Colorectal cancer. ESGE: European Society of Gastrointestinal Endoscopy.

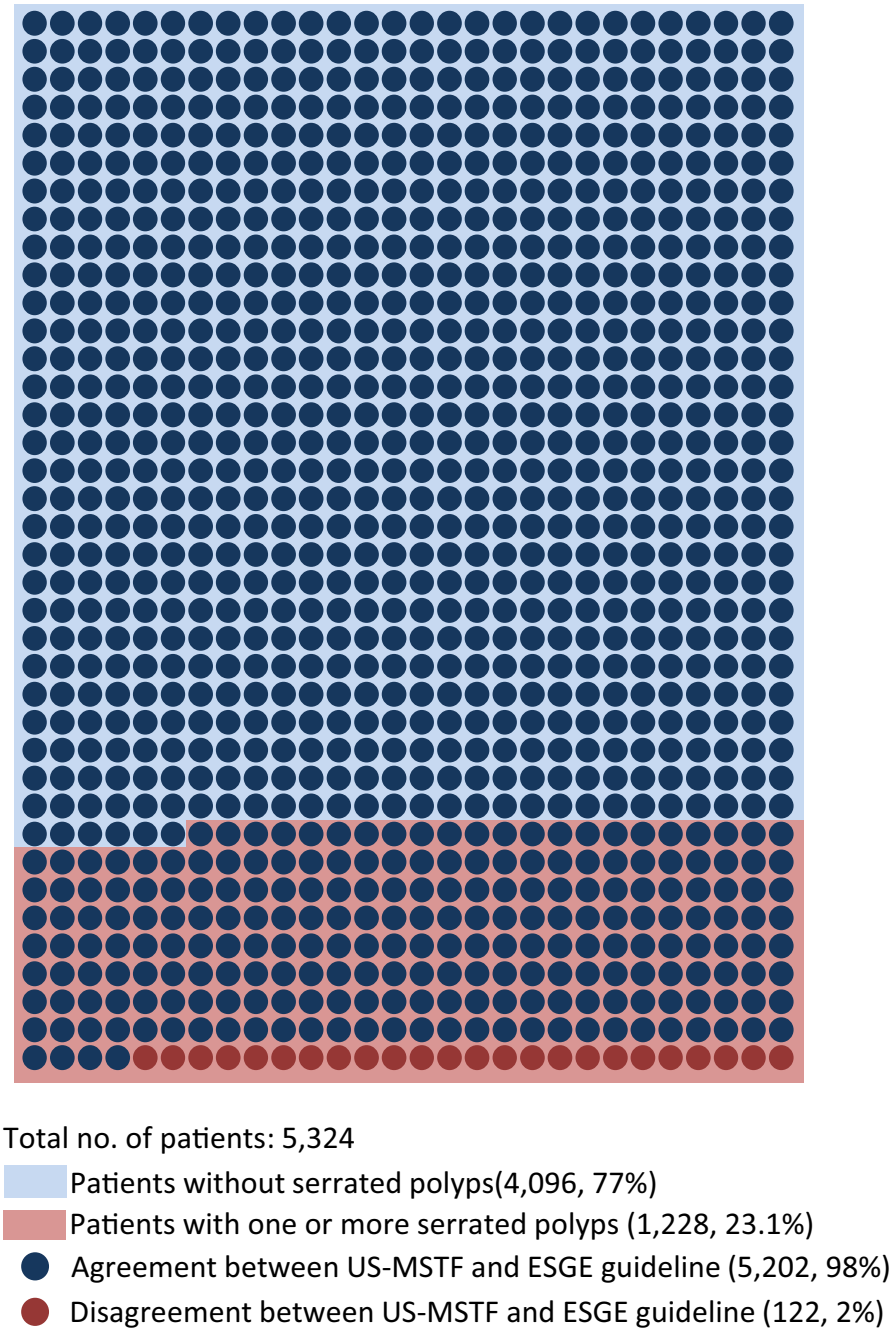

Figure 4. Concordance between ESGE and US-MSTF guideline. Each circle represents five screened individuals.

Participants with serrated polyps

Among the 1,228 patients with SPs, 1,106 (90%) were recommended the same surveillance intervals with the two guidelines (κ=0.80, Table 3). All 371 patients classified as high-risk using the US-MSTF were also classified as high-risk using the ESGE guideline. In the sensitivity analyses in which large HP were treated as low-risk polyps according to the US-MSTF guideline, agreement was 84% between the two guidelines for patients with SP (Supplementary Table 1).

The presence of synchronous conventional adenomas led to a shorter surveillance interval than the SP in 133 of 1,228 subjects (11%) with SPs according to both guidelines. A total of 176 of the 375 subjects with SP (47%) who qualified for a 3-year surveillance interval based on both guidelines, would also have been recommended a 3-year surveillance interval because of co-occurring advanced adenomas or ≥3 non-advanced adenomas, regardless of their advanced SP. Compared to subjects without advanced SP, those with one or more advanced SP (TSA, diameter ≥10mm or presence of dysplasia) were at increased risk of having synchronous non-advanced adenomas (RR 1.75, 95%-CI 1.48-2.05) or advanced adenomas (RR 2.36, 95%-CI 1.69-3.30).

DISCUSSION

In this colonoscopy screening cohort, we compared the recommended surveillance interval based on the ESGE guideline – not considering SP subtype – and the US-MSTF guideline – considering SP subtype – in high-quality screening colonoscopies with reliable histopathologic SP classification. The agreement between the guidelines was strong (90%, κ 0.80) for subjects harbouring one or more SP, and even stronger (98%, κ 0.90) for all screening subjects. All individuals with discordant surveillance intervals between the guidelines had one or more small SSL as the most advanced lesion. Consequently, they received a 5-year surveillance recommendation according to the US-MSTF guideline and a 10-year according to the ESGE guideline.

SP sub-classification is subjected to marked inter-observer disagreement. In one study, three expert gastrointestinal pathologists reviewed proximal SPs previously diagnosed as HPs. Of all HPs, 30-85% was re-diagnosed as SSL after revision, reflecting substantial disagreement both between initial and revised diagnosis, but also between the three expert pathologist.[17] Another study reported that 28% of HPs throughout the colon were re-diagnosed as SSL after expert revision, of which the vast majority was proximally located.[18] To reach more reliable SP sub-classification, training programs for pathologists would be needed.[16] However, our results suggest that such effort would not entail substantial change to clinical care for subjects with SP. In a setting of finite time and resources, improving SP sub-classification might therefore not be the most relevant target for quality improvement programs. Accurate histopathologic differentiation remains, however, important empirically in order to be able to study the natural history, pathophysiology and (epi)genetic aberrations of HP, SSL and TSA.

Another salient finding was the dominance of co-occurring adenomas in subjects with SP. Subjects with SPs had a substantially increased prevalence of synchronous adenomas, as previously shown.[27, 28] As a result, almost half of the subjects with SPs that entailed a 3-year surveillance interval, had co-occurring advanced adenomas or ≥3 non-advanced adenomas. In these prevalent cases where adenomas dictate surveillance intervals, differences between strategies for surveillance after SP resection become irrelevant. Furthermore, this finding should urge endoscopists to look carefully for synchronous adenomas if one or more SPs are detected.

Several limitations of our study should be considered when interpreting our results. First, the current US-MSTF guideline provides no clear guidance on the management of hyperplastic polyps. Surveillance is not recommended for subjects with only small HP <10mm in the rectum or sigmoid, whilst no recommendations are given for proximal and/or large HP. We therefore filled these gaps based on remarks made in the guideline, available literature and expert opinion. In our interpretation of the US-MSTF guideline, all *large* HP (≥10mm) are treated as 'high-risk lesions', and all *small* HP (<10mm) as 'low-risk-lesions', based on the opinion of several expert endoscopists and a recent study showing increased risk of metachronous large SP after resection of SP ≥10mm.[12] With regard to small HP, several studies showed that neither proximal nor distal HPs <10mm are a risk-factor for metachronous (advanced) adenomas, nor were they associated with an increased risk of CRC mortality.[7, 13] Furthermore, the British Society of Gastroenterology recently advised no additional surveillance for any HP <10mm.[29] In contrast to our approach, some might argue that large HP should also be treated as low-risk lesions. We therefore undertook a sensitivity analysis in which not only small, but also large HPs were treated as low-risk polyps in the US-MSTF guideline (supplementary table 1 &2). The agreement between US-MSTF and ESGE was equally high in this alternative scenario. Second, the diagnostic criteria used for the histopathologic revision in our study (the up-to-date WHO guideline[23]) state that at least two SSL-like crypts are needed to classify a polyp as SSL. In contrast, many pathologists used the diagnostic criteria defined by Rex and colleagues for initial diagnosis[4], which state that only one SSL-like crypt is sufficient for SSL diagnosis. Previous studies showed, however, that this difference hardly influences the prevalence of SSL-diagnosis in clinical practice.[30] Furthermore, the agreement between initial and revised diagnosis in this study were high, illustrating that these minor differences in diagnostic criteria did not markedly influence the prevalence of SSL diagnosis. Third, we limited inclusion for this post-hoc analysis to Norwegian participants of the NordICC, which might raise concerns about the external validity of our sample. As explained, this decision was based on the homogeneity and quality of histopathologic diagnosis in Norway. Since our results largely depend on the prevalence of SPs and adenomas, potential differences in prevalence between Norwegian and other NordICC countries could have skewed our results. However, the prevalence of SPs and adenomas in Norway was similar to other countries that participated in the NordICC trial, and we therefore think the inclusion of only Norwegian participants did not jeopardize the external validity of our study.[22] Lastly,

despite the special training pathologists received in classifying SPs, 20% of initial diagnoses was changed after expert revision. Some might argue that an interrater agreement of 80% indicates suboptimal accuracy of initial diagnosis. However, SP classification is notoriously difficult, and previous studies showed that some degree of interrater disagreement is inevitable even in expert hands.[17]

Despite these limitations we consider the external validity of our findings high. First, because substantial effort was made to correctly sub-classify serrated polyps (e.g. initial diagnosis made by trained expert pathologists, revision of a subset of SP), the SP sub-classification can be considered a best-case scenario. This was of paramount importance to study a histology-based surveillance strategy like the US-MSTF's with as little bias as possible. Second, our sample consisted of a homogeneous asymptomatic population, with high-quality prospectively collected colonoscopy data which makes our study representative for primary colonoscopy screening settings. Last, detection rates of both adenomas and SPs are similar to those previously reported in other European and United States' primary colonoscopy screening cohorts, implying our cohort is representative for non-Norwegian populations as well.[22] [18] The most important future issue to be addressed is whether the different SP subclasses indeed predict different risks for metachronous advanced neoplasia. Because most published studies are retrospective and affected by imperfect histopathologic SP classification, available evidence is insufficient to solve this issue. Studies with long-term follow-up after SP resection and expert histopathologic classification, such as the ongoing EPoS trial, are vital to fill this knowledge gap.[31] Such studies will hopefully help us understand the relevance of proximal and/or large HPs.

In conclusion, our study showed high agreement between the US-MSTF and ESGE post-polypectomy guidelines, despite their different approach regarding SPs. Accurate classification of SP sub-classes has limited added value within the current version of these guidelines, and might not be as urgently needed as previously suggested. Until revised editions of the US-MSTF and ESGE become available, the ESGE guideline seems to provide a more simple and practical approach to surveillance after SP resection.

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SUPPLEMENTARY DATA

Supplementary table 1. Sensitivity analysis*
Surveillance intervals according to US-MSTF and ESGE for all subjects (5,323).

All participants (5,323)			
		ESGE surveillance strategy	
		3 years	10 years
US-MSTF surveillance strategy	3 years	563 (11%)	0
	5 years	5 (0.1%)	122 (2.3%)
	10 years	74 (1.4%)	4,559 (86%)
Subjects with one or more serrated polyps (1,228)			
US-MSTF surveillance strategy	3 years	292 (24%)	0
	5 years	5 (0.4%)	122 (10%)
	10 years	74 (6%)	735 (60%)

US-MSTF: United Stated Multi-Society Taskforce on Colorectal cancer. ESGE: European Society of Gastrointestinal Endoscopy.
* The US-MSTF guideline does not provide recommendations for surveillance after resection of large HP due to lack of supporting evidence. In our primary analyses, HP were treated as high-risk polyps with corresponding 3-year surveillance intervals using the US-MSTF guideline (see table 1). In this sensitivity analysis an alternative approach was assessed, in which all HP, regardless their size, are treated as low-risk polyps in the US-MSTF guideline with corresponding 10-year surveillance intervals.

4

Whole exome sequencing of sessile serrated lesions caught in the act of malignant progression reveals *ACVR2A* and *RNF43* as earliest drivers in the MLH1 deficient pathway to colorectal cancer

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Submitted

ABSTRACT

Background and Aims

About 15-30% of colorectal cancer (CRC) develops from sessile serrated lesions (SSLs). After many years of indolent growth, SSLs can suddenly develop dysplasia and rapidly progress into CRC through events that are only partially understood. We studied driver events at very early stages of progression of SSLs via the MLH1 proficient and deficient pathways to CRC.

Methods

We collected a cohort of rare SSLs with a small focus (<10 mm) of dysplasia or cancer. Whole exome sequencing was performed on DNA from non-progressed and progressed components of each SSL, as well as from adjacent normal tissue. Putative driver mutations were identified in known cancer genes that were differentially mutated in the progressed component. All analyses were stratified by MLH1 proficiency.

Results

Forty-five lesions with a focus dysplasia or cancer were included, of which 22 were MLH1 deficient and 23 MLH1 proficient. Tumour mutational burden (TMB) was high in MLH1 deficient lesions (23.9 mutations per MB) compared to MLH1 proficient lesions (6.3 mutations per MB). We identified 34 potential driver genes in MLH1 deficient lesions. Most prominently, *ACVR2A* and *RNF43* were affected in 18/22 lesions, with mutations clustered in three hotspots within microsatellites. In MLH1 proficient lesions *APC* (10/23 lesions) and *TP53* (6/23 lesions) were identified as potential drivers.

Conclusions

Mutational burden is exceptionally high even in the earliest MLH1 deficient lesions. Hotspot mutations in *RNF43* and *ACVR2A* might be responsible for the abrupt malignant transition of SSLs along the MLH1 deficient serrated pathway, while *APC* and *TP53* mutations are early events in the MLH1 proficient pathway.

BACKGROUND

Traditionally, colorectal cancer (CRC) was considered to arise exclusively from adenomas. The molecular alterations involved in the stepwise progression from normal mucosa to adenoma and ultimately to CRC, known as the adenoma-carcinoma pathway, were first described in 1990[1], and have since been elucidated in great detail.

Far less is known about the 'serrated neoplasia pathway', which is now held accountable for 15-30% of all CRCs.[2, 3] CRCs that develop through this alternative pathway predominantly arise from sessile serrated lesions (SSL).[4-7] Recent evidence suggested that these lesions might remain dormant for over 15 years with little change in size, before developing cytological dysplasia. After a brief intermediate stage of dysplasia they rapidly progress into full-blown CRC.[5] The molecular driver events responsible for this 'sudden' malignant progression are poorly understood. A deeper understanding of the driver events responsible for this sudden progression could facilitate development of more accurate (faecal) biomarker tests, improving early diagnosis of advanced SSLs. This is highly warranted, since SSLs give rise to a subset of CRCs with the poorest prognosis of all CRCs (i.e. *BRAF*^{mut}/microsatellite stable CRC).[8-12]

Thus far, several hallmarks of the serrated neoplasia pathway have been identified.[7] An activating *BRAF*^{V600E} mutation is considered the most common initiating mutation.[4, 5, 7] Aside from activating the mitogen activated protein kinase (MAPK) pathway, this mutation also seems to cause genome-wide methylation of CpG-rich promotor regions known as CpG-island methylator phenotype (CIMP).[13, 14] Consequently, tumour suppressor genes (TSGs) are at risk of becoming epigenetically silenced.[4, 5] In 33-75% of SSLs with a focus of dysplasia or cancer, mismatch repair gene *MLH1* is silenced due to methylation of its promoter region, which results in microsatellite instability (MSI).[5, 15-17] MSI causes overt genetic instability, leading to rapid acquisition of mutations and consequently progression into *BRAF*^{mut}/MSI cancer. This pathway will from here-on be referred to as the MLH1 deficient pathway. Although the mechanism for genetic instability along this pathway is clear (i.e. MSI), the specific driver genes and the order by which they become mutated and drive early carcinogenesis are not known.

Alternatively, SSLs can progress into *BRAF*^{mut}/microsatellite stable (MSS) CRC through the MLH1 proficient pathway, in which MLH1 expression is retained. As yet, the mechanisms of this alternative pathway are poorly understood. Among others, somatic mutations in *TP53* and epigenetic silencing of *CDKN2A* (encoding p16) have been suggested to play a role in the progression of SSL into *BRAF*^{mut} /MSS cancer.[5, 18]

To identify the early driver events in both serrated neoplasia pathways we collected a unique cohort of SSLs that are caught in the act of malignant progression, as recognized by the presence of a very small focus of dysplasia or cancer. We compared somatic mutations in matched progressed and non-progressed components of these SSLs.

METHODS

Study design and case selection

We retrospectively retrieved formalin-fixed paraffin embedded (FFPE) samples of SSLs with a focus of dysplasia or cancer from the pathology units of three hospitals in the Netherlands (Amsterdam UMC, Amsterdam; Onze Lieve Vrouwe Gasthuis (OLVG), Amsterdam; Tergooi Hospital, Hilversum). Samples were identified in local pathology databases, and were reviewed by two expert pathologists (CvN and SvE). We applied stringent inclusion criteria to ensure that only SSLs with an early focus of dysplasia or cancer were included. These inclusion criteria were 1) presence of non-dysplastic SSL tissue with an uninterrupted transition to an area with dysplasia and/or cancer (Figure 1A-B), and 2) a maximum diameter of the dysplastic/cancerous focus of ≤10mm. We aimed to compare molecular alterations in the progressed vs. the non-progressed components within these lesions, as described previously.[19, 20]

Because of the observational nature of this study and because only somatic mutations were assessed, the institutional review board of the Amsterdam University Medical Centres (Amsterdam UMC, previously Academic Medical Centre (AMC)) decided that this study fell beyond the legislation regarding Medical Research Involving Human Subjects Act (WMO), and that formal ethical approval was therefore not required. This study was performed in agreement with the Helsinki Declaration.[21]

Clinicopathologic data collection

Patient characteristics and lesion characteristics were retrieved from pathology and patient reports. Data were directly and permanently anonymized, and were stored online using Castor EDC.[22] The rate of dysplasia (low grade dysplasia (LGD), high grade dysplasia (HGD) or cancer) was assigned after revision. During revision, SSL diagnosis was based on the 2010 edition of the World Health Organization Classification of Tumors of the digestive system.[23]

Data analysis & outcome parameters

All data analyses were aimed to identify differences between MLH1-proficient and MLH1-deficient lesions. We therefore stratified all included lesions by MLH1-proficiency, as determined by immunohistochemistry (Figure 1C-D).

Immunohistochemistry

All selected tissue specimens were stained for MLH1, p16, SMAD4 and p53, as described previously.[24] MLH1 deficiency was defined as a complete absence of nuclear MLH1 staining. Complete absence of SMAD4 or p16 staining were considered indicative of TGF-ß pathway or p16 dysfunction, respectively. Dysfunction of p53 was defined as either strongly positive or absent p53 staining in ≥75% of the epithelial nuclei. (Figure 1E-F).

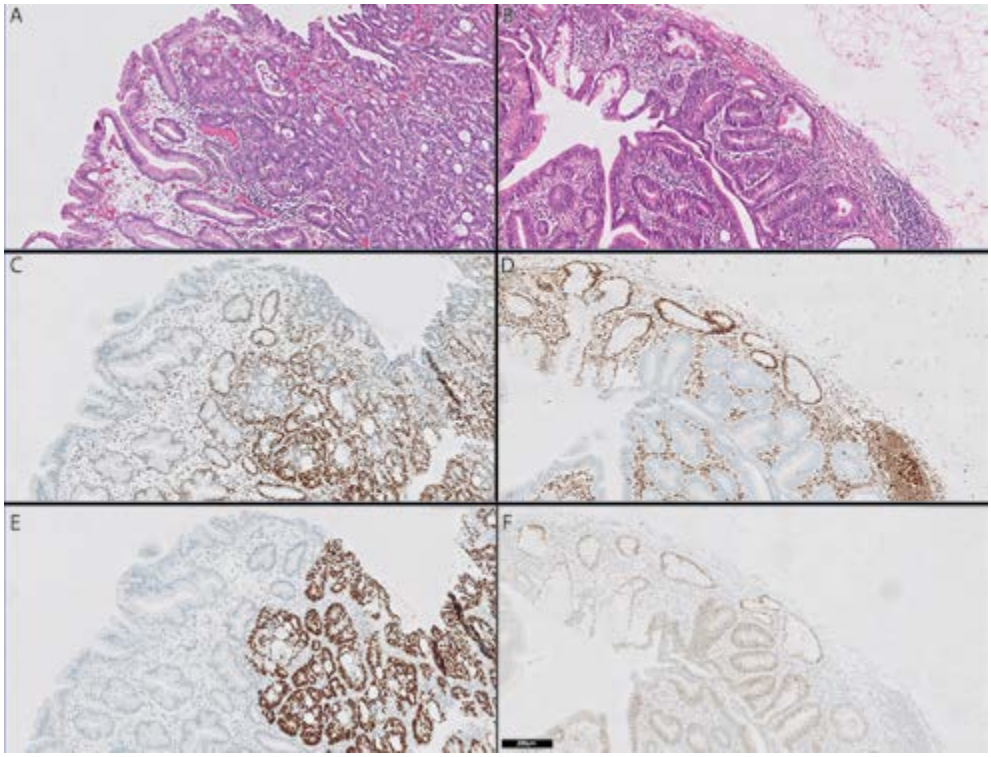

Figure 1 A-E. Example of MLH1 proficient lesion (A, C and E) and MLH1 deficient lesion (B, D and F). A and B: haematoxylin eosin stained sections of transition zone of serrated and progressed components. C and D: MLH1 staining; normal expression in C, loss of expression in D. E and F: p53 staining. Aberrant over-expression in E, normal expression in F.

DNA isolation

DNA was isolated separately from three components within each lesion: **1)** normal colonic mucosa adjacent to the SSL; **2)** non-dysplastic serrated tissue adjacent to the progressed component (from here-on referred to as 'serrated component') and **3)** tissue from the focus of dysplasia or cancer (from here-on referred to as 'progressed component') (Figure 1A-B). DNA was isolated from the three components using manual microdissection of FFPE-tissue slides. DNA was extracted from isolated cells using proteinase K digestion, using 3 µL proteinase K (1mg/mL) in 27 µL proteinase K buffer with an overnight incubation at 50 °C. After incubation, proteinase K was inactivated at 95 °C for three minutes, and samples were then stored at -20 °C until further use.

The percentage of lesional cells in the collected DNA could be assessed for lesions with a BRAF mutation by using the proportion of sequence reads containing a *BRAF* mutation. This mono-allelic mutation has been well described as initiating event in the development of SSLs.

Microsatellite instability

Microsatellite status of the progressed components of each lesion was assessed using MSI analysis system v1.2 (Promega, Madison, USA) according to the manufacturer's instructions. This system uses five microsatellite markers (NR-21, NR-24, MONO-27, BAT25 and BAT26). Lesions were classified as microsatellite instable (MSI-high) if ≥2 markers were instable.

Whole exome sequencing - sequencing protocol

Quality of the DNA samples was assessed using the Fragment Analyzer and expressed as Genomic Quality Number (GQN) score. DNA concentration was determined using Qubit reader (ThermoFisher Scientific, Waltham, USA). Sample preparation and hybridization capture were performed on the SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing Library, protocol v1.8 (G7530-90000), using the Bravo Liquid Handling system (Agilent, Santa Clara, USA). Briefly, 20ng of genomic DNA was fragmented and adapters were added to ends of fragments and PCR amplification of the resulting product was performed. The 67.3 megabase Agilent SureSelectXT Clinical Research Exome v2 capture library (5190-9493) was used (Agilent, Santa Clara, USA).[25] Clustering and DNA sequencing using the NovaSeq6000 (Illumina, San Diego, USA) was performed at GenomeScan B.V. (Leiden, the Netherlands) according to manufacturer's protocols. NovaSeq control software NCS v1.6 was used. A median sequence depth of ≥100 and a horizontal coverage of ≥98% were considered adequate for the current study. Image analysis, base calling, and quality check were performed with the Illumina data analysis pipeline RTA3.4.4 and Bcl2fastq v2.20.

Whole exome sequencing – Analysis pipeline

Obtained sequences were deduplicated using Picard (http://broadinstitute.github.io/picard), adapter sequences were removed with cutadapt 2.9 (https://cutadapt.readthedocs.io/en/stable/) and were aligned to the human reference genome hg19 with GATK (https://gatk.broadinstitute.org/hc/en-us) with subsequent variants calling in the same pipeline. We applied left-normalization with bcftools to the Insertions and Deletions before annotating all variants using Annovar. Only non-synonymous exonic variants (NSVs) and variants at essential splice sites were considered. Samples with more than 5000 rare (MAF <0.01) variants in any of the three sections were deemed of too low quality and were excluded from downstream analysis. This reduced the number of available samples to 45. For downstream analysis, we filtered variants based on minor allele frequency (MAF< 4e-04) in the following databases: Exome Aggregation Consortium (EXaC), Genome Aggregation Database (gnomAD), 1000 Genomes Project (1000G). variants that were present in the germline were removed by excluding all variants that were found in the adjacent normal tissue. Variant annotation was performed on the Lisa computercluster (https://userinfo.surfsara.nl/systems/lisa). Variant filtering and sorting was performed in R version 3.5.1 using packages R.utils, stringr and dplyr.

Identification of driver mutations

Potential driver mutations were identified within 724 known cancer genes listed in the Cancer Gene Census (CGC). The CGC contains an up-to-date list of genes that have been causally implicated in cancer development. The content, structure and curation process are described in detail elsewhere.[26] Potential driver mutations were defined as recurrently mutated genes (i.e. mutated in at least 5 lesions) which were differentially mutated in the progressed vs. the serrated component. We compared the mutation frequency in the progressed vs. the serrated component using a Fisher's exact test. Only mutations in genes with a p-value <0.1 were considered potential drivers.

To adjust for the fact that certain genes are, due to their length and genetic composition and location, more likely to acquire random passenger mutations than others and are thus more prone to be falsely identified as driver gene, we utilized the Genome Aggregation Database (gnomAD) database[27] to control for this background level of expected random mutations. Variants in the gnomAD database were filtered with the same criteria as the variants in our cohort. Differences from background levels of mutations were statistically assessed using a Fisher's exact test for each of the CGC genes. Only genes with a Bonferroni-corrected p-value <.05 were considered as potential drivers.

We utilized the OncoKB database to annotate variants.[28] OncoKB classifies variants as oncogenic, likely oncogenic, likely neutral, inconclusive or unknown. Alterations listed as (likely) oncogenic have either been demonstrated in previous studies to result in gain or loss of function of oncogenes and tumour suppressor genes, respectively, are known hotspot mutations, or are convincingly predicted to result in pathogenic alterations based on the type of mutation.[29]

Tumour mutational burden

Tumour mutational burden (TMB) was defined as the number of mutations per sequenced megabase (Mb). We compared the TMB of MLH1-proficient and deficient lesions, and assessed whether the size of the progressed component or the grade of dysplasia were related with TMB. TMB was also compared with other publicly available cancer cohorts using data from the Cancer Genome Atlas (TCGA) using R package maftools (v2.2.10).[29]

Mutational signatures

Based on the mutational patterns in our cohort, we describe which mutational signatures were most consistent with these mutational patterns in our cohort using the COSMIC database of mutational signatures in human cancers (v3).[30, 31] Mutational signatures are based on the six single base pair substitution (SBS) subtypes: C>A, C>G, C>T, T>A, T>C and T>G. These substitutions are further divided based on their neighbouring 5' and 3' nucleotides, ultimately resulting in 96 possible substitutions (6 types of SBS x 4 types of 5' base x 4 types of 3' base). Based on the relative frequency of these 96 SBS, 60 distinct mutational signatures are listed in the COSMIC database of mutational signatures.[31] As

described previously, we used Rpackage maftools (v2.2.10) to compare mutational patterns in our data to these 60 COSMIC signature.[29] Briefly, the cophenetic correlation was used to calculate how many clusters of signatures could be identified in our samples. Based on the cophenetic plot, we extracted three clusters of mutational signatures from our data and compared these against the aforementioned 60 COSMIC signatures.[30, 31] Similarity between our data and the COSMIC signatures were expressed as cosine similarity, which ranges from 0 (no overlap) to 1 (complete overlap).

Analysis software & R packages

Baseline polyp and patient characteristics were compared using Statistical Package for Social Sciences (SPSS) version 24 (IBM, Somers, New York, USA). All other analyses were performed using RStudio 1.2.1335, with R version 3.6.1, using packages Maftools (v2.2.10), NMF (v0.21.0), BSGenome (v1.54.0), BSgenome.Hsapiens.UCSC.hg19 (v.1.4.0), pheatmap (v1.0.12).

RESULTS

Baseline characteristics

A total of 65 SSLs with a small focus (≤10mm) of dysplasia or carcinoma were included for whole exome analyses. After exome sequencing, 20 lesions had to be excluded due to low sequencing quality as evidenced by an unrealistically high number (i.e. >5000) of rare variants in the normal, serrated and/or progressed component of the sample. After exclusion of these lesions, 45 SSLs from 40 individual patients were included. The SSLs had a focus of LGD in 27/45 (60%), HGD in 10/45 (22.2%) and carcinoma in 8/45 (17.8%; Table 1). These lesions were predominantly detected in females (77.8%) and approximately half (48.7%) occurred in a setting of serrated polyposis syndrome. The majority of polyps (87.2%) was located proximal to the descending colon. Lesions had a median diameter of 10mm (IQR 8-15), while the progressed component had a median diameter of 3.5mm (1.75-4.75mm). Based on immunohistochemistry, 23 (51%) were classified as MLH1-proficient and 22 (49%) as MLH1-deficient. MSI could be assessed for 41/45 samples (91.1%), after exclusion of 4 samples with limited DNA available. All twenty-two lesions from the group of MLH1-deficient lesions were MSI-high, compared to none of the MLH1 proficient lesions.

WES quality metrics and variant calling

A total of 135 DNA samples (45 SSLs x 3 components) were included in our WES analyses. The median Genomic Quality Number (GQN) score of the FFPE DNA input was 1.0 (IQR 0.7-1.4). GQN score was high (>0.7) in 95 samples (70%), medium (0.3-0.7) in 27 samples (20%) and low (<0.3) in 13 samples (9.6%). The median sequence depth was 151 (IQR 108-182), with a median 100x, 50x and 30x coverage of 68.7% (IQR 53.1-76.2%), 88.3% (IQR 80.0-91.9%) and 94.7% (IQR 90.6-96.5%), respectively. The median horizontal coverage was 99.8% (IQR 99.7-99.8%).

Table 1. Baseline patient & polyp characteristics.

	All samples (n=45)	MLH1-proficient (n=23)	MLH1-deficient (n=22)	P-value
Age at diagnosis, median (IQR)	66 (63-70.8)	64 (55-68.3)	68.5 (63.8-72.3)	**.024**
Female gender, n (%)	35/45 (77.8%)	15/23 (65.2%)	20/22 (90.9%)	**.038**
Number of polyps resected (lifetime)				.95
Adenomas, median (IQR)	2 (1-3)	2 (1-3.5)	2 (1-3.5)	**.009**
SPs, median (IQR)	6 (2-11)	3 (1.75-6.25)	6 (6-13.5)	
Missing	*6*	*5*	*1*	
Fulfils diagnostic criteria SPS, n (%)	19/39 (48.7%)	4/18 (22.2%)	15/21 (68.2%)	**.002**
Missing	*6*	*5*	*1*	
Location				**.030**
Proximal to descending colon, n (%)	34/39 (87.2%)	11/18 (61.1%)	19/21 (90.5%)	
Distal to or in descending colon, n (%)	5/39 (12.8%)	7/18 (38.9%)	2/21 (9.5%)	
Missing	*6*	*5*	*1*	
Diameter				.35
Polyp, median (IQR)	10mm (8-15)	10 (7.75-13.0)	10 (8-22.5)	.78
Progressed component, median (IQR)	3.5mm (1.75-4.75)	3mm (1.75-3.0)	3.5 (1.7-4.6)	
Grade of dysplasia/cancer				.30
LGD	27/45 (60%)	15 (65.2%)	12 (54.5%)	
HGD	10/45 (22.2%)	3 (13.0%)	7 (31.8%)	
Cancer	8/45 (17.8%)	5 (21.7%)	3 (13.6%)	
Microsatellite instability (MSI-high)				**<.001**
Microsatellite instable	22/41 (53.7%)	0/19 (0%)	22/22 (100%)	
Missing (insufficient DNA available)	*4*	*4*	*0*	

After applying our filtering pipeline as described above, we found 54,716 variants within the progressed components, of which 3,506 (6.4%) were located within Cancer Gene Census (CGC)[26] genes. The median number of mutations in CGC genes per sample was 72 (IQR 29.5-112.5). A total of 45,215 single-nucleotide polymorphisms (SNPs) were found; 11,639 C>A, 3,698 C>G, 19,282 C>T, 5,223T>C, 2,722 T>A and 2,651 T>G. The median contributions of these SNPs are displayed in Figure 2.

A *BRAF* mutation was identified in 40 (88.9%) of the serrated components and in 40 (88.9%) of the progressed components. The median percentage of sequence reads with a *BRAF* mutation was 27.8% (IQR 22.4-37.4%), corresponding to a median estimated lesional DNA percentage of 55.6% (IQR 44.8—74.8%).

MLH1 proficient vs. MLH1 deficient lesions

Clinicopathologic differences

Comparing the 23 MLH1 proficient with the 22 MLH1 deficient lesions, we found no differences in lesional size (10mm vs. 10mm, p=.35), size of the dysplastic component (3mm vs. 3.5mm, p=.78) and grade of dysplasia (i.e. LGD, HGD or cancer; p=.30), suggesting comparable groups with regard to stage of disease (Table 1). However, compared to MLH1 proficient lesions, MLH1 deficient lesions occurred more often in women (90.9% vs. 65.2%, p=.038), more often in patients that fulfilled the criteria of SPS (68.2% vs. 22.2%, p=.002) and more often in the proximal colon (90.5% vs. 61.1%, p=.03).

Immunohistochemistry

Absence of SMAD4 expression was found in 3/23 (13%) vs. 3/22 (13.6%), p=.65) of MLH1 proficient and MLH1 deficient lesions, respectively. Loss of p16 was found in 7/23 (30.4%) vs. 7/22 (31.8%, p=1.00) of MLH1 proficient and MLH1 deficient lesions, respectively. Aberrant p53 staining was observed in 15/23 (65%) vs. 8/22 (36.4%, p=.076) of MLH1 proficient and MLH1 deficient lesions, respectively.

Driver mutations in malignant transformation

In MLH1 proficient lesions, we identified two potential driver genes that were differentially mutated in the progressed component: *APC* and *TP53* (Table 2, Figure 3, Supplementary table 1A). Seven of 10 (70%) *APC* mutations and 5/6 (83%) *TP53* mutations were classified as (likely) oncogenic by OncoKB.[28, 29] Four lesions harboured previously described hotspot *TP53* mutations (p.C275F, rs863224451; p.R248Q, rs11540652 and p.A138V, rs750600586). [28, 32, 33]

In MLH1 deficient lesions 34 possible driver genes were identified (Table 2, Figure 3 and supplementary table 1B). Most prominently, mutations in *RNF43* and *ACVR2A* each occurred in 18/22 lesions. *ACVR2A* is not listed in the OncoKB database so no prediction about pathogenicity for these mutations was reported, but all the *RNF43* mutations were classified as (likely) oncogenic. The other top-10 possible driver genes were *MN1* (12/22),

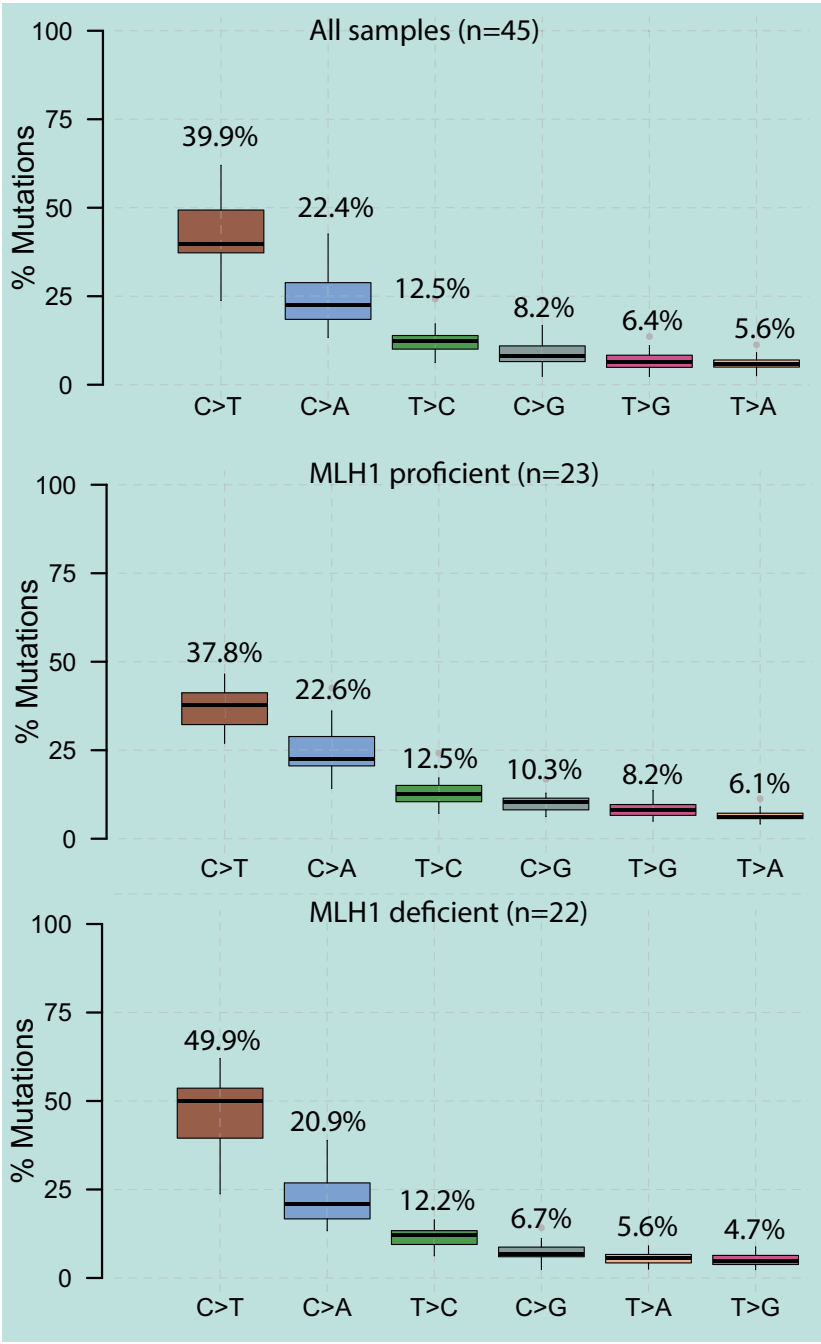

Figure 2. Single nucleotide polymorphisms in the progressed component, in all samples and stratified by MLH1 status. The median proportion of the substitution type for the included lesions is displayed as percentage.

Table 2. Top 10 differentially mutated cancer genes in progressed component compared to non-progressed component of all samples stratified by MLH1 proficiency. Differentially altered genes were identified as follows: proportion of mutations within in progressed vs. non-progressed component, within all CGC genes that are mutated in ≥5 genes.

Hugo symbol	Serrated component	Progressed component	P-value	Adjusted P-value progressed component vs. gnomAD*
MLH1-proficient lesions				
2 Genes with p-value <.10				
APC	3/23 (13.0%)	10/23 (43.5%)	.047	$4.87x10^{-9}$
TP53	1/23 (4.45%)	6/23 (26.1%)	.095	$2.34x10^{-8}$
MLH1-deficient lesions				
34 genes with p-value <.10; only top-10 displayed. See Supplementary table 1 for complete list.				
ACVR2A	1/22 (4.5%)	18/22 (81.8%)	<.001	$7.52x10^{-47}$
RNF43	3/22 (13.6%)	18/22 (81.8%)	<.001	$4.96x10^{-35}$
MN1	0/22 (0%)	12/22 (54.5%)	<.001	$8.21x10^{-19}$
ZNRF3	1/22 (4.5%)	13/22 (59.1%)	<.001	$5.45x10^{-21}$
SMARCA4	1/22 (4.5%)	12/22 (54.5%)	<.001	$1.12x10^{-18}$
KMT2A	1/22 (4.5%)	12/22 (54.5%)	<.001	$1.11x10^{-15}$
FLT4	0/22 (0%)	9/22 (40.9%)	.001	$1.02x10^{-10}$
POLD1	0/22 (0%)	9/22 (40.9%)	.001	$2.65x10^{=10}$
ATM	3/22 (13.6%)	13/22 (59.1%)	.004	$1.32x10^{-14}$
KMT2D	4/22 (18.1%)	14/22 (63.6%)	.005	$9.62x10^{-13}$

* To adjust for the fact that certain genes are, due to their length and genetic composition and location, more likely to acquire random passenger mutations than others and are thus more prone to be falsely identified as driver gene, we utilized the Genome Aggregation Database (gnomAD) database to control for this background level of expected random mutations.

ZNRF3 (13/22), *SMARCA4* (12/22), *KMT2A* (12/22), *FLT4* (9/22), *POLD1* (9/22), *ATM* (13/22) and *KMT2D* (14/22).

Several recurring mutations were identified: 15/18 lesions had a p.G659fs deletion in *RNF43* in a homopolymeric microsatellite (7xG), 4 lesions had a p.R117fs deletion in *RNF43* in a homopolymeric microsatellite (6xG), 17/18 samples had a p.K437fs deletion in *ACVR2A* in homopolymeric microsatellite (8xA) and three lesions had a p.P773fs deletion in *KMT2A* in homopolymeric microsatellite 7xG.

Tumour mutational burden (TMB)

The median TMB was substantially higher for MLH1 deficient lesions (23.9) than for MLH1 proficient lesions (6.27, p<.001). This corresponded with a higher number of mutations across all mutation types (i.e. insertions, deletions, missense, nonsense and splice site mutations; Supplementary table 2). Compared to reference cohorts of the Cancer Genome Atlas (TCGA), MLH1 deficient lesions had higher TMB than the included reference cohorts (Figure 4A), and ranked among the top-5 highest TMB of 166 cancer subtypes based

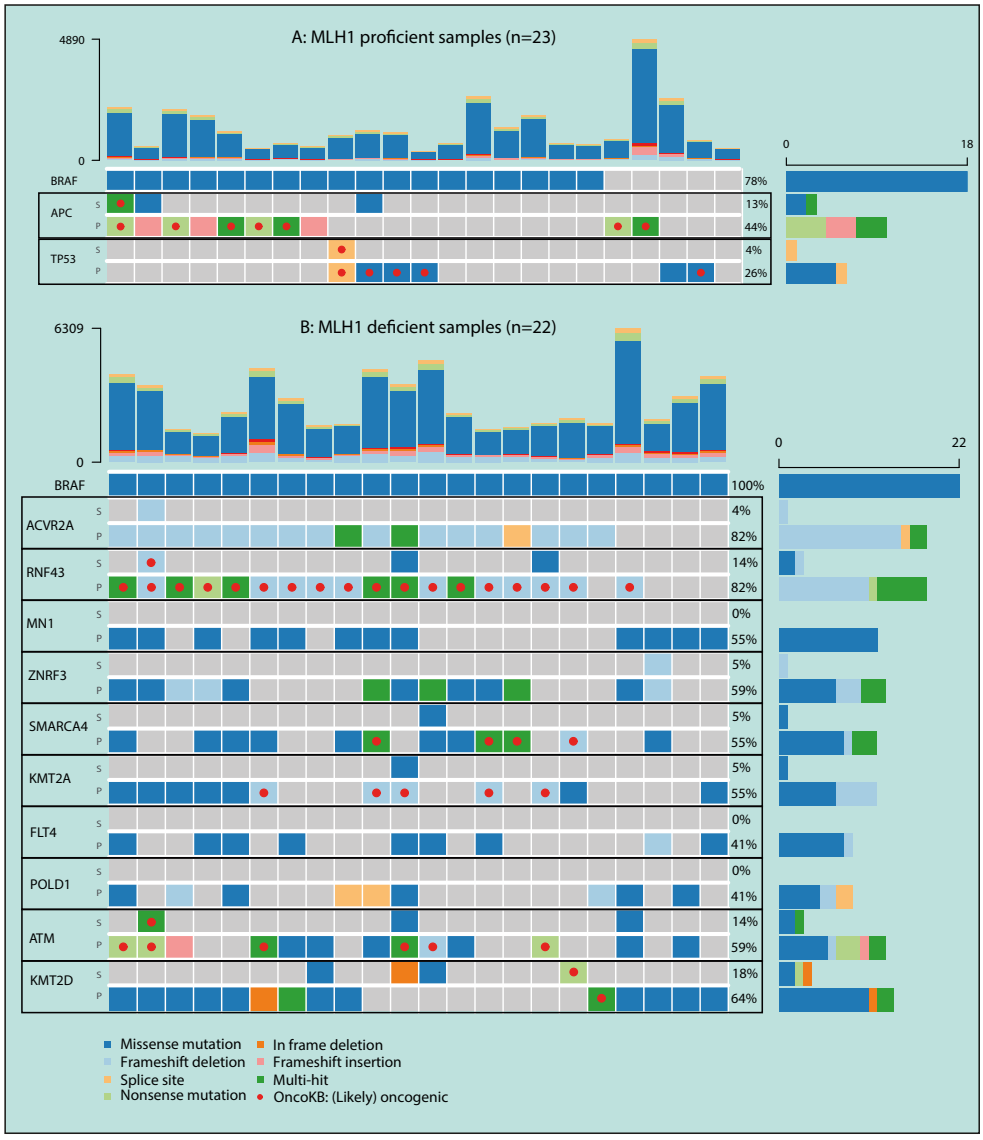

Figure 3. Oncoplots of differentially altered genes ($p<0.1$) in progressed vs. non-progressed component. A: MLH1-proficient lesions; B: MLH1-deficient lesions. For each gene, the top row displays mutations in the serrated component ('S'), while the bottom row displays mutations in the progressed component ('P') of each lesion. Each column corresponds to one lesion.

on a comprehensive analysis of over 100,000 human tumors.[34] In our samples, TMB was not associated with grade of dysplasia (LGD, HGD or CRC) or size of the progressed component (Figure 4 B&C).

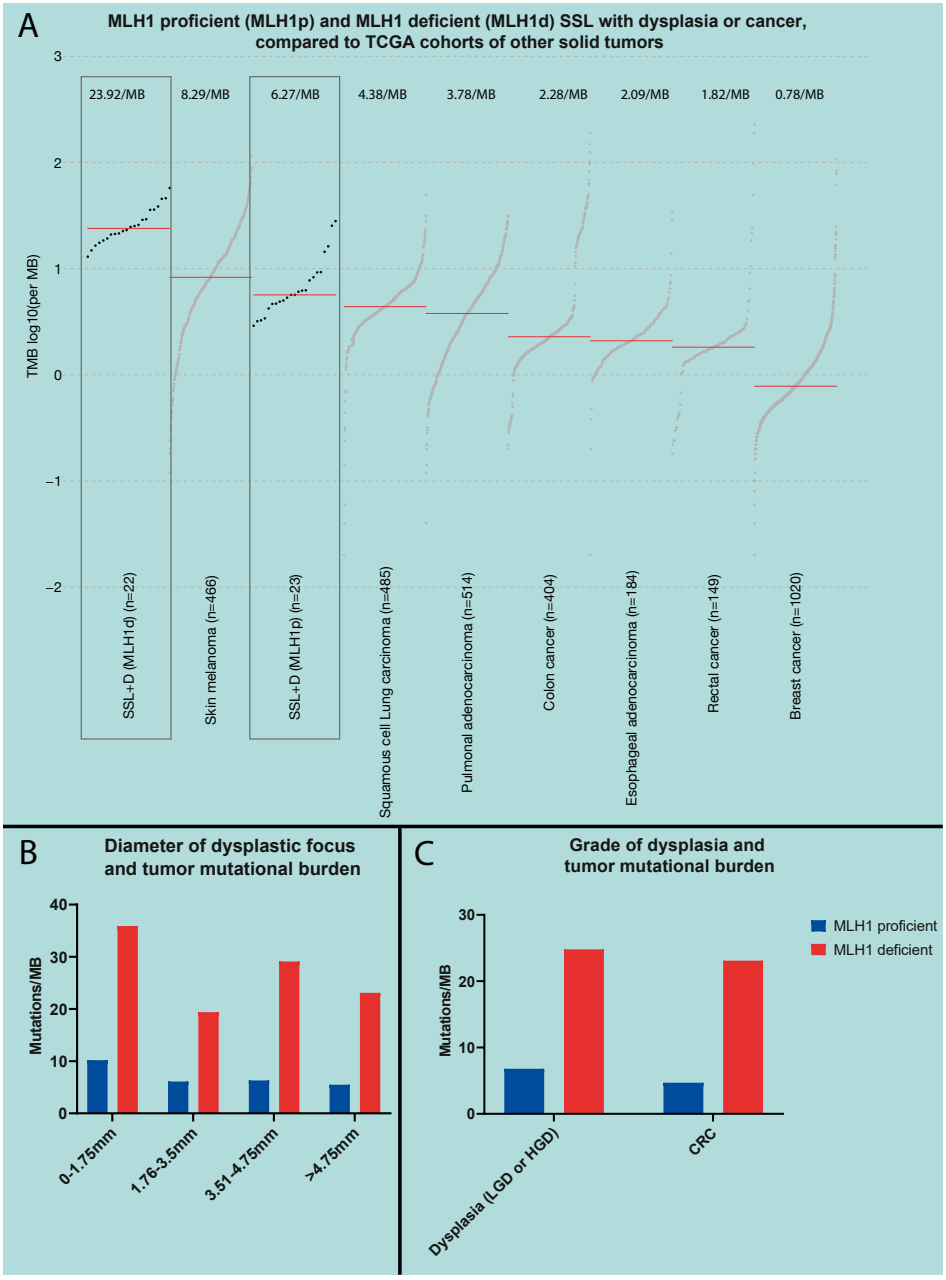

Figure 4. Tumour mutational burden. A: MLH1 proficient (MLH1p) and MLH1 deficient (MLH1d) SSL with dysplasia or cancer, compared to TCGA cohorts of other solid tumours; B: Stratified by diameter of progressed component; C: stratified by grade of dysplasia (LGD/HGD vs. CRC)

Mutational signatures

We identified three distinct mutational signatures, which were enriched in 14 samples (signature 1), 17 samples (signature 2) and 14 samples (signature 3; [Table 3]).

Signature 1 in our samples was most similar to COSMIC signature SBS6, with a cosine similarity score of 0.96. This signature is thought to arise due to defective DNA mismatch repair.[31] All but one (92.8%) of the lesions with this signature were indeed deficient in mismatch repair gene *MLH1* deficient, and were also classified as MSI.

Seventeen samples were enriched for signature 2, which was most similar to COSMIC signature SBS3 with a cosine similarity score of 0.82. The proposed aetiology of this signature is deficiency in DNA double stranded break repair by homologous recombination (HR), known as HR deficiency.[30, 31, 35, 36]

Finally, 14 samples were enriched for signature 3, which was most similar to COSMIC signature SBS29 with a cosine similarity score of 0.85. This signature has a putative aetiology of tobacco chewing habit when found in oropharyngeal carcinomas, but is of uncertain aetiology in other types of cancer.[30]

Table 3. Mutational signatures. Three clusters of mutational signatures were identified in our data (signature 1-3). These were compared to the COSMIC database of mutational signatures.31 Signature 1, 2 and 3 were most similar to COSMIC signature 6, 3 and 29, respectively.

	Signature 1 (n=14)	Signature 2 (n=17)	Signature 3 (n=14)
Most consistent with COSMIC signature	SBS6 - Cosine similarity 0.96	SBS3 - cosine similarity 0.82	SBS29 - cosine similarity 0.85
Proposed aetiology	Mismatch repair deficiency	Deficiency in double-stranded DNA break repair by homologous recombination	Unknown
MLH1 proficiency			
MLH1 proficient	1 (7.1%)	14 (82.3%)	8 (57.1%)
MLH1 deficient	13(92.8%)	3 (17.6%)	6 (42.9%)
TMB, Median (IQR)	21 (16.9-26.16)	6.27 (5.0-12.8)	22.0 (9.8-36.6)
Age, median (IQR)	65.5 (63-72.3)	65 (60.5-69.5)	67 (56.5-71.5)
Female gender, n (%)	12 (85.7%)	12 (70.6%)	11 (78.6%)
Location			
Proximal to descending colon	12 (92.3%)	10 (66.7%)	8 (72.7%)
Distal to or in descending colon	1 (7.7%)	5 (33.3%)	3 (27.3%)
Missing	*1*	*2*	*3*
Smoking history			
Current/previous smoker	7 (53.8%)	6 (46.2%)	4 (36.4%)
Never smoker	6 (46.2%)	7 (53.8%)	7 (63.6%)
Missing	*1*	*4*	3

The most prominent clinicopathologic differences were seen between lesions enriched for signature 1 vs signature 2 (Table 3). Signature 1 lesions were almost exclusively (93%) MLH1 deficient, while signature 2 lesions were almost exclusively (82%) MLH1 proficient. Lesions enriched for signature 1 had a higher TMB (median 21 vs. 6.27, p=.002), and showed a nonsignificant trend for proximal location (92.3% vs. 66.7% proximal, p=.099) and female gender (85.7% vs. 70.6%, p=.32).

DISCUSSION

In this study we aimed to identify the earliest driver events that are involved in the sudden progression of SSLs into dysplasia or carcinoma, both for the MLH1 *proficient* as well as the MLH1 *deficient* serrated pathway. To this end we analysed clinical and mutational characteristics of a unique cohort 45 SSLs with a small focus of dysplasia or early cancer. We were able to identify novel driver events, distinct mutational signatures and differences in clinical characteristics between MLH1 proficient and MLH1 deficient lesions.

Firstly, clinicopathologic characteristics suggested that SSLs occurring in the distal colon, in male patients and those in the absence of comorbid serrated polyposis syndrome, had the highest chance of developing via the MLH1 proficient pathway to dysplasia or early cancer. These findings are consistent with a recent series of 137 SSLs with a focus of dysplasia or cancer and a recent series of 77 *BRAF*^{mut}/MSI and 69 *BRAF*^{mut}/MSS CRCs.[5, 18]

Our whole exome analyses revealed several possible driver genes in the malignant progression of SSLs, which differed substantially between the MLH1 proficient and deficient pathway. P53 was found to be recurrently affected in MLH1 proficient lesions, based on both immunohistochemical as well as mutation analyses. This complies with a recent study that showed that *TP53* mutations were found in 41% of *BRAF*^{mut}/MSS CRC, compared to only 17% of *BRAF*^{mut}/MSI CRC[18]. Also, Bettington *et al*.[5] recently found aberrant p53 staining predominantly in mismatch repair proficient dysplastic SSLs. In addition, we found *APC* to be mutated in 44% of MLH1 proficient SSLs, which suggests that WNT-pathway activation occurs via *APC* mutations in the MLH1 proficient pathway (Table 2 & Figure 3).

Interestingly, neither *TP53* nor *APC* were found as possible drivers in MLH1 deficient lesions. Due to their defective DNA mismatch repair machinery, MLH1 deficient lesions were without exception microsatellite instable and had a much higher number of affected cancer related driver genes (n=34, Table 2 & Figure 3), illustrating how MLH1 loss causes rapid accumulation of hazardous mutations even in these early lesions. To further highlight the impressive magnitude of genomic instability in these early lesions measuring on average only 3,5mm in diameter: the observed median mutational burden of 23.9/MB corresponds to roughly 150 thousand unique somatic mutations genome-wide, based on the (diploid) genomic length of 6.2 billion basepairs.[37] In comparison, healthy colonic stem cells accumulate only ±36 somatic mutations per life year.[38]

This genetic instability most notably manifested with mutations in *RNF43* and *ACVR2A*, which occurred in the progressed component of almost all MLH1 deficient lesions.

Moreover, these mutations were clearly clustered in two hotspots in *RNF43* (p.G658fs and p.R117fs) and one hotspot in *ACVR2A* (p.K437fs), all of which were located in homopolymeric microsatellites. This suggests that specific homopolymeric microsatellites make *RNF43* and *ACVR2A* extremely prone of becoming mutated quickly after MLH1 is lost. Since we included only SSLs that have just progressed into dysplasia or carcinoma (as implicated by the small size of the dysplastic component), it is plausible that *RNF43* and *ACVR2A* mutations are among the first tumour suppressor genes to be affected in the formation of dysplasia in the MLH1 deficient pathway.

Although the role of *RNF43* in the serrated pathway was recently suggested by Bond *et al*.[39], *ACVR2A* mutations have, to the best of our knowledge, never been implicated to play a role in serrated carcinogenesis. However, previous studies provide evidence that these specific hotspot mutations in *ACVR2A* and *RNF43* indeed play a major role in development of MSI cancer. Maruvka recently found in TCGA data that 80% of human MSI cancers harboured a p.K437fs *ACVR2A* mutation, which was found in 77% of our lesions, while 40% of human MSI cancers harboured the p.G659fs *RNF43* mutation that we identified in 68% of our lesions.[40] Moreover, frameshift *ACVR2A* mutations in full-blown MSI CRC have been demonstrated to cause loss of ACVR2A expression[41], which is linked to poor prognosis and metastatic potential of CRC.[42] Interestingly, although *ACVR2A* is involved in the TGFß pathway, our SMAD4 immunohistochemistry did not suggest frequent dysfunction of the TGFß pathway in MLH1 deficient lesions. This might be explained by the fact that loss of ACVR2A loss leads to upregulation of TGFß receptor II, which actually *increases* SMAD4 expression.[43, 44]

Another interesting finding was the co-occurrence of *ZNRF3* mutations which occurred almost exclusively in lesions with *RNF43* mutations. *RNF43* and *ZNRF3* act together in a complex as critical negative feedback regulator of WNT-signaling.[45-47] Tumours with mutations in this *ZNRF3/RNF43* complex are, due to decreased WNT-receptor degradation, hypersensitive to paracrine and autocrine WNT-signaling.[47] WNT-signalling activation can be caused by either mutation independently, but combined knock-out of both enzymes led to rapid formation of intestinal adenomas in a murine model.[46] Based on our results, it seems plausible that SSLs acquire WNT-pathway activation through combined *RNF43* and *ZNRF3* mutations. Tumours with *RNF43/ZNRF3* mutations have been implicated as exceptionally sensitive to upstream WNT inhibitors such as porcupine inhibitors, due to their extreme dependence on WNT signaling.[45] Indeed, an ongoing clinical trial is studying the anti-tumour activity of porcupine inhibitor WNT974 in patients with $BRAF^{mut}/RNF43^{mut}$ CRCs (ClinicalTrials.gov, NCT02278133), which have almost certainly progressed through the MLH1 deficient serrated pathway.

These differences in driver mutations corresponded with distinct mutational signatures in MLH1 proficient and deficient lesions (Table 3).[30] Three signatures were found in our samples, with two of these effectively separating MLH1 proficient from MLH1 deficient lesions. As expected, MLH1 deficient lesions predominantly resembled COSMIC signature SBS6, which is caused by mismatch repair deficiency. [31, 48] More interestingly, however,

the second signature resembling the COSMIC signature SBS3 was found almost exclusively in MLH1 proficient SSLs. The putative aetiology of this signature is defective repair of double stranded DNA breaks by homologous recombination proteins, such as *BRCA1* or *BRCA2*. We did not find *BRCA1* or *BRCA2* to be recurrently mutated in these lesions, nor did we find convincing evidence of other affected genes involved in HR repair (e.g. RAD51 genes). This could mean that, although *resembling* tumours with defective HR repair, MLH1 proficient lesions have other underlying mutagenic mechanisms. This is in line with previous studies, in which a number of cancers with this signature lack mutations genes known to be involved in double-strand break repair.[36] Alternatively and not unlikely, however, epigenetic events such as promoter methylation, which occurs abundantly in SSLs as part of CIMP, could also lead to loss of genes involved in double-strand break repair. Indeed, epigenetic knockout of such genes has been extensively described in other types of cancer.[49-52] If this signature were to be validated in future studies in advanced *BRAF*^{mut}/MSS CRCs, it might have important therapeutic implications. Tumours with signature SBS3 have been demonstrated to show response to platinum-based chemotherapy in various cancer types, and is even used as independent predictor of response to platinum-based therapy in patients with pancreas carcinoma. [53, 54] It deserves notice, however, that COSMIC signature 3 was found in only a very small subset of CRCs according to Alexandrov *et al*.[30], which urges validation of our findings in future studies.

Our results have to be interpreted with prudence, and several limitations have to be considered. First of all, we included only 45 lesions, divided into two small subgroups. This makes our cohort relatively small, and not powered to identify more subtle differences between MLH1 proficient and deficient lesions. Such small sample size also increases the risk of false-discovery, especially in a setting of massively parallel sequencing. To reduce this risk we therefore limited our analyses to 724 known cancer related genes (cancer gene census genes) that were mutated in at least 5 samples. In addition, massively parallel sequencing of FFPE material might lead to an increased number of sequence artefacts due to e.g. formaldehyde-induced crosslinks and DNA fragmentation.[55] Although we cannot rule out that some of our results are the result of such sequence artefacts, the quality (i.e. GQN score) of our FFPE derived DNA was relatively high. Equally reassuring was the fact that many of our results were in line with previous studies. Moreover, WES analyses of matched fresh-frozen and FFPE derived tumour DNA showed high concordance in multiple previous studies. [56-58] Finally, although many of our findings showed high agreement with previously published literature, several novel findings warrant further validation in future studies. Especially the effect of the potential driver mutations on cancer signalling pathways should be assessed in experimental settings. The xenotransplant murine model using CRISPR/CAS9 to genetically engineer serrated organoids that was recently described by Lannagan *et al*. seems to be a very promising model in this regard.[59, 60] Furthermore, the role of COSMIC signature SBS3 should be re-evaluated in other cohorts of advanced MLH1 proficient SSLs or *BRAF*^{mut}/MSS CRC.

In conclusion, our results provide novel insights in the very first driver events that are involved in the malignant progression of SSLs. We found driver hotspot mutations in *RNF43* and *ACVR2A* to be involved in the MLH1 deficient serrated pathway, while *APC* and *TP53* mutations were frequently observed in the MLH1 proficient pathway. We demonstrated that SSLs rapidly acquire extensive microsatellite instability and an exceptional mutational burden following MLH1 loss, even in the earliest stage of dysplasia. Finally, we identified COSMIC signature SBS3 in MLH1 proficient lesions. This signature could potentially be a biomarker for response to platinum-based therapy in advanced tumours deriving from such lesions, and might rely on epigenetic alterations that future studies will hopefully uncover.

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SUPPLEMENTARY DATA

Supplementary table 1A. Differentially altered CGC genes in MLH1 proficient lesions (n=2)

Hugo symbol	Mutations in nonprogressed component	Mutations in progressed component	p-value
APC	3 of 23	10 of 23	0,047175934
TP53	1 of 23	6 of 23	0,095916052

Supplementary table 1B. Differentially altered CGC genes in MLH1 deficient lesions (n=34)

Hugo symbol	Mutations in nonprogressed component	Mutations in progressed component	p-value
ACVR2A	1 of 22	18 of 22	2,31E-07
RNF43	3 of 22	18 of 22	1,16E-05
MN1	0 of 22	12 of 22	6,13E-05
ZNRF3	1 of 22	13 of 22	1,96E-04
SMARCA4	1 of 22	12 of 22	5,67E-04
KMT2A	1 of 22	12 of 22	5,67E-04
FLT4	0 of 22	9 of 22	1,40E-03
POLD1	0 of 22	9 of 22	1,40E-03
ATM	3 of 22	13 of 22	4,05E-03
KMT2D	4 of 22	14 of 22	5,09E-03
PBRM1	0 of 22	7 of 22	8,90E-03
IRS4	1 of 22	9 of 22	9,34E-03
CREBBP	2 of 22	10 of 22	1,57E-02
OLIG2	0 of 22	6 of 22	2,11E-02
BCORL1	1 of 22	8 of 22	2,12E-02
MUC4	11 of 22	19 of 22	2,17E-02
KMT2C	4 of 22	12 of 22	2,68E-02
SETD1B	7 of 22	15 of 22	3,37E-02
RNF213	7 of 22	15 of 22	3,37E-02
BRCA2	2 of 22	9 of 22	3,39E-02
NUMA1	2 of 22	9 of 22	3,39E-02
AXIN2	4 of 22	11 of 22	5,46E-02
CIC	4 of 22	11 of 22	5,46E-02
AR	2 of 22	8 of 22	6,89E-02
FAM47C	2 of 22	8 of 22	6,89E-02
EPAS1	1 of 22	6 of 22	9,46E-02
BAP1	1 of 22	6 of 22	9,46E-02
GRIN2A	1 of 22	6 of 22	9,46E-02
TNFAIP3	1 of 22	6 of 22	9,46E-02
BUB1B	1 of 22	6 of 22	9,46E-02
CDK12	1 of 22	6 of 22	9,46E-02

Supplementary table 1B. (continued)

Hugo symbol	Mutations in nonprogressed component	Mutations in progressed component	p-value
SETBP1	1 of 22	6 of 22	9,46E-02
ERBB2	1 of 22	6 of 22	9,46E-02
ERBB4	1 of 22	6 of 22	9,46E-02

Supplementary table 2. Tumour mutational burden and number of mutations in Cancer Gene Census genes, overall and stratified by MLH1 proficiency

	Overall (n=45)	MLH1-proficient (n=23)	MLH1-deficient (n=22)	P-Value*
Tumour mutational burden (mutations/MB)	13.2	6.27	23.9	<.001
Median number of mutations in CGC genes				
Total	72	30	106.5	<.001
Frameshift deletion	5	1	17	<.001
Frameshift insertion	3	1	6	<.001
In-frame deletion	1	0	2	.141
In-frame insertion	0	0	1	<.001
Missense mutation	56	26	72.5	<.001
Nonsense mutation	3	1	5	<.001
Splice site mutation	1	1	2	.002

*Mann Whitney U test

5

Personalised surveillance for serrated polyposis syndrome: Results from a prospective 5-year international cohort study

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ABSTRACT

Background and aims

Serrated polyposis syndrome (SPS) is associated with an increased risk of colorectal cancer (CRC). International guidelines recommend surveillance intervals of 1-2 years. However, yearly surveillance likely leads to overtreatment for many. We prospectively assessed a surveillance protocol aiming to safely reduce the burden of colonoscopies.

Methods

Between 2013 and 2018 we enrolled SPS patients from nine Dutch and Spanish hospitals. Patients were surveilled using a protocol appointing either a one or two year interval after each surveillance colonoscopy, based on polyp burden. Primary endpoint was the 5-year cumulative incidence of CRC and advanced neoplasia (AN) during surveillance.

Results

We followed 271 SPS patients for a median of 3.6 years. During surveillance, two patients developed CRC (cumulative 5-year incidence 1.3% (95%-CI 0-3.2%)). The 5-year AN incidence was 44% (95%-CI 37-52%), and was lower for patients with SPS type III (26%) than for patients diagnosed with type I (53%) or type I&III (59%, $p<0.001$). Most patients were recommended a 2-year interval, and those recommended a 2-year interval were not at increased risk of AN: AN incidence after a 2-year recommendation was 15.6% compared to 24.4% after a 1-year recommendation (OR 0.57, $p=0.08$).

Conclusion

Risk-stratification substantially reduced colonoscopy burden while achieving CRC incidence similar to previous studies. AN incidence is considerable in SPS patients, but extension of surveillance intervals was not associated with increased AN in those identified as low-risk by the protocol. We identified SPS type III patients as low-risk group that might benefit from even less frequent surveillance.

INTRODUCTION

Serrated polyposis syndrome (SPS) is characterized by the presence of numerous colonic serrated polyps (SPs), and is accompanied by a substantially increased colorectal cancer (CRC) risk.[1-4] Hence, close endoscopic surveillance is essential to prevent CRC development.[1, 2, 5] Although previously considered to be uncommon, recent evidence estimate a prevalence of up to 1:111 (0.9%) individuals in FOBT-based screening cohorts and up to 1:238 (0.42%) in primary screening cohorts fulfil the diagnostic criteria for SPS diagnosis when subsequent surveillance colonoscopies are taken into account, making SPS the most prevalent polyposis syndrome currently known.[6-8]

Since no genetic mutations have been identified to diagnose SPS, diagnosis is based on clinical criteria defined by the World Health Organization (WHO).[5] These include *(1) at least five SPs proximal to the sigmoid, with two or more of these being ≥10mm; (2) any number of SPs proximal to the sigmoid colon in an individual who has a first-degree relative with SPS; or (3) ≥20 SPs of any size, distributed throughout the colon.*

The prevalence of CRC in patients with SPS has been estimated to range between 15%-35%.[1, 2, 4] Two small cohorts reported a higher CRC incidence of 54-70%.[9, 10] Although the majority of CRCs in SPS patients occur prior to, or at the time of SPS diagnosis, there also seems to be an increased risk for CRC during surveillance. In three retrospective and one prospective cohorts, the cumulative 5-year incidence of CRC under endoscopic surveillance ranged between 0-7.0%.[1-4, 11] This has led to stringent surveillance recommendations world-wide, most of them recommending either annual colonoscopy or colonoscopy every 1-2 years.[12-15] For example, the United States Multi-Society taskforce on CRC (US-MSTF) recommends annual surveillance colonoscopies for all SPS patients.[12] Dutch and Spanish guidelines recommend surveillance every

1-2 years[13, 16], but in daily practice clinicians tend to stay on the safe side, with median surveillance intervals in recent cohort studies ranging between 1.1-1.3 years.[1-3] All guidelines mention the scarce evidence to support their stringent recommendations, and many authors have expressed the need for prospective evaluation of these surveillance recommendations.[1-3, 12]

The prevalence of SPS combined with the stringent surveillance regimens lead to substantial colonoscopy burden. Annual surveillance seems appropriate for some patients, but extension of surveillance intervals beyond one year might be safe for the majority. Unfortunately, current guidelines lack risk-stratification and are based on a one-size-fits-all principle.[12-15] Several recent studies suggest that CRC risk depends on patient-specific risk-factors such as a history of advanced SP, advanced adenomas or smoking history.[1-3] Ideally, such risk-factors would facilitate personalised risk-stratification and reduction of colonoscopy burden for patients at low risk of CRC. The importance of such risk-stratification has been advocated by several authors recently.[17, 18]

Therefore, the aim of the current study was to prospectively evaluate the safety and effectivity of a personalised surveillance protocol that uses individual patient characteristics to re-determine the optimal surveillance interval after each surveillance colonoscopy.

METHODS

Patients and study design

All patients fulfilling WHO SPS criterion I and/or III[5] were eligible for inclusion if they underwent endoscopic surveillance after successful clearing of all relevant polyps (see clearing phase below) between January 2013 and April 2018. To reflect a representative clinical setting, both SPS patients that already underwent surveillance before enrolment, as well as patients that had not undergone any surveillance before enrolment, could be included. Patients were recruited in three Spanish and six Dutch centres of expertise. Patients with a history of proctocolectomy or subtotal colectomy, with known CRC-related germline mutations (i.e. *GREM, PTEN, BMPR1A, SMAD4, ENG1,* (bi-allelic) *MutYH* or *APC*) or with inflammatory bowel disease were excluded. Individuals fulfilling only WHO criterion II were excluded from the current study because most experts agree that this criterion is insufficient for diagnosis of SPS, although literature supporting this claim is lacking. By excluding this group we aimed to prevent potential contamination of our cohort with a subgroup of patients with a different CRC risk. Moreover,

This was a prospective international multicentre cohort study. Because the surveillance protocol fell within the surveillance recommendations of Dutch and Spanish guidelines and data were collected as part of routine care, the IRB of the Academic Medical Centre Amsterdam decided that the study fell beyond the legislation regarding Medical Research Involving Human Subjects Act (WMO). Patients were notified about the protocol verbally. The study was registered on the publicly accessible Dutch Trial Register (www.trialregister.nl; trial-ID NTR4609). Data were collected and stored online using Castor EDC.[19]

Histopathologic evaluation

Tissue specimens were routinely processed by gastrointestinal pathologists. SPs were classified as hyperplastic polyp (HP), sessile serrated lesion (SSL, also known as sessile serrated adenoma (SSA) or sessile serrated polyp (SSP)) with or without dysplasia, or traditional serrated adenoma (TSA) based on the WHO classification for SP.[5, 20] Advanced adenomas were defined as adenomas ≥10 mm, with villous structure and/or with high-grade dysplasia. Advanced serrated polyps were defined as any SP >10 mm and/or with presence of dysplasia.

Surveillance protocol

The protocol can be divided into two phases: the clearing phase and the surveillance phase. Surveillance colonoscopies that were scheduled during the study and according to the following protocol will from hereon be referred to as *protocolized surveillance*. All surveillance that took place prior to study inclusion will from hereon be referred to as *non-protocolized surveillance*. A flow-chart of the protocol is presented in Figure 1.

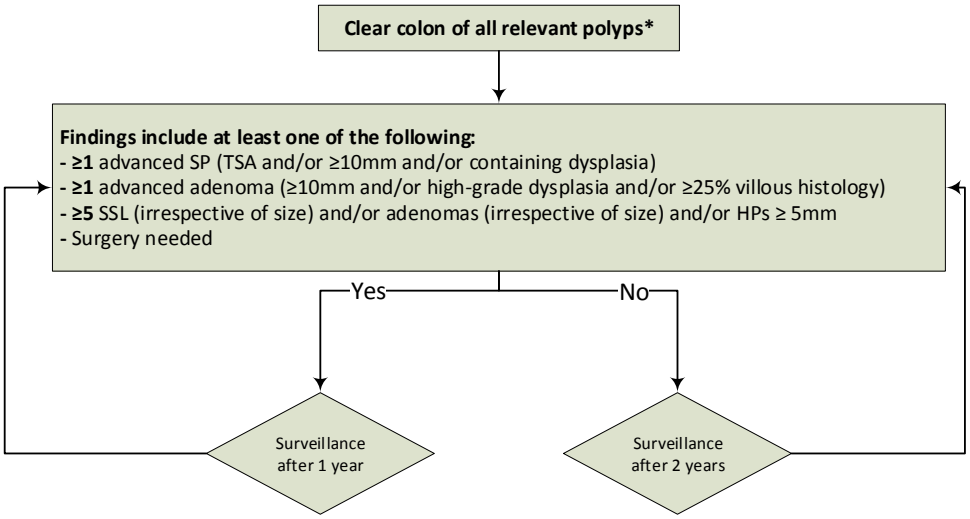

Figure 1. Flow-chart of surveillance protocol. ** Clear colon of all polyps ≥5mm, and all polyps <5mm with the optical aspect of adenoma TSA or SSL. Thus, HPs 1-4mm may be left in situ. Abbreviations: SP, serrated polyp; TSA, traditional serrated adenoma; SSL, sessile serrated lesion.*

Clearing phase

All patients underwent clearing with complete removal of all polyps ≥5mm, and all polyps <5mm with the optical aspect of an adenoma, SSL or TSA (thus, HPs 1-4mm could be left in situ). If endoscopic clearing was not possible during one procedure, colonoscopies were rescheduled within six months until all relevant polyps were removed. A new colonoscopy was also re-scheduled in case the cecum was not intubated and/or when the Boston Bowel Preparation Score (BBPS) was below 6. Patients that had been cleared before the start of this study could be included as well, in case the last recorded clearing colonoscopy was performed according to above mentioned quality criteria.

Surveillance phase

During surveillance, all polyps ≥5mm and all polyps <5mm with the optical aspect of an adenoma or SSLs were removed (HPs 1-4mm could be left in situ). Each surveillance colonoscopy was scheduled with an interval of either one or two years, based on the findings at the previous endoscopy (Figure 1). Thus, the recommended surveillance interval for each patient could vary based on the findings of each subsequent surveillance colonoscopy (Figure 1). In case of multiple colonoscopies in the same surveillance phase (e.g. because of poor bowel preparation, incomplete colonoscopy or multiple colonoscopies due to polyp burden), detected lesions from all colonoscopies were accumulated to determine the subsequent surveillance interval.

Patients were recommended a surveillance interval of one year in case one or more advanced SPs or adenomas were removed, if cumulatively ≥5 relevant polyps were removed (SSL (irrespective of size) and/or adenomas (irrespective of size), and/or HPs ≥5mm), or if surgery was needed during the last surveillance/clearing phase. In all other cases, a two year surveillance interval was recommended.

The criteria used in this protocol were chosen based on consensus of the authors, since no risk-factors had been established at the time this protocol was drafted in 2012.

Protocol violation

Protocol violation was defined as deviation from required surveillance interval of ≥365 days. In case of violation of the surveillance protocol, only the data prior to the protocol violation was used for our analyses, and the patient was censored at the moment the surveillance colonoscopy should have taken place.

Colonoscopy quality

All procedures were performed using standard or high-definition white light endoscopy at dedicated endoscopy programs. Advanced imaging techniques (e.g. (virtual) chromoendoscopy, Endocuff) were not routinely used, but could be used based on the preference of the endoscopist. Bowel preparation was performed according to local practice.

Study endpoints and statistical analyses

Primary endpoint was the cumulative 5-year incidence of CRC alone as well as of AN (CRC, advanced SP or advanced adenoma), calculated using Kaplan-Meier analyses. All risk factors identified in previous studies (smoking, WHO criteria, previously diagnosed with SPs proximal to the splenic flexure, SPs with dysplasia, advanced SPs, advanced adenomas, synchronous large SP and advanced adenoma, and age at SPS diagnosis) as well as history of CRC were assessed in univariate and multivariable (adjusted for age, gender and smoking status) Cox regression analysis and were presented as hazard ratio (HR) with 95% confidence interval (95%-CI). [1-3, 21] A multivariate model was used to perform multiple imputation to adjust for missing variables.

Secondary outcomes were the frequency of one and two year surveillance recommendation and the incidence of conversion to colorectal surgery during the surveillance phase. The risk of AN after a one-year recommendation was compared to the risk of AN after a two-year recommendation using logistical regression analysis, expressed as odds ratio (OR). Survival curves were produced using RStudio version 1.1.453 (Integrated Development for R. RStudio, Inc., Boston, MA, USA) with Survminer package version 0.4.3. All other analyses were performed using Statistical Package for Social Sciences (SPSS) version 24 (IBM, Somers, New York, USA).

RESULTS

Study flow and patient characteristics

Between January 2013 and April 2018, 554 SPS patients were identified in patient registries of the participating centres, and were assessed for eligibility (Figure 2). After review, 141 patients did not meet our in- and exclusion criteria and were thus excluded. Eighty-five patients had to be excluded because they were enrolled too late and thus their first surveillance was scheduled after study closure. Furthermore, patients were excluded because they did not receive any colonoscopy within the study timeframe (31), because of systematic protocol violation (22) or because their colon was not yet completely cleared (4).

The 271 included patients were followed for a median of 3.6 years (IQR 2.3-4.9 years). Mean age at the start of protocolized surveillance was 60 years (±10), and 130 (48%) were male (Table 1). At study inclusion, 99 patients (36.5%) fulfilled WHO criterion I; 99 (36.5%) fulfilled criterion III and 73 (27%) fulfilled criterion I & III. Prior to protocolized

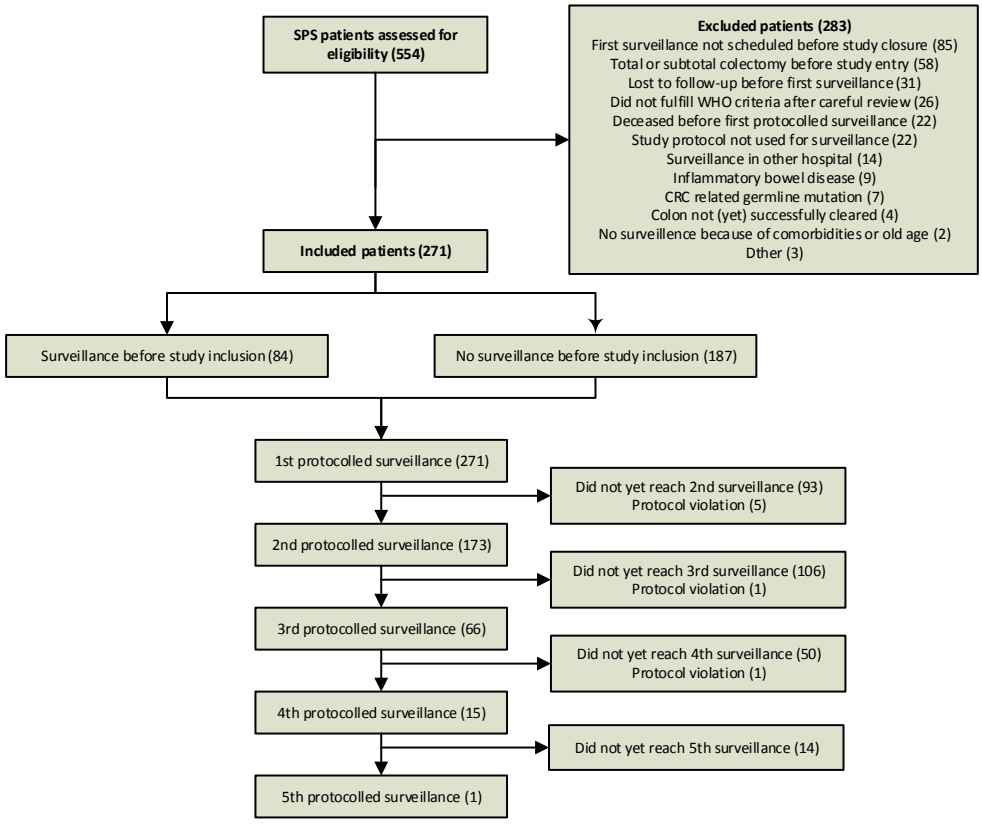

Figure 2. Flow-chart of study inclusions.

Table 1. Baseline characteristics for the 271 included patients

Age at diagnosis SPS, mean (SD)	60 (10)
Age at start of prospective follow-up, mean (SD)	62 (9)
Male, n (%)	130 (48%)
Reason first colonoscopy, n (%)	
FOBT-based screening	79 (29%)
Primary colonoscopy screening	14 (5.2%)
Familial CRC risk	36 (13%)
Symptoms	110 (41%)
Pain/discomfort abdomen	27/110 (25%)
Rectal blood loss	29/110 (26%)
Altered defecation pattern	29/110 (26%)
Anaemia	6/110 (5.5%)
Unexplained weight loss	2/110 (1.8%)
Other/unknown	32 (12%)
Family history	
≥1 FDR with CRC, n (%*)	85/250 (34%)*
≥1 FDR with SPS (WHO 1 and/or 3), n (%*)	11/256 (4.3%)*
WHO SPS classification at inclusion, n (%)	
I	99 (36.5%)
III	99 (36.5%)
I & III	73 (27%)
CRC prior to protocolized surveillance phase, n (%)	67 (25%)
Age at diagnosis, median (range)**	61 (19-79)
Multiple CRC, n (%)	16 (24%)
Synchronous	9/16 (56%)
Metachronous	6 /16 (37.5%)
Synchronous & metachronous	1/16 (6.2%)
Moment of CRC diagnosis, n (%)**	
Prior to clearing phase	40/67 (60%)
During clearing phase	27/67 (40%)
During surveillance, but prior to study inclusion	0
Total no. of colonoscopies prior to clearing	523
Per patient, median (range)	1 (0-15)
Total no. of clearing colonoscopies	543
Per patient, median (range)	2 (1-9)
Total no. of surveillance colonoscopies prior to inclusion	202
Per patient, median (range)	0 (0-7)
Total no. of prospective protocolized surveillance colonoscopies	570
Per patient, median (range)	2 (1-5)
Total years of prospective follow-up	942 patient-years
Per patient, median (IQR)	3.6 (2.3-4.9)

* Percentage refers to patients for whom variable was available

** in case patients had multiple (metachronous) CRCs, these values refer to the first CRC.

SPS, serrated polyposis syndrome; FOBT, faecal occult blood test; CRC, colorectal cancer; FDR, first-degree relative.

surveillance, 67 patients (25%) were diagnosed with CRC, of whom 9 had synchronous CRC, 6 had metachronous CRC and one had both synchronous and metachronous CRC prior to protocolized surveillance.

Of the 271 included patients, 84 (31%) had already undergone surveillance before inclusion while the remaining 69% underwent their first surveillance endoscopy during follow-up. The majority of patients received a two-year surveillance recommendation, which increased from 52% after the first surveillance colonoscopy to 71% after the third surveillance colonoscopy (Figure 3). A two-year interval in the first and second surveillance round was followed by another two-year interval in 79% and 87% of patients, respectively.

All patients underwent at least one protocolized surveillance colonoscopy, which was followed by a second, third, fourth and fifth surveillance colonoscopy in 173, 66, 15 and 1 patients, respectively. Protocol violation led to early drop-out in 7 patients. A detailed overview of relevant findings during surveillance colonoscopies is presented in Table 2.

CRC under protocolized surveillance

Two patients were diagnosed with CRC while under protocolized endoscopic surveillance, corresponding to a cumulative 5-year CRC incidence of 1.3% (95%-CI 0-3.2%, Figure 4-A).

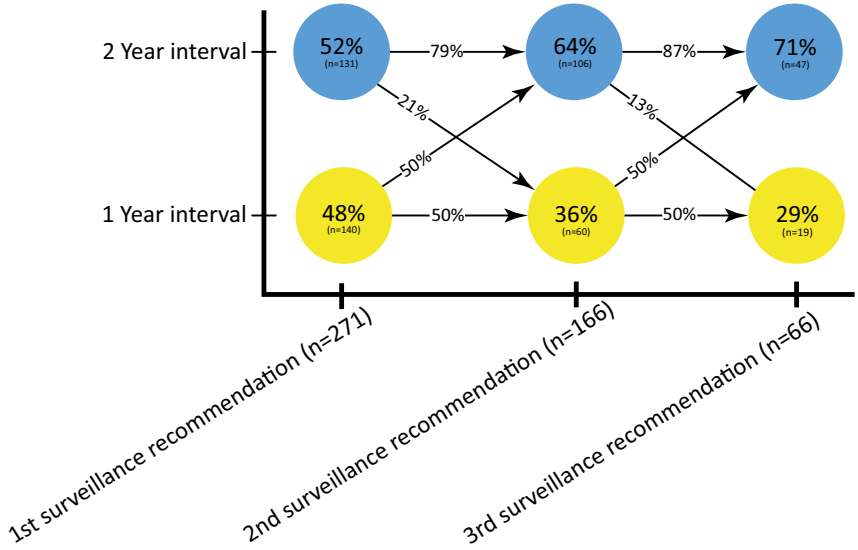

Figure 3. Dynamics of surveillance recommendation 1 year vs. 2 years in surveillance round 1-3. Circles represent the proportion of patients in surveillance round 1, 2 and 3 that received a 1 or 2 year surveillance recommendation. The arrows show the proportion of patients that are recommended the same (horizontal arrows) or a different (diagonal arrows) surveillance interval during subsequent round of surveillance. For example, 79% of the patients recommended a 2-year interval in round 1 are also recommended a 2-year interval in round 2.

Table 2. Prevalence and number of polyps removed prior to or during clearing, during surveillance prior to inclusion and during protocolised surveillance

	During/prior to clearing (n=271)	Surveillance prior to study inclusion (n=84)	Surveillance after study inclusion using personalized surveillance protocol				
			Surveillance 1 (n=271)	Surveillance 2 (n=173)	Surveillance 3 (n=66)	Surveillance 4 (n=15)	Surveillance 5 (n=1)***
Patients with CRC, n (%)	69 (25%)	0	0	1	1	0	0
Patients with ≥1 polyp	271 (100%)	81	214 (79%)	132 (76%)	56 (85%)	15 (100%)	1
No. of polyps per patient , median (range)	22 (1-157)	8 (0-39)	3 (0-19)	2 (0-20)	2 (0-13)	3 (1-9)	2
Patients with ≥1 advanced polyp*	237 (87%)	25 (30%)	73 (27%)	31 (18%)	12 (8%)	6 (40%)	0
Patients with at least one:							
Any SP	271 (100%)	78 (93%)	195 (72%)	121 (70%)	45 (68%)	13 (87%)	1
HP	249 (92%)	77 (92%)	148 (55%)	83 (48%)	25 (38%)	11 (73%)	0
SSL	215 (79%)	37 (44%)	106 (39%)	64 (37%)	27 (41%)	4 (27%)	0
TSA	15 (5.9%)	4 (5%)	1 (0.4%)	2 (1.2%)	1 (1.5%)	0	0
SP≥10mm	192 (71%)	13 (16%)	58 (21%)	28 (16%)	7 (11%)	4 (27%)	0
SP with dysplasia	61 (23%)	7 (8%)	7 (3%)	5 (2.9%)	5 (8%)	0	0
Median no. of SP per patient (range):							
Any SP	17 (1-116)	6 (0-31)	2 (0-17)	2 (0-20)	1.5 (0-13)	2 (0-8)	2
HP	9 (0-114)	4 (0-31)	1 (0-13)	0 (0-10)	0 (0-8)	1 (0-7)	0
SSL	4 (0-55)	0 (0-23)	0 (0-15)	0 (0-11)	0 (0-13)	0 (0-3)	0
TSA	0 (0-7)	0 (0-5)	0 (0-6)	0 (0-1)	0 (0-1)	0 (0-0)	0
SP≥10mm	2 (0-25)	0 (0-5)	0 (0-7)	0 (0-4)	0 (0-3)	0 (0-2)	0
SP with dysplasia	0 (0-14)	0 (0-5)	0 (0-6)	0 (0-2)	0 (0-3)	0 (0-0)	0
Patients with at least one adenoma:	216 (80%)	58 (69%)	113 (42%)	60 (35%)	21 (32%)	7 (47%)	0
Advanced adenoma **	131 (48%)	8 (9%)	20 (7.4%)	3 (2.3%)	1 (1.5%)	2 (13%)	0
Median no. of adenomas per patient	3 (0-41)	1.5 (0-16)	0 (0-10)	0 (0-8)	0 (0-8)	0 (0-4)	0
Advanced adenoma	0 (0-7)	0 (0-1)	0 (0-2)	0 (0-2)	0 (0-1)	0 (0-1)	0

* Advanced polyp: Advanced adenoma (≥25% villous histology, high-grade dysplasia, ≥10mm in diameter) / Advanced SP (TSA, diameter ≥10mm, presence of dysplasia)

** Advanced adenoma: ≥25% villous histology, high-grade dysplasia, ≥10mm in diameter.

*** This column represents only 1 patient. Therefore, no median or range is given.

CRC, colorectal cancer; SP, serrated polyp; HP, hyperplastic polyp; SSL, sessile serrated lesion; TSA, traditional serrated adenoma;

The CRCs occurred in surveillance phase 2 and 3, respectively. Detailed characteristics are presented in Supplementary Table 1. Briefly, the first CRC occurred 1 year after piecemeal removal of a TVA with HGD, and was preceded by a scar inspection 6 months earlier during which the scar could not be located. The second CRC occurred after a 2-year recommendation. It protruded from inside an anastomosis of an earlier right-sided hemicolectomy, and histologically and endoscopically appeared to be a local recurrence of a CRC that had been resected 6 years earlier.

Advanced neoplasia under protocolized surveillance

The cumulative 5-year incidence of AN during follow-up was 44% (95%CI 37-51%, Figure 4B). The risk of developing AN during follow-up was strongly associated with WHO subtype: 53% (95%CI 39-65%), 26% (95%CI 16-35%) and 59% (95%CI 40-72%) for WHO I, III and I&III, respectively. Compared to patients diagnosed with WHO I, patients fulfilling WHO III had a HR of 0.38 (95%CI 0.22-0.63, p <0.001) for developing AN. Adjusted for age at inclusion, smoking status and gender, the HR remained unchanged (Table 3 & Figure 4C). Patients fulfilling only WHO criterion I had the same risk as patients diagnosed with both WHO criteria I&III (HR 0.91, 95%CI 0.58-1.43, p=0.68).

None of the other previously identified CRC risk-factors (gender, smoking status, advanced adenoma, SP with dysplasia, advanced SP, large SP with synchronous advanced adenoma, SP proximal to the splenic flexure or previous history of CRC) were associated with the risk of developing AN during surveillance in univariate and multivariable analyses (Table 3 & Figure 4D).

Because patients that received surveillance prior to study inclusion might have a different risk of AN than those that received their first surveillance during our study, we performed additional sensitivity analyses. These showed that patients that had received one or more surveillance colonoscopy prior to study inclusion had a slightly lower risk of developing AN during our study than patients that received their first surveillance during our study (HR 0.64, 95%CI 0.41-0.99; p=0.047)

Advanced neoplasia after a one-year vs. two-year surveillance interval

In 255 occasions, a surveillance recommendation according to our protocol was followed by a subsequent surveillance endoscopy. AN was detected in 31 of 127 (24.4%) colonoscopies that were performed after a 1-year surveillance recommendation, compared to 20 of 128 (15.6%) colonoscopies performed after a 2-year surveillance recommendation (OR 0.57, 95%CI 0.31-1.07, p=.08). Incidence of other polyp subtypes after 1 vs. 2 year recommendation are displayed in in Supplementary table 2.

Surgery

During protocolized surveillance, surgery was required in three patients. The first patient underwent a laparotomy and adhesiolysis because of bowel obstruction due to previous

Table 3. Risk factors for time to advanced neoplasia during follow-up

	Advanced neoplasia during surveillance		Univariate HR (95%-CI)	P value	Multivariable HR †(95% CI)	P value
	No	Yes				
Gender, n (%)						
Male	83 (64%)	47 (36%)	1.00		1.00	
Female	90 (64%)	51 (36%)	0.95 (0.64-1.41)	0.81	0.99 (0.66-1.50)	0.97
Smoking status						
Never smoker	56 (70%)	24 (30%)	1.00		1.00	
Former smoker	46 (65%)	25 (35%)	1.22 (0.71-2.08)	0.48	1.20 (0.68-2.13)	0.52
Current smoker	58 (69%)	26 (31%)	1.10 (0.62-1.95)	0.75	1.09 (0.61-1.95)	0.76
WHO criterion at study inclusion*						
WHO I	56 (57%)	43 (43%)	1.00		1.00	
WHO III	77 (78%)	22 (22%)	0.38 (0.22-0.63)	<0.001	0.38 (0.22-0.64)	<0.001
WHO I & III	40 (55%)	33 (45%)	0.91 (0.58-1.43)	0.68	0.89 (0.56-1.40)	0.60
Age at SPS diagnosis			0.98 (0.96-1.01)	0.15	0.99 (0.96-1.01)	0.16
≥1 advanced adenomas**						
No	93 (66%)	47 (34%)	1.00		1.00	
Yes	80 (61%)	51 (39%)	1.21 (0.82-1.80)	0.34	1.24 (0.82-1.87)	0.31
≥1 advanced SP**						
No	45 (69%)	20 (31%)	1.00		1.00	
Yes	128 (62%)	78 (38%)	1.46 (0.89-2.39)	0.13	1.46 (0.89-2.40)	0.13
≥1 SP proximal to splenic flexure*						
No	27 (59%)	19 (41%)	1.00		1.00	
Yes	146 (65%)	79 (35%)	0.84 (0.51-1.38)	0.49	0.86 (0.51-1.43)	0.95
≥1 SP with dysplasia**						
No	131 (62%)	79 (38%)	1.00		1.00	
Yes	42 (69%)	19 (31%)	0.82 (0.50-1.36)	0.44	0.84 (0.51-1.39)	0.50
≥1 SP ≥10mm & ≥1 advanced adenoma **						
No	167 (65%)	91 (35%)	1.00		1.00	
Yes	6 (46%)	7 (54%)	1.270 (0.59-2.74)	0.54	1.21 (0.56-2.64)	0.63
CRC**						
No	131 (64%)	73 (36%)	1.00		1.00	
Yes	42 (63%)	25 (37%)	1.14 (0.74-1.80)	0.56	1.28 (0.80-2.07)	0.31

* WHO criterion I: ≥5 SP proximal to sigmoid colon with at least 2 being ≥10mm in diameter; WHO criterion III: ≥20 SP of any size, spread throughout the colon[5] ** Diagnosed prior to or during clearing phase †Adjusted for potential confounders age, gender and smoking status. WHO, World Health Organization; SPS, serrated polyposis syndrome; SP, serrated polyp

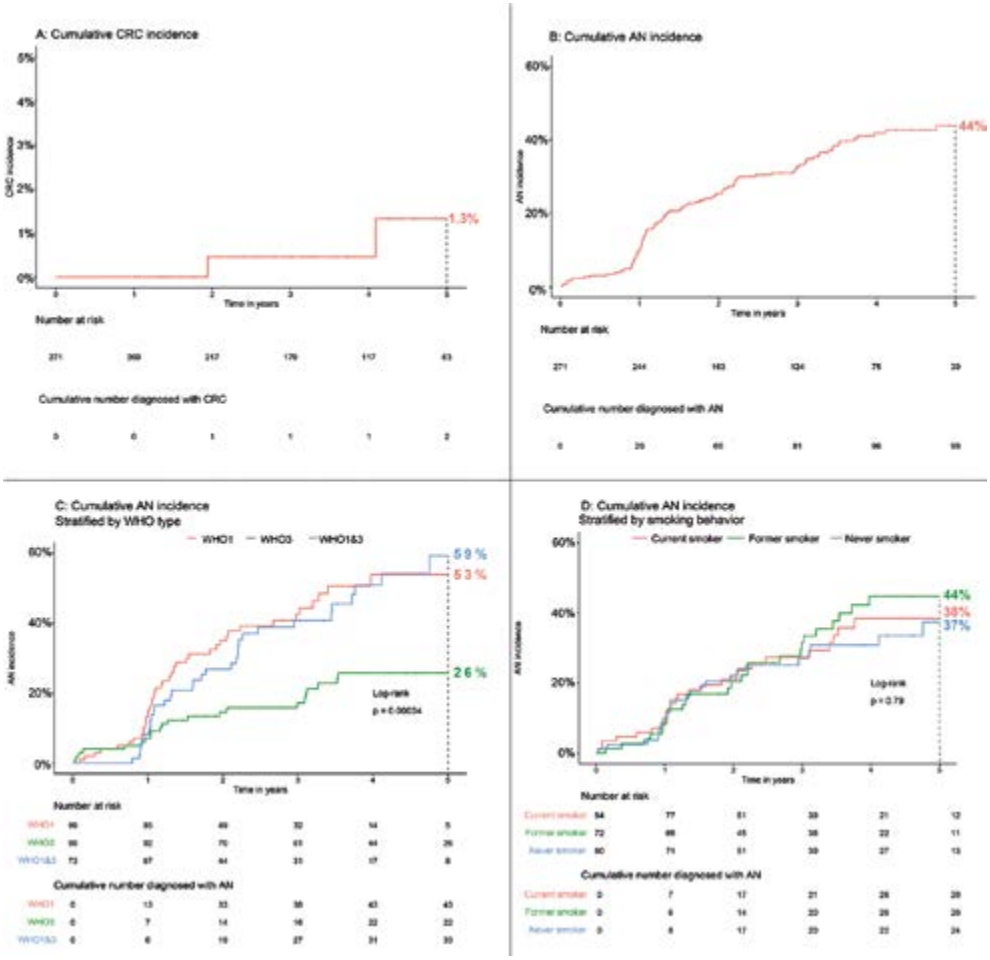

Figure 4. Cumulative 5-year incidence of CRC (A) and advanced neoplasia (B-D). The p-value displayed in panel C and D was calculated using a Log-Rank test, comparing all WHO subtypes (panel C) and smoking behaviour (panel D). *Abbreviations: CRC, colorectal cancer; AN, advanced neoplasia (defined as CRC, advanced adenoma or advanced SP)*

surgery. The other two patients underwent a proctocolectomy and subtotal colectomy, respectively, both because of CRC. Both had undergone a right-sided hemicolectomy for CRC in the past. No surgery was performed for the indication polyp burden or a single endoscopically unresectable polyp.

DISCUSSION

In this large prospective cohort study, we assessed the safety and effectivity of an individualized surveillance protocol for patients diagnosed with SPS. Our protocol showed

to be effective in decreasing colonoscopy burden while at the same achieving low incidence of CRC. During a median follow-up of 3.6 years, the cumulative 5-year incidence of CRC was 1.3%, which is similar to the incidence reported in recent studies,[1, 6, 22] and lower than incidence rates in two retrospective cohort studies.[3, 4] In comparison: this incidence rate is identical to that of a regular postpolypectomy population after resection of intermediate-risk adenomas.[23] The cumulative 5-year incidence of AN, on the other hand, was high (44%) and strongly associated with WHO subtype. Patients diagnosed with WHO criterion I or I&III were more than twice as likely to develop AN during surveillance compared to patients diagnosed with WHO criterion III only. The use of our protocol seems safe and effective with little need for surgical intervention. After three consecutive surveillance colonoscopies, two-thirds of the patients were appointed two-year surveillance intervals according to the protocol. The extended 2-year interval did not result in an increased incidence of AN. Indeed, a non-significant trend was seen for a lower risk of AN after a 2-year recommendation (OR 0.57, p=.08)

The high AN incidence confirms that close endoscopic surveillance of SPS patients is warranted.[3] However, we believe (more) stringent surveillance than the regimen described by our surveillance protocol will not further reduce CRC incidence. After all, one of the two CRCs that occurred during our study occurred after a surveillance recommendation of one year. The other CRC, occurring after a two-year recommendation, was a local recurrence protruding from inside the anastomosis. Although more stringent surveillance could have led to earlier detection of this recurrence, it would not have been prevented. Since none of the CRCs in our cohort can thus be attributed to the lengthened surveillance intervals, extension of the surveillance interval to two years based on our protocol seems safe and feasible. United States guidelines recommend annual surveillance[12], and although European guidelines generally recommend surveillance every 1-2 years, reported median surveillance intervals range from 1.1-1.3 years.[1-3] As our protocol predominantly led to a two-year surveillance recommendation, a substantial reduction in colonoscopy burden could be achieved compared to current practice. As a comparison: in case annual surveillance would have been provided in our cohort, about 942 colonoscopies would have been performed during the 942 patient-years of prospective follow-up. Using our protocol however, only 570 colonoscopies were performed, which means about 372 (39%) fewer colonoscopies were performed using our protocol compared to annual surveillance. Further improvement of risk-stratification should aim to further reduce colonoscopy burden without increasing AN risk. However, no 'acceptable' incidence of AN per surveillance colonoscopy has been established for SPS patients. Although our study might provide benchmarks in this regard, the incidence rate that should trigger early surveillance can be debated. Some might argue that surveillance intervals can be extended up until a point where SPS patients have an AN incidence of 30% or even 40%, whereas others would rather shorten surveillance intervals until AN incidence would be below 15%. In our study we found an AN incidence of 24.4% following a 1-year interval and 15.6% after a 2-year interval. Comparably, a recent large retrospective cohort study recently reported an

average AN incidence of 24% per surveillance colonoscopy.[3] Taken together, this suggests AN incidence rates of 20-25% per surveillance colonoscopy might be inevitable.

To further improve risk stratification, we assessed risk-factors that have previously been identified (smoking status, age at inclusion, fulfilment of WHO criterion I & III, previous diagnosis of an advanced adenoma, advanced SP, synchronous large SP and advanced adenoma, SP proximal to the splenic flexure or an SP with dysplasia).[1-3, 21] Fulfilment of WHO criterion III was inversely associated with a patient's risk of AN during follow-up. This association was remarkably strong, and might suggest that surveillance intervals can be extended for patients that solely fulfil WHO criterion III. None of other risk-factors were associated with AN during surveillance in our cohort. This could be explained by the fact that the studies that initially identified these risk-factors assessed their association with a history of CRC. Almost all CRCs included in those analyses occurred *prior to surveillance*. In contrast, in our study we assessed the association between these risk-factors and the risk of AN *during surveillance*. The incongruence between our and previous findings suggests that risk-factors associated with CRC before surveillance are not necessarily applicable for the risk of developing AN during surveillance. However, although our cohort is large compared to other studies on SPS it is still relatively small, and perhaps was not powered to detect subtle correlations between the assessed risk-factors and AN.

We believe our study has several strengths. First of all, we describe the largest prospective cohort of SPS patients to date, and this study was the first to assess a personalised surveillance protocol for SPS patients, allowing risk stratification for a mixed population with a variable CRC risk. As surveillance guidelines are currently based on low-quality evidence, many authors have expressed the urge of such prospective evaluation of surveillance.[1-3, 12] Our study now provides an evidence-based surveillance strategy for SPS patients which could be of help to establish future surveillance guidelines. The international multicentre design, as well as the fact that both academic and non-academic centres were included, warrants a good external validity. Nonetheless, several limitations have to be acknowledged as well. First, our median follow-up duration of 3.6 years might be considered short to monitor CRC occurrence. Longer follow-up with more rounds of surveillance are needed to assess the long-term CRC incidence. Second, with only two cases of CRC during surveillance, we were not able to assess risk factors for the development of CRC during surveillance because the sample size required for such analyses would be a multitude of our study population. Nonetheless, we were able to evaluate risk factors for AN during follow-up, which is likely to be strongly associated with one's risk of developing CRC. Third, some patients received only one protocolized surveillance colonoscopy, whereas others received five. The more colonoscopies a patient underwent during the 5-year study period, the more likely it is that he or she received several one-year surveillance recommendations instead of two-year surveillance recommendations, owing to advanced polyps or a high number of non-advanced polyps. Therefore, the patients that underwent colonoscopies in surveillance phase 3, 4 or 5 are predominantly high-risk patients that have a tendency to harbour advanced findings. The described prevalence of (advanced)

polyps in surveillance phase 3 to 5 (table 2) might therefore be inflated due to confounder by indication. However, because our survival analyses were not influenced by the number of surveillance colonoscopies, our main outcome measures were not affected by such bias. Finally, some of the patients enrolled in our study (31%) had already received one or more surveillance colonoscopies prior to study inclusion. This is relevant, since AN incidence seemed to decrease after several rounds of surveillance, which can be seen in Table 2 and Figure 3B. Patients that already underwent surveillance prior to study inclusion could therefore be expected to have a lower AN incidence than patients that underwent their first surveillance as part of the study. This effect was confirmed in a sensitivity analysis: patients with surveillance prior to study inclusion were at lower risk of AN than patients without surveillance prior to study inclusion (HR 0.64, 95%CI 0.41-0.99, p=0.047). Since the majority (69%) of our cohort did not undergo surveillance prior to inclusion we expect this effect has not substantially influenced our results. Nonetheless, a slightly higher AN incidence than reported here might be expected in a population without previous surveillance.

All in all, our results suggest that the proposed surveillance protocol can safely be implemented for SPS patients without risking an increase in CRC incidence. Future studies should aim to reduce the colonoscopy burden in true low-risk patients even further. Since a 2-year recommendation was not associated increased AN incidence, perhaps further extension beyond 2 years might be safe for these patients as well. Our results furthermore indicate that patients that only fulfil WHO criterion III might be a group of low-risk, and possibly a 3- or even a 5-year surveillance interval might be safe for this group. However, it is important to note that SPS patients are a group of high-risk patients, and an extension of surveillance intervals from the currently studied protocol should be done with caution and be part of prospective studies in expert centres.

In conclusion, in this largest prospective cohort of SPS patients to date we present and assessed a new personalised surveillance strategy for the treatment and surveillance of this prevalent disease. While achieving a substantial reduction of colonoscopy burden, CRC incidence in our cohort was similar to most previous reported incidence rates. Our protocol will likely lead to substantial reduction in colonoscopy burden. Future efforts will be made to further reduce colonoscopy burden, especially for patients only fulfilling WHO criterion III.

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6

Low incidence of advanced neoplasia in serrated polyposis syndrome after (sub)total colectomy – results of a 5-year international prospective cohort study

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ABSTRACT

Background

Serrated polyposis syndrome (SPS) is accompanied by a substantially increased colorectal cancer (CRC) risk. To prevent or treat CRC in patients with a very high polyp burden, (sub)total colectomy with ileorectal or ileosigmoidal anastomosis is regularly performed. The CRC-risk after (sub)total colectomy might be decreased, but evidence is lacking. We aimed to assess the yield of endoscopic surveillance in SPS patients that underwent (sub) total colectomy.

Methods

For this post-hoc analysis we used prospectively collected data from a large international prospective cohort study. We included patients diagnosed with SPS (WHO type I and/ or III) that underwent (sub)total colectomy. Primary endpoint was the cumulative 5-year incidence of CRC and advanced neoplasia (AN).

Results

Forty-eight patients (mean age 61 (±7.8); 52% male) were included and followed for a median of 4.7 years (interquartile range 4.7-5.1). None of the patients developed CRC during follow-up. Five patients developed AN, corresponding to a cumulative 5-year AN incidence of 13% (95%-CI 1.2-23). In four patients AN was diagnosed at the first surveillance endoscopy after study inclusion, and in one AN was detected during subsequent rounds of surveillance. The risk of AN was similar for patients with ileorectal and ileosigmoidal anastomosis (logrank p=0.83).

Conclusion

(Sub)total colectomy mitigates much of the excess risk of CRC in SPS patients. Advanced neoplasms are mainly detected at the first endoscopy after (sub)total colectomy. Based on these results, after the first surveillance, intervals might be extended beyond the currently recommended 1-2 years.

INTRODUCTION

Serrated polyposis syndrome (SPS) is characterized by the presence of a high number of serrated polyps (SPs) in the colon. With a prevalence of up to 1:111 in FOBT-based screening populations, it is by far the most common colonic polyposis syndrome.[1, 2] SPS is associated with a substantial risk of colorectal cancer (CRC).[3-7] Since no genetic cause for SPS has been identified yet, its diagnosis is based on clinical criteria, defined by the World Health Organization (WHO).[8] These criteria currently include (I) at least five SP proximal to the sigmoid, with two or more of these being ≥10mm; (II) any number of SP proximal to the sigmoid colon in an individual who has a first-degree relative with SPS; or (III) >20 SPs of any size, distributed throughout the colon.

Up to 35% of SPS patients have a personal history of CRC.[3, 4] Since most CRCs in SPS patients occur before or at the time of SPS diagnosis, CRC is almost always the result of undiagnosed and/or untreated or insufficiently treated SPs.[3, 4] In contrast, if SPS patients are adequately treated and systematically surveilled with colonoscopies, the incidence of CRC seems to be low according to the most recently published cohort studies.[3, 4, 9,]

To prevent CRC, the colon of SPS patients should be cleared from pre-malignant polyps. Once cleared of such polyps, endoscopic surveillance is indicated.[5, 9-11] In most patients clearance can be achieved endoscopically, without prophylactic surgery. However, a combination of excessive polyp burden, CRC or unresectable polyps can demand surgical resection of the affected bowel segments.

In two recent cohort studies, 24-27% of SPS patients had undergone surgical intervention during the clearing phase, mostly due to CRC (59-60%) and a high polyp burden (31-41%). In one cohort, 35 of the 79 patients that required surgery underwent a total colectomy with ileorectal anastomosis, which was most often performed because of severe polyposis.[3]

Thus, (sub)total colectomies are commonly performed in the management of SPS. However, the optimal endoscopic surveillance regimen after such extensive surgery is unknown. Although patients with only a short length of rectosigmoid left in situ might not be at increased risk of developing CRC anymore, international surveillance guidelines recommend the same stringent surveillance intervals for patients with or without a history of (sub)total colectomy.[10, 12-15] We hypothesize that SPS patients with a short length of rectosigmoid left in situ are at low risk of developing CRC or advanced polyps during endoscopic surveillance. In a prospective international cohort study we evaluated the yield of endoscopic surveillance in SPS patients after (sub)total colectomy. In addition, we assessed whether the location of the anastomosis (ileorectal or ileosigmoidal) was related to the risk of subsequent AN.

METHODS

Study population and design

This was a post-hoc analysis of a prospective cohort study that was registered on the Dutch Trial Register (www.trialregister.nl; trial-ID 4609). All patients aged 18 years

or older, fulfilling WHO SPS criteria I and/or III[8] were eligible for inclusion. SPS patients were recruited between January 2013 and April 2018. For the current study, only patients with a history of (sub)total colectomy with ileorectal or ileosigmoidal anastomosis that underwent postoperative surveillance were included in the analyses. Patient with SPS that were diagnosed before the start of this study could also be included. For these patients, only the surveillance data prospectively obtained within the timeframe of this study were taken into account for analysis. Patients with CRC-related germline mutations or inflammatory bowel disease (IBD) were excluded.

The Institutional Review Board (IRB) of the AMC decided that this study did not fall under the legislation regarding Medical Research Involving Human Subjects ACT (WMO). Data were collected and stored online using Castor EDC (www.castoredc.com).

Histopathologic evaluation

All polyps were routinely reviewed by dedicated gastrointestinal pathologists. SPs were classified as hyperplastic polyp (HP), sessile serrated lesion (SSL) with or without dysplasia or traditional serrated adenoma (TSA). All SPs with dysplasia or with a diameter of ≥10mm and all TSAs were regarded advanced SPs. Adenomas were diagnosed as tubular adenoma (TA), tubulovillous adenoma (TVA, 25-75% villous histology) or villous adenoma (VA, ≥75% villous histology). All adenomas ≥10mm, with high-grade dysplasia or with ≥25% villous histology were classified as advanced adenomas. Advanced neoplasia was defined as any advanced SPs, advanced adenomas or CRCs.

Surveillance protocol

Before endoscopic surveillance, the colon of each patient had to be cleared from all polyps ≥5mm, and polyps <5mm with the optical aspect of an adenoma, SSL or TSA (thus, HP 1-4mm could be left in situ). Subsequent endoscopic surveillance was scheduled with intervals of either one or two years, based on polyp burden during the last endoscopy. Thus, the surveillance interval was re-determined after each consecutive surveillance endoscopy. An interval of one year was appointed to patients with one or more advanced polyps, or cumulatively ≥5 SSL (irrespective of size), adenomas (irrespective of size) or HP ≥5mm, or if surgery was needed during the previous surveillance/clearing phase. In all other cases, a two-year surveillance interval was recommended (see supplementary figure 1).

In case of violation of this protocol, patients were censored at the date the surveillance colonoscopy should have taken place, and only data prior to the protocol violation was used for our analyses. Violation of the protocol was defined as deviation from required surveillance interval of ≥365 days.

Endoscopy quality

Routinely, standard and high-definition white light endoscopes were used, and endoscopies were routinely performed at dedicated endoscopy programs. Based on the endoscopist's

preference, advanced endoscopic techniques such as (virtual) chromoendoscopy could be used. Bowel preparation was performed according to local practice, and was judged using the Boston Bowel Preparation Scale (BBPS). A BBPS of ≥2 in all bowel segments was considered adequate, and in case of inadequate bowel preparation a new endoscopy was scheduled, preferably within six months.

Outcome measures and statistical analyses

The primary outcome was the cumulative 5-year incidence of AN during post-operative surveillance, calculated with Kaplan-Meier analyses for all patients as well as stratified by type of anastomosis (ileosigmoidal or ileorectal). Differences in AN incidence between anastomosis types were assessed with a logrank test. The location of anastomosis (ileosigmoidal or ileorectal) was extracted from surgical reports or, if surgical reports were not available, from endoscopy reports. Secondary outcome was the yield of non-advanced and advanced polyps for each round of endoscopic surveillance. Statistical analyses were performed using RStudio version 1.1.453 (Integrated Development for R. RStudio, Inc., Boston, MA, USA) with Survminer package version 0.4.3.

RESULTS

Between January 2013 and April 2018, 63 patients with a history of (sub)total colectomy were assessed for eligibility (Figure 1). Fourteen patients were excluded. Nine because they were enrolled too late, and thus their first surveillance was not yet due at study closure, three because they underwent proctocolectomy with ileo-anal pouch reconstruction and two because the study protocol was systematically not followed (2). Forty-eight SPS patients were included in our analyses. They were followed for a median of 4.7 years (IQR 2.4 - 5.1 years; Table 1). Protocol violation led to early drop-out of 3 patients (Figure 1).

Baseline characteristics

The mean age at inclusion was 61 years (±7.8 years), and 52% were male. Most patients fulfilled both WHO criteria I and III (65%). Twenty-two patients had a history of CRC (46%), of whom five had multiple CRCs (3 synchronous and 2 metachronous). Of the 27 detected CRCs, 26 occurred prior to or during the clearing phase (96%), whereas 1 CRC (3.7%) was diagnosed after the clearing phase, but prior to study inclusion. Sixteen of the 27 tumours (59%) were located proximal to the splenic flexure. All but one CRC were diagnosed prior to or at the time of (sub)total colectomy. One patient was diagnosed with CRC after (sub) total colectomy but prior to inclusion. However, in hindsight it appeared that this patient had rather undergone an extended hemicolectomy instead of (sub)total colectomy with ileosigmoidal anastomosis, since the endoscopist located this tumour in the transverse colon at 45cm from the anal verge.

Forty-five out of 48 patients (94%) underwent a (sub)total colectomy prior to or during clearing (Table 1). The remaining 3 patients (6.3%) underwent a (sub)total colectomy after clearing, but prior to study inclusion. Polyp burden was the reason for (sub)total

colectomy in 20 patients (42%), while concurrent CRC or one or more unresectable polyps played a role in 48% and 10%, respectively. Intestinal continuity was restored by ileorectal anastomosis in 29 patients (60%) and by ileosigmoidal anastomosis in 19 patients (40%).

Table 1. Baseline characteristics (n=48)

Age at diagnosis SPS, mean (SD)	58 (±9.6)
Age at start of prospective follow-up, mean (SD)	61 (±7.8)
Male, n (%)	25 (52%)
Reason first colonoscopy, n (%)	
FOBT-based screening	10 (21%)
Primary colonoscopy screening	0
Familial CRC risk	6 (13%)
Symptoms	23 (48%)
Pain/discomfort abdomen	5 (22%)
Rectal blood loss	7 (30%)
Altered defecation pattern	4 (17%)
Anaemia	1 (4.3%)
Other/unknown	6 (26%)
Other/unknown	9 (19%)
Colorectal cancer	
Patients with a history of CRC, n (%)	22 (46%)
Patients with one CRC	17 (35%)
Patients with multiple CRCs	5 (10%)
Synchronous	3 (6.3%)
Metachronous	2 (4.2%)
Total number of CRCs diagnosed	27 (100%)
Time of CRC diagnosis:	
Prior to or during clearing phase	26 (96%)
During surveillance, but prior to study inclusion	1 (3.7%)
During surveillance, after study inclusion	0
Location of CRC	
Cecum	4 (18%)
Ascending colon	5 (23%)
Transverse colon	7 (32%)
Descending colon	3 (14%)
Sigmoid	7 (32%)
Unspecified	1 (4.5%)
WHO SPS classification at study inclusion, n (%)	
I	8 (17%)
III	9 (19%)
I & III	31 (65%)

Table 1. (continued)

Colectomy details	
Moment of colectomy	
Prior to or during clearing phase	45 (94%)
During surveillance**	3 (6.3%)
Length of retained bowel segment in cm, median (IQR)	24 (16-30)
Type of anastomosis	
Ileo-sigmoidal	19 (40%)
Ileo-rectal	29 (60%)
Length of prospective follow-up	
Total number of protocolized surveillance endoscopies	104
Per patient, median (IQR)	2 (1-3)
Total patient-years of prospective follow-up, years	180
Per patient, median (IQR)	4.7 (2.4-5.1)

* Reason for surgery applies to the primary reason. Secondary reasons, such as extensive polyposis accompanying CRC, were not registered.

** All colectomies that were performed during surveillance, were performed prior to study inclusion.

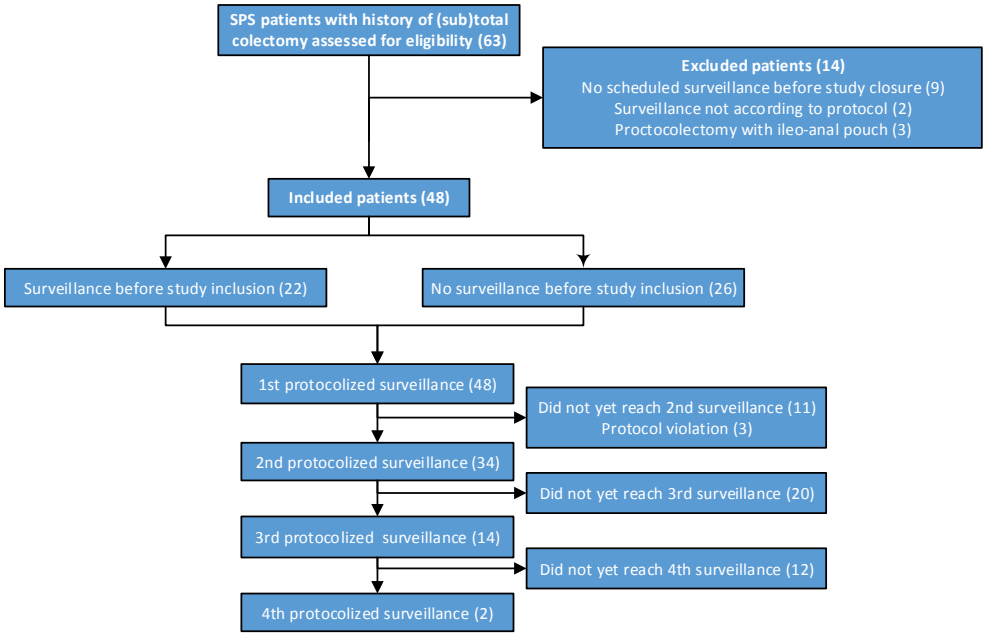


Figure 1. Study flowchart. *Abbreviations: SPS, serrated polyposis syndrome.*

Protocolized surveillance endoscopies

A total of 104 surveillance endoscopies were performed, with a median of two per patient (IQR 1-3). Forty-six percent of patients was diagnosed with SPS before our study started, and had thus undergone one or more surveillance endoscopies before study inclusion. Fifty-four percent was diagnosed with SPS during or shortly prior to our study, and thus underwent their first surveillance endoscopy during the study.. All patients underwent at least one round of protocolized surveillance, followed by a second, third and fourth in 34, 14 and 2 patients, respectively (Figure 1). After the first, second and third surveillance round, 81%, 88% and 93% of patients were surveilled with an interval of 2 years according to the surveillance protocol.

Advanced neoplasia during follow-up

None of the included patients developed CRC in their retained rectosigmoid during follow-up. Five patients developed AN, corresponding to a 5-year cumulative incidence of 13% (95%-CI 1.2-23, Figure 2). AN was detected in the first round of surveillance in 4 patients (80%); one had a TVA, one had a dysplastic SP, two had SPs ≥10mm and one patient harboured three TSAs. The fifth patient had AN (i.e. a dysplastic SP) during the third round of surveillance (Table 2).

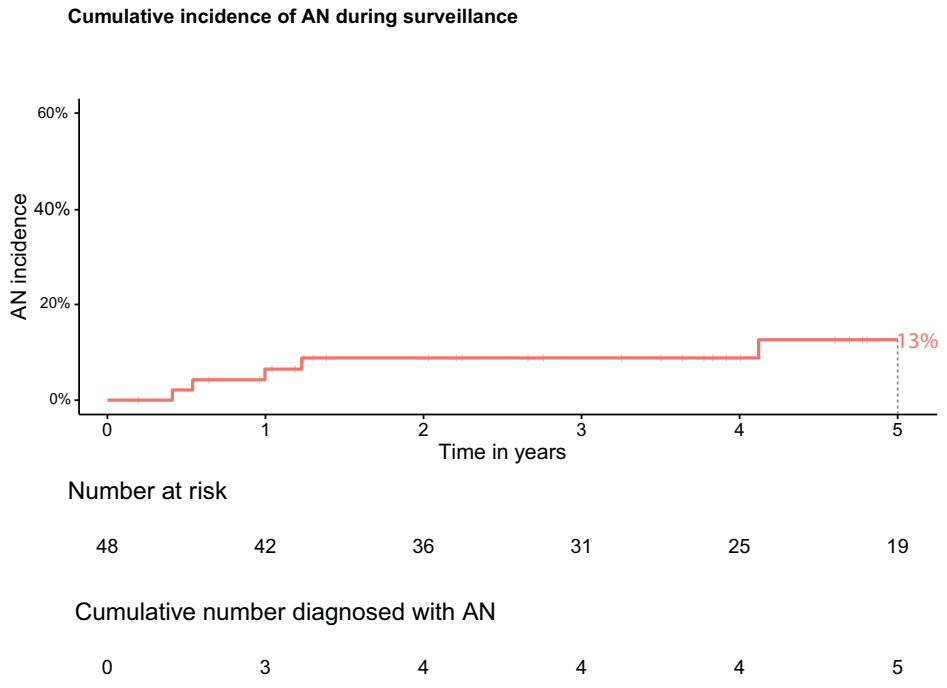


Figure 2. Cumulative incidence of advanced neoplasia. *Abbreviations: AN, advanced neoplasia.*

Table 2. Findings prior to and during surveillance

	During/prior to clearing (n=48)	Surveillance prior to study inclusion (n=22)	Findings during prospective follow-up			
			Surveillance 1 (n=48)	Surveillance 2 (n=34)	Surveillance 3 (n=14)	Surveillance 4 (n=2)
Patients with CRC, n (%)	22 (46%)	1 (9.1%)	0	0	0	0
Patients with ≥1 polyp, n (%)	48 (100%)	20 (80%)	23 (48%)	17 (50%)	7 (50%)	1 (50%)
No. of polyps per patient , median (range)	39 (10-285)	3 (0-24)	0 (0-10)	0.5 (0-11)	0.5 (0-3)	1.5 (0-3)
Patients with ≥1 advanced polyp*	41 (85%)	5 (23%)	4 (8.3%)	0	1 (7.1%)	0
Patients with at least one:						
Any SP	48 (100%)	17 (77%)	23 (48%)	16 (47%)	7 (50%)	1 (50%)
HP	44 (92%)	14 (64%)	17 (35%)	14 (41%)	6 (43%)	1 (50%)
SSL	42 (88%)	5 (23%)	1 (2.1%)	3 (8.8%)	2 (14%)	0
TSA	1 (2.1%)	0	1 (2.1%)	0	0	0
SP≥10mm	32 (67%)	3 (14%)	3 (6.3%)	0	0	0
SP with dysplasia	19 (40%)	2 (9.1%)	0	0	1 (7.1%)	0
Median no. of SP per patient (range):						
Any SP	37 (8-281)	3 (0-22)	0 (0-10)	0 (0-11)	0.5 (0-3)	1.5 (0-3)
HP	12.5 (0-126)	2 (0-22)	0 (0-3)	0 (0-3)	0 (0-3)	1.5 (0-3)
SSL	11.5 (0-207)	0 (0-4)	0 (0-1)	0 (0-11)	0 (0-1)	0 (0-0)
TSA	0 (0-2)	0 (0-0)	0 (0-3)	0 (0-0)	0 (0-0)	0 (0-0)
SP≥10mm	2 (0-31)	0 (0-4)	0 (0-2)	0 (0-0)	0 (0-0)	0 (0-0)
SP with dysplasia	0 (0-23)	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-0)
Patients with at least one adenoma:	43 (90%)	3 (14%)	2 (4.2%)	2 (5.9%)	0	0
Advanced adenoma **	22 (46%)	1 (4.5%)	1 (2.1%)	0	0	0
Median no. of adenomas per patient (range):	3 (0-50)	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-0)	0 (0-0)
Advanced adenoma**	0 (0-10)	0 (0-1)	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)

* Advanced polyp: Advanced adenoma (≥25% villous histology, high-grade dysplasia, ≥10mm in diameter) / Advanced SP (TSA, diameter ≥10mm, presence of dysplasia)

** Advanced adenoma: ≥25% villous histology, high-grade dysplasia, ≥10mm in diameter.

CRC, colorectal cancer; SP, serrated polyp; HP, hyperplastic polyp; SSL, sessile serrated lesion; TSA, traditional serrated adenoma;

Ileosigmoidal vs. ileorectal anastomosis

Three of the 29 patients (10%) with an ileorectal anastomosis developed AN (10%), compared to two out of 19 (10.5%) with an ileosigmoidal anastomosis. The hazard of developing AN did not differ between these groups: the 5-year cumulative AN incidence was 11% (95%-CI 0-25) for patients with ileosigmoidal anastomosis, compared to 12% (95%-CI 0-25%) for those with an ileorectal anastomosis (p=0.83; supplementary figure 2).

Polyps detected during surveillance round 1-4

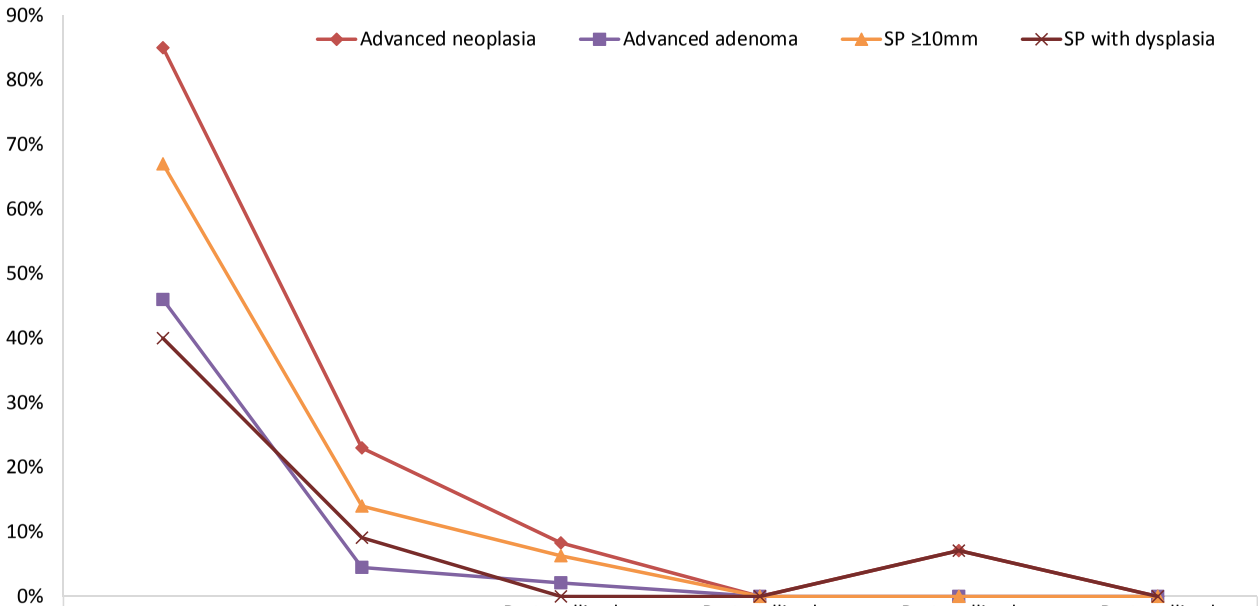
During surveillance rounds 1-4, a total of 124 polyps were removed. Of these, 110 were classified as SP (89%) and 5 as adenoma (4.0%). The 110 SPs consisted of 66 HPs (60%), 24 unspecified SPs (22%), 17 SSLs (15.5%) and 3 TSAs (2.7%). Seven (6.4%) of these were advanced SPs, 56 (51%) were non-advanced but relevant SPs (i.e. HPs ≥5mm or SSLs of any size) and 47 (43%) were non-relevant SPs (i.e. HPs <5mm). Of the five resected adenomas, four were classified as TA and one as TVA. During each round of surveillance, about half of the patients had one or more polyps removed (Table 2). The yield of advanced polyps was highest during the first round of surveillance (8.3%), while only one patient developed an advanced polyp during later rounds of surveillance (Table 2 and Figure 3).

DISCUSSION

In this largest prospective cohort study to date, we assessed the yield of endoscopic surveillance in SPS patients that underwent a (sub)total colectomy. We hypothesized that patients with little colorectum left in situ would have a limited risk of AN. After a median follow-up of 4.7 years, indeed none of the included patients developed CRC, and AN incidence was low (5-year cumulative incidence 13% (95%-CI 1.2-21%)). This contrasts with the high risk of AN in patients *without* a history of subtotal colectomy which was estimated to be over 50% in a recent cohort study.[16]. All but one AN were found at the first surveillance endoscopy, suggesting an even lower long-term CRC risk after the first round of surveillance. Our results furthermore suggest similar risk of AN for patients with ileosigmoidal and ileorectal anastomosis. Finally, our results show that a large proportion of the detected polyps after a (sub)total colectomy are small hyperplastic polyps. The incidence of relevant lesions such as SSLs or conventional adenomas was low.[1, 17]

These results confirm that SPS patients that underwent a (sub)total colectomy are at low risk for AN during surveillance. This contrasts with the high risk of AN in patients *without* a history of (sub)total colectomy, recently estimated to be over 50%.[16] Similar to our results, the same cohort study presented a subgroup analysis of postsurgical surveillance in SPS patients, in whom AN incidence was much lower than in SPS patients that did not undergo a (sub)total colectomy.[16]

In our subgroup analysis of ileorectal vs. ileosigmoidal anastomosis, we found no difference in terms of AN risk during follow-up. Although compromised by the small size of these subgroups (29 and 19 patients, respectively), this is an important finding, as a longer



	Prior to/during clearing (n=48)	Surveillance before inclusion (n=22)	Protocollized surveillance round 1 (n=48)	Protocollized surveillance round 2 (n=34)	Protocollized surveillance round 3 (n=14)	Protocollized surveillance round 4 (n=2)
Advanced neoplasia	85%	23%	8%	0%	7%	0%
Advanced adenoma	46%	5%	2%	0%	0%	0%
SP ≥10mm	67%	14%	6%	0%	0%	0%
SP with dysplasia	40%	9%	0%	0%	7%	0%

Figure 3. Incidence of advanced neoplasia per surveillance phase. Advanced neoplasia: CRC, advanced adenoma, advanced SP; Advanced adenoma: diameter ≥10mm, high-grade dysplasia, ≥25% villous histology; Advanced SP: diameter ≥10mm, presence of dysplasia, traditional serrated adenoma. *Abbreviations: SP, serrated polyp*

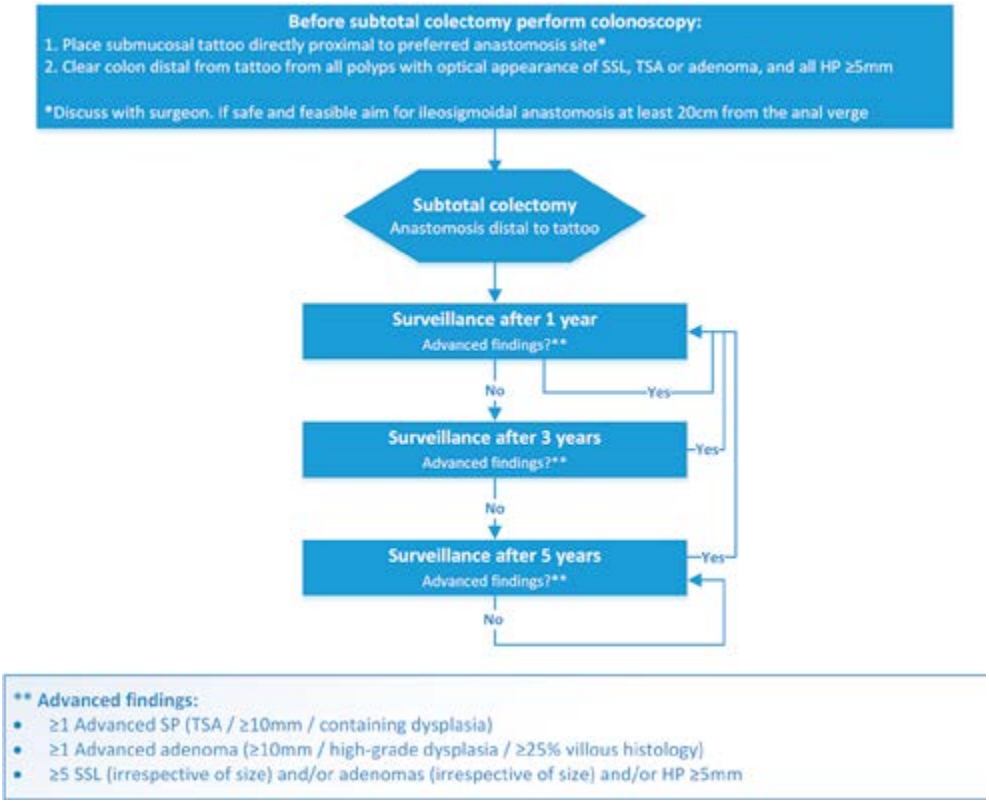


Figure 4. Proposed stepwise treatment and surveillance strategy for SPS patients undergoing (sub) total colectomy. *Abbreviations: SSL, sessile serrated lesion; TSA, traditional serrated adenoma; HP, hyperplastic polyp*

bowel segment in situ may help to achieve better postoperative bowel function. Previous studies suggested that a postoperative rectosigmoid length of ≥20cm was needed for a patient to acquire satisfactory postoperative bowel function[18], and ileosigmoidal anastomosis has been linked to superior postoperative bowel function compared to ileorectal anastomosis.[19] Therefore, if (sub)total colectomy is indicated for SPS patients, if feasible it seems preferable to create an ileosigmoidal anastomosis and leave a segment of rectosigmoid of at least 20cm in situ.

Given the prophylactic effect of (sub)total colectomy on CRC in SPS patients, some might argue that surgery should have a more prominent place in the standard treatment of SPS. However, these benefits have to be weighed against the inherent risk of surgery-related morbidity and mortality. In the modern era of minimal invasive surgery, perioperative morbidity after colorectal surgery has been reduced substantially and mortality rates are low. In a recent cohort study of elective laparoscopic colorectal surgery in the Netherlands,

mortality and morbidity rates were 2.4% and 19% respectively, which was substantially lower than after open colorectal surgery.[20] Furthermore, functional outcome after laparoscopic (sub)total colectomy, particularly in patients with ileosigmoidal anastomosis, is good.[19] Nevertheless, morbidity rates after surgery still exceed the risk of morbidity after endoscopic management of SPS and functional outcomes are not jeopardized when SPS is managed endoscopically.[4, 9] Hence, despite its beneficial effect on CRC risk, we believe that prophylactic (sub)total colectomy should not serve as a standard treatment for SPS, but be reserved for patients with exceptional polyp burden, CRC or endoscopically unresectable polyps. The potential benefits and risks of surgery versus multiple endoscopic interventions should be weighed in shared decision with each individual patient.

Although our study was not designed to evaluate the optimal surveillance interval, we think our results give reason to believe that the currently recommended interval of 1-2 years might be too stringent, given the relatively low incidence of AN. Although extension of surveillance intervals should be carried out with caution and as part of prospective cohort studies, we propose a concept for a surveillance strategy, which includes the aforementioned recommendation to preserve a longer segment of rectosigmoid and extended surveillance intervals up to five years (Supplementary figure 3). Because the yield of AN was highest during the first round of surveillance, we propose that the first surveillance takes place one year after the clearing phase. In case no AN and less than five non-advanced polyps are detected during any round of surveillance, intervals can be extended to three and then to five years (supplementary figure 3). Before implementation in clinical practice, this proposed surveillance strategy should be evaluated empirically.

We believe our study has several strengths. First, this was the largest study to date assessing the risk of CRC and AN during follow-up in SPS patients after a total or (sub) total colectomy. All endoscopies were performed in centres with years-long experience in the management of SPS, and were routinely performed on dedicated endoscopy programs. Second, all patients were surveilled with the same surveillance protocol, eliminating the potential bias of varying surveillance intervals. Several limitations have to be acknowledged as well. Some clinicians in participating centres indicated that they regarded SPS patients with a history of (sub)total colectomy as a group of low-risk patients, and therefore deviated from the study protocol, extending surveillance intervals to 3 or 5 years, in some patients. Although we were aware of only two of such cases (see Figure 1), we cannot rule out that more patients were excluded for this reason, which might have resulted in a selection bias. Second, patients with an ileorectal anastomosis underwent more extensive surgery than those with ileosigmoidal anastomosis, and hence they might have harboured a more severe SPS phenotype than patients with ileosigmoidal anastomosis at baseline. However, in a sensitivity analysis both groups had similar incidence of CRC ($p=0.72$) and harboured a similar number of advanced polyps prior to their (sub)total colectomy ($p=0.50$). Third, some patients already received endoscopic surveillance before inclusion, whereas others did not. These two groups might theoretically differ in their risk of AN during our prospective follow-up. However, in a sensitivity analyses there was no

difference in AN incidence in patients with, compared to patients without surveillance prior to study inclusion (Log-rank p=0.571). Last, in our subgroup analysis of ileorectal vs. ileosigmoidal anastomosis, our sample size was relatively small (26 vs. 17 patients, respectively), and was not sufficiently powered to detect subtle differences in AN between ileorectal and ileosigmoidal anastomosis.

In conclusion, much of the excess CRC risk in SPS patients is mitigated by (sub)total colectomy. Based on the low AN incidence in these patients, we propose extension of surveillance intervals, which we incorporated in a newly proposed surveillance strategy (supplementary figure 3). Such extension of surveillance should be carried out with caution, in experienced centres and preferably as part of high-quality prospective cohort studies. Last, ileosigmoidal anastomosis seems to result in the same AN risk as ileorectal anastomosis. Considering the beneficial outcome after ileosigmoidal anastomosis and a retained rectosigmoid length of ≥20cm[18, 19], surgeons and gastroenterologists should make a joint effort to preserve a maximum length of retained rectosigmoid.

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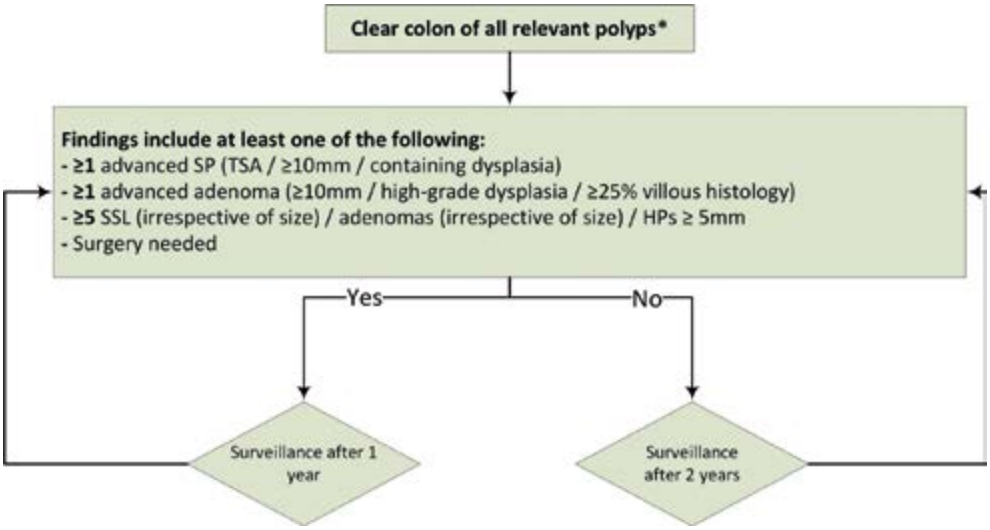
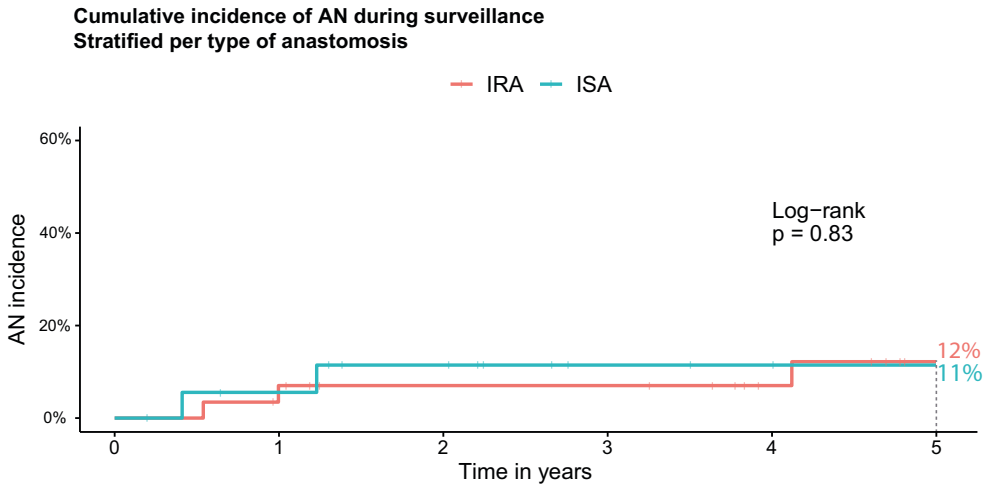
SUPPLEMENTARY DATA



Supplementary figure 1. Personalised surveillance protocol used during study. *Abbreviations: SP, serrated polyp; TSA, traditional serrated adenoma; SSL, sessile serrated lesion.* * Relevant polyps: all polyps ≥5mm, and all polyps <5mm with optical aspect of adenoma, TSA or SSL. Thus, HPs 1-4mm may be left in situ.



Number at risk

IRA	29	26	23	23	18	13
ISA	19	16	13	8	7	6

Cumulative number diagnosed with AN

IRA	0	2	2	2	2	3
ISA	0	1	2	2	2	2

Supplementary figure 2. Cumulative incidence of advanced neoplasia, stratified by site of anastomosis.
Abbreviations: AN, advanced neoplasia; IRA, ileorectal anastomosis; ISA, ileosigmoidal anastomosis

7

The (ir)relevance of the abandoned criterion II for the diagnosis of serrated polyposis syndrome: A retrospective cohort study

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ABSTRACT

Background and study aims

The World Health Organization (WHO) recently updated the diagnostic criteria for serrated polyposis syndrome (SPS). One of the 3 previous diagnostic criteria (criterion II^{2010}) is now abandoned: *≥1 serrated polyp (SP) proximal to the sigmoid in a first-degree relative (FDR) of a patient with SPS*. Individuals fulfilling this abandoned criterion now receive the same surveillance recommendations as all FDRs of patients with SPS. We aimed to compare the incidence of advanced neoplasia (AN) in FDRs with vs. without fulfilment of the abandoned criterion II^{2010}.

Patients and methods

We retrospectively recruited FDRs of patients with SPS who underwent a colonoscopy, and stratified them according to fulfilment of criterion II^{2010} at baseline. Our primary and secondary outcomes were AN incidence during surveillance and at baseline, respectively.

Results

We included 224 FDRs of patients with SPS, of whom 36 (16%) fulfilled criterion II^{2010} at baseline. One hundred and five underwent surveillance after baseline. Criterion II^{2010}-positive FDRs were at increased risk of AN, both during surveillance (hazard ratio 8.94, 95%CI 2.15-37.1, p=.003) as well as at baseline (adjusted odds-ratio 9.30, 95%CI 3.7-23.3, p<.001).

Conclusions

FDRs of patients with SPS that underwent colonoscopy and fulfilled the abandoned criterion II^{2010} for SPS diagnosis were at increased risk of AN at baseline and during surveillance in this small, retrospective cohort study. Our results should be interpreted with caution but suggest that adherence to surveillance recommendations for all FDRs of patients with SPS is important, especially for those that would have fulfilled the now abandoned criterion II^{2010}.

INTRODUCTION

Serrated polyposis syndrome (SPS) is characterized by the presence of numerous serrated polyps (SPs), and is associated with an increased colorectal cancer (CRC) risk.[1-3] A genetic basis of the syndrome has not been identified and so the diagnosis is based solely on clinical criteria, which have been redefined several over the past decades.[4-6] Due to a scarcity of supporting data these clinical criteria can be considered somewhat arbitrary. As such, one particular clinical criterion (2010's criterion II) has been topic of continuous debate ever since its introduction.[4] The WHO's 2010 clinical criteria [4] included **I)** *at least five SPs proximal to the sigmoid colon, with two or more of these being >10mm;* **II)** *any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative (FDR) with serrated polyposis syndrome*, or **III)** *>20 serrated polyps of any size, but distributed throughout the colon*. Recently, the World Health Organization (WHO) published the updated 2019 clinical criteria for SPS diagnosis, abandoning this controversial second criterion.[7] The updated 2019 criteria now only include two diagnostic criteria: **I)** *≥ 5 serrated lesions/polyps proximal to the rectum, all being ≥ 5 mm in size, with ≥ 2 being ≥ 10 mm in size;* or **II)** *> 20 serrated lesions/polyps of any size distributed throughout the large bowel, with ≥ 5 being proximal to the rectum.*

Unfortunately, the debate about the 2010's criterion II has been held mostly 'offline' and has seen limited empirical support due to an almost complete lack of evidence on patients fulfilling this criterion. This lack of evidence, in turn, has been a result of the consistent exclusion of criterion II patients from most recent cohort studies, which extensively described CRC risk and risk-factors in criterion I and/or III patients, but not in criterion II patients.[1, 2, 8-10]

Thus, evidence supporting both the introduction of criterion II in 2010, as well its abandonment in 2019, is lacking. Nevertheless, there are two important rational arguments to support the abandonment. First, the presence of a serrated polyp proximal to the sigmoid in a FDR of a patient with SPS might often be a manifestation of a naturally occurring serrated polyp, rather than a manifestation of a polyposis syndrome. After all, with a prevalence of 4.7-12.2%, serrated polyps proximal to the descending colon are common in healthy individuals without SPS.[11-13] The prevalence of SPs proximal to the sigmoid, following the WHO's definition of proximal, is probably even somewhat higher. Exposing all such cases to the stringent endoscopic surveillance regimens imposed on 'true' serrated polyposis patients might lead to overtreatment. Second, *all* FDRs of patients with SPS are recommended to undergo regular endoscopic surveillance (mostly 5 yearly) according to several guidelines and experts.[8, 10, 14-17] Such recommendations are based on several studies demonstrating an increased CRC risk in first-degree relatives of patients with SPS.[16-18] Therefore, even after abandonment of criterion II, all criterion II individuals who would formerly have been diagnosed with SPS will still receive regular endoscopic surveillance if these recommendations are obeyed, simply because they are by definition FDRs of a patients with SPS.

The clinical implication of abandonment of criterion II is that those who were formerly diagnosed with SPS based on criterion II only, will no longer be considered 'true' SPS, and will fall under the same recommendations as all other FDRs of patients with SPS. Although we and many others support the WHO's decision to abandon 2010's criterion II, this decision would ideally have been supported by cohort studies demonstrating that, indeed, criterion II patients harbour a similar CRC risk compared to other FDRs of patients with SPS. Such studies are unfortunately lacking. In an attempt to bridge this empirical gap, we carried out this first retrospective cohort study focusing on patients fulfilling the abandoned WHO's 2010 criterion II.

METHODS

To prevent confusion between WHO's 2010 and updated 2019 criteria, we have superscripted '^{2010}' or '^{2019}' each time one of the WHO's criteria is mentioned, referring to the 2010 WHO guideline[4] and 2019 WHO guideline[6], respectively.

Study aim

We aimed to compare risk of advanced neoplasia (AN) in FDRs of patients with vs. *without* fulfilment of criterion II^{2010}.

Study design and population

We retrospectively collected a cohort of FDRs of patients with SPS and fulfilled WHO criterion I^{2010} and/or III^{2010}.[4] Probands were identified through patient records in the participating Spanish, British and Dutch hospitals and their FDRs who had undergone at least one colonoscopy were included in the study. Because of the retrospective nature of this study, the Institutional Review Board (IRB) of the Academic Medical Centre (AMC) in Amsterdam decided that this study did not fall under the legislation regarding Medical Research Involving Human Subjects ACT (WMO). Data were collected and stored online using Castor EDC.[19]

Inclusion criteria

All FDRs (siblings, children or parents) of index SPS cases were eligible for inclusion if they underwent at least one colonoscopy in their lifetime. We excluded all FDRs with known CRC-related germline mutations or an inflammatory bowel disease. We also excluded FDRs that fulfilled WHO criteria I^{2010} and/or III^{2010} at baseline colonoscopy. FDRs were divided into two groups according to their fulfilment of criterion II^{2010} at baseline, allowing us to compare II^{2010}-positive FDRs vs. II^{2010}-negative FDRs.

Outcome parameters

Our primary endpoint was the risk AN encountered during surveillance colonoscopies after baseline, comparing II^{2010}-positive vs. II^{2010}-negative FDRs. Secondary endpoints

were polyp burden at baseline in II^{2010}-positive vs. II^{2010}-negative FDRs, and progression into 'true' SPS phenotype (i.e. fulfilment of WHO criterion I^{2010} and/or III^{2010}) based on cumulative polyp count during consecutive surveillance colonoscopies.

Serrated polyps were divided into three subtypes: sessile serrated lesion (SSL) with or without dysplasia, hyperplastic polyp (HP) and traditional serrated adenoma (TSA). Advanced SPs (ASPs) were defined SPs ≥10mm in diameter, with dysplasia (any grade) or TSAs. Advanced adenomas (AAs) were defined as adenomas ≥10mm in diameter, with high-grade dysplasia (HGD) and/or a villous component of ≥25%. Advanced neoplasia was defined as any ASP, AA or CRC.

Statistical analyses

Baseline characteristics were compared using independent samples t-tests, chi-square tests, Fisher's exact tests and Mann-Whitney U tests, where appropriate. Risk of AN during surveillance was calculated using Kaplan Meier survival analyses and expressed as cumulative hazard and 5-year cumulative incidence. FDRs were censored at the date of their most recent colonoscopy. The AN hazard for criterion II^{2010}-positive and II^{2010}-negative FDRs was compared in univariate and multivariable Cox-regression analyses and expressed as hazard ratio (HR). Baseline polyp burden was compared between criterion II^{2010}-positive and II^{2010}-negative FDRs with univariate and multivariable logistic regression analyses. Co-variates for multivariable Cox- and logistic regression analyses were selected based on baseline differences between the two groups (age, gender and smoking status). We restricted the number of variables for multivariable analyses to 1 variable per 10 events to prevent overfitting in our dataset.[20]

Survival curves were produced using RStudio version 1.1.453 (Integrated Development for R. RStudio, Inc., Boston, MA, USA) with Survminer package version 0.4.3. All other analyses were performed using Statistical Package for Social Sciences (SPSS) version 24 (IBM, Somers, New York, USA).

RESULTS

Baseline characteristics

Two hundred and twenty four FDRs fulfilled our inclusion criteria. At baseline, 36 (16%) were II^{2010}-positive and 188 (84%) were II^{2010}-negative. The mean age at the time of baseline colonoscopy was 50 years (±13), and 42% were male (Table 1). The majority (65%) of FDRs was a sibling to the index patient, while 29% was a child and 4.9% was a parent of the proband. The indication of baseline colonoscopy was familial screening in 184 (82%), symptoms in 27 (12%) and population screening in 3 (1.3%) individuals. Age, gender and reason for baseline colonoscopy did not differ between II^{2010}-positive and II^{2010}-negative FDRs. Smoking or former smoking was more common within II^{2010}-positive FDRs (44% vs. 20%, p=.006), although smoking status was missing for 54% of included individuals.

Table 1. Baseline characteristics stratified according to fulfilment of WHO's criterion II[2010] for SPS diagnosis at baseline colonoscopy. Characteristics of the entire cohort are presented in the upper half of the table; the baseline characteristics of the subcohort of patients with surveillance colonoscopies are presented in the bottom half of the table.
Abbreviations: FDR, first-degree relative; SD, standard deviation; IQR, interquartile range

All patients (n=224)				
	Overall (n=224)	Criterion II[2010]-positive FDR (n=36)	Criterion II[2010]-negative FDR (n=188)	P-value
Age at index colonoscopy, mean (SD)	50 (13)	53 (12)	50 (13)	.11
Male, n (%)	95 (42%)	16 (44%)	79 (42%)	.86
Relation to index patient				.97
Sibling	145 (65%)	23 (64%)	122 (65%)	
Parent	11 (4.9%)	2 (5.6%)	9 (4.7%)	
Child	65 (29%)	11 (31%)	54 (29%)	
Unknown	3 (1.3%)	0	3 (1.6%)	
Reason index colonoscopy				.66
Familial screening	184 (82%)	31 (86%)	153 (81%)	
Community screening	3 (1.3%)	1 (2.8%)	2 (1.1%)	
Symptoms	27 (12%)	3 (8.3%)	24 (13%)	
Other/unknown	10 (4.5%)	1 (2.8%)	9 (4.8%)	
Smoking history				.006
Previous/current smoker	54 (24%)	16 (44%)	38 (20%)	
Never smoker	49 (22%)	4 (11%)	45 (24%)	
No information	121 (54%)	16 (44%)	105 (56%)	
Subcohort of patients with one or more surveillance colonoscopies after index (n=105)				
	Overall (n=105)	Criterion II[2010]-positive FDR (n=15)	Criterion II[2010]-negative FDR (n=90)	
Age at index colonoscopy, mean (SD)	50 (12.7)	54 (11.7)	49 (12.1)	.13

Table 1. (continued)

	Overall (n=105)	Criterion II[2010]-positive FDR (n=15)	Criterion II[2010]-negative FDR (n=90)	P-value
Male, n (%)	44 (42%)	7 (47%)	37 (41%)	.71
Relation to index patient				.95
Sibling	80 (76%)	12 (80%)	68 (76%)	
Parent	6 (5.7%)	1 (6.7%)	5 (5.6%)	
Child	18 (17%)	2 (13%)	16 (18%)	
Unknown	1 (1%)	0%	1 (1.1%)	
Reason index colonoscopy				.29
Familial screening	89 (85%)	14 (93%)	75 (83%)	
Community screening	2 (1.9%)	1 (6.7%)	1 (1.1%)	
Symptoms	9 (8.6%)	0%	9 (10%)	
Other/unknown	5 (4.8%)	0%	5 (5.5%)	
Smoking history				.070
Previous/current smoker	29 (28%)	7 (47%)	22 (24%)	
Never smoker	29 (28%)	2 (13%)	27 (30%)	
No information	47 (45%)	6 (40%)	41 (46%)	
Number of surveillance colonoscopies after baseline				.18
1 colonoscopy	51 (49%)	5 (33%)	46 (51%)	
2 colonoscopies	24 (23%)	3 (20%)	21 (23%)	
3 colonoscopies	13 (12%)	4 (27%)	9 (10%)	
4 colonoscopies	5 (4.8%)	1 (6.7%)	4 (4.4%)	
5 colonoscopies	5 (4.8%)	2 (13%)	3 (3.3%)	
>5 colonoscopies	7 (6.7%)	0 (0%)	7 (7.8%)	
Median interval (years) between surveillance colonoscopies (IQR)	1.91 (1.09 – 2.59)	1.49 (1.10 – 2.27)	1.97 (1.09-2.72)	
Number of surveillance colonoscopies, median (IQR)	2 (1-3)	2 (1-3)	1 (1-3)	.18
Median follow-up duration after baseline, years (IQR)	5.3 (2.9-7.7)	3.5 (1.3-6.0)	5.6 (3.1-8.4)	.026

Of the 224 included FDRs, 105 underwent one or more surveillance colonoscopies after baseline, of whom 15 (13%) were II^{2010}-positive and 90 (78%) were II^{2010}-negative. The median follow-up duration was 5.3 years (IQR 2.9-7.7), and was slightly longer for II^{2010}-negative than II^{2010}-positive FDRs (3.5 vs. 5.6 years, p=.026). In this subcohort of 105 FDRs, II^{2010}-positives were slightly older at baseline (54 vs. 49 years, p=.13), and were more often (former) smokers (47% vs. 24%, p=.070).

Advanced neoplasia during surveillance

The risk of AN during surveillance could be assessed in the subcohort of 105 patients who underwent surveillance colonoscopies after baseline. None of the included patients developed CRC during surveillance. AN during surveillance occurred in 9/90 II^{2010}-negative (10%) FDRs; four had an AA, four had an ASP and one had both an AA and an ASP. In comparison, AN occurred in 4/15 (27%) II^{2010}-positive FDRs; three had an ASP while one had both an AA and an ASP (Table 2).

The cumulative 5-year incidence of AN during surveillance was 7.2% (95% CI 1.4-12.6%, Figure 1A). The 5-year cumulative AN incidence was significantly higher for criterion II^{2010}-positive FDRs (30.4%, 95%CI 0-51.5%) than for II^{2010}-negative FDRs (3.2%, 95%CI 0-7.4%) (Figure 1B & Table 2), with a corresponding univariate HR of 8.94 (95%CI 2.15-37.1, p=.003). Because only 13 events occurred in our cohort, no multivariable regression analysis could be performed.

Polyp burden at baseline

CRC prevalence and polyp burden at baseline could be assessed in the entire cohort of 224 patients. None of the II^{2010}-positive FDRs presented with CRC at baseline colonoscopy (0%, 95%CI 0-9.7%), compared to 4 of the 188 II^{2010}-negative FDRs (2.1%, 95% CI 0.58-5.4%; Table 3). Thirteen out of 36 II^{2010}-positive (39%) FDRs presented with AN at baseline colonoscopy, compared to 11 of the 188 II^{2010}-negative (5.9%) FDRs (OR 10.2, 95%CI 4.1-25, p<.001; Table 3). Adjusted for age at baseline colonoscopy, this difference remained statistically significant (OR 9.30, 95%CI 3.7-23.3, p<.001). Aside from overall AN incidence, both advanced SPs (11/36, 31% vs. 0/188, 0%; p<.001) as well as advanced adenomas (7/36, 19% vs. 11/188, 5.9%; p=.01) were more common in II^{2010}-positive than II^{2010}-negative FDRs (Table 3).

Progression to fulfilment of WHO criterion I^{2010} and/or III^{2010} during surveillance

Progression to WHO criterion I^{2010} and/or III^{2010} positive SPS during surveillance could be assessed in the subcohort of 105 individuals who underwent surveillance after baseline. A total of five patients (2.2%) developed sufficient numbers of polyps over time to fulfil criterion I^{2010} and/or III^{2010}. This occurred more often in criterion II^{2010}-positive FDRs (3/15, 20%) than in II^{2010}-negative FDRs (2/90, 2.2%, Fisher's Exact p=.03). Detailed patient

Table 2. Cox regression analysis of advanced neoplasia during surveillance, comparing first-degree relatives of patients with SPS with vs. without fulfilment of WHO's criterion II[2010] for SPS diagnosis. Abbreviations: AN, advanced neoplasia; HR, hazard ratio.

		Cumulative 5-year AN incidence	Unadjusted HR (95%CI)	P-value
AN detected during surveillance, n (%)				
II[2010]-negative	9/90 (10%)	7.2% (1.4-12.6%)	1.00	
II[2010]-positive	4/15 (27%)	26.4% (8.0-41.1%)	8.94 (2.15-37.1)	0.003

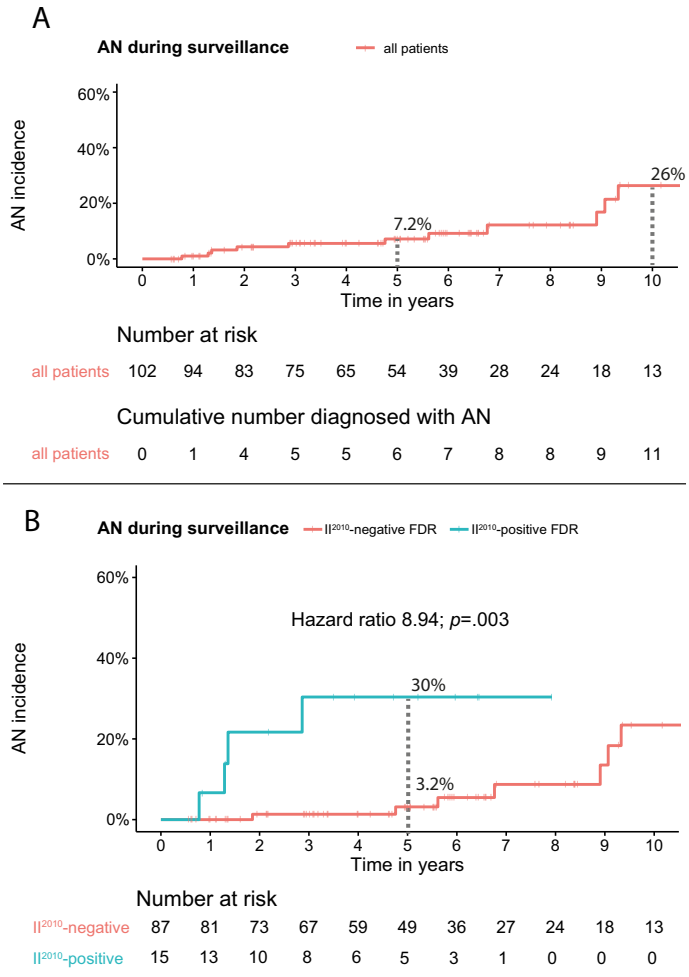

Figure 1. Cumulative hazard curve for advanced neoplasia during surveillance for all first-degree relatives (Panel A), and stratified by fulfilment of WHO criterion II[2010] (Panel B).Abbreviations: AN, advanced neoplasia; FDR, first-degree relative

Table 3. Logistic regression analysis of findings at index colonoscopy, comparing first-degree relatives of patients with SPS with vs. without fulfilment of WHO's criterion II[2010] for SPS diagnosis. Abbreviations: OR, odds ratio; CRC, colorectal cancer; AN, advanced neoplasia; ASP, advanced serrated polyp; AA, advanced adenoma

		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)[a]	P-value
CRC detected at baseline colonoscopy, n (%)					
II[2010]-negative	4/188 (2.1%)				
II[2010]-positive	0/36	n/a[b]	.37[b]	n/a[b]	n/a[b]
AN detected at baseline colonoscopy, n (%)					
II[2010]-negative	11/188 (5.9%)	1.00		1.00	
II[2010]-positive	14/36 (39%)	10.2 (4.1-25)	<.001	9.30 (3.7-23.3)	<.001
ASP detected at baseline colonoscopy, n (%)					
II[2010]-negative	0/188 (0%)				
II[2010]-positive	11/36 (31%)	n/a**	<.001[b]	n/a[b]	n/a[b]
AA detected at baseline colonoscopy, n (%)					
II[2010]-negative	11/188 (5.9%)	1.00		1.00	
II[2010]-positive	7/36 (19%)	3.88 (1.39-10.83)	.010	3.43 (1.21-9.68)	.020

[a]Adjusted for Age at index colonoscopy.

[b] Logistic regression analysis not possible because no events occurred in one of the strata; p-value calculated using Chi-squared test.

characteristics and polyp burden at baseline and during surveillance are displayed in supplementary table 1.

Fulfilment of WHO criterion II[2010] during surveillance

Among the 90 FDRs who were II[2010]-negative at baseline and underwent subsequent surveillance, 20 (22.2%) were diagnosed with a SP proximal to the sigmoid during follow-up, thus fulfilling WHO criterion II[2010]. Compared to the 70 FDRs who did not fulfil II[2010] during surveillance, those who did were at increased risk for AN (HR adjusted for age at baseline 7.1, 95%CI 1.34=37.6, p=.021). Based on the 49 FDRs for whom smoking history was known, FDRs that fulfilled criterion II[2010] during surveillance were significantly more often (former) smokers than FDRs who did not fulfil II[2010] during surveillance (73.3% vs. 32.4%, p=.012).

DISCUSSION

Based on the updated WHO criteria in 2019, patients that were formerly diagnosed with SPS based on criterion II[2010] are no longer considered to have SPS anymore. Therefore,

these individuals now receive surveillance recommendations identical to all other FDRs of patients with SPS. [8, 14-17, 21] However, in this retrospective international multi-centre cohort we show that those FDRs that fulfil the WHO criterion II^{2010} seem to be at increased risk of developing AN during surveillance (HR 8.94, p=.003) as compared to WHO criterion II^{2010} negative FDR. In addition, we demonstrate that II^{2010}-positive FDRs more often presented with AN at baseline colonoscopy (adjusted OR 9.30, p<.001). Furthermore, 22.2% of FDRs who were II^{2010}-negative at baseline and underwent surveillance, fulfilled II^{2010} at a later stage based on polyps removed during surveillance. Those who did were at increased risk of AN during surveillance (p=.021). Lastly, and perhaps not surprisingly, FDRs that were II^{2010}-positive at baseline were more likely to acquire enough polyps to fulfil WHO criterion I^{2010} and/or III^{2010} during surveillance. This demonstrates the concept of 'SPS in evolution': since SPS diagnosis is based on cumulative metachronous polyp count, patients that will later be diagnosed with SPS I^{2010} and/or III^{2010} might first fulfil only criterion II^{2010}. Although several important limitations have to be taken into account, these results suggest that criterion II^{2010}-positive patients might in fact be at increased AN risk as compared to criterion II^{2010}-negative FDRs.

There are several potential explanations for this difference. First of all, proximal SPs are strong predictors of metachronous AN in a non-SPS population.[22, 23] This creates an important á priori difference: criterion II^{2010}-positive FDRs have, by definition, proximal SPs at baseline while II^{2010}-negative FDRs, by definition, do not. Erichsen and colleagues demonstrated an OR of 12.42 for future CRC development after baseline resection of a proximal SP, while Schreiner and colleagues reported an OR of 3.37 for metachronous AN after resection of proximal SPs. Since II^{2010}-positive FDRs presented with proximal SPs at baseline while II^{2010}-negatives did not, the increased metachronous AN risk seems comparable with the data in literature on metachronous risk after resection of sporadic SPs. This explanation implies that the increased risk of metachronous AN in II^{2010}-positive FDRs might rather be a result of the predictive value of baseline proximal SPs for metachronous AN, which would also be seen in an average-risk population with proximal SPs at baseline. Second, criterion II^{2010}-positive FDRs more often had advanced adenomas at baseline than II^{2010}-negatives (19% vs. 5.9%, adjusted p=.020). Anderson and colleagues recently reported that metachronous AN during surveillance was most common in patients with synchronous high-risk adenomas and serrated polyps at baseline colonoscopy.[24] This combination of baseline SPs and high-risk adenomas was much more common among II^{2010}-positives, further increasing their a priori risk of metachronous AN. A final possible explanation for the observed difference between II^{2010}-positive and negative FDRs is the difference in smoking behaviour. II^{2010}-positive FDRs were more often (former) smokers, and FDRs who were II^{2010}-negative at baseline but later fulfilled II^{2010} were far more likely to be (former) smokers. Since smoking is a known risk-factor for AN, our results also imply that smoking might be a risk-factor for both fulfilment of II^{2010} as well as development of metachronous AN in FDRs of patients with SPS. [25]

Several limitations in our study design and sample size have to be acknowledged to enable proper interpretation of the data. First, the number of included patients was small. Most notably, our primary outcome measure could be assessed in 105 patients, of whom only 15 were criterion II[2010] positive. This small sample-size unfortunately impeded multivariable Cox-regression analyses, despite important baseline differences between the II[2010]-negative and positive groups with regard to smoking behaviour and age. 7 Criterion II[2010]-positive FDRs more often had a history of smoking (44% vs. 20%, p=.006) and were of older age at baseline (53 years vs. 50 years, p=.11). Since smoking behaviour could not be adjusted for due to our small sample-size and high proportion of missing data, this confounder might have significantly influenced our results. Second, we report as secondary outcome measures ASP incidence at baseline and fulfilment of WHO I and/or III during surveillance. However, since the II[2010] positive group was defined by the presence of one or more proximal SPs at baseline, this group was by definition at increased risk of both ASPs at baseline and fulfilment of WHO I and/or III during surveillance. Although we feel these outcome measures are informative and relevant to report, this 'circularity' should be kept in mind when interpreting these outcome measures. Furthermore, due to the retrospective design of this study, only FDRs who underwent surveillance colonoscopy after baseline could be included in our primary analysis. This might have led to a selection-bias towards high-risk patients, since surveillance is more likely to be provided to those FDRs who were considered to be at risk of metachronous neoplasia. Due to the retrospective design patients also did not receive colonoscopies at identical surveillance intervals, data were missing and quality parameters were more difficult to interpret.

To our opinion these limitations illustrate how complicated studying criterion II[2010] is, which is probably why no previous studies have focused on this group of patients. Adequate study design is complicated and acquiring sufficient numbers of FDRs is difficult, even in a large collaborative network. In the absence of superior prospective studies, however, we think our study has several noteworthy strengths that make our data clinically useful. First, since neither introduction nor abandonment of criterion II[2010] has been supported by convincing evidence, empirical data on this group of patients was urgently needed. We are the first to provide such data. Second, this study was performed within five high volume expert centres in three different countries, which increases external validity of the data.

Due to the above-mentioned limitations, results from this study should be interpreted with caution and, to our opinion, do not encourage a debate about re-introduction of criterion II[2010] as a separate SPS diagnostic criterion. We believe adequate CRC prevention can be provided for both criterion II[2010]-positive as well as II[2010]-negative FDRs by adhering to the currently recommended regular endoscopic surveillance for *all* FDRs of patients with SPS (most guidelines: 5 yearly). In case (advanced) lesions are detected, surveillance should be adjusted if indicated according to the current post-polypectomy guidelines.

In conclusion, FDRs of patients with SPS that would have fulfilled the abandoned criterion II[2010] for SPS diagnosis might have a higher risk of AN during surveillance than

FDRs not fulfilling criterion II[2010], although firm conclusions are impeded by our small and retrospective study design. As is applicable to all FDRs of patients with SPS, regular endoscopic surveillance should be provided to prevent CRC.[8, 10, 14-17] Future prospective studies are needed to validate our findings.

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8

The long-term outcomes and natural disease course of Serrated Polyposis Syndrome: over 10 years of prospective follow-up in a specialized centre

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ABSTRACT

Background and aims

Serrated polyposis syndrome (SPS) is the most prevalent colonic polyposis syndrome known and is associated with a high risk of colorectal cancer (CRC) if left untreated. Treatment consists of clearance of initial polyp burden, followed by life-long stringent endoscopic surveillance. However, the long-term safety and efficacy of surveillance and the natural disease course after initial clearance have not been described in detail yet.

Methods

We analysed a single-centre cohort of SPS patients with over 10 years of prospective follow-up. Outcome measures were 1) CRC incidence, 2) post-colonoscopy complication rates and 3) trends in polyp recurrence during endoscopic surveillance.

Results

A total of 142 patients were included, who underwent a median of 6 colonoscopies with a median of 47 months of prospective follow-up after initial polyp clearance. During surveillance (every 1-2 years), one CRC occurred (5-year CRC incidence 1.0% (95% confidence interval 0-2.9%)). During 447 surveillance colonoscopies with 1,308 polypectomies, one episode of post-polypectomy bleeding, one post-polypectomy syndrome and no perforations occurred (complication rate 0.45% per colonoscopy). During up to 9 rounds of surveillance, no upward or downward trend in polyp recurrence was observed.

Conclusions

In this prospective cohort with over 10 years follow-up, endoscopic surveillance was effective and safe, with a low risk of CRC and colonoscopy-related complications. Furthermore, we show that the disease course of SPS is such that the polyp burden remains more or less equal during long-term surveillance, which advocates lifelong adherence to (personalised) surveillance guidelines and discourages de-intensifying surveillance intervals after multiple rounds of surveillance.

INTRODUCTION

Serrated polyposis syndrome (SPS) is characterized by the presence of numerous serrated polyps, and is associated with an increased risk of colorectal cancer (CRC). Although previously considered rare, SPS is now believed to be the most prevalent polyposis syndrome known, with a prevalence of up to 1:111 in FIT-based screening cohorts.[1, 2] Recent cohort studies report that about 15-29% of SPS patients are diagnosed with CRC at a certain point.

SPS diagnosis is based on clinical criteria defined by the World Health Organization (WHO): I) ≥5 serrated polyps (SPs) proximal to the rectum, all being ≥5mm in size, with at least 2 being ≥10mm in size; or II) >20 SPs of any size distributed throughout the large bowel, with ≥5 being proximal to the rectum.[3] Current recommendations for the management of SPS separate a clearing and a surveillance phase. During the clearing phase, a patient is cleared from initial polyp burden by removal of all potentially hazardous polyps, preferably by endoscopy or, if required, by surgical resection of affected bowel segments. The clearing phase is followed by endoscopic surveillance every 1-2 years.[4-8]

Recent cohort studies have helped to develop evidence-based treatment and surveillance strategies. [4-8] However, most studies were retrospective, and the few prospective cohort studies were limited by the short follow-up duration [5, 9-11]. This has impeded analysis of the long-term risks (e.g. CRC development, colonoscopy-related complications) of endoscopic surveillance after initial polyp clearance. In addition, in the absence of long-term follow-up studies, the disease course of SPS has not been described in detail yet. It is therefore unknown whether patients should be advised the same stringent surveillance regimen for the rest of their lives, or whether the natural disease course of SPS is such that polyp recurrence will gradually fade away after several years of surveillance have passed.

To address these questions, we analysed a cohort of SPS patients that we have prospectively followed for over ten years. In this prospective cohort study, we aimed to describe the long-term risks and effectiveness of endoscopic surveillance. In addition we aimed to describe the long-term natural disease course during endoscopic surveillance of SPS in terms of polyp burden after the initial clearing phase.

METHODS

Study design & population

This was a post-hoc analysis of a prospective cohort of SPS patients in the Amsterdam University Medical Centres (Amsterdam UMC) in the Netherlands. All SPS patients in our centre have been surveilled in the context of two prospective cohort studies, unless they met the exclusion criteria described below. [5, 11] The data were prospectively collected by four consecutive research fellows (KB, YH, JIJ, AB).

The first prospective cohort study consecutively enrolled SPS patients from January 2007 until December 2012, and assessed the yield of annual endoscopic surveillance after

initial polyp clearance (Dutch Trial Register: www.trialregister.nl ID NL2629).[11] Subsequently, from January 2013 until April 2018, all patients were included in a second prospective cohort study, which assessed safety and feasibility of a new individualized surveillance protocol (Dutch Trial Register: www.trialregister.nl, ID NL4476).[5] Prospective data-entry in our database has continued until June 2019 awaiting initiation of new studies.

All patients were enrolled from our dedicated, weekly hereditary CRC outpatient clinic. This outpatient clinic has been staffed uninterrupted by a professor in gastrointestinal oncology (ED) who is specialized in hereditary CRC syndromes, a staff gastroenterologist specialized in hereditary CRC syndromes (BB), a research nurse specialized (SO) in hereditary CRC syndromes and a research fellow specialized in SPS. This has ensured uninterrupted specialist care for all our SPS patients. The attendance of the specialized research fellows ensured adherence to the surveillance protocols described below. Colonoscopies were performed by (or under direct supervision of) ED or BB, or by a selected group of other GI endoscopists who had performed at least 1000 colonoscopies.

Because of the non-interventional nature of this cohort study, this study fell beyond the legislation regarding Medical Research Involving Human Subjects Act. In addition, the Amsterdam UMC's institutional review board (IRB) decided that both Hazewinkel's[11] as well as Bleijenberg's[5] study fell beyond the WMO, and were therefore exempt from formal ethical approval.

Inclusion and exclusion criteria

Patients were included in case they fulfilled the 2010 WHO's diagnostic criteria I (*≥5 SPs proximal to the sigmoid, with ≥2 being >10mm)* and/or III(>20 SPs of any size distributed throughout the colon.[12] Patients only fulfilling 2010's criterion II (*i.e. any number of SPs proximal to the sigmoid colon in a first-degree relative of a SPS patient*) were not included.

Patients were eligible for inclusion if they were undergoing treatment in the Amsterdam UMC between January 2007 and June 2019, with at least one surveillance colonoscopy after initial polyp clearance. Initial polyp clearance was defined as clearance of all relevant polyps (i.e. all sessile serrated lesions (SSLs), traditional serrated adenomas (TSAs) and conventional adenomas, as well as all hyperplastic polyps (HPs) ≥5mm in diameter). We excluded patients with inflammatory bowel disease or known CRC-related germline mutations (e.g. *GREM, PTEN, BMPR1A, SMAD4, ENG1,* (bi-allelic) *MutYH* or *APC*).

Surveillance protocols

All patients have been treated using uniform surveillance protocols. Between January 2007 and December 2012, patients were treated according to a surveillance protocol described by Hazewinkel et al.[11] Briefly, once clearing of initial polyp burden was achieved, patients underwent annual endoscopic surveillance.

Between January 2013 and June 2019 all patients were treated using an updated protocol, in which patients were appointed to surveillance intervals of either 1 or 2 years,

based on their most recent polyp burden. This protocol was described in detail elsewhere.[5] Briefly, each consecutive surveillance colonoscopy was scheduled with an interval of 1 year in case the most recent colonoscopy yielded ≥1 advanced SP, ≥1 advanced adenoma or ≥5 non-advanced clinically relevant lesions. In case no advanced polyps and <5 clinically relevant lesions were found, patients were appointed a 2 year interval. Clinically relevant lesions were defined as all polyps ≥5mm and all polyps <5mm but with the optical aspect of adenoma, TSA or SSL. All colonoscopies had to be of sufficient quality, with a Boston bowel preparation score of 6 or higher and cecum intubation. If these criteria were not met, a repeated procedure was scheduled within 6 months.

Histopathology

Histopathological assessment of all polyps was routinely performed by dedicated gastrointestinal pathologists. In line with the most up-to-date WHO guideline and with a recent review regarding nomenclature of serrated polyps[3, 13], serrated polyps were classified as HPs, SSLs with/without dysplasia and TSAs. Of note, SSL is synonymous with the frequently used terms 'sessile serrated polyp (SSP)' and 'sessile serrated adenoma/polyp (SSA/P)'. Advanced SPs (ASPs) were defined as any SP with dysplasia and/or with a diameter ≥10mm, as well as all TSAs. Adenomas were classified as tubular adenomas or (tubulo)villous adenomas. Advanced adenomas (AAs) were defined as all adenomas ≥10mm, with (tubulo)villous histology or high-grade dysplasia. Advanced neoplasia (AN) was defined as any ASP, AA or CRC.

Aims and outcome parameters

We primarily aimed to describe the long-term safety and feasibility of endoscopic surveillance. To this end we describe **1)** the risk of CRC development and **2)** the risk of colonoscopy-related complications per colonoscopy and per polypectomy. Our second aim was to assess whether or not polyp burden (AN, ASPs and AAs) during surveillance followed a distinct upward or downward trend during the multiple rounds of surveillance following the initial polyp clearance phase. We performed these trend analyses in the entire cohort, as well as after stratification into **1)** WHO subtype (fulfilment criterion I, III or I & III), **2)** smoking status (i.e. non-smoker or current/former smoker), **3)** gender and **4)** indication of first colonoscopy (i.e. screening or symptoms).

Colonoscopy-related complications were defined as events related to the colonoscopy occurring during or after the procedure (e.g. perforation, bleeding or post-polypectomy syndrome), leading to a change in management after the colonoscopy (e.g. re-colonoscopy, surgery, admission or antibiotic treatment). Perforations or bleedings that were resolved during the procedure and did not require additional treatment or admission were not considered colonoscopy-related complications. Complications were registered routinely in the complication registry of the Dutch society of Gastroenterology) until 2015. From January 2016, this registry was replaced by the Dutch Registration of Complications in

Endoscopy (DRCE). Occurrences of complications were identified by querying these two registries as well as the patient's medical charts.

Statistical analyses

The presence of statistically significant trends in polyp burden was analysed using a mixed effects logistic regression model in a per-colonoscopy database, with the presence of any AN, any ASP or any AA as independent variable. The numerical variable "round of surveillance" (1-9) was included in the model as fixed effect; individual patients were included as random effect to account for the repeated measurement for each subject. We defined a trend as a statistically significant association between the round of surveillance and the presence of AN, ASPs or AAs, with a two-sided p-value of ≤.05. We present the results of this logistic regression model as Odds Ratio (OR) with 95%CI, which should be interpreted as the OR of detecting AN for each additional round of surveillance. ORs >1.00 suggest an upward trend, ORs <1.00 suggest a downward trend.

All analyses were performed using RStudio[14] with lme4 package[15]. Figures were produced using GraphPad Prism (version 7.03, GraphPad Software, La Jolla California USA).

RESULTS

Baseline characteristics

A total of 212 potentially eligible patients was identified in our database, of whom 70 were excluded (Figure 1). Patients were most frequently excluded because they had not yet received their first surveillance colonoscopy after the clearing phase (30) or because they did not fulfil WHO's 2010 criterion I and/or III after careful review (11). Thus, 142 patients were include in the present study (Table 1).

The mean age at SPS diagnosis was 60.8 (± 9.7) years, and 65 (45.8%) were male. SPS diagnosis was made prior to 2010 in 39 (34.5%), between 2010 and 2014 in 68 (47%) and from 2015 onwards in 25 (17.6%) patients. At the end of the clearing phase, 56 (39.4%) patients had a diagnosis of WHO criterion I, 50 (35.2%) had a diagnosis of WHO criterion III and 36 (25.4%) fulfilled both criterion I & III. About one third (52, 36.7%) of patients was diagnosed with CRC. Nine patients were additionally diagnosed with a second synchronous CRC while 6 were later diagnosed with a second metachronous CRC. The median age at diagnosis of the first CRC was 59.5 years (range 25-75). Included patients underwent a median of 3 (IQR 1-4) surveillance colonoscopies after clearing, with a median follow-up duration of 47 months (IQR 15-71.5).

CRC during surveillance

One patient developed CRC while under endoscopic surveillance in our centre, corresponding to a cumulative 5-year CRC incidence of 1.0% (95%CI 0-2.9%). This CRC was diagnosed in 2015 in a 55-year old female at surveillance colonoscopy 23 months after initial polyp clearance. She had a history of cervical cancer 17 years earlier, which

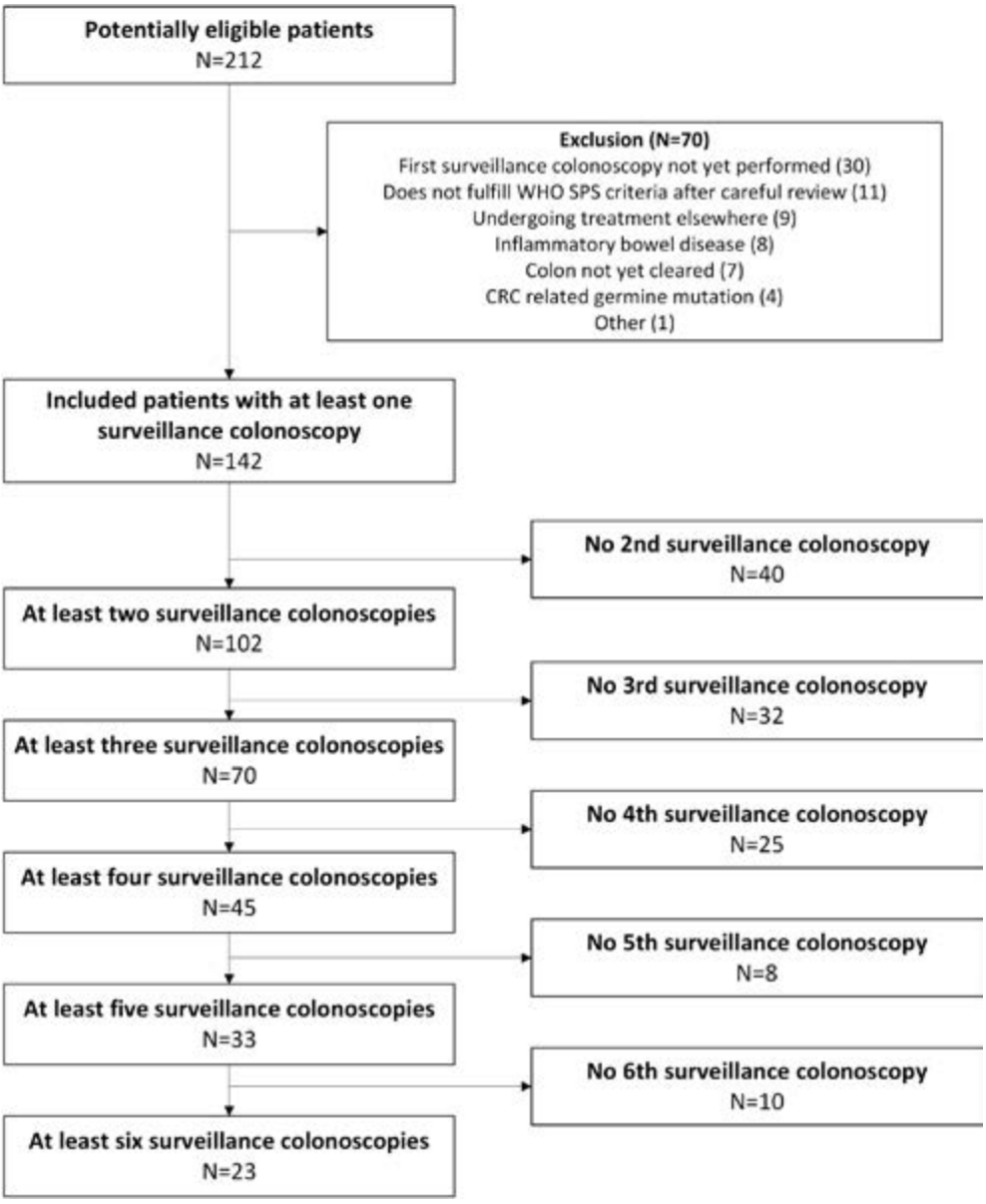

Figure 1. Study flow chart.

was treated with abdominal radiotherapy. This patient already underwent a right-sided hemicolectomy for a T4N0M0 cecal CRC that was diagnosed during the clearing phase. During the first surveillance colonoscopy after clearing, a tubulovillous adenoma with high-grade dysplasia was removed piecemeal from the transverse colon. During scar

Table 1. Baseline characteristics (n=142)

Age at diagnosis SPS, mean (SD)	60.8 (SD 9.7)
Year of SPS diagnosis, n (%)	
Prior to 2010	39 (34.5%)
2010-2014	68 (47.0%)
2015-2019	25 (17.6%)
Male, n (%)	65 (45.8%)
Indication first colonoscopy, n (%)	
FOBT-based screening	19 (13.4%)
Primary colonoscopy screening	7 (4.1%)
Familial CRC risk	14 (9.9%)
Symptoms	83 (58.5%)
Pain/discomfort abdomen	21/83 (25.3%)
Rectal blood loss	22/83 (26.5%)
Altered defecation pattern	24/83 (28.9%)
Anaemia	4/83 (4.8%)
Unexplained weight loss	3/83 (3.6%)
Other/unknown symptoms	9/83 (10.8%)
Other/unknown	19 (13.4%)
Smoking history	
Never smoker	54 (38.0%)
Former/current smoker	85 (59.9%)
No information	3 (2.1%)
WHO SPS classification*, n (%)	
I	56 (39.4%)
III	50 (35.2%)
I & III	36 (25.4%)
Patients diagnosed with CRC, n (%)	52 (36.7%)
One CRC	37/52 (71.1%)
Multiple CRCs	15/52 (28.9%)
Synchronous	9/15 (60%)
Metachronous	6/15 (40%)
Timing of CRC diagnosis, n (%)	
Prior to or during clearing phase	66/67** (98.5%)
During surveillance	1/67** (1.5%)
Age at CRC diagnosis, median (range)***	59.5 (25-75)
Median no. of colonoscopies per patient (IQR)	6 (4-10)
Colonoscopies prior to or during clearing	3.5 (2-5)
Surveillance colonoscopies after clearing	3 (1-4)
Total years of follow-up after clearing, median (range)	47 (4-144 months)

* Fulfilment of WHO criterion I and/or III was assessed directly after the clearing phase

** A total of 67 CRCs were diagnosed in 52 patients.

*** Refers to age at diagnosis of first CRC in case of a history of multiple metachronous CRCs.

inspection 6 months later, the scar could not be located. The subsequent surveillance colonoscopy 6 months thereafter revealed a T3N0M0 CRC in the transverse colon, for which a proctocolectomy was performed. All surveillance colonoscopies were scheduled according to the surveillance protocol.

Adverse events during surveillance

In the 142 included patients, a total of 447 surveillance colonoscopies were performed and 1,308 polypectomies. Two complications occurred, which corresponds to an overall complication risk of 0.45% (95%CI 0-1.6%) per colonoscopy and 0.15% (95%CI 0-0.6%) per polypectomy.

One complication was a post-polypectomy bleeding occurring 5 days after endoscopic mucosal resection (EMR) of a 15mm flat lesion, which required hospital admission, blood transfusion and re-colonoscopy with clip placement. Histopathology showed this lesion was a tubular adenoma with low-grade dysplasia. This corresponds to a bleeding risk of 0.22% (95%CI 0-1.2%) per colonoscopy or 0.08% (95%CI 0-0.4%) per polypectomy.

The second complication was a post-polypectomy syndrome consisting of fever and severe abdominal pain, which was managed conservatively with intravenous antibiotics during a 4-day hospital-admission.

No patients presented with a perforation, resulting in a per-colonoscopy perforation rate of 0% (95%CI 0-0.8%) and a per-polypectomy perforation rate of 0% (0-0.28%).

Polyp burden per surveillance round

ASP incidence differed per surveillance round between 2.9% (95%CI 0.35-9.9%) and 21.2% (9.0-39.9%, Table 2), but the incidence did not follow a clear upward or downward trend over time (OR 1.00, 95%CI 0.82-1.23; Figure 2A & Table 3). The incidence of AAs was lower, and varied between 3.9% (95%CI 1.1-9.7%) and 12.9% (95%CI 6.1-23.0%). Similar to ASP incidence, no distinct upward or downward trend was seen in AA incidence over time (OR 1.12, 95%CI 0.73-1.74, Figure 2A & Table 3). When stratified into fulfilment of WHO subtype, smoking status and gender, ASP and AA incidence did not follow a distinct upward or downward trend either (Figure 2B-D & Table 3). More detailed incidence rates for each surveillance round, stratified by WHO subtype, smoking status, gender and indication of first colonoscopy are included in supplementary table 1.

When stratifying our cohort for the indication of their first colonoscopy, figure 2E suggests a downward trend in ASP incidence in patients who underwent their first colonoscopy for a screening indication (FOBT-based, familial or primary screening). Logistic regression analyses resulted in non-significant downward trend with an OR of 0.42 (p=.10, Figure 2E & Table 3). This trend was not observed for AA incidence.

DISCUSSION

In this post-hoc analysis of a large prospective cohort of SPS patients in a tertiary referral centre with a dedicated team of endoscopists and nurses, we assessed the long-term

Table 2. Polyp burden per surveillance round 1-6

	Round of surveillance					
	1st	2nd	3rd	4th	5th	6th
	N=142	N=102	N=70	N=45	N=33	N=23
Patients with advanced neoplasia, (%)	21 (14.8% [9.4-21.7])	11 (10.8% [5.5-18.5])	11 (15.7% [8.1-26.4])	6 (13.3% [5.1-26.8])	8 (24.2% [11.1-42.3)	2 (8.7% [1.1-28.0])
Advanced SP[b]	18 (12.7% [7.7-19.3])	8 (7.8% [3.5-14.9])	2 (2.9% [.35-9.9])	4 (8.9% [2.5-21.2])	7 (21.2% [9.0-38.9])	2 (8.7% [1.1-28.0)
Advanced adenoma[c]	7 (4.9% [2.0-9.9])	4 (3.9% [1.1-9.7])	9 (12.9% [6.1-23.0)	3 (6.7% [1.4-18.3])	3 (9.1% [1.9-24.3)	1 (4.3% [0.1-22.0])
CRC	0 (0% [0-2.6])	1 (1.0% [0-5.3])	0 (0% [0-5.1])	0 (0% [0-7.9])	0 (0% [0-10.6])	0 (0% [0-14.8])
Patients with at least one, (%)						
≥1 SP	109 (76.8%)	66 (64.7%)	43 (61.4%)	31 (68.9%)	26 (78.8%)	13 (56.5%)
≥1 HP	76 (53.5%)	50 (49.0%)	33 (47.1%)	23 (51.1%)	19 (57.6%)	9 (39.1%)
≥1 SSL	54 (38.0%)	33 (32.4%)	19 (27.1%)	16 (35.6%)	14 (42.4%)	7 (30.4%)
≥1 TSA	0	1 (1.0%)	2 (2.9%)	0	0	0
≥1 SP with dysplasia	3 (2.1%)	2 (2.0%)	5 (7.1%)	1 (2.2%)	1 (3.0%)	1 (4.3%)
≥1 SP ≥10mm	16 (11.3%)	7 (6.9%)	6 (8.6%)	3 (6.7%)	6 (18.2%)	1 (4.3%)
≥1 Adenoma	47 (33.1%)	33 (32.4%)	26 (37.1%)	19 (42.2%)	13 (39.4%)	5 (21.7%)

[a]Advanced neoplasia: advanced adenoma, advanced SP or CRC.

[b]SP with dysplasia, diameter of ≥10mm or traditional serrated adenoma.

[c]Adenoma with high-grade dysplasia, villous histology or a diameter of ≥10mm

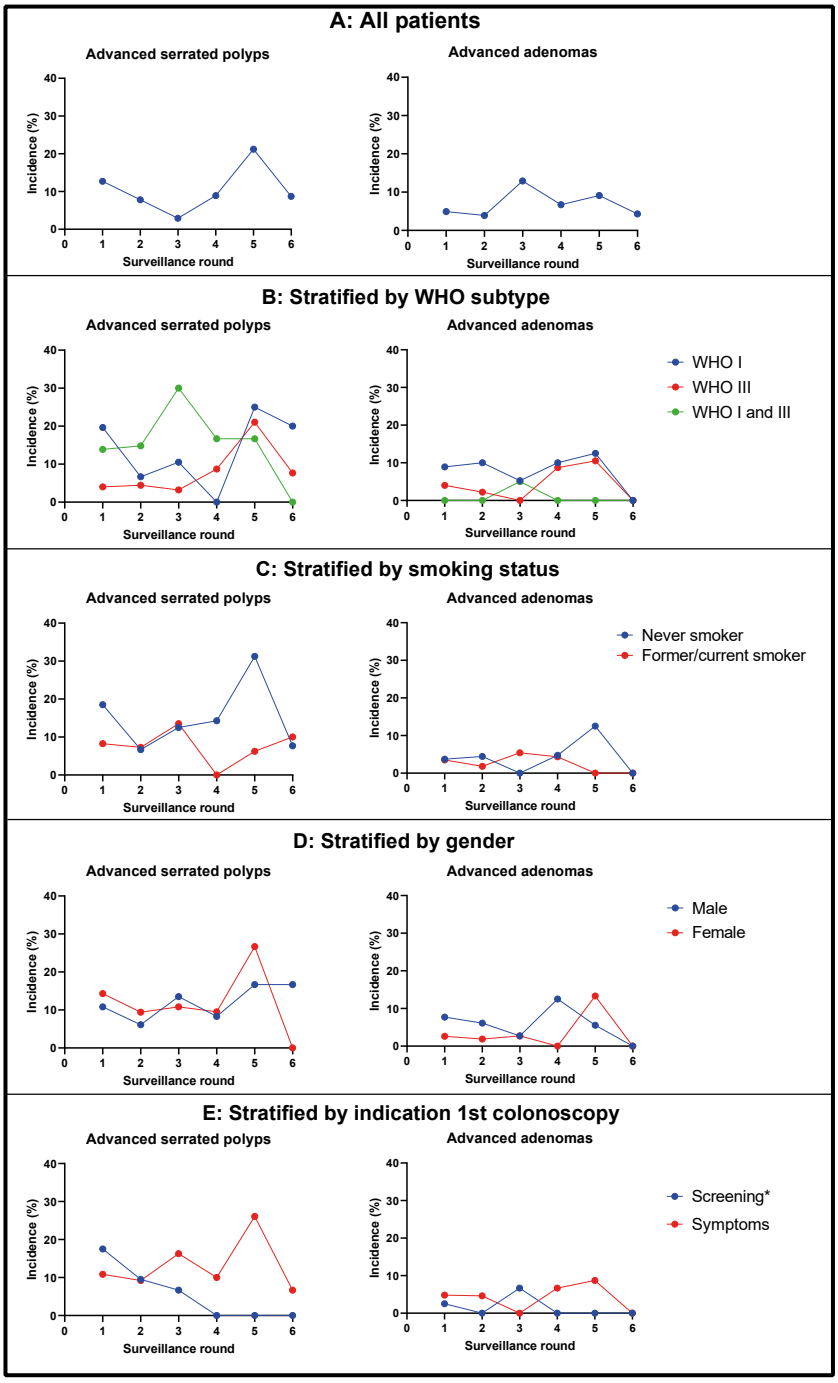

Figure 2. Polyp burden per surveillance round. * FIT-based, primary or familial screening. WHO, World Health Organization.

Table 3. Trend analysis Based on mixed logistic regression analyses with fixed effect for the round of surveillance. The odds-ratios should be interpreted as follows: Odds-ratios >1.0 suggest a positive trend, i.e. increasing polyp burden over multiple rounds of surveillance. Odds-ratios <1.0 suggest a negative trend, i.e. fading polyp burden over multiple rounds of surveillance.

	Any advanced neoplasia		Advanced serrated polyps		Advanced adenomas	
	Odds-ratio* (95% CI)	P	Odds-ratio* (95% CI)	P	Odds-ratio* (95% CI)	P
Overall	0.99 (0.83-1.9)	.94	1.00 (0.82-1.23)	.98	1.12 (0.73-1.74)	.60
By WHO subtype						
WHO I	.99 (0.75-1.31)	.95	1.00 (0.71-1.41)	.99	1.07 (0.53-2.18)	.85
WHO III	1.23 (0.89-1.69)	.21	1.36 (0.87-2.13)	.18	1.45 (0.71-2.97)	.31
WHO I and III	0.90 (0.62-1.29)	.56	0.89 (0.60-1.33)	.58	1.06 (0.41-2.76)	.90
By Smoking status						
Never smoker	1.29 (0.75-1.29)	.90	0.95 (0.71-1.27)	.75	1.30 (0.65-2.58)	.46
Current/former smoker	0.99 (0.77-1.27)	.92	1.00 (0.73-1.35)	.98	0.96 (0.50-1.44)	.55
By gender						
Male	1.00 (0.80-1.25)	.99	1.08 (0.84-1.39)	.55	0.87 (0.50-1.52)	.63
Female	0.96 (0.71-1.31)	.81	0.84 (0.55-1.30)	.44	1.08 (0.70-1.67)	.74
By indication of 1st colonoscopy						
Screening*	0.54 (2.6-1.13)	.10	0.42 (0.15-1.18)	.10	0.92 (0.31-2.76)	.88
Symptoms	1.01 (0.82-1.25)	.92	1.02 (0.79-1.30)	.90	1.01 (0.82-1.25)	.92

* Screening: FOBT-based, primary or familial CRC

outcomes of endoscopic surveillance during more than 10 years follow-up. First, we demonstrated that long-term endoscopic surveillance is effective, resulting in a low incidence of CRC. Second, we demonstrate that long-term endoscopic surveillance in such setting is safe, with only one colonoscopy-related bleeding and no perforations in 447 procedures. These findings are reassuring for clinicians and newly diagnosed SPS patients. Additionally, our results suggest that the natural disease course of SPS is such that the polyp burden remains more or less constant over many rounds of surveillance following initial polyp clearance. This finding supports life-long adherence to current surveillance recommendations.

The median number of colonoscopies that was required to clear the colon of relevant polyps, prior to initiating endoscopic surveillance, was 3.5 in our study. This is in line with a recent prospective study by MacPhail and colleagues, in which 2-3 colonoscopies were needed to achieve initial polyp control.[16] Furthermore, the low cumulative 5-year CRC incidence (1.0%, 95%CI 0-2.9%) in our cohort was comparable to most recent cohort studies.[5, 9, 11, 17, 18] Only one recent retrospective cohort study by Alcalde and colleagues, including 152 SPS patients, report a substantially higher incidence rate of 6.4% (95%CI 0-13.6). Although we cannot explain the discrepancy between the study of Alcalde and our and other recent cohort studies, our results support the assumption that endoscopic surveillance every 1-2 years is effective in CRC prevention. These findings can be used to inform SPS patients about a relatively favourable long-term prognosis once their initial polyp burden has been cleared, provided that surveillance protocols are adhered to, and surveillance is performed in an experienced centre with high quality colonoscopies (i.e. adequate bowel preparation and cecum intubation). The low CRC risk during surveillance sharply contrasts with the high CRC prevalence prior to diagnosis and/or initial polyp clearance (Table 1). The fact that CRC prevalence is high in case SPS is undiagnosed and thus untreated, while CRC risk is low once proper treatment and surveillance are provided, suggests that CRC is mostly preventable in SPS patients as long as proper treatment and surveillance are provided. This hypothesis is supported by the markedly lower CRC incidence of 15% in our patients if we analyse the subgroup who was diagnosed with SPS after a regular (FIT-positive) screening colonoscopy. This highlights why early diagnosis of SPS before these patients develop CRC is so important. Therefore, adequate detection of SPs and careful application of the WHO's diagnostic criteria are warranted.

Besides the low CRC-risk, the procedure-related risks of endoscopic surveillance were also reassuring. We demonstrate that surveillance procedures were safe, with a bleeding risk of only 0.22% (95%CI 0-1.6%) per colonoscopy, and a perforation rate of 0% (95%CI 0-0.82%). Considering the large number of colonoscopies SPS patients are exposed to, the risks and benefits of such intensive surveillance regimens should be carefully weighed. This low complication rate is in line with a recent report of MacPhail and colleagues, in which no perforations or delayed bleedings occurred in the management of 115 SPS patients during clearing and subsequent surveillance procedures.

Finally, our study was the first to describe the disease course in SPS patients following initial polyp clearance. We hypothesized that polyp burden would gradually decrease after several rounds of endoscopic surveillance. This hypothesis was based on the assumption that the first few surveillance colonoscopies after initial polyp clearance are more likely to detect polyps that were missed during initial polyp clearance, while during later surveillance colonoscopies predominantly newly developed, less advanced polyps would be detected. Following this hypothesis, the incidence of larger, more advanced polyps would gradually become lower after multiple rounds of surveillance colonoscopies. However, our results do not suggest such a downward trend. Instead, we demonstrate that polyp burden fluctuates from surveillance round to surveillance round without following a distinct upward or downward pattern. It remains unclear whether these advanced polyps occurring during later surveillance rounds represent polyps that were missed during earlier surveillance rounds, when they were still small, or whether these polyps newly developed between two surveillance colonoscopies. Regardless of the underlying mechanism, it seems plausible that SPS patients are at increased risk of having advanced polyps long after their initial polyp burden has been cleared. This advocates life-long adherence to surveillance guidelines for all patients, and discourages de-intensifying surveillance after several rounds of surveillance.

Our study has several strengths. We utilized a large single-centre cohort of SPS patients with over 10 years of prospective follow-up. In addition, all included patients have been treated according to standardized surveillance protocols as part of two consecutive prospective cohort studies.[5, 11] We could thus describe the natural disease course of SPS (while under endoscopic surveillance) with minimal risk of interfering factors such as variable treatment protocols and inter-endoscopist variation in polyp detection.

Nevertheless, several limitations have to be noted. First, we included SPS patients over a large time-span. This is of importance: Since 1:111 individuals with a positive FIT-result might fulfil the diagnostic criteria of SPS, the introduction of the national FIT-based bowel cancer screening program in the Netherlands in 2014 has likely led to an increased number of asymptomatic SPS patients.[2] Indeed, this hypothesis is can be confirmed by stratifying our cohort into patients that underwent their first surveillance prior to 2014 or from 2014 onwards: 3.8% vs. 25.4% received their first colonoscopy because of a positive FIT result, respectively. The CRC risk of FIT-positive, asymptomatic individuals may differ from patients that undergo their first colonoscopy because of symptoms. Another effect of including patients over such a large time-span results from the fact that the serrated polyp detection of participating endoscopists might have improved over the past 10 years, which might have made both clearing as well as surveillance colonoscopies more effective over the past few years. Second, although the single-centre design of our study has minimized interference from inter-hospital and inter-endoscopist variation, the external validity would have been superior in a multi-centre design. Thirdly, all patients in our cohort were diagnosed according to the WHO's 2010 diagnostic criteria, while the WHO recently (summer 2019) published their updated diagnostic criteria[19]. These are almost identical

to the 2010 criteria[19], except for the fact that 2010's criterion II has been abandoned in the 2019 criteria. Since we excluded all patients fulfilling 2010's criterion II in this study, our cohort is representative for future SPS patients diagnosed according to 2019's criteria as well.

In conclusion, in this large single-centre cohort study with over 10 years of prospective follow-up we demonstrate that the long-term outcomes of SPS patients under surveillance are favourable, with a low risk of CRC development and a low risk of colonoscopy-related complications. In addition, we demonstrate that polyp burden remains rather stable throughout long-term surveillance and as such life-long adherence to surveillance recommendations seems warranted. Our findings can be used to inform patients about their long-term prognosis during surveillance that will follow clearance of their initial polyp burden.

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9

Thesis summary and future perspectives

SUMMARY

Colorectal cancer (CRC) remains the second leading cause of cancer-related death worldwide.[1] Its aetiology is heterogeneous, and CRC can develop through multiple distinct pathways. One of these pathways is the serrated neoplasia pathway. This pathway has only gradually come into focus over the past two decades, and we now know that serrated precursor lesions are responsible for about 15-30% of all CRCs. [2-5] Unfortunately, our understanding as well as the clinical management of serrated polyps leaves much room for improvement. This is highlighted by the fact that the serrated neoplasia pathway seems to be the cancer pathway for a relatively high percentage of post-colonoscopy CRCs (PCCRCs).[6, 7]

The recent discovery of the substantial role of the serrated neoplasia pathway in CRC incidence has fuelled a dramatic increase in academic attention for serrated polyps, as is illustrated by the still increasing number of publications about this type of polyps (**Figure 1**, source: *PubMed*). In parallel to this publication surge, international guidelines have attempted to provide guidance for the management of serrated polyps, both for those occurring sporadically as well as those occurring in patients with serrated polyposis syndrome (SPS). This academic attention has greatly increased our understanding of serrated polyps. Nevertheless, the clinical management of patients with serrated polyps still poses several important challenges.

The most important challenges can be broadly categorized into **1)** High SP miss rates and suboptimal awareness among endoscopists; **2)** Poor performance of colonoscopy triage tests (i.e. faecal biomarker tests such as faecal immunochemical tests (FIT)), **3)** poor prognosis of CRCs that arise via the serrated neoplasia pathway; **4)** lack of evidence-based surveillance guidelines for patients with serrated polyps or serrated polyposis syndrome.

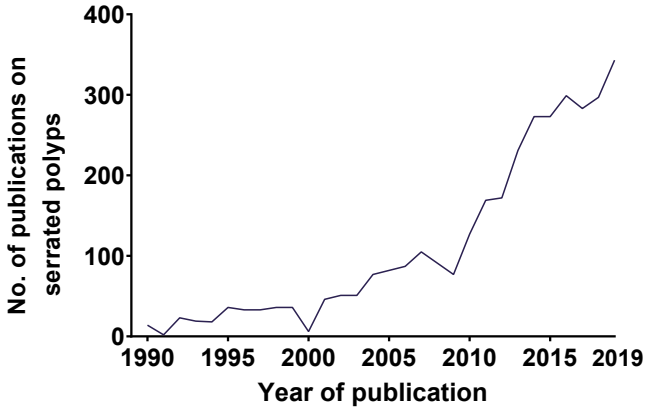

Figure 1. Number of publications about serrated polyps per year - 1990-2019

The studies described in this thesis have been performed to address these challenges, aiming to reduce the burden of disease caused by serrated polyps and their malignant successors.

Part I – Serrated polyps

Chapter 2 of this thesis describes a study in which we trained a group of endoscopists face-to-face in the detection of serrated polyps. We then compared their post-training proximal serrated polyp detection rate (PSPDR) with their detection rate at the start ("baseline PSPSDR"), as well as the PSPDR of 100 randomly selected untrained endoscopists around the country. We demonstrated that two training sessions of 45 minutes achieved a long-lasting increase in PSPDR from 9.3% at baseline to 15.4% after our training sessions, while the untrained endoscopists retained a stable PSPDR around 10% throughout the follow-up period of 4 years. Based on these findings, we concluded that improvement of SP detection can be achieved with a relatively simple intervention.

We deliberately chose the PSPDR as most suitable detection indicator since it does not rely on histopathologic discrimination between different SP subtypes. Theoretically, parameters such as 'SSL detection rate' or 'clinically relevant SP detection rate' could be a more precise indicator of an endoscopist's capability of detecting the most relevant serrated polyps. However, this requires reliable histopathologic discrimination between HPs, SSLs and TSAs and this discrimination is renowned unreliable, with high interrater variability between pathologists and pathology laboratories.[8-10] The PSPDR is not affected by such diagnostic uncertainty. Since the majority of 'clinically relevant' serrated polyps are located in the proximal colon, the PSPSDR is an attractive alternative to histology-based quality parameters.

The diagnostic uncertainty has important implications for choosing post-polypectomy surveillance intervals as well. Different countries choose different strategies to cope with this uncertainty. Therefore, in Chapter 3, we describe a study in which we compared two different strategies for determining the optimal surveillance interval after SP resection. The European Society for Gastrointestinal endoscopy (ESGE) recommends surveillance intervals purely based on size of serrated polyps and presence of dysplasia. This one-size-fits-all approach differs from the United States Multi-society taskforce on CRC (US-MSTF), who recommend more tailored surveillance strategies based on the various SP subtypes (e.g. patients with SSLs are surveilled more stringently than those with HPs). We demonstrate that the net-effect of this difference between guidelines is very modest. Based on a simulated application of the ESGE and US-MSTF strategies on over 5,000 individuals undergoing a screening colonoscopy, we demonstrate that 98% of them would receive an identical surveillance recommendation based on US-MSTF and ESGE guidelines. For the subgroup of 1,228 individuals with serrated polyps, concordance was 90%. Based on these results we concluded that surveillance recommendations not necessarily need to consider SP subtypes, since this does not substantially influence clinical management on a patient-level.

If serrated polyps are not timely detected and resected, they might eventually develop dysplasia and soon thereafter progress into CRC.[11] This progression from non-dysplasia to dysplasia or cancer is a critical step in the serrated neoplasia pathway, since non-dysplastic SSLs are relatively harmless and do not cause complaints, while their dysplastic or malignant successors give rise to *BRAF*-mutant full-blown CRC.[5] In **chapter 4** we describe a comprehensive cohort of SSLs with an early focus of dysplasia or cancer, which have thus *just* passed this critical step in the serrated neoplasia pathway. In combined analyses of clinical, immunohistochemical and whole exome sequencing (WES) results, we further elucidate two distinct serrated neoplasia pathways. We demonstrate that *APC* and *TP53* mutations might be involved in malignant progression of microsatellite stable, MLH1-proficient SSLs while SSLs following the microsatellite instable (MSI), MLH1-deficient pathway have often acquired mutations in certain hotspot regions of *ACVR2A* and *RNF43*, among many other affected genes. In addition, we describe two distinct mutational signatures between these two pathways, and describe clinical differences in age and gender distribution of patients with MLH1 proficient versus MLH1 deficient lesions.

Part II – Serrated polyposis syndrome

Chapter 5 describes a prospective cohort study in which we developed, implemented and evaluated a new, individualized surveillance protocol for patients with SPS. The goal of this individualized approach was to decrease the colonoscopy burden for patients at low risk of advanced neoplasia. We show that implementation of this personalised protocol might result in a substantial 39% decrease in colonoscopy burden for SPS patients compared to a strategy that recommends annual colonoscopies for all SPS patients, such as the US-MSTF's guideline.[12] This decreased colonoscopy burden was not associated with an increased CRC risk. The 5-year cumulative incidence of CRC under surveillance of 1.3% was low compared to other recent cohort studies.[13-16]

In **chapter 6** we report the risk of CRC or advanced neoplasia of SPS patients that underwent a (sub)total colectomy. Since SPS manifests as a colorectal disease, surgical removal of the colon would hypothetically mitigate most of the excess CRC risk of SPS patients. We prove this hypothesis to be true, based on 48 SPS patients with only rectum or rectosigmoid in situ. After a median of 4.7 years of prospective follow-up, none of the included patients developed CRC and the 13% 5-year cumulative AN incidence was low compared to patients that did not undergo a (sub)total colectomy. Surgical colon resection should to our opinion be averted by endoscopic polyp control whenever possible. However, for those patients in whom such extensive surgery could not be averted due to excessive polyp burden or concurrent CRC, the frequency of post-surgical endoscopic surveillance can likely be reduced.

One group of SPS patients was systematically excluded from the studies in chapter 5 and chapter 6. This concerns patients only fulfilling the World Health Organization's (WHO) criterion II of the 2010 diagnostic criteria for SPS (i.e. "presence of ≥1 SP proximal to the sigmoid in an individual with a first-degree relative (FDR) with SPS").[17] In **chapter 7**

we describe the first cohort study focusing specifically on patients fulfilling this criterion, which was abandoned in the updated WHO diagnostic criteria in 2019.[18, 19] Unlike our hypothesis that all FDR, regardless of the presence of "≥1 SP proximal to the sigmoid", would have a similar risk of CRC or advanced neoplasia, the results of this study suggest a somewhat increased risk among FDRs fulfilling the (currently abandoned) criterion II. This unexpected finding should be interpreted in the light of several potential biases, inherent for the retrospective study design. Fortunately, as is the case for all other FDRs of SPS patients, current guidelines already recommended surveillance colonoscopies every 5 years for these individuals.

Finally, in **chapter 8** we describe the prospective follow-up of our SPS patients in the Amsterdam University Medical Centers (Amsterdam UMC), which has been ongoing for over 10 years. As part of two consecutive prospective trials, patient data has been collected since 2007. Based on this long-term follow-up, we describe the disease course of SPS patients that are under long-term surveillance. We demonstrate that endoscopic surveillance is associated with a low risk of colonoscopy-related complications, and that CRC incidence is low. Furthermore, we found no evidence for our hypothesis that polyp burden of SPS patients slowly diminishes after several rounds of endoscopic surveillance. Instead, we show that polyp burden remains rather stable through many rounds of surveillance.

FUTURE PERSPECTIVES

The era in which adenomas were considered the sole precursor lesion for CRC development has been left far behind us. Serrated polyps are now widely accepted as significant contributor to CRC development. The ever-increasing academic attention for serrated polyps has brought us a wealth of novel insights, but also an equal number of new, unanswered questions. While this thesis contributes in improving care for patients with serrated polyps and serrated polyposis syndrome, its content is equally important in guiding future studies that might answer these questions.

Serrated polyp detection

We showed in this thesis that the suboptimal detection of serrated polyps by endoscopists can be improved by simple face-to-face training sessions (chapter 2). Wide-spread implementation in the Netherlands and abroad would hypothetically prevent a large number of PCCRCs. However, such large-scale provision of live training sessions, held by competent and experienced trainers, is challenging. Recruiting sufficient numbers of (trained) trainers will be difficult and expensive, and the quality of the intervention would strongly rely on the quality of the trainers. An alternative, cheap and scalable solution for this problem would be to provide the training as an online learning module. This would facilitate immediate availability for endoscopists around the world, even in the most remote areas. With minimal funding, training could easily be offered free of charge to

low-resource countries as well. Hence, development and validation of a digitalized version of our (or similar) training sessions could be a valuable next step in improvement of SP detection and thus reduction of PCCRC. However, this strategy does rely on improving the *endoscopist's* ability to detect polyps and thus, the endoscopists have to be motivated to do so. This is essential *at present*, but we live in an era in which artificial intelligence (AI) is rapidly replacing human tasks in all industry sectors, including healthcare. In gastrointestinal endoscopy too, attention from academics and clinicians in AI is rapidly increasing. Lecture sessions during international gastroenterology conferences focusing on AI in endoscopy are overcrowded, and the number of AI-related publications is rapidly increasing. Promising algorithms for polyp detection, polyp differentiation, automated endoscopy reports, oesophageal dysplasia detection and many other applications have been reported over the past years.[20-22] The performance of polyp detection algorithms is impressive[23-27], with some authors already describing higher detection rates for computers than for endoscopists. It is only a matter of time before these algorithms will be a part of our 'everyday' endoscopy equipment. This urges us to question: What is the added value of training endoscopists to detect serrated polyps if their endoscopy machines will automatically detect polyps *for* them with the push of a button?

First of all, we firmly believe that the role of AI algorithms should never be to *replace* the endoscopist's skills, but rather to supplement them. Endoscopists are ultimately responsible for the quality of care they provide, and should thus always be capable of critically interpreting the output of the machines they work with. To be able to do so, they should be capable and trained in the detection of serrated polyps without the help of AI. Secondly, the accuracy of AI algorithms for detection as well as differentiation of SPs lags behind compared to the accuracy for adenomas.[28] This means that although AI algorithms might soon outperform the endoscopist's eye in detection and differentiation of adenomas, it might still take years to develop algorithms that can outperform endoscopists with regard to SP detection and differentiation. Admittedly, this hypothesis might underestimate the speed of technical advances that are currently being made. Finally, once AI algorithms are capable of SP detection and differentiation, their (expensive) adoption might be delayed for many years due to commercial, logistical and financial obstacles. This is especially true for low- and middle-income countries, who will remain dependent on the endoscopist's eyes for much longer than high-income countries.

Complete reliance on AI for improvement of SP detection and differentiation in the near future therefore seems overly optimistic, and might lead to deterioration of valuable knowledge and skills of endoscopists. Instead, in our opinion traditional methods to train endoscopists remain of importance, and are still an important topic of future research. Simultaneous improvement of AI algorithms to detect and differentiate serrated polyps will ultimately lead to a synergistic collaboration between man and machine.

Serrated polyp detection: the more the better?

If human and artificial intelligence will jointly increase serrated polyp detection, a new problem arises. Only a fraction of all serrated polyps will actually progress into CRC, and thus the majority of detected and resected serrated polyps could have been left in situ without ever progressing into CRC. The problem is that, at present, it is impossible to identify which polyp will develop into CRC and which one will not. And therefore, we are currently advised to resect *all* polyps (except for diminutive rectal HPs). We do know, however, that some serrated polyps (e.g. large or dysplastic SSLs), are more likely to progress into CRC than others (e.g. diminutive HPs). If SP detection increases due to AI algorithms and improved training of endoscopists, the additionally detected polyps would likely be predominantly small lesions with a lower risk of malignant progression. It is highly questionable whether resection of such lesions actually reduces CRC incidence, while it is accompanied with procedure related complication risks, longer colonoscopy times and higher costs for excess histopathologic assessment. In other words, there might be limits as to which extent improvement of SP detection is beneficial.

In an ideal world, only polyps that will progress into CRC would be resected during colonoscopy, while patients without such polyps would not even undergo a colonoscopy in the first place. This would require highly accurate triage tests for colonoscopy, and real-time differentiation between polyps which will progress into CRC and which will not. Although it is debatable whether this will ever be possible, we are far from that ideal world at present. Nevertheless, important steps towards this goal have been taken over the past decade.

Faecal biomarker tests have emerged as an effective tool to identify patients with high-risk polyps or CRC. Concerningly however, these biomarkers detect high-risk adenomas with increasing accuracy, but adequate biomarkers for patients with high-risk serrated polyps are lacking. The sensitivity of the faecal immunochemical test (FIT), the most widely used biomarker test, for the detection of large SSLs is estimated to be between 5-10%, which is multi-fold lower than the sensitivity for advanced adenomas.[29, 30] Even the Cologuard, which has the highest sensitivity for advanced serrated polyps, leaves much room for improvement with a sensitivity of only 42,4%.[30] In order to improve the accuracy of (faecal) biomarker tests, a better understanding of the molecular aberrations in high-risk serrated polyps is therefore urgently needed.

Molecular biology of the serrated neoplasia pathway(s)

In this thesis we described several novel driver events in the serrated neoplasia pathway. As already suggested by other authors [11, 31], our results confirm that there are actually *two* serrated neoplasia pathways: the MLH1-proficient and MLH1-deficient pathway. The biological characteristics of these pathways were found to be so different that we should abandon references to "THE serrated neoplasia pathway". Instead, it seems more logical to refer to either the MLH1 proficient (microsatellite stable, MSS) or MLH1 deficient (microsatellite instable, MSI) serrated pathway. Since these pathways each have their own

distinct driver mutations and mutational signatures, and even occur in different types of patients, diagnosis and treatment of both CRC subtypes likely differ accordingly. This is an important concept to keep in mind in all future studies aiming to identify novel biomarkers or targeted treatment strategies for serrated pathway CRCs.

In our study described in chapter 4 of this thesis we identified hotspot mutations in *ACVR2A* and *RNF43* in over 80% of MLH1 deficient lesions. The downstream effects of these mutations should be assessed in preclinical models, such as the CRISPR-CAS9 based organoid model described by Lannagan and colleagues.[32, 33] Regardless of their biological effect, however, they might be detectable in stool samples. If this would be the case, addition of these biomarkers to currently available tests (e.g. Cologuard [30]) could improve sensitivity for these high-risk lesions, which are on the verge of becoming full-blown CRC.

ACVR2A and *RNF43* hotspot mutations carry another interesting implication. Frameshift mutations in microsatellite regions result in the production of faulty proteins, which are recognized as 'neoantigens' by the immune system. Such neoantigens resulting from frameshift mutations are known as frameshift peptides.[34, 35] Previous studies have demonstrated that Lynch patients with a history of MSI CRC often had detectable levels of antibodies against certain frameshift peptides in their blood. These frameshift peptides originated from recurrently mutated genes in MSI CRC (e.g. *AIM2* and *TGFBR2*).[34] Theoretically, if antibodies against frameshift peptides of *ACVR2A, RNF43* or other genes would be detectable in the serum of patients with an MLH1-deficient advanced SSL as well, they could also serve as a serum biomarker for prevention or early diagnosis of CRC in such patients. This seems an attractive topic for future research. Such biomarkers could for example play a future role in surveillance of patients with serrated polyposis syndrome, who are clearly at increased risk of developing (dysplastic) SSLs.

Unfortunately, the molecular biology driving cancer development in MLH1 proficient serrated polyps remains much less understood. In our study we identified a mutational signature that suggested a deficiency in double stranded DNA break repair, a repair mechanism that is often ascribed to *BRCA1* and *BRCA2,* among other genes. In our study, however, we could not identify which genetic or epigenetic aberrations could explain the signature we observed. Perhaps comprehensive methylation analyses of serrated polyps with dysplasia would be helpful in this regard, since promotor methylation plays a major role in serrated tumorigenesis. The role of chromosomal instability such as copy-number alterations (CNAs) also deserves attention. This has hardly been studied in serrated polyps, despite the fact that CNAs play a major role in the traditional adenoma-carcinoma pathway.[36, 37]

Part II – Serrated polyposis syndrome

Reducing colonoscopy burden

At present, SPS patients are often under stringent surveillance regimens, with some guidelines even recommending annual surveillance.[12] These strict recommendations were based on early, retrospective cohort studies reporting up to 7% 5-year CRC risk.[38]

However, most of the recently published cohort studies have shown much lower risks of CRC once initial polyp burden was cleared and regular endoscopic surveillance was provided.[13, 14, 16, 39-41] Indeed, based on the only four prospective cohort studies published to date, the true 5-year CRC incidence during surveillance is more likely to range between 0 and 1.3%.[16, 39-41] Taking into account that SPS is a very heterogeneous disease with variable CRC risk, the currently recommended surveillance intervals of 1 year or 1-2 years are probably too stringent for many (if not most) SPS patients.

The potential benefits of further reducing colonoscopy burden can be illustrated by applying the estimated prevalence of 1:111 to the Netherlands. Between 2020 and 2030, an estimated 66,000 FIT positive colonoscopies will be performed in Dutch individuals per year (source: RIVM). This means about 595 new individuals fulfil the diagnostic SPS criteria *each year*, or roughly 6,000 new SPS patients in the coming decade. An annual increase of 6,000 colonoscopies solely for SPS surveillance (in a scenario of annual surveillance) would not only mean an excessive burden for patients, but also for our health care system as a whole, which is already constrained by limited time and financial resources. If we would be able to effectively identify low-risk SPS patients and decrease their surveillance frequency to once every 3-5 years, enormous benefits could be achieved.

Although this calculation illustrates the need for reducing colonoscopy burden, another urgent reason for reduction results from the fact that management of SPS patients should take place in one of the few dedicated expert centres according to current Dutch guidelines.[42] The capacity of these few centres is at risk of becoming exceeded in the coming years.

This leads us to question whether surveillance of SPS should be performed *exclusively* in these expert centres, or whether certain cases could also be surveilled in non-expert centres. The reassuringly low estimates of CRC incidence during surveillance in prospective studies (i.e. 0-1.3% per 5 years) mostly stems from cohorts undergoing surveillance in expert centres.[16, 39-41] Surveillance of SPS by non-expert endoscopists might result in higher CRC incidence rates due to lower serrated polyp detection rates and unfamiliarity with SPS and surveillance guidelines. However, with additional safety measures, cases with a mild phenotype could in our opinion safely be performed in non-expert centres as well, increasing our nationwide capacity to provide care for SPS patients.

New Zealand poses an interesting example in this regard. In New Zealand, treatment and surveillance of SPS patients is performed in non-expert centres, but under coordination of a tertiary centre with longstanding expertise in management of gastrointestinal cancer syndromes (i.e. the New Zealand Familial Gastrointestinal Cancer Service, NZFGICS). After each procedure, the results are discussed with a central team of clinical advisors of the NZFGICS. This team subsequently provides the non-expert centres with recommendations about further surveillance and management.[41] Following this approach, a 5-year CRC incidence of 0% was observed in 96 individuals with SPS in New Zealand.[41]

A similar strategy could be considered in the Netherlands as well, with the current centres of expertise coordinating SPS care in their respective regions, in collaboration

with selected endoscopists with high SP detection rates in non-expert centres. This exchange of expertise could be facilitated by introducing a central online registry, in which recommendations about e.g. surveillance intervals could be provided by the expert centres. An additional benefit of this approach would be the possibility of nationwide coverage of prospective cohort studies, as has been done in New Zealand.[41] Such large prospective cohorts are indispensable in evaluating new surveillance strategies. Chapter 5 and 6 of this thesis might be useful in guiding the design of these studies.

In chapter 5 of this thesis we described a personalised surveillance protocol which aims to reduce colonoscopy burden by allocating patients for a prolonged 2-year surveillance interval in case CRC risk is estimated to be low. We demonstrated that this prolonged interval was not associated with an increased CRC risk. The opposite seemed to be true: in case our protocol allocated a patient to the prolonged 2-year surveillance interval, the subsequent surveillance colonoscopy yielded 16% advanced neoplasia compared to 25% in case our protocol led to a 1-year recommendation. This indicates that further extension of surveillance intervals to 3 years (or even 5 years) might be possible for individuals that are allocated to the 2 years interval according to the present protocol. In addition, patients only fulfilling diagnostic criterion II (i.e. >20 serrated lesions of any size, distributed throughout the large bowel, with at least 5 proximal to the rectum [19], formerly known as criterion III in the 2010 diagnostic criteria [43]) were less than half as likely to develop advanced neoplasia during surveillance compared to other SPS patients. This suggests that patients only fulfilling criterion II could also be surveilled at lower intensity. Lastly, we found patients with a history of (sub)total colectomy had a very low risk of advanced neoplasia (chapter 6). Safety of stepwise prolongation of surveillance intervals to 5 years could be assessed.

The serrated polyposis syndrome puzzle: Nature or Nurture?

Another urgent topic for future studies, which has seen many unsuccessful attempts, is unravelling the aetiology of SPS. Aside from a few rare cases that are caused by germline mutations in *RNF43*[44-47], the underlying cause of SPS is unclear. Smoking has been implicated as causative by some studies, and an increased SPS prevalence among first-degree family members suggests a hereditary aetiology in some cases as well.[13, 14, 48, 49] Whereas these factors might indeed be associated with SPS development, they seem responsible for a minority of cases. Future studies will hopefully shed light on the aetiology of the remaining majority.

Since multiple researchers have failed to identify causative germline mutations (aside from rare cases caused by *RNF43* mutations), other possible epigenetic and environmental aetiologies have to be explored as well. For example, the methylome of healthy colon tissue of SPS patients should be compared to that of non-SPS patients. Indeed, a yet unpublished study by a Spanish research group, presented during the digestive disease week in Washington in 2018, reports a differential methylation pattern of healthy colonic

mucosa in SPS patients, which suggests a predisposing field effect for SP formation.[50] Such colon-wide aberrant methylation patterns could explain the relation between tobacco smoking and SPS (and sporadic serrated polyps): tobacco smoking has been found to cause hypermethylation of CpG-islands in healthy colon tissue of smokers.[51] Other potential underlying mechanisms, such as the microbiome, chronic (viral) infections or exposure to environmental influences (e.g. chemotherapy, radiation, dietary habits) should also be explored.

One difficulty in solving the puzzle is the heterogeneity of SPS. A patient with 50-100 mainly distal hyperplastic polyps and another patient with 5 large, proximal SSLs will both receive the same diagnosis of SPS. It seems likely, however, that the underlying disease mechanism for these two patients is completely different. Thus, future studies should be adequately powered to perform subgroup analyses, since newly discovered aetiologies will probably be responsible for only a subgroup of SPS patients. The ever-increasing prevalence of SPS, resulting from increased detection, better diagnosis and growing academic attention, reassures us that sufficiently powered, large and international cohort studies will be able to further unravel the multiple aetiologies of SPS.

In summary, our results and the directions for future research provided in this final chapter share the same goal: reducing the number of CRC developing from serrated polyps, without exposing patients to unnecessary colonoscopies. With the promising recent technical advances, proper training of endoscopists, improved biomarker accuracy and an increased understanding of SPS, we are confident that such reduction of CRC can be achieved in the near future, while simultaneously *reducing* the number of (unnecessary) colonoscopies.

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Nederlandse samenvatting

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Dankwoord

NEDERLANDSE SAMENVATTING

Wereldwijd is darmkanker, na longkanker, de meest voorkomende oorzaak van kanker gerelateerde sterfte. Darmkanker ontstaat niet van de ene op de andere dag, maar groeit vanuit een voorloperstadium van darmpoliepen. Dit proces, van poliep tot darmkanker, duurt waarschijnlijk meer dan tien jaar.(1) Er kan onderscheid worden gemaakt tussen twee soorten poliepen: adenomen en serrated poliepen. Middels een kijkonderzoek van de dikke darm (coloscopie) kunnen darmpoliepen worden opgespoord en voortijdig worden verwijderd. Op die manier kan darmkanker worden voorkomen.(2)

Tot kortgeleden werd gedacht dat alléén adenomen konden uitgroeien tot darmkanker, en dat serrated poliepen goedaardig waren. In de afgelopen jaren is echter duidelijk geworden dat serrated poliepen óók een aanzienlijk deel, tot wel 30%, van alle gevallen van darmkanker veroorzaken. Dit heeft voor een verschuiving gezorgd in de strategieën om darmkanker te voorkómen: werden maag- darm- leverartsen (MDL-artsen) vroeger nog geacht om alleen adenomen te verwijderen, nu dienen zij óók serrated poliepen te detecteren en te verwijderen.

Helaas blijkt uit meerdere onderzoeken dat serrated poliepen nog (te) vaak over het hoofd worden gezien tijdens een coloscopie. Dit heeft waarschijnlijk te maken met het subtiele en gecamoufleerde uiterlijk van deze poliepen: ze hebben vaak dezelfde kleur als de omliggende darmwand, zijn overwegend vlak en bedekt onder een laagje slijm of ontlasting.(3). Wat waarschijnlijk ook een rol speelt, is dat er bij de opleiding van MDL-artsen van oudsher, tot ongeveer 10-20 jaar geleden, veel aandacht was voor het detecteren van adenomen, maar nauwelijks voor het detecteren van serrated poliepen. (4, 5) Serrated poliepen die over het hoofd worden gezien tijdens een coloscopie krijgen de kans om uit te groeien tot darmkanker. Op basis van de moleculaire eigenschappen en de voorkeurslocatie van darmkanker die optreedt ná een coloscopie (zgn. post-coloscopie colorectaal carcinoom, PCCRC) blijkt inderdaad dat serrated poliepen relatief vaak verantwoordelijk zijn voor de ontwikkeling van PCCRCs.(6, 7)

Een aanvullend probleem is dat mensen met serrated poliepen niet goed worden geïdentificeerd in het bevolkingsonderzoek. Patiënten die een coloscopie ondergaan in het Nederlandse bevolkingsonderzoek worden geselecteerd op basis van de fecale immunochemische test (FIT). De FIT detecteert bloed in de ontlasting, en is daardoor zelden afwijkend bij patiënten met serrated poliepen. Dit is waarschijnlijk een gevolg van het feit dat serrated poliepen niet snel bloeden, hetgeen bij adenomen veel sneller gebeurt. Daardoor worden patiënten met hoog-risico serrated poliepen, zoals grote of dysplastische laesies, vaak ten onrechte gerustgesteld door een gunstige FIT-uitslag. Dit heeft geleid tot een roep om andere biomarker testen die niet alleen adenomen, maar ook serrated poliepen kunnen detecteren.

De ontdekking en erkenning van de substantiële bijdrage van serrated poliepen aan het ontstaan van darmkanker heeft in de afgelopen 20 jaar tot een enorme toename in wetenschappelijk onderzoek geleid. Deze toename in wetenschappelijk onderzoek heeft

ons veel geleerd over serrated poliepen. Toch loopt de kennis over serrated poliepen fors achter op onze kennis over adenomen, en valt er zoals beschreven nog veel winst te behalen in het verbeteren van de detectie.

De belangrijkste uitdagingen laten zich samenvatten in een aantal punten: 1) De suboptimale detectie van serrated poliepen door MDL-artsen, 2) De matige sensitiviteit van coloscopie triage tests (e.g. fecale biomarkers, zoals de FIT), 3) de slechte prognose van darmkanker die voortkomt uit (gemiste) serrated poliepen en 4) een gebrek aan wetenschappelijk onderbouwde richtlijnen voor de behandeling van patiënten met het zogenaamde serrated polyposis syndroom.

De studies in dit proefschrift hebben allen ten doel gehad om deze uitdagingen aan te gaan, en op die manier het aantal patiënten met darmkanker te verminderen.

Deel I – Serrated poliepen

In **hoofdstuk 2** van dit proefschrift beschrijven we een studie waarin een groep MDL-artsen klassikaal werd getraind in het detecteren van serrated poliepen. Hun detectiegraad voor serrated poliepen werd vergeleken met een onafhankelijke, willekeurige groep MDL-artsen die niet werd getraind. De resultaten van deze studie lieten zien dat de detectiegraad voor serrated poliepen is het proximale deel van het colon (proximal serrated polyp detection rate, PSPDR) van 9.3% naar 15.4% steeg na onze training sessies, terwijl de ongetrainde endoscopisten een detectiegraad van circa 10% behielden gedurende de volledige 4-jaar durende follow-up. Uit deze resultaten concluderen wij dat endoscopisten goed 'trainbaar' zijn in het detecteren van serrated poliepen middels eenvoudige klassikale trainingen.

Als uitkomstmaat in deze studie kozen wij juist de PSPDR, omdat serrated poliepen die kunnen uitgroeien tot darmkanker (met name het subtype sessiel serrated laesie, SSL) veelal proximaal gelokaliseerd zijn, en omdat er voor de PSPDR geen rekening gehouden hoeft te worden met het type serrated poliep. Het is voor pathologen namelijk erg moeilijk om het subtype SSL te onderscheiden van het subtype hyperplastische poliep (HP), welke niet uitgroeit tot darmkanker en zich veelal in het distale colon bevindt. Dat maakt de PSPDR een aantrekkelijke graadmeter voor de detectiegraad van relevante serrated poliepen. Immers, de PSPDR is onafhankelijk van de patholoog, en wordt dus ook niet beïnvloed voor pathologische misclassificatie.

Deze pathologische misclassificatie heeft daarnaast ook implicaties voor het kiezen van de optimale termijn van controle coloscopieën (surveillance coloscopieën) nadat er bij een patiënt poliepen zijn verwijderd. Patiënten met SSLs zouden idealiter met een korter interval gecontroleerd moeten worden middels een surveillance coloscopie dan patiënten met HPs, maar omdat de pathologische classificatie niet betrouwbaar is, is dergelijke stratificatie complex. Er zijn aanmerkelijke verschillen tussen internationale richtlijnen om met deze onzekerheid om te gaan. In **hoofdstuk 3** beschrijven we daarom een studie waarin we de twee grootste internationale richtlijnen voor postpoliepectomie surveillance met elkaar vergelijken: de Europese en de Amerikaanse richtlijn. De Europese richtlijn

adviseert een 'one-size-fits-all' aanpak, waarbij alleen de grootte en de aanwezigheid van dysplasie worden gebruikt voor het bepalen van de surveillance termijn na resectie van serrated poliepen, ongeacht het subtype. In de Amerikaanse richtlijn daarentegen wordt *wel* gestratificeerd op basis van subtype: patiënten met SSLs worden strenger gecontroleerd dan patiënt met alleen HPs. Om te onderzoeken of dit verschil in aanpak ook echt tot ander beleid leidt, simuleerden wij bij meer dan vijfduizend patiënten welk surveillance beleid er geadviseerd zou worden bij gebruik van de Amerikaanse, dan wel de Europese richtlijn. In 98% van alle patiënten bleken de Europese en Amerikaanse richtlijn tot exact hetzelfde advies te komen. In de subgroep van patiënten met serrated poliepen was de overlap 90%. Op basis van deze resultaten concludeerden wij dat het subtype serrated poliep niet per sé gebruikt hoeft te worden bij het bepalen van surveillance termijnen, aangezien dit op patiëntniveau nauwelijks verschil maakt.

Bovenstaande studies hebben zich met name gericht op het tijdig detecteren van serrated poliepen, vóórdat ze uit zijn gegroeid tot darmkanker. Als serrated poliepen niet tijdig worden verwijderd, krijgen zij de kans om dysplasie te ontwikkelen, een voorloper van darmkanker. Deze dysplasie kan vervolgens in korte tijd ontaarden in darmkanker.(8) Deze overgang van goedaardige naar kwaadaardige poliep is een kritieke stap in de zogenaamde 'serrated neoplasia pathway', waarmee de stapsgewijze progressie van serrated poliep tot darmkanker wordt aangeduid. In **hoofdstuk 4** hebben wij een groep serrated poliepen onderzocht met een zeer klein gebied van dysplasie of kanker. Deze poliepen hebben deze kritieke stap dus *net* doorlopen, en kunnen worden beschouwd als de vroegste kwaadaardige serrated poliepen. Er is nog weinig bekend over de moleculaire achtergrond van deze overgang, al is wel bekend dat de progressie naar darmkanker in ongeveer de helft van alle gevallen volgt op epigenetische uitschakeling van het gen *MLH1*. Dit leidt tot microsatelliet instabiliteit, hetgeen gekarakteriseerd wordt door razendsnelle accumulatie van mutaties. Door middel van uitvoerige klinische en moleculaire analyses, laten wij zien dat de genen *APC* en *TP53* vaak gemuteerd zijn in het ontstaan van MLH1-proficiente darmkanker, terwijl *ACVR2A* en *RNF43* gemuteerd bleken in vrijwel alle laesies die uitgroeiden tot MLH1-deficiente darmkanker. Verder bleken deze MLH1-proficiente en MLH1-deficiente tumoren een geheel eigen mutatie patroon te hebben, en ook klinische eigenschappen zoals leeftijd en geslacht verschilden aanmerkelijk tussen de twee groepen.

Deel II – Serrated polyposis syndrome

In **hoofdstuk 5** beschrijven we een prospectieve cohortstudie waarin we een nieuw, gepersonaliseerd behandelprotocol evalueren voor endoscopische controle van patiënten met serrated polyposis syndroom (SPS). Het doel van deze gepersonaliseerde aanpak was het veilig reduceren van het aantal coloscopieën dat SPS-patiënten ondergaan. We laten zien dat de gepersonaliseerde aanpak leidde tot een 39% reductie van het aantal controle coloscopieën ten opzichte van jaarlijkse surveillance, zoals bijvoorbeeld de Amerikaanse richtlijn adviseert.(9) Belangrijk was uiteraard dat dit niet gepaard ging met een verhoogd

risico op darmkanker: het 5-jaars risico op darmkanker was in onze studie 1.3%, wat lager is dan in de meeste andere recente cohort onderzoeken.(10-13) Daarmee lijkt dit gepersonaliseerde surveillance protocol veilig en effectief.

In **hoofdstuk 6** onderzoeken we een cohort van SPS-patiënten die een (sub)totale colectomie ondergingen (d.w.z. chirurgische resectie van vrijwel de gehele dikke darm). Bij hen is er hypothetisch een zeer laag risico op darmkanker, aangezien SPS zich uitsluitend in de dikke darm en de endeldarm manifesteert. Deze hypothese werd bevestigd in onze studie, waarbij na 4,7 jaar follow-up geen van de 48 SPS-patiënten darmkanker ontwikkelde, en waarbij het 5-jaars risico op hoog-risico poliepen slechts 13% bleek te zijn. Deze resultaten laten zien dat patiënten bij wie in het verleden een (sub)totale colectomie is verricht, het aantal postoperatieve surveillance coloscopieën mogelijk aanmerkelijk kan worden gereduceerd.

In bovenstaande studies excludeerden we telkens een specifieke groep SPS-patiënten. Dit waren SPS-patiënten die alléén voldeden aan het tweede diagnostische criterium van de World Health Organization (WHO) richtlijn uit 2010: "tenminste één serrated poliep proximaal van het sigmoïd, in een individu met een eerstegraads familielid bij wie SPS is gediagnosticeerd". (14) In **hoofdstuk 7** beschrijven we het eerste cohort onderzoek naar deze specifieke groep patiënten, over wie al meerdere decennia discussie wordt gevoerd. Meerdere experts vonden dit criterium niet stringent genoeg, wat zou kunnen leidden tot overdiagnose van SPS. Dit criterium is dan ook verlaten in de nieuwe WHO-richtlijn die in 2019 uit kwam. In deze retrospectieve cohortstudie onderzochten wij de hypothese dat *alle* eerstegraads familieleden van SPS-patiënten een vergelijkbaar risico op darmkanker zouden hebben, ongeacht of ze zouden voldoen aan het verlaten tweede criterium. In tegenstelling tot wat wij verwachtten, bleken de eerstegraads familieleden die wél voldeden aan dit criterium echter een iets verhoogd risico te hebben op hoog-risico poliepen tijdens surveillance vergeleken met eerstegraads familieleden die hier niet aan voldeden. Deze bevinding moet echter voorzichtig worden geïnterpreteerd in verband met een aantal beperkingen in het retrospectieve studie-design.

Tenslotte beschrijven we in **hoofdstuk 8** een groot cohort SPS-patiënten dat al meer dat 10 jaar prospectief wordt gevolgd in het Amsterdam UMC. Wij gebruikten hierbij data die was verzameld in twee opeenvolgende prospectieve studies in ons centrum, waarvan de eerste in 2007 van start ging. Omdat wij daarmee beschikking hadden cohort dat wij al lange tijd opvolgden, konden we onderzoeken hoe SPS zich gedraagt gedurende vele jaren van endoscopische surveillance. De resultaten van deze studie laten zien dat langdurige endoscopische surveillance effectief is met een laag risico op darmkanker, en tevens veilig, met een zeer laag risico op coloscopie-gerelateerde complicaties. Verder lieten we zien dat (hoog-risico) poliepen ook na vele jaren surveillance nog worden gedetecteerd. Deze laatste bevinding suggereert dat SPS-patiënten endoscopisch gesurveilleerd moeten worden zolang leeftijd en comorbiditeiten dit toelaten.

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PHD PORTFOLIO

PhD Candidate: A.G.C. Bleijenberg
PhD period: 2016-2020
PhD Supervisors: Prof. dr. E. Dekker
Prof. Dr. C.J. van Noesel
PhD Co-supervisors Dr. J.E.G. IJspeert
Dr. B. Carvalho

PhD Training	Year	Workload (ECTS)
General courses		
Practical Biostatistics	2017	1.1
Observational clinical epidemiology, effects & effictiveness	2017	0.6
Clinical Epidemiology, Systematic Reviews	2016	0.7
AMC World of Science	2016	0.7
Clinical Data Management	2017	0.7
Scientific Writing in English for Publication	2017	1.5
DNA Technology	2017	2.1
Basic Course Legislation and Organization for Clinical Researchers (BROK)	2017	1.0
Seminars, workshops and masterclasses		
Weekly research seminars Gastroenterology	2016-2020	3
Biweekly colorectal cancer group meeting	2016-2020	2
AGM PhD Retreat	2017-2018	1
Bi-monthly Gut Club meetings	2016-2020	1
Oral presentations		
Digestive Disease Week (2x)	2018 & 2019	1
United European Gastroenterology Week	2018	0.5
Digestive Disease Days	2019	0.5
International Society for Gastrointestinal Hereditary Tumours	2019	0.5
Castor Research Award	2019	0.5
Poster presentations		
Digestive Disease Week	2018	0.5
International Society for Gastrointestinal Hereditary Tumours (3x)	2017 & 2019	1.5
United European Gastroenterology Week	2019	0.5
(Inter)national conferences		
WEO CRC Screening meeting (4x) *Washington, Vienna, San Diego, Barcelona*	2016-2019	1
Digestive Disease Week (2x) *Washington, San Diego*	2018-2019	2
United European Gastroenterology Week (2x) *Vienna, Barcelona*	2018-2019	1.5
International Society for Gastrointestinal Hereditary Tumours (2x) *Florence, Auckland*	2018-2019	1.5
Digestive Disease Days *Veldhoven*	2019	0.5

PhD Portfolio. (continued)

Teaching	Year	Workload (ECTS)
Teaching		
Tillots masterclass in colonoscopy (8x)	2016-2019	1
Supervision group of 2nd Year medical students during research internship	2017	0.2
Mastercourse Biomedical Sciences (lecture entitled "from polyp to colorectal cancer")	2018	0.2
AGM Course (lecture entitled "Pathophysiology of colon cancer, from clinic to cell biology")	2020	0.2
Netwerkbijeenkomst Bevolkingsonderzoek Darmkanker (*Lecture entitled: Serrated poliepen, wolf in schaapskleren")*	2017	0.2
Face-to-face training for gastroenterologists (*aiming to increaseserrated polyp detection; UMCG, ErasmusMC, AVL, Ms Twente, Catharina ZKH, MUMC)*	2016	1
Seminars in Gastroenterology *(Lecture entitled "Serrated polyps and serrated polyposis syndrome")*	2020	0.2
Supervising		
Eva Bocharewiz (Bachelor thesis)	2017	1
Julia Felsborg (Bachelor thesis)	2017	1
Jade Jansen (Master thesis)	2017	1
Maurits Visser (Master thesis)	2017	1
Willemijn de Klaver (Master thesis)	2017	1

ABOUT THE AUTHOR

Arne Bleijenberg was born on October 1st 1988 in Warnsveld, the Netherlands. In 2007 he graduated from the Baudartius College in Zutphen, after which he continued to study Medicine at the VU University in Amsterdam. He obtained his medical master degree in September 2014, after which he started working as a resident in the Alrijne hospital in Leiderdorp for the departments of internal medicine, gastroenterology, pulmonology and cardiology. Pursuing a long-term passion for child and adolescent psychiatry, he subsequently worked as a resident at the department of Child and Adolescent psychiatry of the Erasmus Medical Center, where he was involved in the care of youths with severe developmental disorders. After this intermezzo, he continued his career as a PhD candidate at the department of Gastroenterology and Hepatology at the Amsterdam UMC in 2016. Under supervision of prof. dr. Dekker and prof. dr. van Noesel, and co-supervision of dr. IJspeert and dr. Carvalho, he coordinated several clinical and translational studies on serrated polyps and serrated polyposis syndrome, many of which were in collaboration with Spanish, Norwegian and British colleagues. During his PhD trajectory he also participated in the clinical care of all serrated polyposis syndrome patients of the Amsterdam UMC. The results of these studies are presented in this thesis. In March 2021 he will start his specialist training in Gastroenterology and Hepatology at the Amsterdam UMC.

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&

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