FANNY E.R. VUIK

THE GASTROINTESTINAL TRACT

FROM HEALTHY MUCOSA TO COLORECTAL CANCER

The Gastrointestinal Tract

From healthy mucosa to colorectal cancer

Fanny Vuik

The Gastrointestinal Tract
Form healthy mucosa to colorectal cancer

ISBN/EAN: 978-94-6423-978-2

The printing of this thesis had been financially supported by the Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center, Rotterdam; Erasmus University Rotterdam, Nederlandse Vereniging voor Gastrenterologie; Dr. Falk Pharma Benelux B.V., Erbe Nederland B.V., Yakult Nederland B.V.

Cover Design: Rhodri van Dooren, Hoenenenvandooren
Lay-out: Eefke Schreur-Meilink, ProefschriftMaken.nl
Printing: ProefschriftMaken.nl

The Gastrointestinal Tract

From healthy mucosa to colorectal cancer

Het maagdarmkanaal

Van gezond mucosa tot darmkanker

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag van de
Rector magnificus

Prof. dr. A.L. Bredenoord

en volgens het besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Vrijdag 2 december 2022 om 13.00 uur

door

Fanny Elselina Rudina Vuik
geboren te Dordrecht, Nederland

Erasmus University Rotterdam

Promotiecommissie

Promotor
Prof. dr. M.C.W. Spaander

Overige leden
Prof. dr. C.J. van der Woude
Prof. dr. G.A. Meijer
Prof. dr. M.E. van Leerdam

Paranimfen
S.A.V. Nieuwenburg
C.A.M. Roumans

Table of contents

Part I

Introduction

Chapter 1.1
General introduction

Chapter 1.2
Aims and outline of this thesis

Chapter 1.1

General Introduction

General Introduction

Gastrointestinal (GI) problems are a common reason for patients to attend the primary care clinic as well as the outpatient clinic of hospitals. In the United States, more than 40.7 million visits were registered for GI symptoms (1). In The Netherlands, 3.7 million people were known with a GI disease in 2017 and the prediction is that by 2030 the percentage of people with a GI disease will increase to 10% of the population (2). One third of people with GI symptoms will consult a general practitioner (3). If we look at the prevalence of GI diseases then the prevalence is higher in adults above the age of 65 years old. Data, however, on the prevalence of GI mucosal abnormalities in an asymptomatic population are scarce.

The intestinal microbiome may play an important role in the different GI diseases. The microbiome encompasses ten times more bacterial cells than human cells (5). In the last decades, it was recognized that the diversity of microbes observed by microscopy far exceeded that of organisms recovered with the use of traditional cultural based approaches. Other tools for analyzing microbes are for example biomarker sequencing (16S rRNA) and metagenomics. Since the use of these techniques, the complexity, diversity and interaction between microbial communities are now better understood (6). The microbiome profile of each person is unique, and is affected by internal and external factors. Each location of the GI tract differ in pH, flow rates and secreted fluids. Therefore, the intestinal microbiome differs per anatomical region (7). Also, a difference exits between the mucosal and the intestinal microbiome (8). Characterization of the microbiome in the different parts of the GI tract in healthy individuals is still not fully clarified. The homeostasis of the microbiome and its host, the human gut, needs to be further explored before fully understanding the effect of the microbiome on disease development. What we do know is that intestinal dysbiosis could lead to various diseases. For example, presence of several bacterial species have been associated with CRC, like Streptococcus bovis and Fusobacterium nucleatum (5).

CRC mostly affects the 50- 75 years of age individuals (9). While the CRC incidence in this age group has decreased in the past decades presumably due to screening, the incidence of early onset CRC (EOCRC) increased. EOCRC is generally defined as CRC diagnosed before the age of 50 years (6). In the United States, colon cancer incidence rate increased by 1.0-3.4% annually since the mid-1980s in patients aged 20-39 years old. Rectal cancer incidence rates have been increasing longer and faster: 3.2% annually since 1974-2013 in patients aged 20-29 years old (7). A trend that was not only observed in the United States, but also in other parts of the world (8). Clinicopathological features of EOCRC tumours differ from late-onset CRC. EOCRC is more often located in the

rectum. Patients are also more likely to be diagnosed at an advanced stage. Histopathological features associated with EOCRC are a poor tumour differentiation and signet-ring cell differentiation (10, 11). The reason for the increase in EOCRC incidence is not fully understood. Possible explanations could be change in diet, obesity, more frequent use of antibiotics (12).

Different screening methods to detect GI lesions are available. Colon capsule endoscopy (CCE) is a non-invasive technique that enables to image the whole colon. No sedation or gas insufflation is needed and the procedure could be performed at home (14). The colon capsule has two cameras on each side of the capsule and is able to acquire images with a frame rate of 4 to 35 frames per second. The capsule transmits data to a recorder that the patients wear on a belt. The data can then be downloaded on the computer in the form of a video. Optimal bowel preparation is needed to allow adequate visualization of the gastrointestinal mucosa (15). The ESGE guideline recommends a liquid diet the day before and on the day of the procedure and 2L polyethylene glycol electrolyte (PEG) solution in the evening before and the morning of the procedure in split dose (16). Besides cleaningness, also the capsule transit time is important. This has to be fast enough to achieve completion within the battery time, but not too fast that it may miss lesions. The ESGE recommends to use a promotility agent if gastric emptying is longer than one hour and two boosters of low-dose sodium-phosphate to propel the capsule through the small bowel (16).

CCE is able to accurately detect colonic abnormalities, like colonic polyps and CRC. Colon capsule has a sensitivity of 87% for the detection of polyps >10mm and a specificity of 95% (17). Its diagnostic accuracy exceeds the accuracy of CT-colonography (18, 19). However, studies on the accuracy of CCE as a CRC screening tool are scarce. Though the colon capsule is approved to image the colon, it can be used as pan-endoscopy (20). One of the disadvantages of CCE is the labour-intensive reading time per video and the inter-observer variability. Automated reviewing could be a possible solution, reducing reading time and generating an objective outcome.

Screening is appealing for CRC as the disease is common, has a long pre-clinical phase, and in case of early detection survival improves. Therefore, CRC screening programs have been implemented across the world (21). One screening tool is the faecal immunochemical test (FIT). FIT detects human globin using an antibody-based assay, which leads to an quantitative measurement. In the Netherlands, a biennal fecal immunochemical test (FIT) is offered to individuals aged between 55-75 years of age, with a follow-up colonoscopy for those with a positive test result (22). Recently, a study was published showing that few years after the introduction of the national FIT based CRC

screening program the CRC incidence has decreased (23). Limited information, however, is available regarding the effect of FIT screening on CRC mortality.

References

1. Peery AF, Crockett SD, Murphy CC, Lund JL, Dellon ES, Williams JL, et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018. Gastroenterology. 2019;156(1):254-72 e11.
2. Available from: https://www.volksgezondheidenzorg.info/onderwerp/maag-darm-en-leveraandoeningen/overzicht.
3. Ehlin AG, Montgomery SM, Ekbom A, Pounder RE, Wakefield AJ. Prevalence of gastrointestinal diseases in two British national birth cohorts. Gut. 2003;52(8):1117-21.
4. Dumic I, Nordin T, Jecmenica M, Stojkovic Lalosevic M, Milosavljevic T, Milovanovic T. Gastrointestinal Tract Disorders in Older Age. Can J Gastroenterol Hepatol. 2019;2019:6757524.
5. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLOS Biology. 2016;14(8):e1002533.
6. Lynch SV, Pedersen O. The Human Intestinal Microbiome in Health and Disease. N Engl J Med. 2016;375(24):2369-79.
7. Ruan W, Engevik MA, Spinler JK, Versalovic J. Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration. Dig Dis Sci. 2020;65(3):695-705.
8. Watt E, Gemmell MR, Berry S, Glaire M, Farquharson F, Louis P, et al. Extending colonic mucosal microbiome analysis—assessment of colonic lavage as a proxy for endoscopic colonic biopsies. Microbiome. 2016;4(1):61.
9. Wong MCS, Huang J, Lok V, Wang J, Fung F, Ding H, et al. Differences in Incidence and Mortality Trends of Colorectal Cancer Worldwide Based on Sex, Age, and Anatomic Location. Clin Gastroenterol Hepatol. 2021;19(5):955-66 e61.
10. Chang DT, Pai RK, Rybicki LA, Dimaio MA, Limaye M, Jayachandran P, et al. Clinicopathologic and molecular features of sporadic early-onset colorectal adenocarcinoma: an adenocarcinoma with frequent signet ring cell differentiation, rectal and sigmoid involvement, and adverse morphologic features. Mod Pathol. 2012;25(8):1128-39.
11. Willauer AN, Liu Y, Pereira AAL, Lam M, Morris JS, Raghav KPS, et al. Clinical and molecular characterization of early-onset colorectal cancer. Cancer. 2019;125(12):2002-10.
12. O'Sullivan DE, Sutherland RL, Town S, Chow K, Fan J, Forbes N, et al. Risk Factors for Early-Onset Colorectal Cancer: A Systematic Review and Meta-analysis. Clinical Gastroenterology and Hepatology. 2021.
13. Wolf AMD, Fontham ETH, Church TR, Flowers CR, Guerra CE, LaMonte SJ, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin. 2018;68(4):250-81.
14. Pasha SF. Applications of Colon Capsule Endoscopy. Curr Gastroenterol Rep. 2018;20(5):22.
15. Spada C, Hassan C, Costamagna G. Colon Capsule Endoscopy. Gastrointestinal Endoscopy Clinics of North America. 2015;25(2):387-401.
16. Spada C, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2012;44(5):527-36.
17. Spada C, Pasha SF, Gross SA, Leighton JA, Schnoll-Sussman F, Correale L, et al. Accuracy of First- and Second-Generation Colon Capsules in Endoscopic Detection of Colorectal Polyps: A Systematic Review and Meta-analysis. Clinical Gastroenterology and Hepatology. 2016;14(11):1533-43. e8.

18. González-Suárez B, Pagés M, Araujo IK, Romero C, Rodríguez de Miguel C, Ayuso JR, et al. Colon capsule endoscopy versus CT colonography in FIT-positive colorectal cancer screening subjects: a prospective randomised trial-the VICOCA study. BMC Med. 2020;18(1):255.
19. Spada C, Hassan C, Barbaro B, Iafrate F, Cesaro P, Petruzziello L, et al. Colon capsule versus CT colonography in patients with incomplete colonoscopy: a prospective, comparative trial. Gut. 2015;64(2):272-81.
20. Romero-Vázquez J, Caunedo-Álvarez Á, Belda-Cuesta A, Jiménez-García VA, Pellicer-Bautista F, Herrerías-Gutiérrez JM. Extracolonic findings with the PillCam Colon: is panendoscopy with capsule endoscopy closer? Endosc Int Open. 2016;4(10):E1045-E51.
21. Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJY, Young GP, et al. Colorectal cancer screening: a global overview of existing programmes. Gut. 2015;64(10):1637-49.
22. Toes-Zoutendijk E, van Leerdam ME, Dekker E, van Hees F, Penning C, Nagtegaal I, et al. Real-Time Monitoring of Results During First Year of Dutch Colorectal Cancer Screening Program and Optimization by Altering Fecal Immunochemical Test Cut-Off Levels. Gastroenterology. 2017;152(4):767-75 e2.
23. Breekveldt ECH, Lansdorp-Vogelaar I, Toes-Zoutendijk E, Spaander MCW, van Vuuren AJ, van Kemenade FJ, et al. Colorectal cancer incidence, mortality, tumour characteristics, and treatment before and after introduction of the faecal immunochemical testing-based screening programme in the Netherlands: a population-based study. Lancet Gastroenterol Hepatol. 2021.

Chapter 1.2

Aims and outline of this thesis

Aims and outline of this thesis

In this thesis, we aim to provide insights in prevalence of mucosal abnormalities of the gastrointestinal (GI) tract and colorectal cancer (CRC). We will provide strategies that may contribute to optimizing diagnostic modalities for GI symptoms and CRC screening (**Part I)**.

Part II of this thesis focuses on the GI disease in a general population. **Chapter 2** will describe the prevalence of GI disease in an asymptomatic healthy elderly population. On microscopic level, the bacterial composition was explored along nine mucosal sites within the GI tract (**Chapter 3).** So far, most studies focused only on the composition of the microbiome in the colon. To elucidate the role of the microbiome in disease, it is necessary to unravel the composition of the microbiome in the entire GI tract in individuals without GI disease.

The aim of **Part III** is to provide more insights in the trend of the rising incidence of sporadic early onset colorectal cancer (EOCRC). In **Chapter 4** we focus on the EOCRC incidence and mortality in Europe over the last 25 years. EOCRC tumors have different clinical and pathological features compared to late-onset CRC. However, former published studies showed conflicting results and often included patients with Lynch syndrome (LS). In **chapter 5,** we investigated the clinicopathological features of sporadic EOCRC patients, stratified per age group and LS-patients were excluded.

Colon capsule endoscopy (CCE) is a new noninvasive technique that images the whole colon. In **Part IV** possible applications of the colon capsule will be discussed as well as suggestions for improvement. An overview of the use of CCE in a CRC screening population will be discussed in **Chapter 6.** Hence the colon capsule is primarily designed to review the colon, it images the entire GI tract. The applicability of the CCE as pan-endoscopy is described in **Chapter 7.** Some pitfalls previously described using CCE are long transit times through the GI tract which may result in a high percentage of incomplete examinations. We aimed to investigate risk factors for long transit times in **Chapter 8.** Furthermore, reading the images of the colon capsule is a time- consuming activity. Automated reading of the CCE images could be time saving. Besides the interobserver variability could be reduced (**Chapter 9**)**.**

In **Part V** the effectiveness of CRC screening using faecal immunochemical test (FIT) was investigated. Many previous studies used guaiac fecal occult blood test (gFOBT). However, the effect of FIT on CRC incidence and especially mortality remained uncleair. Within this scope, **Chapter 10** is a population- based study evaluating the effect of FIT

screening on the CRC incidence and CRC-related mortality. The effectiveness of FIT screening may also be influenced by the use of anticoagulant medication. Therefore, we aimed to investigate the effect of NSAIDS on the accuracy of FIT in a screening population in **Chapter 11**.

Part II

Gastrointestinal disease in a general population

Chapter 2
Population-based prevalence of gastrointestinal abnormalities at colon capsule endoscopy

Chapter 3
Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals

Chapter 2

Population-based prevalence of gastrointestinal abnormalities at colon capsule endoscopy

F.E.R. Vuik, S.A.V. Nieuwenburg, S. Moen, E.H. Schreuders, M.D. Oudkerk-Pool, E.F.P. Peterse, C. Spada, O. Epstein, I. Fernandez-Urien, A. Hofman, E.J. Kuipers, M.C.W. Spaander

Clinical Gastroenterology and Hepatology, October 2020

Abstract

Introduction The population prevalence of gastrointestinal (GI) disease is unclear and difficult to assess in an asymptomatic population. The aim of this study was to determine prevalence of GI lesions in a largely asymptomatic population undergoing colon capsule endoscopy (CCE).

Methods Participants aged between 50-75 years were retrieved from the Rotterdam Study, a longitudinal epidemiological study, between 2017-2019. Participants received CCE with bowel preparation. Abnormalities defined as clinically relevant were Barrett segment >3cm, severe ulceration, polyp >10 mm or ≥3 polyps in small bowel (SB) or colon, and cancer.

Results Of 2800 invited subjects, 462 (16.5%) participants (mean age 66.8 years, female 53.5%) ingested the colon capsule. A total of 451 videos were analyzed, and in 94.7% the capsule reached the descending colon. At least 1 abnormal finding was seen in 448 (99.3%) participants. The prevalence of abnormalities per GI segment, and the most common type of abnormality, were as follows: Esophageal 14.8% (Barrett's esophagus <3 cm in 8.3%), gastric 27.9% (fundic gland polyps in 18.1%), SB abnormalities 33.9% (erosions in 23.8%), colon 93.3% (diverticula in 81.2%). A total of 54 participants (12%) had clinically relevant abnormalities, 3 (0.7%) in esophagus/stomach (reflux esophagitis grade D, Mallory Weiss lesion and severe gastritis), 5 (1.1%) in SB (polyps > 10 mm; n = 4, severe ulcer n = 1,) and 46 (10.2%) in colon (polyp > 10 mm or ≥3 polyps n = 46, colorectal cancer n = 1).

Conclusions GI lesions are very common in a mostly asymptomatic Western population, and clinically relevant lesions were found in 12% at CCE. These findings provide a frame of reference for the prevalence rates of GI lesions in the general population.

Introduction

A considerable proportion of patients with gastrointestinal (GI) abnormalities remain undiagnosed because they do not always present with symptoms for which endoscopy is deemed necessary. Therefore, prevalence rates of GI diseases in the general population are unknown. What we do know is that GI diseases increase with age and that life expectancy is steadily expanding leading to an increased elderly population (1). For this reason, it is expected that the prevalence of GI disease will rise (2, 3). Learning the prevalence rates of GI mucosal abnormalities in an asymptomatic population will help to set a frame of reference of GI lesions that may be found during endoscopy, which is of interest especially in a screening setting. Furthermore, it may help to better inform patients about the (non-relevant) lesions found during endoscopy, when this could be compared against a general asymptomatic population.

Multigenerational prospective cohort studies with healthy participants that are followed throughout life are of paramount importance. In order to assess the etiology, contributing factors and burden of a certain disease, a frame of reference within a healthy population is essential. For example, the Framingham Heart Study has already shown us that monitoring healthy participants provided breakthroughs on the occurrence and natural course of cardiovascular diseases (4). Further, biobank studies such as the Lifelines cohort are becoming the core of clinical research worldwide (5). Nowadays, research that focuses not only on the disease, but also the healthy individual is just as important for unraveling pathologies.

The Rotterdam study is a prospective cohort study including healthy individuals 45 years of age and older that are followed throughout their lives (6). The current study is embedded within this cohort study. By the use of colon capsule endoscopy (CCE), we were able to image the entire GI tract of the participants. The colon capsule has 2 cameras on each side of the capsule and is able to acquire images with a frame rate of 4–35 frames/s. The CCE can be adequately used as pan-endoscopy (7, 8). The aim of this study was to assess the prevalence of any GI lesion in a general asymptomatic population-based study using CCE.

Materials and Methods

Study Design

This trial is embedded within the Rotterdam study. The rationale and design of the Rotterdam study have been described previously (6). The current study aims to evaluate the

prevalence of GI lesions in a largely asymptomatic population using CCE between 2017 and 2019. The study has been approved by the Medical Ethics Committee of Erasmus MC (registration number MEC-2015-453). The protocol was registered in the Netherlands Trial Register (NTR6321). All participants signed written informed consent before participation in the study. The authors of this manuscript had access to the study data and have read and approved this manuscript.

Participants

In the Rotterdam study, participants were recruited from 1990 onward (6). People participating in the Rotterdam study were eligible to participate in this study if between 50 and 75 years of age and able to give informed consent. Participants were excluded when meeting 1 of the following criteria: (1) unable or unwilling to sign written informed consent, (2) severe or terminal disease with a life expectancy <5 years, (3) allergy or known contraindication to the medications used in this study, (4) chronic heart failure New York Heart Association functional class III or IV, (5) severe kidney insufficiency (glomerular filtration rate<30 ml/min/1.73 m3), (6) dysphagia or swallowing disorder, (7) increased risk for capsule retention (M. Crohn, prior abdominal surgery likely to cause bowel obstruction), (8) pacemaker or other implantable cardioverter-defibrillator, (9) magnetic resonance imaging scheduled within 14 days after ingestion of the capsule, (10) risk of congenital extended QT syndrome or medication known to extend the QT interval, and (11) diabetes mellitus with use of insulin.

Participants received an announcement by post, followed by an invitation 2 weeks later, which included the patient information letter. In case of nonresponse, a reminder was sent after 6 weeks. Positive responders were invited for an interview to explain the CCE procedure and sign informed consent. A second appointment was made for the ingestion of the capsule. Both appointments took place in the study center, a specialized research facility in Ommoord, the Netherlands .

CCE Procedure

The second-generation colon capsule (PillCam COLON 2; Medtronic, Minneapolis, MN) was used. The ingestion of the capsule took place between 9 Am and 11 Am in the presence of a physician. After successful ingestion of the capsule, participants went home. The sensor belt, which is attached to the participant before ingesting the colon capsule and receives transmission data from the colon capsule, was taken off by participants at 8 pm or earlier when the capsule had left the body before 8 pm (for a detailed description of the CCE device, see the Supplementary Methods). Bowel preparation regimen for CCE consisted of 2 L of polyethylene electrolyte glycol plus ascorbic acid (Moviprep; Norgine, Amsterdam, the Netherlands) plus 2 L of water in split dose. A sulfate-based solution

(Eziclen, Zambon, the Netherlands) was used as booster. After the capsule exited the stomach, the participant ingested the booster, which propelled the capsule through the small bowel and added fluid to the colon. The exact bowel preparation regimen is shown in Supplementary Table 1.

Reading Technique

CCE reading and evaluation was performed by a specially trained Erasmus MC study team, which consisted of 1 certified gastroenterologist, 3 medical doctors, and 1 endoscopy nurse. After a 2-day CCE masterclass, the participating readers practiced with an e-learning program. In total, they spent 30 hours evaluating videos each. Finally, the study team followed a course for 3 days at the Royal Free Hospital in London, United Kingdom. They were required to identify pathological features of the entire digestive tract in the videos and indicate the type, location, and size of the lesions.

In case of uncertainty, an international external reading expert team was consulted (C.S., I.F.-U., O.E.). The first 20 videos of each reader were re-evaluated by a second, experienced reader for quality control. All findings were saved as thumbnails, with a detailed description of each finding. The upper GI tract was defined as esophagus, stomach, and small bowel. The lower GI tract was defined as all segments of the colon and rectum. Each video was evaluated within 3 weeks of receipt.

Cleansing of the stomach, small bowel, and colon was graded according to 3 different grading scales (Supplementary Table 2). Colon cleansing grades of good and excellent were considered adequate bowel preparation, and grades of poor and fair were considered inadequate. A video was considered complete when the anal verge was observed.

Findings and Follow-Up

All findings are listed in Supplementary Table 3. In case an abnormality was found with potential clinical consequences, the finding was shared with the participant and the general practitioner. Only in those cases in which a clinically relevant finding was found was an endoscopy with or without biopsies or polypectomy performed. Clinically relevant findings were defined as the following: Barrett's segment >3 cm, severe ulceration >1 cm, polyp >10 mm or ≥3 polyps in the small bowel, or polyp >10 mm or ≥3 polyps in the colon and cancer (Supplementary Table 4). Barrett's esophagus (BE) will only be ascertained when the Z-line is visible. The participant received an appointment at the gastroenterology outpatient clinic of the Erasmus MC or another hospital in the Netherlands, where—in accordance with the participant—further investigations were planned. Prevalence rates were based upon the findings of CCE and the additional endoscopy in cases of clinically relevant findings found by CCE.

Statistical Analysis

To assess prevalence estimates with a good and acceptable precision, the sample size must be large enough. For diseases with an estimated prevalence under 10%, it is advised to use a precision of half the prevalence.9,10 For a valid estimate of prevalence rates of ≥3.3% with a precision of 0.0165, a sample size of 450 participants is needed. Descriptive statistics were used to describe the results. Statistical analyses were performed with SPSS software version 25 (IBM Corporation, Armonk, NY).

Results

Study Population

A total of 2800 subjects between 50 and 75 years of age were invited to participate, of whom 462 (16.5%) ingested the colon capsule (Figure 1). No difference in sex, age, tobacco use, and alcohol intake was found between participants and nonparticipants (Supplementary Table 5). However, participants had a lower body mass index, more often had a paid job, and were more often highly educated compared with the nonparticipants. Owing to a technical failure, 11 videos could not be assessed, resulting in a total of 451 participants for further analyses. The majority of participants were Caucasian with a mean age of 67.4 ± 4.9 years, and 53.7% were female (Table 1). A medical history of GI disease was reported in 17.7% of the participants, most commonly colon polyps removed in the past (8.9%), hemorrhoids (2.4%), and diverticulosis (2.2%) (Table 1). In 84.8% of the participants, no GI symptoms or complaints were present at time of the interview. Some participants (15.2%) presented with only minor symptoms for which they would not seek a doctor: heartburn, changed defecation pattern, and gastric complaints.

Prevalence of All GI Findings

In this study cohort, 448 (99.3%) participants had any abnormality in the GI tract. In total, 1948 abnormalities were found, with a mean number of 4.3 ± 2.5 abnormalities per participant (Figure 2A). Both men and women were equally affected, 99.5% of all men had any abnormality vs 99.2% of all women. However, the distribution of abnormalities was different between men and women (Figure 2B and C). In 304 of the 451 (67.4%) participants, abnormalities were found in the upper GI tract, with a total of 553 abnormalities. In the lower GI tract 1395 abnormalities were found in 419 (93.3%) participants

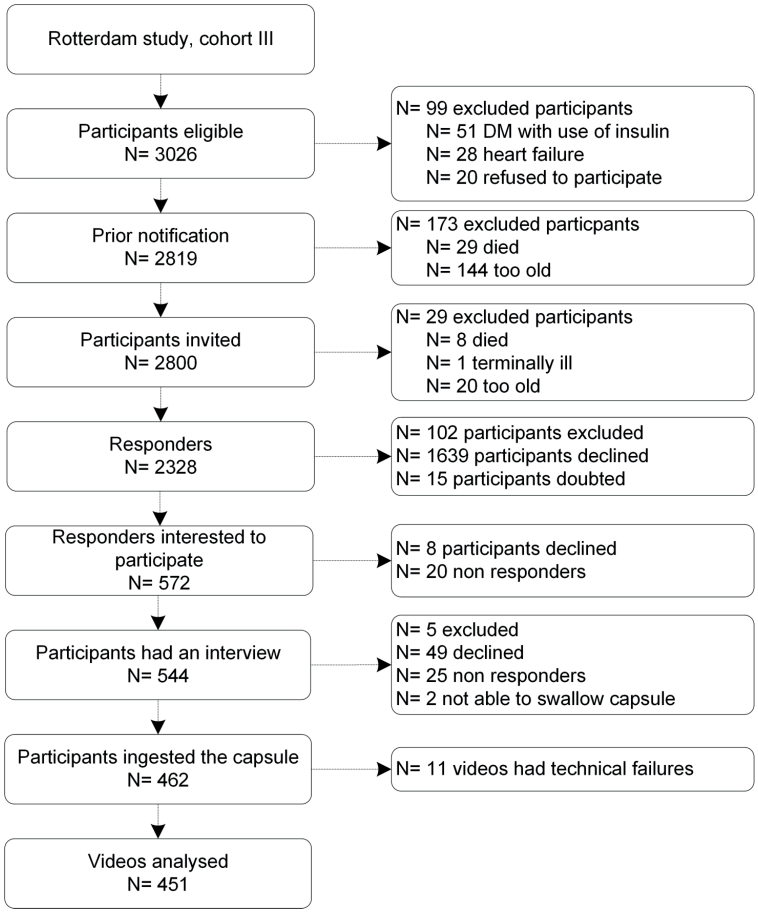


Figure 1 Study flow chart. DM, diabetes mellitus

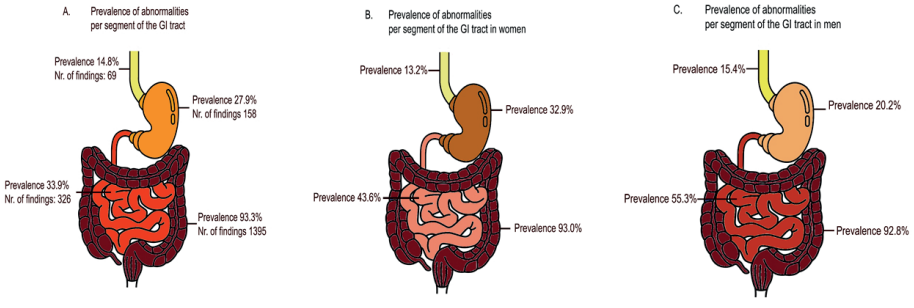


Figure 2 Heatmap of the prevalence rates of abnormalities per segment of the GI tract observed by CCE. **(A)** Prevalence rate per GI segment of all 451 participants with total number of findings per segment. Prevalence rate per segment in **(B)** women (n = 243) and **(C)** men (n = 208).

Table 1 Medical history of participants (N = 462) *GI, gastrointestinal; NSAID = nonsteroidal anti-inflammatory drug.*

	Total	N	%
Male/female	462	214 / 248	46.3 / 53.7
Mean age, years (SD)	462	67.4 (4.9)	
Ethnicity	462		
European		400	86.6
East-Asian		2	0.4
African		8	1.7
Mixture		5	1.1
Missing		47	10.2
GI symptoms	454		
None		385	84.8
Heartburn		20	4.4
Changed defecation pattern		15	3.3
Gastric complaints		10	2.2
Other		24	5.3
Medical history	462		
None		205	44.4
GI disease		82	17.7
Cardiac disease		95	20.6
Pulmonary disease		35	7.6
Cerebral disease		20	4.3
Endocrine		41	8.9
Malignancy in the past		44	9.5
Medication use	459		
Antihypertensive		159	34.6
Proton pomp inhibitor		108	23.5
Statin		106	23.1
Platelet aggregation inhibitor		43	9.3
β_2 adrenergic receptor agonist		35	7.6
Laxative		27	5.9
NSAID		27	5.9
Antidiabetic		17	3.7
Grading general health	411		
Poor		3	0.7
Fair		33	8.0
Good		257	62.5
Very good		95	23.1
Excellent		23	5.6

Upper GI Tract

Esophageal abnormalities were found in 64 (14.8%) participants, with a total number of 69 findings. BE <3 cm and esophagitis were the most common abnormalities, with prevalence rates of 8.3% and 5.5%, respectively (Figure 3 and Supplementary Tables 6 and 7). Gastric abnormalities were found in 122 (27.9%) participants. In total, 158 abnormalities were found in the stomach. Most frequent abnormalities were fundic gland polyps (FGP) (prevalence of 18.1%) and end erosions (prevalence of 6.6%). In total, 326 small bowel abnormalities were found in 151 (33.9%) participants with erosions (23.8%) being the most common lesions. Although not defined as an abnormality, lymphangiectasis was observed in 30.7% of the participants.

Lower GI Tract

Colon abnormalities were present in 419 (93.3%) participants, with a total of 1395 abnormalities. (Figure 3 and Supplementary Tables 6 and 7). Abnormalities were found less frequently in the cecum (25.4% of the participants). In 44.8% of the participants, any abnormality was found in the ascending colon, and in 41.8% of participants, any abnormality was found in the transverse colon. Compared with the other segments of the colon, most abnormalities were found in the descending colon (82.7% of the participants). Most common findings were diverticula (prevalence of 71.4%) and polyps (prevalence of 34.0%), both having a specific distribution (Figure 4). In the rectum, 181 abnormalities were found in 127 (50.8%) participants. Most frequent findings were hemorrhoids (36.4%) and polyps (16.0%).

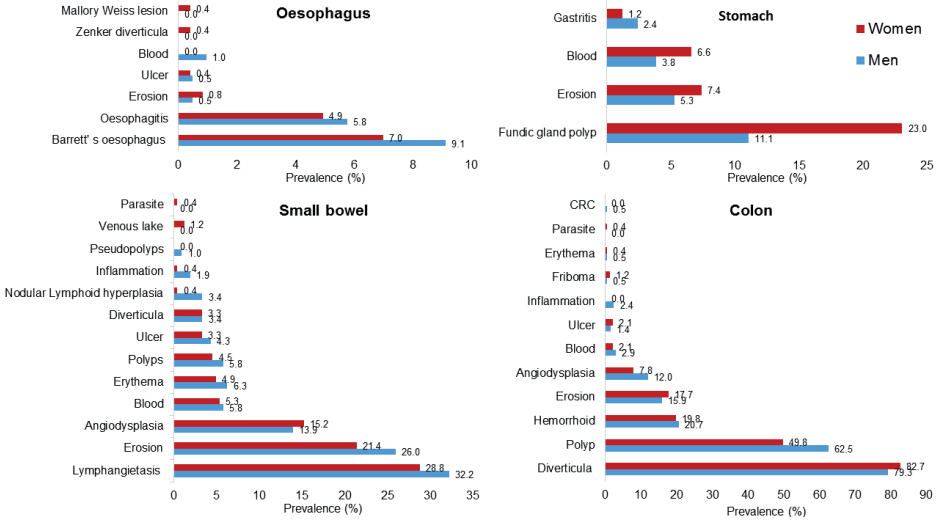


Figure 3 Prevalence rates of any abnormality in the GI tract divided by men (blue) and women (pink).

Prevalence of Clinically Relevant Findings and Clinical Follow-Up

A total of 54 (12%) participants had clinically relevant abnormalities, 3 (0.7%) findings in the stomach, 5 (1.1%) findings in the small bowel and 46 (10.2%) findings in the colon (Table 2 and Supplementary Table 8). In 2 participants, bleeding in the stomach was detected by CCE. At endoscopy, it was found that a Mallory-Weiss lesion and reflux esophagitis grade D had caused the bleeding. The third participant had a severe gastritis. Of the 5 participants with clinically relevant findings in the small bowel, 1 participant had a severe ulcerative lesion and 4 participants had a polyp larger than 10 mm. Of the 46 participants with clinically relevant abnormalities in the colon, 46 participants had 1 polyp larger than 10 mm or 3 or more polyps and 1 participant had also a colorectal carcinoma (CRC).

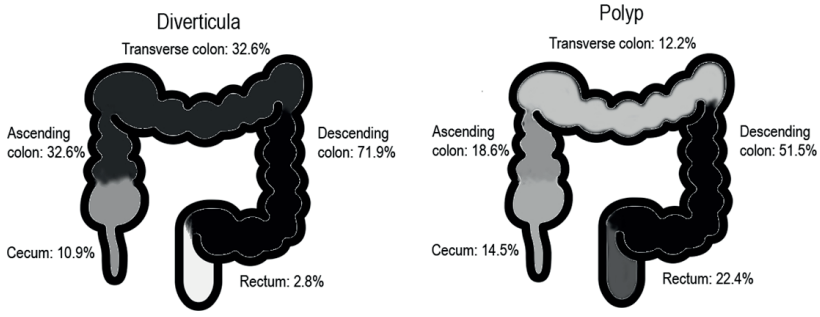


Figure 4 Distribution of colonic diverticula and polyps among participants.

Additional Findings

In the participants with clinically relevant findings, additional imaging tests were performed. Findings observed at upper endoscopy and not by CCE were a reflux esophagitis grade D and a Mallory-Weiss lesion in the esophagus. In the small bowel, no additional findings were observed by magnetic resonance imaging and follow-up CCE. In the colon, 53 additional polyps were found at colonoscopy (OC), of which 45 were ≤9 mm and 8 were >10 mm (Table 2).

One participant was diagnosed with a CRC in the sigmoid 6 months after the CCE procedure. CCE had missed the CRC due to the fact that the battery life of the colon capsule had ended in the descending colon, and therefore, the CRC located in the sigmoid was not visualized.

Quality Parameters of Colon Capsule

The gastric cleansing was considered good in 304 (69.6%) participants, the small bowel cleansing was good or excellent in 442 (99.1%) participants, and the overall colon cleansing was adequate in 344 (76.6%) participants. The Z-line, the gastroesophageal junction,

was observed in 44.8% of the participants. The capsule reached the descending colon in 94.7% and completion was achieved in 51.9% of the participants. The number of visualized segments of the GI tract are described in Supplementary Table 9. No difficulties in swallowing the capsule were observed. No procedure-related serious adverse events occurred.

Table 2 – Clinical follow-up of clinical relevant findings at CCE by endoscopy and histology

Esophagus, stomach and small bowel				
	CCE	**Endoscopy**		**Histology**
		Type	Finding	
Esophagus/Stomach, N = 3	Bleeding	Gastroscopy	Reflux esophagitis grade D	-
	Bleeding	Gastroscopy	Mallory Weiss lesion and erythema of antrum and corpus with two small erosions	Chronic active inflammation. Helicobacter pylori organisms
	Severe gastritis	Gastroscopy	Mild erosive antrum gastritis	Intestinal metaplasia antrum
Small bowel, N = 3*	Ulcer >10mm	MRI	Hyperaemia and bowel wall thickening	NA
	Polyp >10mm	MRI	No abnormalities	NA
	Polyp >10mm	CCE follow up	No change in size or appearance	NA
Total polyps detected, N	135	Colonoscopy	163	
Size, N (%)				
≤5 mm	49 (36.3)		69 (42.3)	HP 16, SSA 1, TA 38, TVA 2 No dysplasia 18, LGD 42
6-9 mm	45 (33.3)		41 (25.2)	HP 7, SSA 4, TA 24, TVA 1 No dysplasia 11, LGD 26, HGD 1
≥10 mm	41 (30.4)		37 (22.7)	HP 1, SSA 8, TA 15, TVA 11, CA 1 No dysplasia 6, LGD 27, HGD 1
Location, N (%)				
Cecum	17 (12.6)		18 (11.0)	SSA 4, TA 8, TVA 1 No dysplasia 3, LGD 9, HGD 1
Ascending colon	23 (17.0)		32 (19.6)	HP 1, SSA 3, TA 15, TVA 3 No dysplasia 4, LGD 19
Transverse colon	23 (17.0)		37 (22.7)	HP 7, SSA 1, TA 26, TVA 1 No dysplasia 8, LGD 26, HGD 1

Descending colon/ sigmoid	54 (40.0)	Colonoscopy	51 (31.3)	HP 11, SSA 3, TA 27, TVA 6 No dysplasia 12, LGD 34
Rectum	18 (13.3)		25 (15.3)	HP 8, SSA 1, TA 5, TVA 4, CA 1 No dysplasia 10, LGD 8, HGD 1
Appearance, N (%)				
Sessile	94 (69.6)		92 (56.4)	HP 20, SSA 18, TA 41, TVA 4 No dysplasia 25, LGD 57, HGD 1
Pedunculated	39 (28.9)		21 (12.9)	HP 1, SSA 1, TA 10, TVA 8 No dysplasia 2, LGD 17, HGD 1
Flat	1 (0.7)		23 (14.1)	HP 4, SSA 1, TA 9, TVA 2 No dysplasia 5, LGD 10, HGD 1

Values are n (%). CA, carcinoma; CCE, colon capsule endoscopy; HGD, high-grade dysplasia; HP, hyperplastic polyp; LGD, low-grade dysplasia; MRI, magnetic resonance imaging; NA, not applicable; OC, colonoscopy; SSA ¼ sessile serrated polyp; TA, tubular adenoma; TVA, tubulovillous adenoma.*= small intestine polyps had no follow-up.

Discussion

True population prevalence data of GI disease are scarce, as most prevalence studies are based on select, often symptomatic populations. This study provides prevalence rates of GI lesions in a general mostly asymptomatic population. GI lesions appeared to be a very common condition in a Western population. Prevalence of BE was 8.3%, esophagitis 5.8%, FGP in 18.1%, and diverticula in 81.6%, and prevalence of colon polyps was 56%. In 12%, clinically relevant findings were detected. The most common clinically relevant lesions found were colon polyps >10 mm.

GI diseases are usually detected when patients undergo a diagnostic procedure because of symptoms. Prevalence of GI lesions in asymptomatic population are difficult to assess. Most people perceive endoscopies as burdensome and invasive and are therefore reluctant to undergo such procedure in case no symptoms are present. Therefore, studies assessing prevalence of GI lesions are mainly performed in screening or symptomatic patients who already have to undergo an endoscopy.

Our findings are not in line with previous literature. One Swedish study has assessed the prevalence of BE in a general population and found a rate of 1.6% (11). Other studies have reported significantly higher prevalence rates of BE, ranging from 6.8% to 25% (12, 13). We found a prevalence rate of 8.3% in the adult general population. On the one hand, this prevalence may be underestimated because the Z-line was observed in only

44.8% of the participants. On the other hand, BE was defined on macroscopic findings only. The difference in prevalence rates could be explained by time, as the Swedish publication was in 2005. It is known that the prevalence of gastroesophageal reflux disease, which is often accompanied with BE, has increased over the last 20 years (14).

An Italian study focused on gastroesophageal reflux symptoms and esophagitis in a general Italian population and found prevalence of esophagitis of 11.8%. The prevalence of reflux symptoms in their population was 44.3%, which could explain the higher prevalence numbers in comparison to our findings (prevalence of 5.5%) (15).

We found an FGP prevalence rate of 18.1% in our population, and 40% of them used a proton pump inhibitor. True prevalence of gastric polyps is not well known, as they are rarely symptomatic (16). The prevalence rates of all gastric polyps range between 0.5% and 14%, of which FGP are the most common types, with prevalence rates varying from 21% to 47% in symptomatic populations (17, 18).

In a study from the United States among Kaiser Permanente members, the colon adenoma prevalence was estimated based on 20.792 patients undergoing a screening OC. They found an adenoma prevalence of 20.2% in women and 30.6% in men (19). A meta-analysis reporting on the prevalence of colon adenomas and CRC in an average risk population by OC concluded that the pooled prevalence of adenomas was 30.2% (range, 22.2–58.2%) (20). In our study, the prevalence of all polyps was 57% and the prevalence of polyps >10 mm was 10%. Our polyp detection rate (PDR) is higher compared with the adenoma detection rate (ADR) found with OC (20) This difference could be explained by 2 reasons. First, it is known that the detection rate of polyps by CCE is different from OC. A Danish study reported that the PDR was significantly higher in CCE vs OC (74% vs 64%, respectively) (21). Second, to assess the ADR, it is essential to have pathology results, which cannot be performed by CCE. A recently performed meta-analysis calculated a conversion factor of 0.68 to calculate the ADR from PDR (22). If we apply this to our data, then an ADR of 38% is found, which is then in line with previously mentioned literature.

Finally, colonic diverticula was the most common clinically non-relevant finding in our study. Although it is generally known that diverticulosis is common and more prevalent at older ages, the true prevalence of diverticula is difficult to determine because most estimates were subjected to selection bias (23). In a recently performed study from the United States, it was shown that in a screening population older than 60 years of age, diverticula were present in 58% of the screened individuals. The prevalence of diverticula was the highest in the sigmoid (24). Our study reported a prevalence of 81.2%. The difference in distribution of diverticula between the study from the United States

and our study was remarkable. In the former study, most diverticula were found in the sigmoid, with <11% in other segments of the GI tract, while in our study the highest prevalence was found in the descending colon and around 30% in the ascending and transverse colon. The difference in distribution could be explained by the difference in diagnostic tool: in the U.S. study, OC was used vs CCE in our study.

CCE is a noninvasive method to assess the mucosal surface of the entire GI tract. Multiple studies have reported on the usefulness of the colon capsule, especially in the detection of colonic polyps and to observe the colonic mucosa of patients with inflammatory bowel disease (25, 26). Two studies assessed the use of CCE in evaluating the mucosal surface of the entire GI tract. The first study included 21 symptomatic patients and concluded that a CCE is a feasible method (7). The second study included 165 patients to rule out pathology and used both first and second generation colon capsules (8).

The strength of this study is that this study is the first to set a frame of reference of the prevalence of GI abnormalities in the entire GI tract within 1 person. This study also has several limitations. First, 16.5% of the invited subjects participated in our study, which could lead to a selection bias. However, when inviting Dutch individuals, 50–75 years of age, for primary CRC screening with colonoscopy, the participation rate was comparable (22%) (27). Second, the completion rate of CCE was only 51.9%. However, the descending colon was seen in 94.7% of the participants and therefore almost the entire GI tract was observed. Also, the sleep mode (the default setting of the colon capsule in order to save battery life to observe the entire colon by taking only 4 pictures/min in the stomach) was not turned off. Therefore, the stomach was in some participants less accurately visualized. The sleep mode saves battery allowing an almost complete evaluation of the colon. CCE is not the preferred method to observe the esophagus; in our study, the Z-line was observed in 44.7% of the participants. The 3 previously mentioned limitations may have led to an underestimation of prevalence rates found. Third, the prevalence rates are dependent on the experience of the reader of the videos. Special attention was given to train the readers. An expert team (O.E., C.S., I.F.-U.) was installed and advised when reviewers were having doubts. Fourth, owing to the design of the study, not all abnormalities were confirmed by histopathology, unless clinically relevant lesions were found and the participant had to undergo an endoscopy. This may have overestimated the prevalence of BE. Last, the CCE software has an polyp estimation tool to measure polyps in the colon. For this study, the tool was used to measure all abnormalities, which may have affect the accurate size of findings.

In conclusion, this study provides an overview of the prevalence of GI findings in a largely asymptomatic average-risk population. GI findings are commonly found in a Western

population, with 12% having a clinically significant abnormality. This study has set a frame of reference for the prevalence and distribution of GI abnormalities in a general Western population.

Acknowledgments

The authors thank Sophia van Baalen-Van Daalen and the endoscopy nurses of Camerapil for their contribution reviewing the colon capsules videos. They also thank all employees of the Rotterdam Study working in health care center Ommoord for their help in the logistics of this study.

References

1. Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science. 2002;296(5570):1029-31. Epub 2002/05/11.
2. Goldacre MJ. Demography of aging and the epidemiology of gastrointestinal disorders in the elderly. Best Pract Res Clin Gastroenterol. 2009;23(6):793-804. Epub 2009/11/28.
3. Dumic I, Nordin T, Jecmenica M, et al. Gastrointestinal Tract Disorders in Older Age. Can J Gastroenterol Hepatol. 2019;2019:6757524. Epub 2019/02/23.
4. Mahmood SS, Levy D, Vasan RS, et al. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet. 2014;383(9921):999-1008. Epub 2013/10/03.
5. Scholtens S, Smidt N, Swertz MA, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015;44(4):1172-80. Epub 2014/12/17.
6. Ikram MA, Brusselle GGO, Murad SD, et al. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol. 2017;32(9):807-50. Epub 2017/10/25.
7. Remes-Troche JM, Jimenez-Garcia VA, Garcia-Montes JM, et al. Application of colon capsule endoscopy (CCE) to evaluate the whole gastrointestinal tract: a comparative study of single-camera and dual-camera analysis. Clin Exp Gastroenterol. 2013;6:185-92. Epub 2013/09/27.
8. Romero-Vazquez J, Caunedo-Alvarez A, Belda-Cuesta A, et al. Extracolonic findings with the PillCam Colon: is panendoscopy with capsule endoscopy closer? Endosc Int Open. 2016;4(10):E1045-E51. Epub 2016/10/18.
9. Daniel W. Biostatistics: a foundation for analysis in the health sciences. New York: John Wiley & Sons; 1999.
10. Lwanga SK, Lemeshow, Stanley, World Health Organization. Sample size determination in health studies : a practical manual Geneva: World Health Organization 1991.
11. Ronkainen J, Aro P, Storskrubb T, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology. 2005;129(6):1825-31. Epub 2005/12/14.
12. Gerson LB, Shetler K, Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. Gastroenterology. 2002;123(2):461-7. Epub 2002/07/30.
13. Rex DK, Cummings OW, Shaw M, et al. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. Gastroenterology. 2003;125(6):1670-7.
14. Kuipers EJ, Spaander MC. Natural History of Barrett's Esophagus. Dig Dis Sci. 2018;63(8):1997-2004.
15. Zagari RM, Fuccio L, Wallander MA, et al. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. Gut. 2008;57(10):1354-9. Epub 2008/04/22.
16. Martin FC, Chenevix-Trench G, Yeomans ND. Systematic review with meta-analysis: fundic gland polyps and proton pump inhibitors. Aliment Pharmacol Ther. 2016;44(9):915-25. Epub 2016/09/17.
17. Borch K, Skarsgard J, Franzen L, et al. Benign gastric polyps: morphological and functional origin. Dig Dis Sci. 2003;48(7):1292-7.
18. Stolte M, Sticht T, Eidt S, et al. Frequency, location, and age and sex distribution of various types of gastric polyp. Endoscopy. 1994;26(8):659-65. Epub 1994/10/01.
19. Corley DA, Jensen CD, Marks AR, et al. Variation of adenoma prevalence by age, sex, race, and colon location in a large population: implications for screening and quality programs. Clin Gastroenterol Hepatol. 2013;11(2):172-80. Epub 2012/09/19.

20. Heitman SJ, Ronksley PE, Hilsden RJ, et al. Prevalence of adenomas and colorectal cancer in average risk individuals: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2009;7(12):1272-8. Epub 2009/06/16.
21. Kobaek-Larsen M, Kroijer R, Dyrvig AK, et al. Back-to-back colon capsule endoscopy and optical colonoscopy in colorectal cancer screening individuals. Colorectal Dis. 2018;20(6):479-85. Epub 2017/11/23.
22. Niv Y. Polyp detection rate may predict adenoma detection rate: a meta-analysis. Eur J Gastroenterol Hepatol. 2018;30(3):247-51.
23. Barroso AO, Quigley EM. Diverticula and Diverticulitis: Time for a Reappraisal. Gastroenterol Hepatol (N Y). 2015;11(10):680-8.
24. Peery AF, Keku TO, Martin CF, et al. Distribution and Characteristics of Colonic Diverticula in a United States Screening Population. Clin Gastroenterol Hepatol. 2016;14(7):980-5 e1. Epub 2016/02/14.
25. Spada C, Hassan C, Galmiche JP, et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2012;44(5):527-36. Epub 2012/03/06.
26. Pasha SF. Applications of Colon Capsule Endoscopy. Curr Gastroenterol Rep. 2018;20(5):22.
27. Stoop EM, de Haan MC, de Wijkerslooth TR, et al. Participation and yield of colonoscopy versus non-cathartic CT colonography in population-based screening for colorectal cancer: a randomised controlled trial. Lancet Oncol. 2012;13(1):55-64.

Supplementary files

Methods

Technical features of colon capsule endoscopy

Colon capsule endoscopy consists four main components: PillCamTM COLON2 capsule (Medtronic), a sensor belt which is worn, a data-recorder and a workstation with RAPID™ 7.0 software (Supplementary image 1). The colon capsule is 11.6 × 31.5 mm^2 in size and equipped with two head cameras with 168° angle of view. The colon capsule has a feature of an adaptive frame rate (AFR). The AFR is activated once the capsule is in the small bowel and alternates between 4 images per second when the capsule is stationery and changes to 35 images per second when the capsule is moving. The data recorder allows real-time review of images during examinations. The RAPID™ software includes a graphical interface tool for polyp size estimation which allows the reviewer to measure polyps. Furthermore, the capsule provides feedback through the recorder and when capsule enters the small bowel, the recorder provides a notification (1).

Supplementary figure 1 An image of the colon capsule and data recorder (left image), the sensor belt (image in the middle) and the Rapid™ software (right image).

Supplementary table 1 Bowel preparation schedule for colon capsule endoscopy. PEG = polyethylene electrolyte glycol solution. OSS = oral sulphate solution.

Day	Time	Bowel preparation and booster
Day -2	8 p.m.	1 bisacodyl 5 mg tablet
Day -1		Light breakfast + lunch
	1 p.m.	Clear liquid diet
	6 – 8 p.m.	1L PEG+ 1L clear liquid diet
Day 0	6 – 8 a.m.	1L PEG + 1L clear liquid diet
	~ 9 a.m.	Ingestion capsule
	1 hour after ingestion capsule	10 mg metoclopramide *(only if capsule is still in stomach)*
	Small bowel detection	250ml OSS + 0.5L clear liquid diet
	3 hours after small bowel detection	250ml OSS + 0.5L clear liquid diet
	8 p.m.	Sensor belt removed by participant

Supplementary table 2 Definition of the cleansing grading scales of the stomach, small bowel and colon

Gastric grading scale	
Good	>90% of the mucosa was observed
Fair	70%-90% of the mucosa was observed
Poor	<70% of the mucosa was observed
Small bowel grading scale	
Proportion of visualized mucosa	
Excellent	> 75%
Good	50-75%
Fair	25-50%
Poor	<25%
The degree of bubbles, debris and bile	
Excellent	<5%, no obscuration
Good	5-25%, mild obscuration
Fair	25-50%, moderate obscuration
Poor	>50%, severe obscuration
Colon grading scale	
Cleansing level grading scale	
Poor	Large amount of fecal residue precluding a complete examination
Fair	Enough feces or dark fluid present to prevent a reliable exam
Good	Small amount of feces or dark fluid not interfering with examination
Excellent	No more than small bits of adherent feces
Bubbles interfering effect scale	
Significant	Bubbles/content/blurry images that interfere with the examination More than 10% of surface area is obscured
Insignificant	No bubbles/content/blurry images or so that they do not interfere with the examination. Less than 10% of surface area is obscured

Supplementary table 3 List of gastrointestinal lesions.

All findings	Definition
Barrett's esophagus	Distal esophagus is lined with columnar epithelium with a minimum length of 1 cm
Esophagitis	Mucosal break of the esophagus
Erosion	Circumscribed area of mucosal disruption
Ulcer	Large erosion with a central area with exudates
Inflammation	Redness and/or swelling of the tissue
Polyp	Protuberance into the lumen above the surrounding of the mucosa
Blood	Free intraluminal blood
Zenker diverticulum	Diverticulum of the mucosa and submucosal layers above the pharyngoesophageal junction
Mallory Weiss Lesion	Linear mucosal lacerations of distal esophagus or upper stomach
Fundic gland polyp	Sessile, shiny, translucent and pale polyp
Gastritis	Inflammation of the lining of the stomach
Erythema	Reddening of the mucosa
Angiodysplasia	Aberrant blood vessel
Diverticula	Sac-like protusion of the colonic wall
Nodular Lymphoid Hyperplasia	Multiple small nodules
Pseudopolyp	Projecting mass of granulation tissue
Vascular lesion	Vascular lesion consisting of arterioles, capillaries and venules
Venous Lake	Dilated veins
Parasite	An organism living in the gastrointestinal tract
Hemorrhoid	Abnormal swelling of the anal vascular cushions
Fibroma	Benign tumors composed of fibrous tissue

Supplementary table 4 Definition of significant lesions.

Significant Lesions	Definition
Long segment Barrett's esophagus	Segment ≥ 3cm
Severe ulceration of the digestive tract	Segment > 1cm, whether or not containing sigs of blood loss
Marked villous atrophy in the small intestine	-
Polyps in the small bowel or colon	Polyp ≥ 10mm, or three or more polyps
Esophagus tumour, gastric tumour or intestinal tumour	-

Supplementary table 5 Baseline characteristics participants and non-participants . N=number , SD = standard deviation , BMI = body mass index

	Participants		Non-participants		P-value
	Total	N (%)	Total	N (%)	
Male/female	462	214 (46.3) / 248 (53.7)	2327	970 (41.7) / 1357 (58.3)	0.066
Mean age, years (SD)	462	67.4 (4.9)	2323	67.1 (4.8)	0.158
Mean BMI, kg/m^2 (SD)	462	26.9 (4.0)	2323	27.6 (4.6)	**0.003**
Smoking, ever	460	331 (67.6)	2321	1560 (67.2)	0.869
Total alcohol intake in g/day, mean (SD)	456	8.5 (8.9)	2321	8.0 (9.0)	0.432
Ethnicity	462		2323		**0.039**
European		400 (86.6)		2010 (86.5)	
East-Asian		2 (0.4)		39 (1.3)	
African		8 (1.7)		30 (1.3)	
Mixture		5 (1.1)		7 (0.3)	
Missing		47 (10.2)		237 (10.2)	
Job	428		2119		**0.001**
Paid job		334 (78.0)		1394 (65.8)	
Unemployed		10 (2.3)		76 (3.6)	
Housewife/househusband		41 (9.6)		342 (16.1)	
Incapacitated		20 (4.7)		128 (6.0)	
Annuitant		0 (0.0)		9 (0.4)	
Early retirement		20 (4.7)		158 (7.5)	
Retirement		3 (0.7)		10 (0.5)	
unknown		0 (0.0)		2 (0.0)	
Education	428		2119		**0.012**
Elementary school		28 (6.5)		174 (8.2)	
Primary vocational school		54 (12.6)		336 (15.9)	
General secondary school		69 (16.1)		415 (19.6)	
Secondary vocational school		103 (24.1)		471 (22.2)	
General higher education		22 (5.1)		122 (5.8)	
Higher vocational education		108 (25.2)		465 (21.9)	
University education		42 (9.8)		121 (5.7)	
Different		2 (0.5)		15 (0.7)	

Supplementary table 6 Prevalence rates of gastrointestinal (GI) disease and total number of findings observed with colon capsule endoscopy (CCE). N = number. * = lymphangiectasis were presented in this table but not defined as abnormal and therefore not included in the calculation of the number of participants with findings and total number of findings. CRC = colorectal cancer

Segment GI tract, Number of observed segments, n	**Esophagus,** N =433		**Stomach,** N=437		**Small bowel,** N=446		**Cecum,** N=449		**Ascending colon,** N=442		**Transverse colon,** N=433		**Descending colon,** N=427		**Rectum,** N=250	
TOTAL																
Number of participants with findings, n (%)	64 (14.8)		122 (27.9)		151 (33.9)		114 (25.4)		198 (44.8)		180 (41.6)		353 (82.7)		127 (50.8)	
Total number of findings	69		158		326		144		248		212		610		181	
TYPE OF FINDING																
	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n
Barrett's esophagus <3cm	36 (8.3)	36														
Esophagitis	24 (5.5)	24														
Erosions	3 (0.7)	3	29 (6.6)	29	106 (23.8)	119	10 (2.2)	10	9 (2.0)	9	6 (1.4)	6	42 (10.7)	48	16 (6.4)	16
Ulcer	2 (0.5)	2			17 (3.8)	19	5 (1.1)	7	1 (0.2)	1	1 (0.2)	1	3 (0.7)	3	1 (0.4)	1
Inflammation					5 (1.1)	5	2 (0.4)	2					2 (0.5)	2	1 (0.4)	1
Polyp					23 (5.2)	27	56 (12.5)	65	73 (16.5)	82	44 (10.2)	53	145 (34.0)	220	40 (16)	56
CRC													1 (0.2)	1		
Blood	2 (0.5)	2	24 (5.5)	24	25 (5.6)	25	3 (0.7)	3	2 (0.5)	2	2 (0.5)	2	3 (0.7)	3	4 (1.6)	4
Zenker diverticulum	1 (0.2)	1														
Mallory Weiss lesion	1 (0.2)	1														
Fundic gland polyp			79 (18.1)	97												
Gastritis			8 (1.8)	8												
Erythema					25 (5.6)	25							2 (0.8)	2		

Supplementary table 6 Prevalence rates of gastrointestinal (GI) disease and total number of findings observed with colon capsule endoscopy (CCE). N = number. * = lymphangiectasis were presented in this table but not defined as abnormal and therefore not included in the calculation of the number of participants with findings and total number of findings. CRC = colorectal cancer (*continued*)

Segment GI tract, Number of observed segments, n	**Esophagus,** N =433		**Stomach,** N=437		**Small bowel,** N=446		**Cecum,** N=449		**Ascending colon,** N=442		**Transverse colon,** N=433		**Descending colon,** N=427		**Rectum,** N=250	
	TYPE OF FINDING															
	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n	Nr. of participants with findings, n(%)	All findings, n
Lymphangiectasis*					137 (30.7)	190										
Angiodysplasia					66 (14.8)	75	6 (1.3)	7	10 (2.3)	10	9 (2.1)	9	22 (5.2)	24	1 (0.4)	1
Diverticula					15 (3.4)	16	49 (10.9)	49	144 (32.6)	144	141 (32.6)	141	304 (71.4)	307	(3.2)	7
Nodular lymphoid hyperplasia					8 (1.8)	8										
Pseudo polyps					2 (0.4)	3										
Venous lake					3 (0.7)	3										
Parasite					1 (0.2)	1	1 (0.2)	1								
Hemorrhoids															91 (36.4)	91
Fibroma															4 (1.6)	4

Supplementary table 7 Distribution of relevant findings based on gender and age

Clinical relevant findings	Male/Female	55-60 years	60-65 years	65-70 years	70-75 years
Barret segment >3cm	-				
Severe ulceration >1cm	1/0			1	
Polyp >10mm or ≥3 polyps in the small bowel	3/1	1		1	2
Polyp >10mm or ≥3 polyps in the colon	26/20	5	5	17	19
Colon cancer	1/0			1	
Total	31/21	6	5	20	21

Supplementary table 8 Observed segments of the gastrointestinal tract (total of 451 videos), observed Z-line and transit times of colon capsule endoscopy. GI = gastrointestinal. CCE = colon capsule endoscopy. IQR = inter quartile range

	N	%
Z-line observed	202	44.8
Completion rate	234	51.9
Number of visualized segments of the GI tract		
Esophagus	433	96.0
Stomach	437	96.9
Small bowel	446	98.9
Colon	449	99.6
Cecum	449	99.6
Ascending colon	442	98.0
Transverse colon	433	96.0
Descending colon	427	94.7
Rectum	250	55.4
Reach		
Stomach	1	0.2
Small bowel	1	0.2
Cecum	5	1.1
Ascending colon	10	2.2
Transverse colon	7	1.6
Descending colon	118	26.2
Sigmoid	59	13.1
Rectum	15	3.3

Reference

1. Pasha SF. Applications of Colon Capsule Endoscopy. *Curr Gastroenterol Rep* 2018; **20**(5): 22.

Chapter 3

Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals

F. E.R. Vuik*; J. Dicksved*, S.Y. Lam, G. M. Fuhler, L. J.W. van der Laan, A. van de Winkel, S. R. Konstantinov, M.C.W. Spaander, M. P. Peppelenbosch, L. Engstrand, E.J. Kuipers

*Both authors contributed equally

United European Gastroenterology Journal, August 2019

Abstract

Introduction Homeostasis of the gastrointestinal tract depends on a healthy bacterial microbiota, with alterations in microbiota composition suggested to contribute to diseases. To unravel bacterial contribution to disease pathology, a thorough understanding of the microbiota of the complete gastrointestinal tract is essential. To date, most microbial analyses have either focused on faecal samples, or on the microbial constitution of one gastrointestinal location instead of different locations within one individual. We aimed to analyse the mucosal microbiome along the entire gastrointestinal tract within the same individuals.

Methods Mucosal biopsies were taken from nine different sites in 14 individuals undergoing antegrade and subsequent retrograde double-balloon enteroscopy. The bacterial composition was characterised using 16 S rRNA sequencing with Illumina Miseq.

Results At double-balloon enteroscopy, one individual had a caecal adenocarcinoma and one individual had Peutz-Jeghers polyps. The composition of the microbiota distinctively changed along the gastrointestinal tract with larger bacterial load, diversity and abundance of Firmicutes and Bacteroidetes in the lower gastrointestinal tract than the upper gastrointestinal tract, which was predominated by Proteobacteria and Firmicutes.

Conclusions We show that gastrointestinal location is a larger determinant of mucosal microbial diversity than inter-person differences. These data provide a baseline for further studies investigating gastrointestinal microbiota-related disease.

Introduction

In recent years, an increasing level of knowledge on the interaction between host and bacteria has made us come to regard the gut microbiota as a separate entity (1). The microbiota has important immunological, structural, metabolic and defence functions in the gut. Alterations in microbiota composition have been linked to intestinal disease, including colorectal cancer and inflammatory bowel disease (IBD). Unravelling the microbiota composition and its distribution along the gastrointestinal (GI) lining in healthy individuals is important to understand the role of the microbiota in disease (2).

Characterization of the microbiota in the entire GI tract is hampered by the fact that some locations are more difficult to access than others and most research has focused on the colonic faecal microbiota (1). The mucosal microbiome is arguably the more relevant compartment, as such mucosa-associated flora lives in close contact with the GI tract lining. The microbial composition of the colonic mucosa has been most often investigated. While it is clear that the composition and abundance of mucosal microbiota of the oesophagus and stomach in healthy individuals differ from that in the colon, information about the microbial composition in the jejunum and ileum is scarce because of the inaccessibility of these sites (3–5).

Nevertheless, differences in the physiological functions of GI sites logically predict regional bacterial differences. The colonic microbiota for example, is driven by complex carbohydrates whereas simple carbohydrates fuel the microbiota in the small intestine (2, 6). Furthermore, the composition of the mucus layer protecting the epithelial barrier from excessive bacterial contact differs along the intestinal tract (7, 8).

Given the limited information about mucosal microbiota in the entire GI tract, we aimed to characterise the mucosal microbiota along the length of the entire GI tract within the same subjects.

Methods

Subject recruitment

Subjects, all inhabitants of The Netherlands, had abdominal symptoms of unknown cause requiring diagnostic antegrade and subsequent retrograde double-balloon enteroscopy (DBE). Exclusion criteria were: patients younger than 18 years, use of antibiotics three months before DBE, IBD, and failure to understand written Dutch. The study was conducted in accordance with the Declaration of Helsinki Principles and approved by

the ethical committee of the Erasmus University Medical Center, Rotterdam (MEC-2017-151) on 3 April 2017.

Sampling

Mucosal samples were obtained endoscopically using antegrade and subsequent retrograde double balloon enteroscopy (DBE) at the Erasmus Medical Center using Fujinon EN-450P5 and EN-450T5 (Fujinon Inc., Saitama, Japan) endoscopes. Endoscopes were disinfected before use. Mucosal biopsies using standard biopsy forceps were taken at nine different sites of the GI tract (Figure 1). Upper GI biopsies (oesophagus to proximal ileum) were collected using antegrade endoscopy and lower GI biopsies (distal ileum to rectum) with retrograde endoscopy. Between the antegrade and retrograde endoscopy the canal of the endoscope was cleaned with sterile water. All patients used bowel preparation before DBE consisting of macrogol and electrolytes (Klean-Prep (Norgine BV, Amsterdam, The Netherlands)).

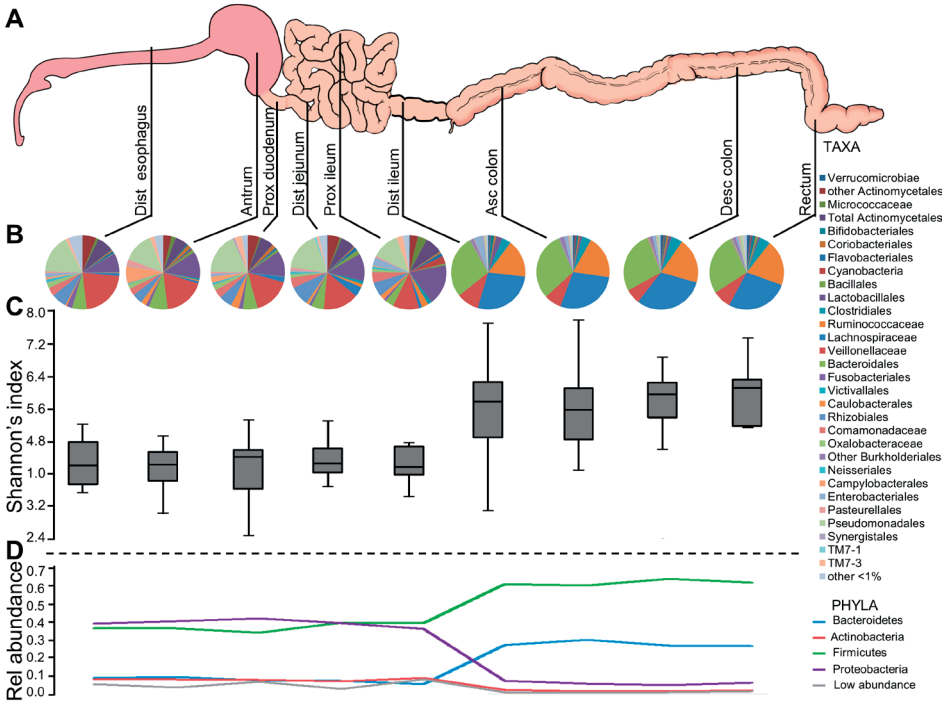


Figure 1 Overview of the study. (a) Location of the retrieved mucosal biopsies of the gastrointestinal (GI) tract. (b) Marked differences in bacterial taxa are present between different GI locations as indicated by boxplot of the median Shannon's index of the different locations. (c) Diversity as measured by Shannon's index is higher in the distal ileum, ascending colon, descending colon and rectum as compared to distal oesophagus, antrum, proximal duodenum, distal jejunum and proximal ileum. (d) Relative abundance of the major phyla fluctuates along the GI tract. Asc: ascending; Desc: descending; Dist: distal; Prox: proximal.

Samples were stored in Eppendorf cups (0.2 ml) with a stabilising reagent Allprotect (Qiagen Gmbh, Hilden, Germany). The samples were homogenised using the MagNA Lyser machine (Roche Diagnostics, Mannheim, Germany), stored in Trizol tubes (Invitrogen, Groningen, The Netherlands) and immediately frozen and stored at –80° C for subsequent analyses. DNA was isolated from the samples using QIAamp DNA mini kit (Qiagen) with an initial bead beating step added to the protocol, as described previously (9).

Generation of 16 S rRNA gene amplicons

Sequencing libraries were prepared by amplifying the V3–V4 region of the 16 S rRNA gene using the 341f-805 r primers, as described earlier (10). After the initial amplification, PCR (Polymerase chain reaction) products were confirmed with gel electrophoresis and purified using Agencourt AMPure XP magnetic beads (Beckham Coulter Inc., Bromma, Sweden). A second PCR was performed to attach Illumina adapters and barcodes that allow for multiplexing and the products were purified as above, quantified and pooled into equimolar amounts. Samples were sequenced using the Illumina MiSeq platform at Science for Life Laboratory, Solna, Sweden. From the generated sequence data, primer sequences were trimmed away and the paired-end reads produced by the sequencing instrument were merged using SeqPrep version 1.1 (https://github.com/jstjohn/SeqPrep) with default parameters and thereafter the merged sequences were processed with QIIME 1.8 pipeline (Quantitative Insights into Microbial Ecology) (11). A de novo operational taxonomic unit (OTU) strategy was used to assign sequences to OTUs. Using the UCLUST algorithm built into the QIIME pipeline, sequences were clustered at 97% identity against the Greengenes reference database (12,13).

PCR analysis

Conventional PCR was performed for the confirmation of bacterial and human DNA isolation of biopsies. While analysing the results of this study, we noticed that the family Helicobacteraceae were present not only in the antrum, but also in other parts of the GI tract. However, sequencing did not allow us identify this feature on species level. To improve our understanding, we performed additional analyses by PCR. DNA amplification was executed with the Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, USA) using 16 S (different from sequencing PCRs), Helicobacter pylori (HP) specific UreA and VacA S1/S1, and human ACTB primers (Supplementary Table 1). For HP genes, the reaction mixture contained GoTaq buffer (Promega, Madison, Wisconsin, USA), 1.25 mM MgCl2 (Promega), 0.167 mM (each) deoxynucleotides (Roche Diagnostics), 2.5 U GoTaq polymerase (Promega), 333 nM of each primer (Sigma-Aldrich, St Louis, Missouri, USA) and 2 µl un-normalised stock DNA. PCR cycle consisted of four minutes 95°C, several cycles of 30 s denaturing at 95°C, 30 seconds annealing and one minute extension at

72°C, followed by the final extension for 10 min at 72°C. Annealing temperature was 60°C for 16 S, UreA and VacA and 60.5°C for ACTB. Number of cycles was 40 for HP genes, and 35 for 16 S and ACTB. Amplicons were analysed by gel electrophoresis using 2% agarose gel in 1X TBE (Tris-borate-EDTA) buffer and bacterial DNA load was quantified using Image J software.

Statistical analysis

The similarity between two samples was calculated using weighted Unifrac distances. Biodiversity within a sample was measured using the Shannon index. All diversity calculations were also performed for a least detectable relative abundance of 0.1%, corresponding to 1000 sequences in a sample, but this did not alter the results (data not included). Principal coordinate analysis (PCoA) using Bray Curtis metrics based on abundance data from sequences classified to genus level was performed to determine clustering patterns among the subjects.

Differences in diversity and similarity indices were tested with Mann-Whitney or Kruskal-Wallis test using the IBM SPSS statistics 21 software (Chicago, Illinois, USA). For differences in relative abundance of specific bacterial taxa we used Wilcoxon tests and linear regressions using the r statistical framework, version 3.0.1.

Results

Subject population

Fourteen subjects undergoing an antegrade and subsequent retrograde DBE were included. In 13 patients, the mucosal samples were also studied by histology. Twelve subjects had no relevant anomalies found with DBE and histology (Table 1). One patient had Peutz-Jeghers polyps in the distal jejunum and one patient had a caecum tumour in the distal ileum (Supplementary Table 1). Written informed consent was obtained from each patient included in the study.

Table 1 Baseline characteristics of subjects. BMI: body mass index, DBE: double-balloon enteroscopy, GI: gastrointestinal, IQR: interquartile range, SB: small bowel, SD: standard deviation.

Characteristics	Numbers
Mean age, mean (IQR) (year)	51 (42-60)
Sex, N (%)	
Male	7 (50%)
Race, N (%)	
Caucasian	10 (71%)
Other	4 (29%)
BMI, mean (SD) (kg, m^2)	22,9 (5,4)
Unknown, N	5
Current smoker, N (%)	
Yes	8 (58%)
No	3 (21%)
Unknown	3 (21%)
Alcohol, N (%)	
Yes	6 (43%)
No	5 (36%)
Unknown	3 (21%)
Medication use, N (%)	
Yes	11 (79%)
No	3 (21%)
Medical history, N (%)	
Hypertension	1 (7%)
Diabetes	2 (13%)
Cardiac disease	1 (7%)
Peripheral arterial disease	2 (13%)
Stroke	1 (7%)
Chronic pulmonary disease	1 (7%)
Liver disease	1 (7%)
Resection part of GI tract	2 (13%)
Other	2 (13%)
No medical history	2 (13%)
Presenting symptoms, N (%)	
Iron deficiency anaemia	5 (29%)
Diarrhea	4 (24%)
Abdominal complaints	4 (24%)
Weight loss	3 (18%)
Rectal blood loss	1 (5%)
Findings DBE, N (%)	
No abnormal findings	10 (71%)
Ulcerative lesions in small bowel	1 (7%)
Polyps in small bowel	2 (14%)
Polyps in colon	1 (7%)
Pathology finding, N (%)	
No abnormal findings	9 (64%)
Reflux esophagitis	1 (7%)
Chronic inflammation antrum	1 (7%)
Chronic inflammation SB	1 (7%)
Peuthz Jeghers polyps	1 (7%)
Ulcerative changes	1 (7%)

Overview of sequencing data generated from the samples

A total of 118 mucosal samples were retrieved from nine locations of the GI tract in 14 individuals. Eight samples could not be sequenced due either to inability to analyse the retrieved samples or inability to reach the site.

First, we confirmed bacterial DNA isolation from all samples by conventional PCR. While human genomic DNA content was similar in all samples (Supplementary Figure 1), the bacterial load decreased from oesophagus to proximal ileum, but increased again in the lower GI tract (Figure 2). Samples were subsequently subjected to 16S rRNA gene amplicon sequencing using a V3-V4 specific primer set, resulting in a total of 4.369.079 high-quality sequences, with 37.026 sequences per sample (range: 17.294–68.696).

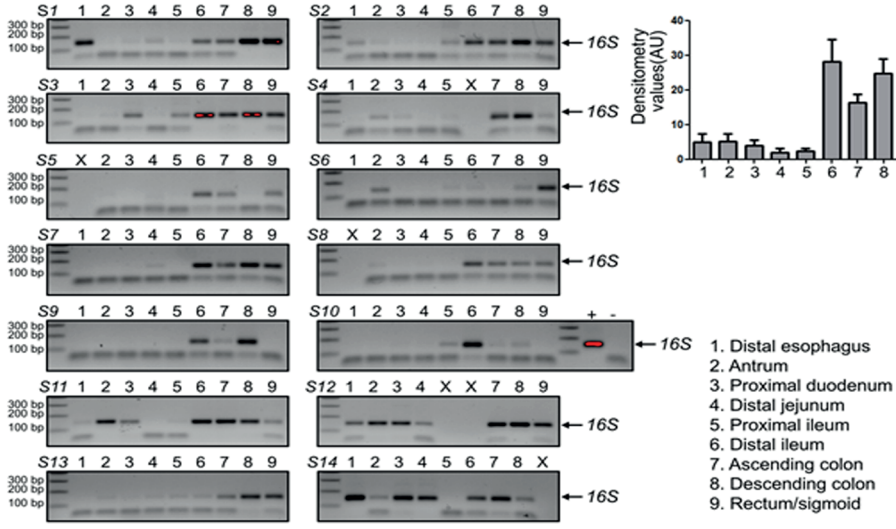


Figure 2 Differential bacterial load at the mucosa along the gastrointestinal tract. Bacterial abundance at all locations of the 14 included subjects was determined by 16 S PCR and electrophoresis results are shown for all samples. Missing samples are indicated by 'X'. +: positive control (DNA isolated from human faecal sample); -: negative control (water). For semi-quantitative analysis, bands were quantified and for each patient, the data was normalised to the total intensity per gel to adjust for differences between gel compositions and staining intensity. Mean ± standard error of the mean (SEM) is shown in bar graph.

Diversity of the microbiota along the GI tract

To estimate the diversity of the microbial communities of the biopsies in the entire GI tract, analysis of alpha diversity, represented by Shannon's index, was performed (Figure 1). The location of sampling had a significant influence on the alpha diversity of the microbiota, with samples taken from oesophagus to proximal ileum harbouring a lower level of microbial diversity than samples obtained from terminal ileum to rectum ($p < 0.05$). When comparing the average alpha-diversity of the individual locations from

individual subjects, a wide spread in the mean Shannon index between individuals became apparent with, in particular, subject 12 showing a low diversity in all samples (Figure 3(a)). This patient was diagnosed with a caecum tumour. Nevertheless, all participants, except subject 10, showed a higher alpha-diversity in lower GI locations (Figure 3(b)) as compared to upper GI locations.

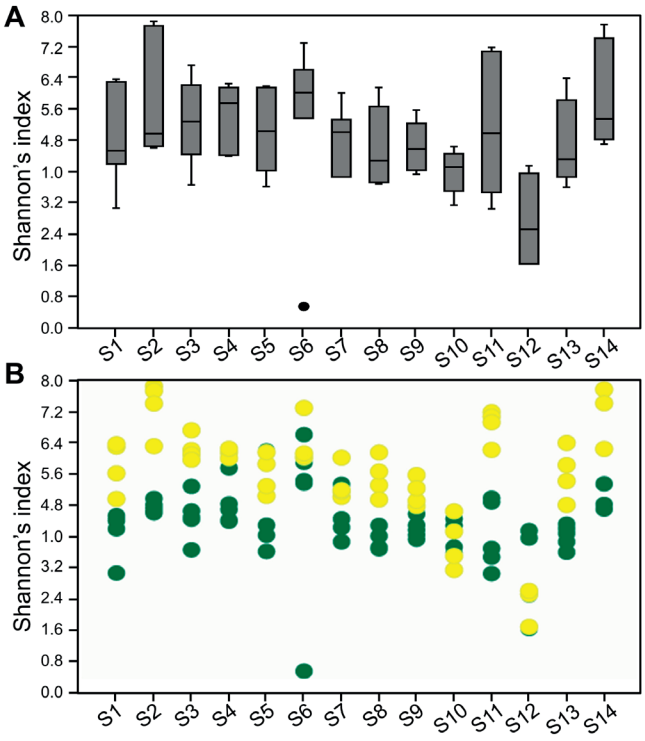


Figure 3 The α-diversity of the microbiota of the gastrointestinal (GI) tract (a) Boxplot of the median Shannon's index over all locations within each subject (S1–S14). Subject S12 shows a low α-diversity. The outlier for subject S6 represents the antrum biopsy. (b) The same data, but represented in a Jitter plot, with each dot representing a location in the GI tract. Green-coloured dots represent the distal oesophagus, antrum, proximal duodenum, distal jejunum and proximal ileum (upper GI tract) and the yellow coloured dots represent the distal ileum, ascending colon, descending colon and rectum (lower GI tract samples). All subjects, except S10 show a higher α-diversity in samples obtained from the lower GI tract as compared to the upper GI tract.

Differential microbial composition along the GI tract

We further searched for clustering patterns among samples according to their microbial population structure by PCoA based on Bray Curtis distance metrics. Again, a distinct separation of bacterial community structure was observed, with samples from the distal oesophagus to the proximal ileum clustering together, separately from distal ileum to rectum (Figure 4). Several samples clustered neither with the upper nor the lower GI

samples, but belonged to the patient diagnosed with a caecum tumour. These samples from this patient appeared to be dominated by Enterobacteriaceae. (Supplementary Figure 2).

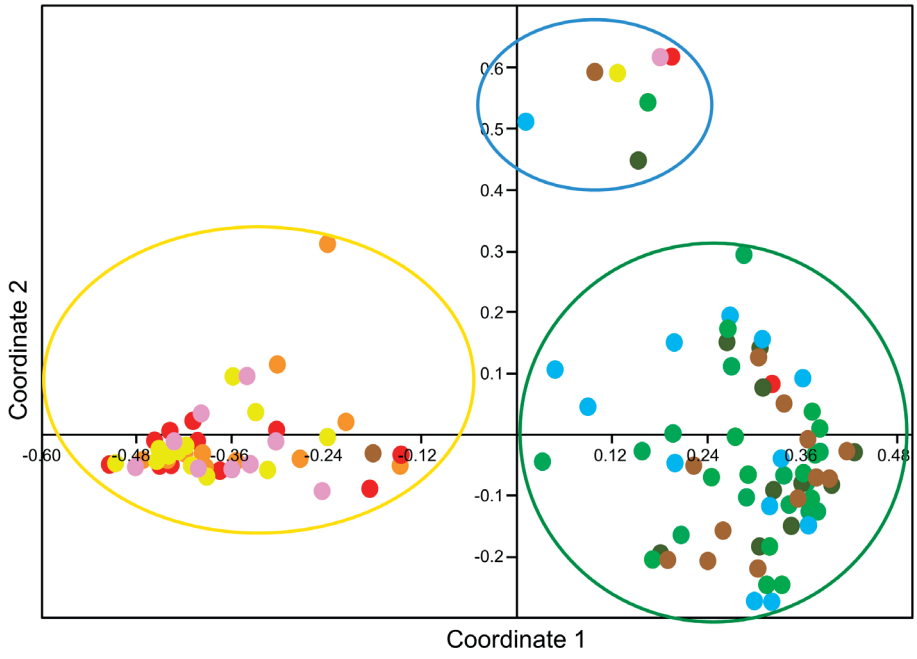


Figure 4 Principal coordinate analysis (PCoA) plot illustrates a clear difference between gut location and composition of the microbiota. Different coloured dots represent different locations of the gastrointestinal (GI) tract. The green circle contains mainly oesophagus,antrum, proximal duodenum, distal jejunum and proximal ileum samples (upper GI tract), the yellow circle contains only distal ileum, ascending colon, descending colon and rectum samples (lower GI tract). The blue circle highlights samples dominated by Enterobacteriaceae which were all derived from one patient with a caecum tumour (S12).

Cluster analysis using Euclidian distance at family level was used to visualise these data in a different way, which again demonstrates the separate clustering of this patient with a caecum tumour and the lower and upper GI tract samples (Supplementary Figure 3). Samples from individual patients appear to cluster more closely together in lower GI samples than upper GI samples (Supplementary Figures 3 and 4).

The similarity in microbiota composition between different sites in the GI tract was also visualised using weighted UniFrac distances, which showed that the microbial composition in the rectum was a good predictor for the microbial composition in the ascending and descending colon and – to a somewhat lesser extent – the distal ileum (Figure 5(a)). The composition of the microbiota in the distal oesophagus was also compared to the other locations in the GI tract. However, the microbiota in the distal oesophagus was not

as good a predictor for the other locations in the upper GI tract as the rectum was for the lower GI tract (Figure 5(b)).

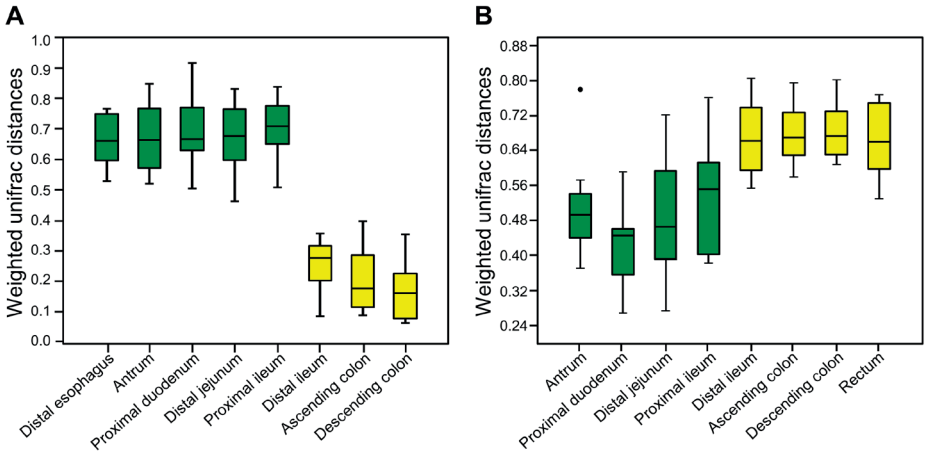


Figure 5 Similarity between different sites in the gastrointestinal (GI) tract analysed using weighted UniFrac distances. (a) The microbiota in the rectum was compared to the eight other locations, and is a good proxy for other lower GI locations. (b) The microbiota of the distal oesophagus was compared to the eight other locations, and is a less efficient predictor for the microbiota of the other locations.Green: upper GI tract; Yellow: lower GI tract.

Characterization of mucosa-associated microbiota

All regions in the GI tract were dominated by three major bacterial phyla: Bacteroidetes, Firmicutes and Proteobacteria. Although ubiquitously dominant within the entire GI tract, each of the three phyla revealed distinct profiles along the length of the GI tract (Figure 1(d)). The mucosa-associated microbiota of the upper GI tract was dominated by Proteobacteria (mean abundance of 40 ± 2.1%) and Firmicutes (38 ± 2.3%). However, in the lower GI tract the level of Proteobacteria decreased consistently (distal colon (5.3 ± 0.4%)). Firmicutes, already highly abundant in the upper GI tract, dominated the large intestine with the highest level in the distal colon (mean abundance 64 ± 7%). Bacteroidetes was present at low levels in the upper GI tract (8 ± 1.6%), but became a dominant phylum in the lower GI tract (mean abundance in ascending colon 28 ± 1.6%).

The most prevalent bacterial families in the upper GI tract were Veillonellaceae, Pseudomonadaceae and Streptococcaceae (Figure 6). In contrast to other sites in the GI tract, Prevotellaceae (relative abundance of 8%) and Helicobacteraceae (relative abundance of 8%) were dominant in the antrum. Helicobacter species were detected in nine subjects, and predominated the antrum of one subject (S6) to the extent that other species were almost not found (Supplementary Figure 3). PCR analysis of the UreA and

VacA gene confirmed that the Helicobacteraceae detected by sequencing were indeed Helicobacter pylori (Figure 7(a)). Helicobacter was present across the entire upper GI tract, and some lower GI tract locations in three subjects, which confirms data that this bacterium may spread beyond the stomach (Supplementary Figure 5). Interestingly, subject S14 showed high levels of Helicobacteraceae in the proximal duodenum, while not detected in the antrum (Figure 7(b)).

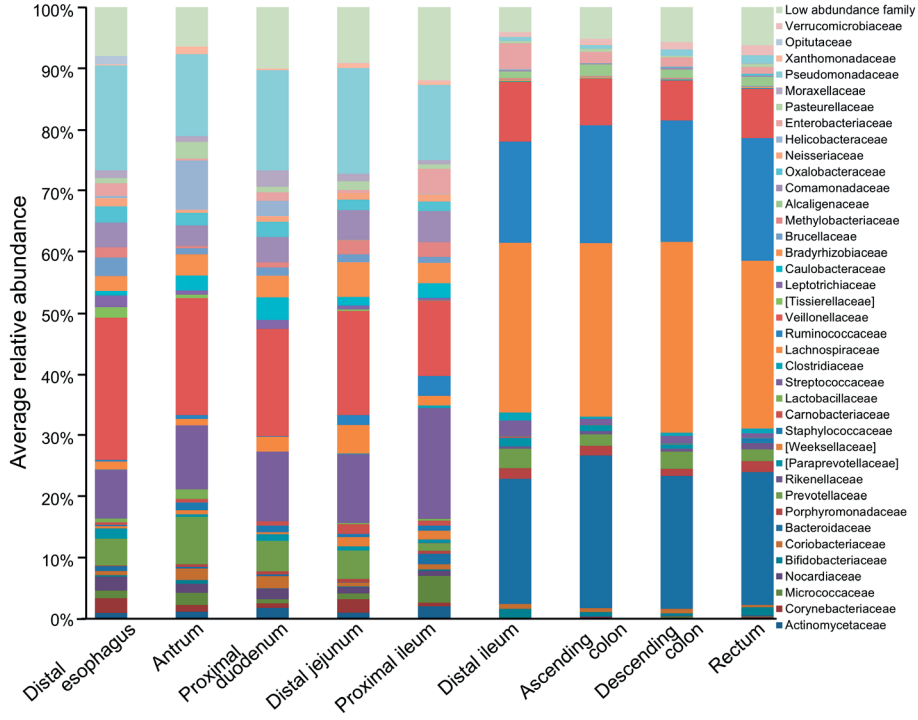


Figure 6 Most important bacteria at family level (>1% abundance) per location. Samples from patient 12, which were predominated by Enterobacteriaceae and showed low α-diversity, were excluded from this analysis.

In the distal jejunum, Bradyrhizobiaceae (relative abundance of 6%) occurred more often compared to other parts of the GI tract. The same applies to Micrococcaceae (relative abundance of 4%) in the proximal ileum. The lower GI tract was dominated by Lachnospiraceae, Bacteroidaceae, Ruminococcaceae and Veillonellaceae. The highest abundance of the bacterial family Clostridiaceae (relative abundance of 1%) was seen in the distal ileum. Rikenellaceae was only seen with a higher relative abundance than 1% in the rectum.

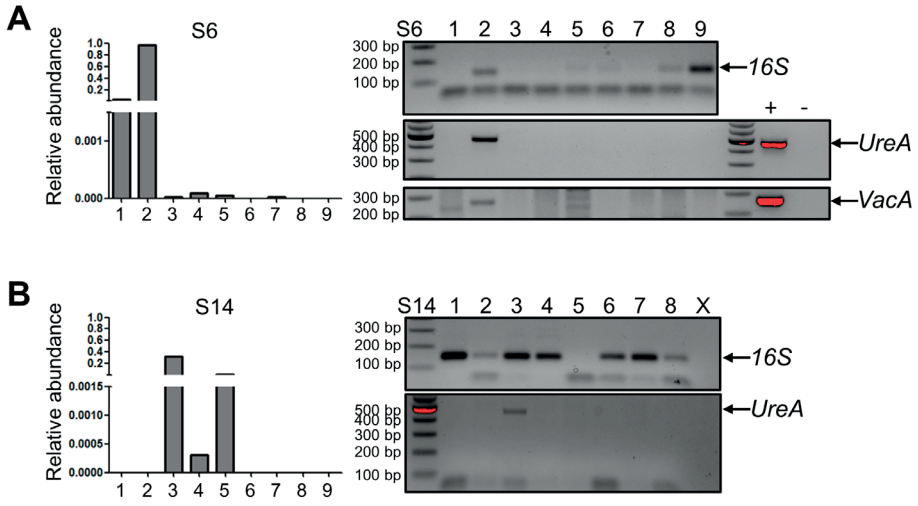


Figure 7 Helicobacter pylori predominates in the antrum from one patient, and extends beyond the stomach. (a) Relative abundance of Helicobacter species across the nine different gastrointestinal (GI) sites in subject S6 as determined by sequencing. Identity of Helicobacter pylori at species level was confirmed by PCR in the high Helicobacter abundant samples by *UreA* and *VacA*. The antrum was dominated by H. pylori, resulting in a low diversity in this sample (see Figure 2 and Supplementary Figure 4). 16 S PCRs, similar to Figure 2, are shown here to allow comparison of total bacterial abundance in these samples. (b) Relative abundance of Helicobacter species across the different GI sites in subject S14 as determined by sequencing. Identity of H. pylori at species level was confirmed by PCR of *UreA*. Numbers are as described above, X represents a missing samples. While H. pylori was not detected in the antrum, high levels were present in the proximal duodenum. 1: distal oesophagus; 2: antrum; 3: proximal duodenum; 4: distal jejunum, 5: proximal ileum; 6: distal ileum; 7: ascending colon; 8: descending colon; 9: rectum; +: positive control of pure H. pylori culture strain ATCC®43504 (American Type Culture Collection, Rockville, Maryland, USA); –: negative control (water).

Discussion

This study describes the composition of the microbiota along the entire GI tract in the same individuals without significant pathology. In agreement with earlier reports, the bacterial load decreases from the oesophagus to the proximal ileum, but drastically increases again in the lower GI tract, starting from the distal ileum. The composition of the microbiota markedly changes along the GI tract, with the most prevalent bacterial families present in the upper GI tract Veillonellaceae, Pseudomonadaceae and Streptococcaceae, while the lower GI tract is dominated by Lachnospiraceae, Bacteroidaceae and Ruminococcaceae.

Our findings to a large extent reflect data obtained from other studies comparing only partly matched samples, but probing multiple locations within one patient may provide

better accuracy. One report comparing only duodenal and rectal content from healthy individuals reported higher Shannon diversity values in both mucosa and luminal content from the duodenum, while others support our findings of a less complex luminal microbiota in the small intestine compared to the colonic content (6, 14, 15).

Arguably, the least studied GI sites in the current literature are the jejunum and distal ileum. In the jejunum, Proteobacteria and Firmicutes were the most dominant phyla, and at family level Veillonellaceae, Pseudomonadaceae and Streptococcaceae dominated. A previous study retrieving mucosal biopsies from the proximal jejunum of 19 healthy individuals also observed Proteobacteria, Bacteroidetes and Firmicutes as the predominant phyla, although family level classification indicated Brevibacteriaceae, Barnesiellaceae and Leuconostocaceae (16). Possible explanations for these discrepancies could be the difference in individual populations (Taiwanese versus Dutch population) as well as alternative methodologies used for sampling, preparation and analysis of the samples.

In terms of the proximal and distal ileum, our samples were found to have large differences in composition. In the proximal ileum, Proteobacteria and Firmicutes dominated, whereas Firmicutes and Bacteroidetes were the dominant phyla in the distal ileum. It is conceivable that the distal ileum was contaminated from the colon, either due to sampling or through bowel movements. At present the only comparison that can be made in this context comes from animal studies. A study comparing 10 paired GI locations in mice showed that the largest difference between two locations in terms of bacterial diversity was seen between ileum and proximal cecum, with lower GI samples clustering away from upper GI samples (17, 18) In pigs, a similar clear separation between the upper and lower GI could be seen, although in this case the dividing line appeared to lie between jejunum and ileum (17, 18).

A further notable finding in our study was that a patient who had a caecum tumour showed a significant dysbiosis predominated by Enterobacteriaceae in all other GI sites tested. A role for Enterobacteriaceae in carcinogenesis has been suggested before, as several enterobacterial strains are known to produce DNA-damaging genotoxins and may therefore cause mutations (19, 20).

The major strength of this study is that we collected nine mucosal samples along the entire GI tract of 14 different individuals allowing us to study the composition of the microbiota along the length of the gut. Since all individuals underwent an antegrade DBE followed directly by a retrograde DBE, no bias could have occurred based on the timeframe.

There are also a number of limitations. Firstly, the same endoscope was used for anterograde and retrograde DBE. Although the canal of the endoscope was cleaned with sterile water between the antegrade and retrograde DBE, it is impossible to exclude contamination from the upper GI tract to the lower GI tract using this methodology (21). However, the low level of similarity of the microbial composition in the upper and lower GI tract suggests that this is not a major issue in our study. Secondly, the subjects in our study underwent DBE for unexplained symptoms and therefore may not fully represent healthy individuals. However, ethical considerations preclude performing DBE in individuals without clinical indication and thus we consider our study the best that can be achieved with current technical approaches. Third, neither DBE nor histopathology of the retrieved biopsies showed clinical abnormalities except for one patient with a caecum tumour and one patient with Peutz-Jeghers polyps. Fourth, patients were treated with colonic lavages prior to DBE, which could potentially have diminished the diversity of the mucosa-associated microbiota. Unfortunately, a DBE cannot be performed without bowel preparation (22). Finally, stool samples were not collected of these patients and therefore the faecal microbiota could not be analysed. Whether stool and mucosal microbiome correlate well is somewhat debated in literature, and having stool samples would have been of value (1, 14). With the exception of the patient with a caecum tumour, the data represented here could be conceived as representing the 'normal' mucosal microbiome. While it is already well described that education of the immune system depends on the intestinal microbiome, to what extent local mucosal differences affect local immunological responses is less well elucidated. Diseases like IBD are largely driven by an altered immunological response towards intestinal microbes. Thus a comparison of disease-location specific mucosal microbial changes to normal microbiome signatures at these sites may be of use (23). The use of faecal microbiota transplantation for IBD has been advocated, and it is thought that optimal donor selection is important for clinical efficacy, although more research is needed to identify which components of the gut microbiome constitute key member (24).

In conclusion, we have generated a first overview of the composition of the microbiota along the entire GI tract. This study is of particular importance in helping us to understand the interactions between bacterial communities and human cells and takes us to the next step in describing the impact of the microbiota on health and its involvement in diseases.

References

1. Watt E, Gemmell MR, Berry S, Glaire M, Farquharson F, Louis P, et al. Extending colonic mucosal microbiome analysis-assessment of colonic lavage as a proxy for endoscopic colonic biopsies. Microbiome. 2016;4(1):61.
2. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2016;65(2):330-9.
3. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human distal esophagus. Proc Natl Acad Sci U S A. 2004;101(12):4250-5.
4. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology. 2009;137(2):588-97.
5. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103(3):732-7.
6. Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CC, Troost FJ, et al. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. ISME J. 2012;6(7):1415-26.
7. Moran C, Sheehan D, Shanahan F. The small bowel microbiota. Curr Opin Gastroenterol. 2015;31(2):130-6.
8. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol. 2013;10(6):352-61.
9. Dicksved J, Lindberg M, Rosenquist M, Enroth H, Jansson JK, Engstrand L. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. J Med Microbiol. 2009;58(Pt 4):509-16.
10. Hugerth LW, Wefer HA, Lundin S, Jakobsson HE, Lindberg M, Rodin S, et al. DegePrime, a program for degenerate primer design for broad-taxonomic-range PCR in microbial ecology studies. Appl Environ Microbiol. 2014;80(16):5116-23.
11. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.
12. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460-1.
13. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010;26(2):266-7.
14. Li G, Yang M, Zhou K, Zhang L, Tian L, Lv S, et al. Diversity of Duodenal and Rectal Microbiota in Biopsy Tissues and Luminal Contents in Healthy Volunteers. J Microbiol Biotechnol. 2015;25(7):1136-45.
15. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol. 2015;21(29):8787-803.
16. Chung CS, Chang PF, Liao CH, Lee TH, Chen Y, Lee YC, et al. Differences of microbiota in small bowel and faeces between irritable bowel syndrome patients and healthy subjects. Scand J Gastroenterol. 2016;51(4):410-9.
17. Suzuki TA, Nachman MW. Spatial Heterogeneity of Gut Microbial Composition along the Gastrointestinal Tract in Natural Populations of House Mice. PLoS One. 2016;11(9):e0163720.
18. Kelly J, Daly K, Moran AW, Ryan S, Bravo D, Shirazi-Beechey SP. Composition and diversity of mucosa-associated microbiota along the entire length of the pig gastrointestinal tract; dietary influences. Environ Microbiol. 2017;19(4):1425-38.

19. Yurdakul D, Yazgan-Karatas A, Sahin F. Enterobacter Strains Might Promote Colon Cancer. Curr Microbiol. 2015;71(3):403-11.
20. Allen-Vercoe E, Jobin C. Fusobacterium and Enterobacteriaceae: important players for CRC? Immunol Lett. 2014;162(2 Pt A):54-61.
21. Walker MM, Talley NJ. Review article: bacteria and pathogenesis of disease in the upper gastrointestinal tract--beyond the era of Helicobacter pylori. Aliment Pharmacol Ther. 2014;39(8):767-79.
22. Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. Gastroenterology. 2014;146(6):1449-58.
23. Ueno A, Jeffery L, Kobayashi T, Hibi T, Ghosh S, Jijon H. Th17 plasticity and its relevance to inflammatory bowel disease. J Autoimmun. 2018;87:38-49.
24. Woodworth MH, Carpentieri C, Sitchenko KL, Kraft CS. Challenges in fecal donor selection and screening for fecal microbiota transplantation: A review. Gut Microbes. 2017;8(3):225-37.

Supplementary Table 1 Baseline characteristics per individual subject. na = information is not available; - = no specialties, *no biopsies were taken during DBE, the mass was diagnosed as a cecum tumor during follow up endoscopy, m = male, f = female, DBE = double balloon enteroscopy

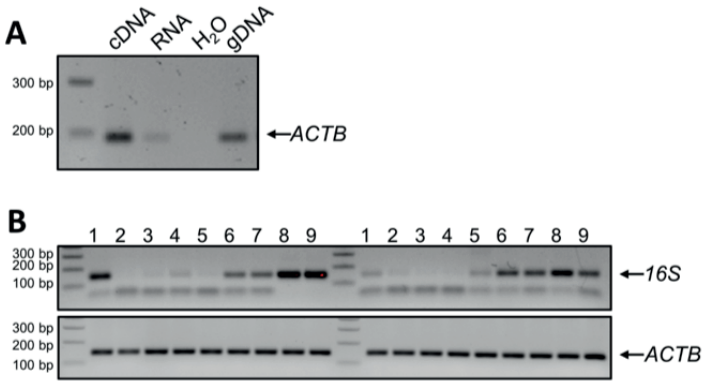
Subjects	Age (y)	Sex	Race	BMI	Alcohol (units/time)	Smoking	Medical history	Medication use	Presenting symptoms	Findings DBE	Findings Pathology
1	50	M	Caucasian	20	4-5 units/ month	-	1964: resection of small bowel	Methadone	Blood loss	Multiple polyps in proximal small bowel	Peutz Jeghers Polyps
2	38	F	Caucasian	21	-	15 cigarettes /day	BCRA1 gene mutation	Pancreatic enzymes Eye drops	Diarrhea	-	-
3	44	F	na	na	na	na	-	-	Abdominal pain	-	-
4	61	F	Caucasian	24	3 units/ day	-	Atrial fibrillation	Proton pump inhibitor, Antiplatelet drug Paracetamol Acenocoumarol Benzodiazepine	Diarrhea; weight loss	Polyp in proximal duodenum	-
5	26	F	Caucasian	na	-	1-10 cigarettes/ day	-	-	Abdominal pain	-	-
6	39	M	Black	na	na	na	Diabetes type 2	Metformin Proton pump inhibitor Antiplatelet drug	Abdominal pain	-	-
7	67	F	Caucasian	16	-	Yes	1997: liver failure based on hepatitis B infection wherefore liver transplantation 2005: osteopenia Mild COPD	Proton pump inhibitor Immunosuppressive (Ciclosporin) Calcium/Vitamin D	Iron deficiency anemia	-	-
8	47	F	Caucasian	15	-	-	Fibromyalgia Endometriosis	Budesonide H_2 antagonist Antispasmodic	Diarrhea; weight loss; abdominal pain	-	-

Supplementary Table 1 Baseline characteristics per individual subject. na = information is not available; - = no specialties, *no biopsies were taken during DBE, the mass was diagnosed as a cecum tumor during follow up endoscopy, m = male, f = female, DBE = double balloon enteroscopy (*continued*)

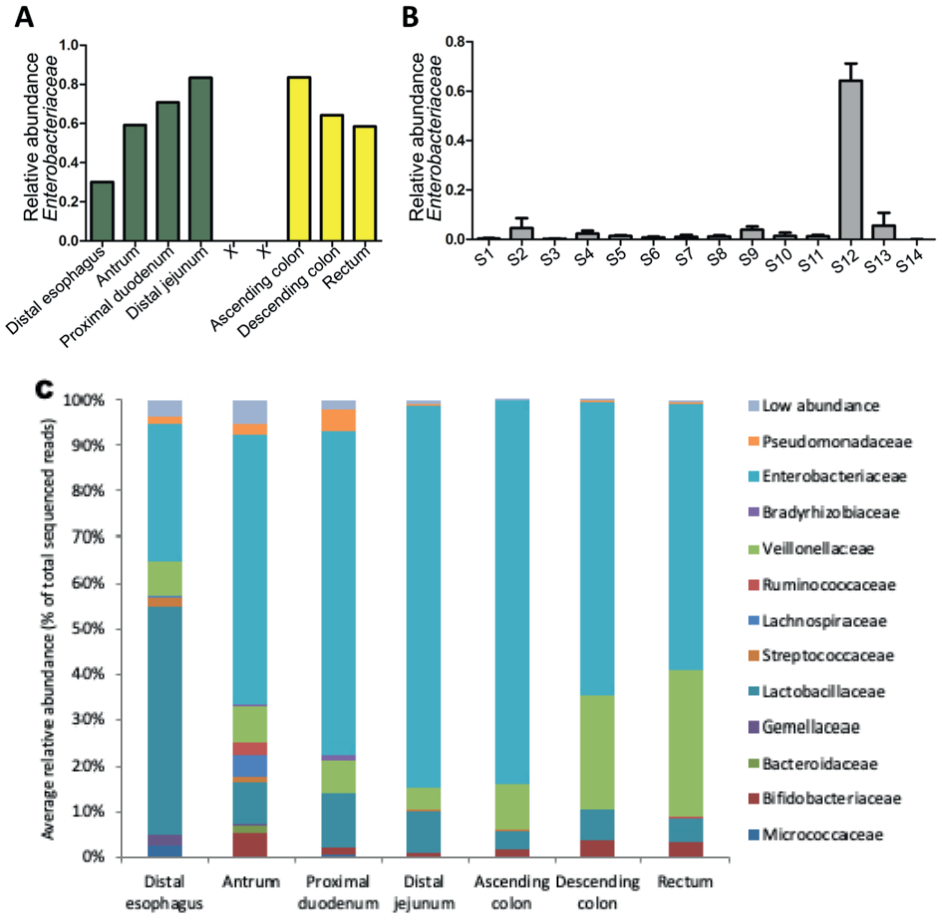
Subjects	Age (y)	Sex	Race	BMI	Alcohol (units/time)	Smoking	Medical history	Medication use	Presenting symptoms	Findings DBE	Findings Pathology
9	54	M	Caucasian	32	6 units/ week	-	Hypertension Cholecystectomy Peripheral arterial disease	-	Diarrhea	Erosive abnormalities in jejunum	Reflux esophagitis
10	53	M	Caucasian	na	2 units/ day	35 cigarettes/ day	2007: cerebrovascular accident 80% stenosis of a. carotid	Antiplatelet drug Proton pump inhibitor Cholesterol inhibitor Iron supplement	Iron deficiency anemia	Venectasia	-
11	50	M	Caucasian	21	2 units/ day	23 cigarettes/ day	Barrett's esophagus	Cholesterol inhibitor Proton pump inhibitor	Weight loss	Small polyp in colon	-
12	55	M	Caucasian	29	-	23 cigarettes/ day	Diabetes type 1 hypertension	Insulin pump Proton pump inhibitor Antiplatelet drug Fludrocortisone ACE inhibitor Cholesterol inhibitor Hydrocortisone	Iron deficiency anemia	-	-
13	74	F	Caucasian	na	na	na	Jejunoileal bypass surgery for obesity	Na	Iron deficiency anemia	Extensive ulcerative abnormalities in distal ileum obstructive mass in cecum	Ulcerative lesion in distal ileum *
14	62	M	na	27	1 unit/ day	10 cigarettes/ day	Peripheral arterial disease	Antiplatelet drug Proton pump inhibitor Iron supplements Cholesterol inhibitor Tramadol	Iron deficiency anemia	-	-

Supplementary Table 2 Primers used for DNA amplification

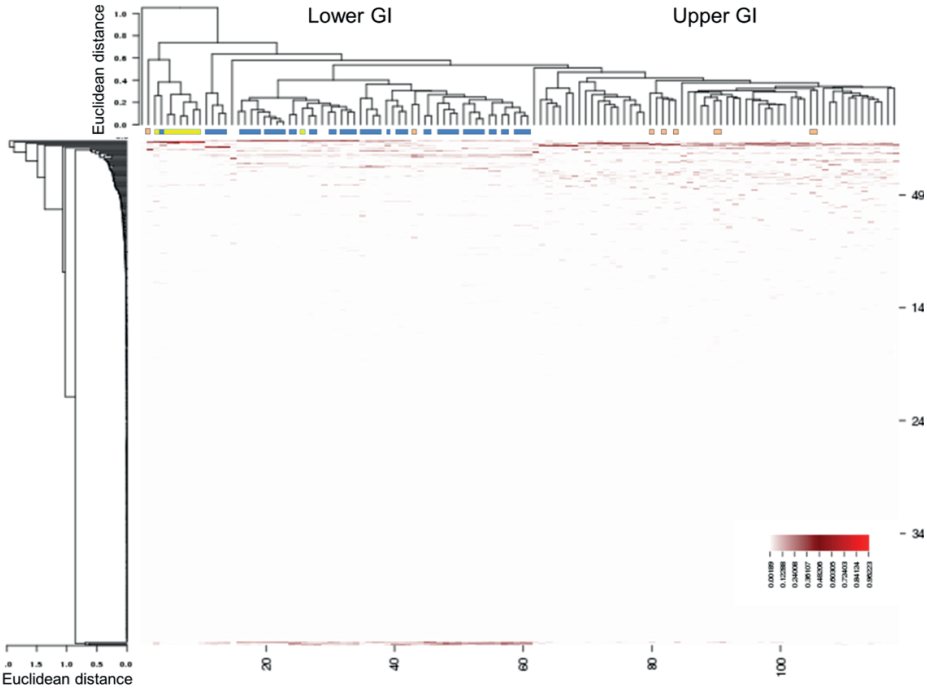
Target	Forward	Reverse	Reference
16S	5′-CGGTGGAATACGTTCCCGG-3′	5′-TACGGCTACCTTGTTACGACTT-3′	(1-3)
UreA	5′-ATGAAACTCACCCCAAAAGA-3′	5′-TTCACTTCAAAGAAATGGAAGTGTGA-3′	(4, 5)
VacA S1/S1	5′-ATGGAAATACAACAAACACAC-3′	5′-CTGCTTGAATGCGCCAAAC-3′	Adapted from (6)
ACTB	5′-CTGGAACGGTGAAGGTGACA-3′	5′-AAGGGACTTCCTGTAACAATGCA-3′	(7)



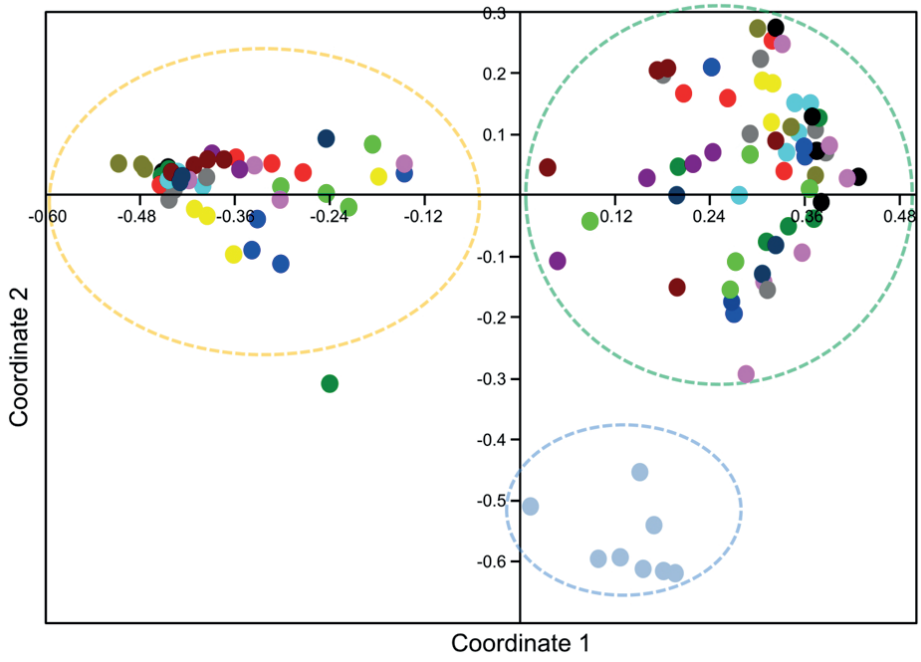
Supplementary Figure 1. Bacterial abundance, unlike human genomic content, fluctuates along the intestinal tract. (A) Human *ACTB* primers identify the gene encoding beta-Actin in both human copyDNA (cDNA) and genomic DNA (gDNA) isolated from human colorectal epithelial cancer cell lines CACO2. **(B)** Two representative examples of comparison of bacterial DNA (16S) and human DNA (ACTB) along the intestinal tract from two subjects (S1 and S2). 1: distal oesophagus; 2: antrum; 3: proximal duodenum; 4: distal jejunum, 5: proximal ileum; 6: distal ileum; 7: ascending colon; 8: descending colon; 9: rectum.



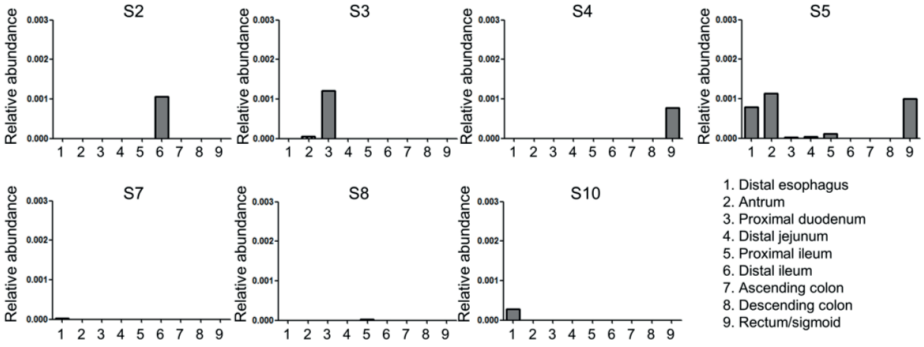
Supplementary Figure 2: Abundance of *Enterobacteriaceae* at family level along the gastrointestinal tract (A) Relative abundance of enterobacteriaceae in mucosal biopsies from a patient with a cecum tumor (S12) is shown. X: missing sample. Green: upper gastrointestinal locations. Yellow: lower gastrointestinal locations (B) Comparison of abundance of *Enterobacteriaceae* at family level between patients, mean±SEM of all the GI locations are shown for subjects 1-14. (C) Most important bacteria at family level (>1% abundance) per location in patient with a cecum tumor.



Supplementary Figure 3: Cluster analysis of taxonomy at family level demonstrating the clustering per patients and the upper and lower digestive tract. Samples indicated with yellow box were from patient 12, who was characterized *Enterobacteriaceae* dominance. The utmost left sample was a Helicobacter-dominated sample from patient 6 (indicated in orange). Blue boxes indicate clustering of two or more samples from one individual patient.



Supplementary Figure 4: Principal coordinate analysis (PCoA) plot of Bray curtis distances. Similar to **Figure 4**, but now the different coloured dots represent different patients. Egg blue dots circled in blue indicate subject S12, dominated by by *Enterobacteriaceae*. In the left cluster (lower GI, circled in yellow), individual patient samples appear to lie closer together than in the right cluster (upper GI, circled in green).



Supplementary Figure 5. Relative abundance of *Helicobacter* species across the different GI sites. Helicobacter was detected in 9 subjects. The relative abundance of *Helicobacteraceae* as detected by sequencing are shown here for individual GI locations of 7 subjects. Subjects S6 and S14 are shown in Figure 7.

References

1. Suzuki MT, Taylor LT, DeLong EF. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Appl Environ Microbiol. 2000;66(11):4605-14.
2. Rodes L, Saha S, Tomaro-Duchesneau C, Prakash S. Microencapsulated Bifidobacterium longum subsp. infantis ATCC 15697 favorably modulates gut microbiota and reduces circulating endotoxins in F344 rats. Biomed Res Int. 2014;2014:602832.
3. Furet JP, Firmesse O, Gourmelon M, Bridonneau C, Tap J, Mondot S, et al. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. FEMS Microbiol Ecol. 2009;68(3):351-62.
4. van Vliet AH, Kuipers EJ, Stoof J, Poppelaars SW, Kusters JG. Acid-responsive gene induction of ammonia-producing enzymes in Helicobacter pylori is mediated via a metal-responsive repressor cascade. Infect Immun. 2004;72(2):766-73.
5. van Vliet AH, Kuipers EJ, Waidner B, Davies BJ, de Vries N, Penn CW, et al. Nickel-responsive induction of urease expression in Helicobacter pylori is mediated at the transcriptional level. Infect Immun. 2001;69(8):4891-7.
6. Kim JW, Kim JG, Chae SL, Cha YJ, Park SM. High prevalence of multiple strain colonization of Helicobacter pylori in Korean patients: DNA diversity among clinical isolates from the gastric corpus, antrum and duodenum. Korean J Intern Med. 2004;19(1):1-9.
7. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7):RESEARCH0034

Part III

Early onset colorectal cancer

Chapter 4
Increasing incidence of colorectal cancer in young adults
in Europe over the last 25 years

Chapter 5
Clinicopathological characteristics of early onset colorectal cancer

Chapter 4

Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years

F.E.R. Vuik, S.A.V. Nieuwenburg, M. Bardou, I. Lansdorp-Vogelaar, M. Dinis-Ribeiro, M.J. Bento, V. Zadnik, M. Pellisé, L. Esteban, M.F. Kaminski, S. Suchanek, O. Ngo, O. Májek, M. Leja, E.J. Kuipers, M.C.W. Spaander

Gut, October 2019

Abstract

Introduction The incidence of colorectal cancer (CRC) declines among subjects aged 50 years and above. An opposite trend appears among younger adults. In Europe, data on CRC incidence among younger adults are lacking. We therefore aimed to analyse European trends in CRC incidence and mortality in subjects younger than 50 years.

Methods Data on age-related CRC incidence and mortality between 1990 and 2016 were retrieved from national and regional cancer registries. Trends were analysed by Joinpoint regression and expressed as annual percent change.

Results We retrieved data on 143.7 million people aged 20–49 years from 20 European countries. Of them, 187 918 (0.13%) were diagnosed with CRC. On average, CRC incidence increased with 7.9% per year among subjects aged 20–29 years from 2004 to 2016. The increase in the age group of 30–39 years was 4.9% per year from 2005 to 2016, the increase in the age group of 40–49 years was 1.6% per year from 2004 to 2016. This increase started earliest in subjects aged 20–29 years, and 10–20 years later in those aged 30–39 and 40–49 years. This is consistent with an age-cohort phenomenon. Although in most European countries the CRC incidence had risen, some heterogeneity was found between countries. CRC mortality did not significantly change among the youngest adults, but decreased with 1.1%per year between 1990 and 2016 and 2.4% per year between 1990 and 2009 among those aged 30–39 years and 40–49 years, respectively.

Conclusion CRC incidence rises among young adults in Europe. The cause for this trend needs to be elucidated. Clinicians should be aware of this trend. If the trend continues, screening guidelines may need to be reconsidered.

Introduction

The overall crude incidence of colorectal cancer (CRC) increased in most European countries over the last decade. The annual increase ranged in different countries between 0.4% and 3.6% (1). The recent introduction of CRC screening in most European countries will likely reverse this trend (2, 3). These screening programmes typically target subjects aged 50 years and above. In several parts of the world, the CRC incidence has also risen in individuals below 50 years of age. In the USA, the incidence of colon cancer increased since 1974 with 1.0%–2.4% annually and the incidence of rectal cancer with 3.2% (4).The possible reasons for this increasing incidence are unknown, but may be related to the increasing prevalence of obesity, lack of exercise and to dietary factors such as alcohol and processed meat (3). Furthermore, urbanisation and pollution have been implicated in the overall increase in cancer incidence (5). CRC in young adults is in part due to hereditary cancer syndromes, but most cases are sporadic (6). The changing epidemiology of CRC may also have practical implications, in particular for age to start screening. With the use of the Microsimulation Screening Analysis simulation model, we previously showed that screening initiation at age 45 years had in the US population a favourable balance between screening benefits and burdens (7). This finding supported the American Cancer Society to recommend starting screening at age 45 years instead of 50 years (8). Whether the incidence of CRC also increases among young adults in Europe has not been investigated. We therefore analysed trends in CRC incidence in this population.

Methods

Study design and data source

Data on age-specific incidence and mortality of CRC by year of diagnosis were retrieved from national and regional European cancer registries with a time frame of at least 10 years (online supplementary table 1). We evaluated incidence and mortality of CRC, colon cancer (ICD-O-3 codes C18) and rectal cancer (C20) between 1990 and 2016. Data were collected for subjects aged 20–49 years. Five-year incidence and mortality rates were collected and expressed per 100 000 persons.

Statistical analysis

Temporal trends in CRC incidence within the study period were investigated using Joinpoint regression analyses, applying an algorithm to define significant changes in temporal trends on a logarithmic scale. The annual percent change (APC) in each Joinpoint segment represents the rate of change in cancer incidence per year in a given time period. The analyses were performed using the Joinpoint Regression Programme 4.5.0.1,

National Cancer Institute. All tests of statistical significance were two-sided; a p value of <0.05 was considered significant. Incidence rates were calculated for three age groups (20–29, 30–39, 40–49 years), presented per 100 000 persons and adjusted to population numbers for each country.

As not all countries could provide data over the entire time period, a sensitivity analysis was performed with data from countries that covered the entire time frame.

We set out to distinguish between a period effect and a cohort effect. While a period effect results from external factors that equally affect all age groups at a particular time period, a cohort effect represents variations resulting from unique exposure of a specific birth cohort. To this aim, we identified for each age group the year in which the increase in CRC incidence, if any, had started. If it were to be the same for the three age groups, the increase in incidence was considered to be a period effect. If the starting year were to be more recent in the older age groups, the increase was considered to be a cohort effect.

Results

Incidence data were available from 20 European countries (figure 1); mortality data from 16 of those (not including Belgium, France, the UK and Ireland). In 2009, the population of these 20 countries numbered 91 842 346 individuals aged 20–39 years, of whom 47 364 were diagnosed with CRC from 1990 to 2016, and 51 868 457 individuals aged 40–49 years, of whom 140 554 were diagnosed with CRC from 1990 to 2016.

Incidence of colorectal cancer

Age group 20-29 years

For both sexes combined, CRC incidence increased from 0.8 to 2.3 cases per 100.000 persons between 1990 and 2016. This increase was 1.7% per year between 1990 and 2004, and then rose to 7.9% increase per year between 2004 and 2016 (Figure 2). In men, the CRC incidence increased with 2.6% per year between 1992 and 2005. This increase rose to 7.4% per year between 2005 and 2016. In women, the CRC incidence increased with 1.8% per year between 1990 and 2003 and with 8.1% per year between 2003 and 2016. The incidence of colon cancer rose more markedly (2.7% per year between 1990 and 2005 and 9.3% per year between 2005 and 2016) than the incidence of rectal cancer. The latter increased with 3.5% annually throughout the whole period without an acceleration over time.

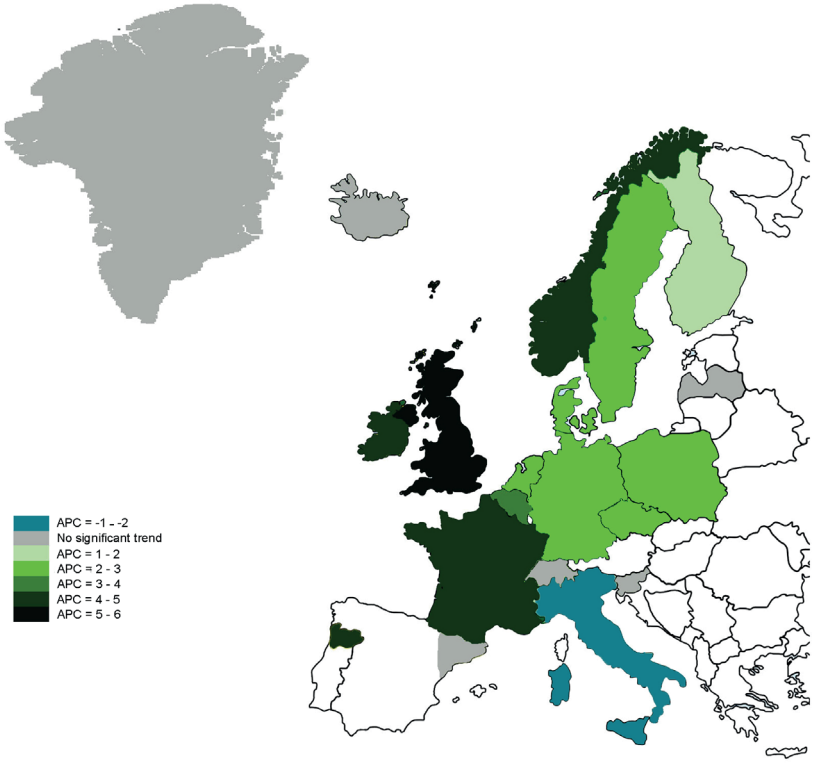


Figure 1 Annual percent change (APC) in colorectal cancer (CRC) incidence from the European countries included in the analysis in adults aged 20–39 years, 1990–2016. Light green to dark green: significant increase in CRC incidence rate; blue: significant decrease in CRC incidence rate; grey: no significant trend.

Age group 30–39 years

For both sexes combined, in age group 30–39 years the CRC incidence increased, although less steeply than in age group 20–29 years (Figure 2). In men, the CRC incidence increased with 3.4% per year between 2001 and 2016 (from 3.7 to 7.1 cases per 100 000 persons between 1990 and 2016). In women, no significant change in trend was observed between 1990 and 2005, but the CRC incidence increased with 6.8% annually between 2005 and 2016 (from 2.8 to 6.4 cases per 100 000 persons between 2006 and 2016). The colon cancer incidence increased between 2006 and 2016 with 6.4% per year; that of rectal cancer with 1.6% per year between 1990 and 2016.

Age group 40–49 years

In age group 40–49 years, the CRC incidence decreased with 0.8% between 1990 and 2004, but increased with 1.6% per year between 2004 and 2016 (incidence increased from 15.5 to 19.2 cases per 100 000 persons between 2005 and 2016). The same trend was observed for colon cancer: the incidence decreased with 1.3% per year between

1990 and 2004 and then increased with 1.6% annually between 2004 and 2016. No significant change in trend was observed for rectal cancer (Figure 2).

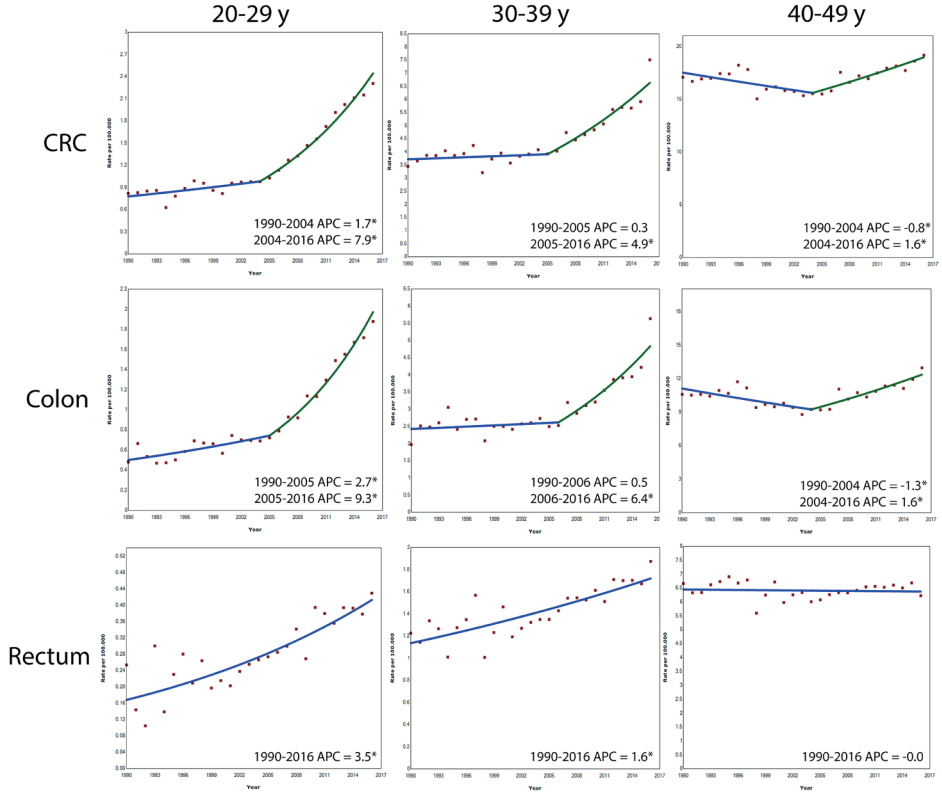


Figure 2 Annual percent change (APC) in age-specific colorectal cancer (CRC), colon cancer and rectal cancer incidence rates in Europe, 1990–2016. *Indicates that APC is statistically significant different from zero

Country-specific trends

Trends in incidence of CRC per European region are shown in figure 1. CRC incidence increased significantly among subjects aged 20–39 years in 12 countries: Belgium, Germany, the Netherlands, the UK, Norway, Sweden, Finland, Ireland, France, Denmark, Czech Republic and Poland. Italy showed a decrease in incidence in this age group. No significant change was observed in the remaining six countries (online supplementary figure 1).

CRC incidence increased significantly among subjects aged 40–49 years in eight countries: the UK, Greenland, Sweden, Slovenia, Germany, Finland, Denmark and the Netherlands. Only Czech Republic showed a significant decrease in incidence from

1997 to 2015. No significant change was observed in the remaining 11 countries (online supplementary figure 2).

Sensitivity analyses

Not all countries could provide data over the entire time period of 1990 to 2016. We therefore performed sensitivity analyses for the longest possible time frame: 1991 to 2014. Data from nine countries were included: Denmark, Finland, Norway, Sweden, the Netherlands, Greenland, Slovenia, Czech Republic and Switzerland. The outcomes indicated increases in the incidence of both colon and rectal cancer in all age groups (Figure 3).

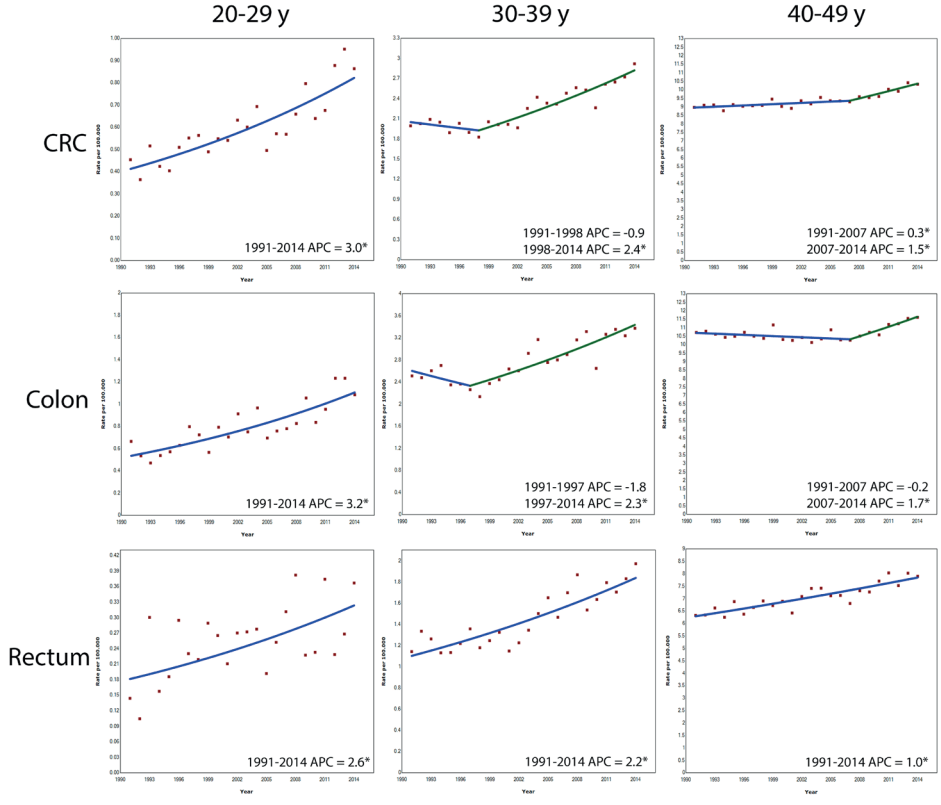


Figure 3 Annual percent change (APC) in age-specific colorectal cancer (CRC), colon cancer and rectal cancer incidence rates in nine European countries, 1991–2014. Analyses on trend in incidence of CRC was based on nine countries: Slovenia, Norway, Denmark, Sweden, Finland, the Netherlands, Czech Republic, Switzerland and Greenland. Analyses on trend of incidence of colon cancer and rectum cancer was based on eight countries: Slovenia, Norway, Denmark, Sweden, Finland, the Netherlands, Czech Republic and Greenland. *Indicates that APC is statistically significant different from zero

We assessed by means of sensitivity analysis whether the increase in incidence was a period or a cohort effect (Figure 3). This showed that adults aged 20-29 years had an increase in CRC incidence from 1991 to 2014. In age group 30-39 years, a rise in incidence started in 1998 and exactly 10 years later (2007) a rise in incidence was observed among those aged 40-49 years. This difference in starting points is compatible with a cohort effect.

Mortality due to colorectal cancer

Age group 20–39 years

The mortality rate for CRC did not significantly change in the age group 20–29 years. In the age group 30–39 years, the mortality decreased with 1.1% per year (Figure 4). The mortality rate of colon cancer decreased with 9.7% per year between 1990 and 1993, and with 0.5% per year between 1993 and 2014, to remained stable from 2014 onwards. No significant change in mortality was observed for rectal cancer.

Age group 40–49 years

The overall mortality of CRC in the age group 40–49 years decreased with 2.4% per year between 1990 and 2009, but increased with 1.1% per year between 2009 and 2016 (Figure 4).

The mortality rate of colon cancer decreased with 2.4% per year between 1990 and 2010, and remained stable between 2010 and 2016. The mortality rate of rectal cancer decreased with 2.6% per year between 1990 and 2006, and remained stable between 2006 and 2016.

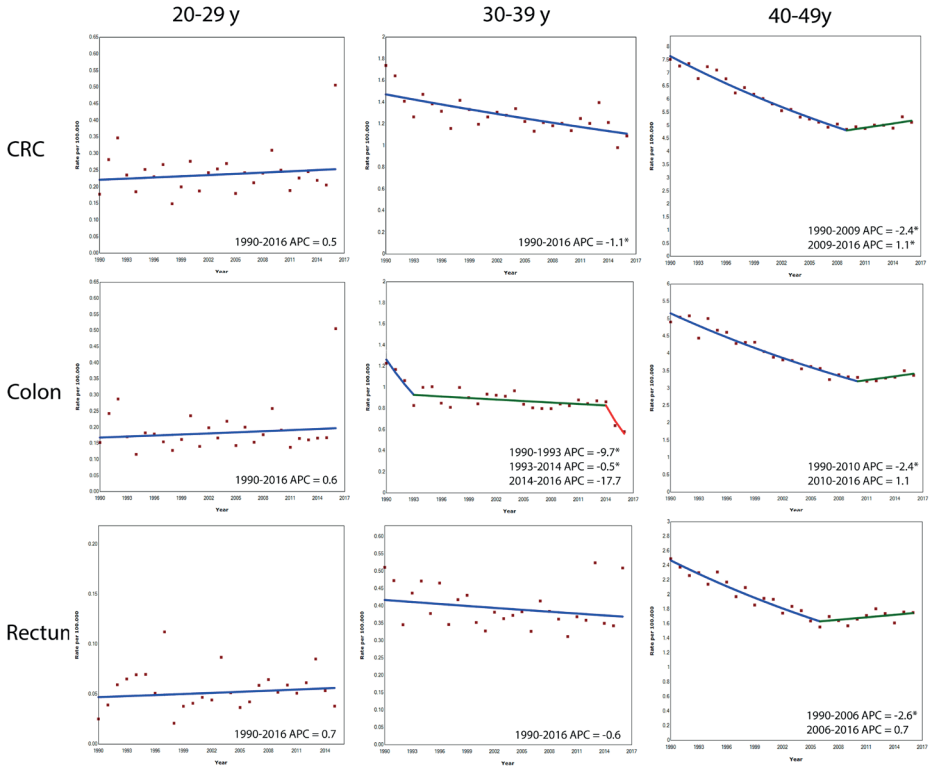


Figure 4 Annual percent change (APC) in age-specific colorectal cancer (CRC), colon cancer and rectal cancer mortality rates in Europe, 1990–2016. *Indicates that APC is statistically significant different from zero.

Discussion

Our study showed an increase in CRC incidence in adults aged 20–49 years in Europe. The largest increase in CRC incidence occurred among subjects aged 20–39 years. The incidence of colon cancer increased with 6.4%–9.3% annually; that of rectal cancer with 1.6%–3.5% per year. The causes of this increase are yet unknown. Awareness of this trend is relevant to identify patients at risk. Further research is needed to determine whether the trend can be reversed, among others by lowering the age to start screening.

In the past years, an increase in CRC incidence in young adults has been observed in different parts of the world, such as the USA.4 In Canadian subjects aged 20–29 years, the incidence of colon cancer rose faster than that of rectal cancer (APC 6.2%, respectively 1.5%). CRC incidence in young adults also rises in Australia and China. In the latter country, adoption of a Western lifestyle is thought to contribute to this trend (9, 10).

In the USA, the increase in CRC incidence was explained by a cohort effect. Our data support a similar effect in Europe. The incidence started to rise exactly 10 years earlier in the age groups 30–39 years than in the group of 40–49 years. CRC incidence also rose among those aged 20–29 years, however, with no turning point during the study period. This suggests that the turning point already occurred before 1990. The cause of this trend is unknown. A combination of factors is likely to have contributed. This includes the increasing prevalence of obesity. The latter parallels the increase in CRC incidence in young adults (11). A meta-analysis showed that weight gain is associated with an increased risk of CRC (12). Excess nutrients may initiate a chronic low-grade inflammatory response in metabolic cells (13). Also, other risk factors such as lack of physical activity, increased alcohol intake and cigarette smoking may play a role (14–17).

We found that the rate of increase differed for colon and rectal cancer, ranging from 1.6% to 9.3% for colon cancer vs 0% to 3.5% for rectal cancer. Although the above-mentioned risk factors apply to both colon and rectal cancer, some factors are strongly associated with colon cancer only. Lifestyle factors such as diet, physical activity and alcohol have been associated with risk of colon cancer, but not with rectal cancer (18). Also, a meta-analysis showed that obesity was in particular associated with an increased risk of colon cancer. For rectal cancer this association was less apparent in men, and absent in women (19). This might in part be explained by the greater susceptibility of the colon to the effects of insulin in comparison with the rectum (20). The increasing use of colonoscopy for diagnostic and screening purposes may have been responsible for a proportion of the detected CRCs in young adults. Nevertheless, detection bias is probably not the driving factor for this trend, since young adults are less likely to be screened for CRC, the rise was most marked in the youngest age group and the turning points differed between age groups.

Current guidelines in Europe recommend CRC screening from the age of 50. In 2018, the American Cancer Society recommended to start screening at the age of 45. This recommendation was based on the burden of disease, the increasing incidence among younger subjects, the results of modelling and the assumption that screening the age group 45–49 years will have preventive effect as screening those 50 years and above. The American Cancer Society's analyses showed a favourable benefit-to-burden balance with an expected reduction in CRC mortality and incidence (8). For several reasons, the results of our study provide no argument for starting screening at the age of 45 years in Europe. First, the largest increase in CRC incidence rate was observed in the age group of 20–39 years. Second, the rate of change in CRC incidence differed between countries. Third, the absolute numbers of CRC in these age groups still remain low in comparison with elderly subjects. Fourth, most European countries struggle to find the resources

to properly screen the age group of 50–75 years, or are in the process of implementing screening for this group. For these reasons, it is too early to use our data to support screening for those aged 45–50 years. However, it is relevant to research to monitor this trend, and repeatedly assess whether screening practice needs to be adapted. Furthermore, we should find underlying causes, and identify high-risk subjects who might benefit from earlier screening. A first step to reach this goal is to make clinicians aware that the CRC incidence in young adults is rising quite rapidly.

Italy is the only country that showed a significant decrease in CRC incidence among subjects aged 20–39 years. This occurred at a rate of 1.8% per year from 1998 onwards. We should be careful with data interpretation though, because the observation might be due to selection bias. The Italian data were retrieved from the AITRUM database, covering only nine regions from 1996 to 2009 instead of the entire country over a longer period. The incidence trend did not significantly change in Green-land, Iceland, Slovenia, Catalonia, Latvia and Switzerland. This can likely be explained by the low population numbers in these countries, affecting power of our calculations.

This study is the first to give an overview of CRC incidence and mortality rates in younger adults in Europe. A major strength is the use of data from 20 European counties. Still, several limitations need to be addressed. First, not all European Union member countries could be included, either because of the lack of a national cancer registry or inaccessibility of the data. Also, for some countries (Portugal, Spain and Italy), data were only available for only a limited number of regions. Second, not all countries could provide data over a period of 25 years, because some national cancer registries were set up in a later year. In all countries, however, data were available for at least 10 years. The analysis of data from countries with a longer observation period (1991–2014) consistently showed the same trends. Third, the quality of data differed between countries. Data quality was estimated in terms of microscopically verified (MV) and death certificate only (DCO). The German data, for example, had an MV rate of 85.6% and a DCO rate of 13%. The Latvian data had an MV rate of 80.7% and a DCO rate of 5.5%. Fourth, the national cancer registries from Switzerland and Germany present estimated nationwide data on CRC incidence, because not all regions can provide CRC incidence and mortality rates. Fifth, individual data were not accessible. It was not possible, therefore, to differentiate between left and right colon cancers and pathological characteristics of patients with CRC could not be retrieved.

In conclusion, the incidence of CRC is rising in Europe among subjects aged 20–49 years. If this trend continues, screening guidelines may need to be reconsidered. Until the

underlying cause of this trend is clarified, it would be commendable to raise clinicians' awareness and identify factors possibly associated with this trend.

References

1. Arnold M , Sierra MS , Laversanne M , et al . Global patterns and trends in colorectal cancer incidence and mortality. Gut2017;66:683–91.
2. Doubeni CA , Corley DA , Quinn VP , et al . Effectiveness of screening colonoscopy in reducing the risk of death from right and left colon cancer: a large community-based study. Gut 2018;67:291–8.
3. Rosato V , Bosetti C , Levi F , et al . Risk factors for young-onset colorectal cancer. Cancer Causes Control2013;24:335–41.
4. Siegel RL , Fedewa SA , Anderson WF , et al . Colorectal cancer incidence patterns in the United States, 1974-2013. J Natl Cancer Inst2017:109.
5. Fitzmaurice C , Dicker D , Pain A , et al . The Global Burden of Cancer 2013. JAMA Oncol2015;1:505–27.
6. Connell LC , Mota JM , Braghiroli MI , et al . The rising incidence of younger patients with colorectal cancer: Questions about screening, biology, and treatment. Curr Treat Options Oncol2017;18:23.
7. Peterse EFP , Meester RGS , Siegel RL , et al . The impact of the rising colorectal cancer incidence in young adults on the optimal age to start screening: Microsimulation analysis i to inform the american cancer society colorectal cancer screening guideline. Cancer2018;124:2964–73.
8. Wolf AMD , Fontham ETH , Church TR , et al . Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin2018;68:250–81.
9. Troeung L , Sodhi-Berry N , Martini A , et al . Increasing incidence of colorectal cancer in adolescents and young adults aged 15-39 years in western australia 1982-2007: Examination of colonoscopy history. Front Public Health 2017;5.
10. Deng Y . Rectal Cancer in Asian vs. Western Countries: Why the Variation in Incidence? Curr Treat Options Oncol2017;18:64.
11. Patel P , De P . Trends in colorectal cancer incidence and related lifestyle risk factors in 15-49-year-olds in Canada, 1969-2010. Cancer Epidemiol2016;42:90–100.
12. Karahalios A , English DR , Simpson JA . Weight change and risk of colorectal cancer: a systematic review and meta-analysis. Am J Epidemiol2015;181:832–45.
13. Gregor MF , Hotamisligil GS . Inflammatory mechanisms in obesity. Annu Rev Immunol2011;29:415–45.
14. Wolin KY , Yan Y , Colditz GA , et al . Physical activity and colon cancer prevention: a meta-analysis. Br J Cancer2009;100:611–6.
15. Yuhara H , Steinmaus C , Cohen SE , et al . Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am J Gastroenterol2011;106:1911–21.
16. Fedirko V , Tramacere I , Bagnardi V , et al . Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol2011;22:1958–72.
17. Botteri E , Iodice S , Bagnardi V , et al . Smoking and colorectal cancer: a meta-analysis. JAMA2008;300:2765–78.
18. Wei EK , Giovannucci E , Wu K , et al . Comparison of risk factors for colon and rectal cancer. Int J Cancer2004;108:433–42.
19. Larsson SC , Wolk A . Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr2007;86:556–65.
20. Giovannucci E . Insulin and colon cancer. Cancer Causes Control1995;6:164–79.

Supplementary table 1 Data source for the age-standardized incidence and mortality rates of colorectal cancer and population data. -: data was not available; * information retrieved from GLOBOCAN; º information retrieved from country specific database mentioned in column 2 (Incidence); NA = not applicable.

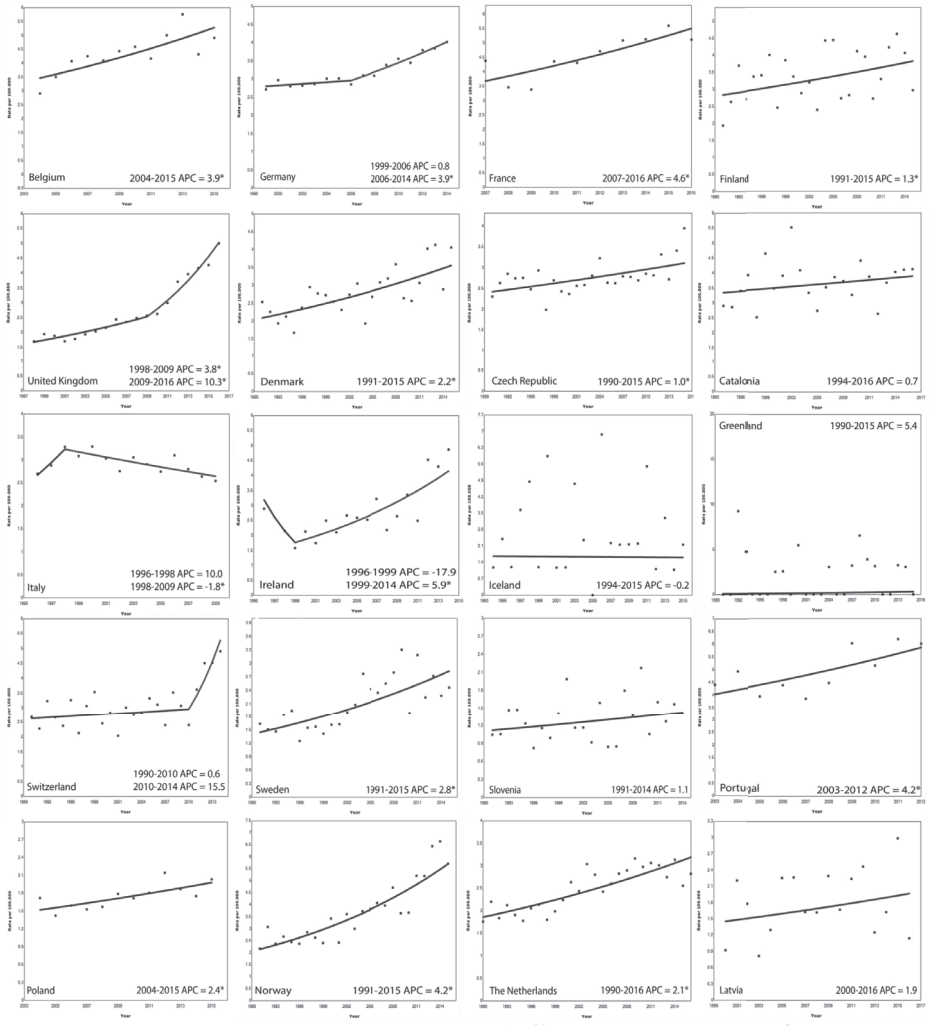
Country	Incidence	Mortality	Population	Microscopically verified (MV) (%)*	DCO (death certificate only) rate (%)*	Level of completeness º	National coverage º
The Netherlands	The Netherlands Cancer Registry, IKNL, 2018. https://www.cijfersoverkanker.nl	The Netherlands Cancer Registry, IKNL, 2018. https://www.cijfersoverkanker.nl	United states CENSUS bureau https://www.census.gov/en.html	97.3	-	100%	All data available from 1989
Germany	The German Centre for Cancer Registry Data (ZfKD) www.krebsdaten.de/database	The Federal Statistical Office Germany	United states CENSUS bureau https://www.census.gov/en.html	85.6	13	90%	All data available from 1999
Belgium	The Belgium cancer registry http://www.kankerregister.org/Home_en	NA	United states CENSUS bureau https://www.census.gov/en.html	99.0	-	-	95%
Ireland	National cancer registry Ireland https://www.ncri.ie	NA	United states CENSUS bureau https://www.census.gov/en.html	93.5	1.0	98.2	All data available from 1994
Italy	AIRTUM ITACAN: Cancer in Italy, Version 2.0. Italian Association of Cancer Registries http://www.registri-tumori.it	AIRTUM ITACAN: Cancer in Italy, Version 2.0. Italian Association of Cancer Registries http://www.registri-tumori.it	United states CENSUS bureau https://www.census.gov/en.html	87.9	2.5	-	70% of data available from 2006
Denmark	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	95.7	0.3	100%	-

Country	Incidence	Mortality	Population	Microscopically verified (MV) (%)*	DCO (death certificate only) rate (%)*	Level of completeness°	National coverage°
Sweden	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	-	-	100%	All data available from 1960
Norway	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	94.6	0.8	100%	All data available from 1953
Finland	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	-	-	100%	All data available from 1953
Iceland	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	>97.9	<0.3	100%	All data available from 1955

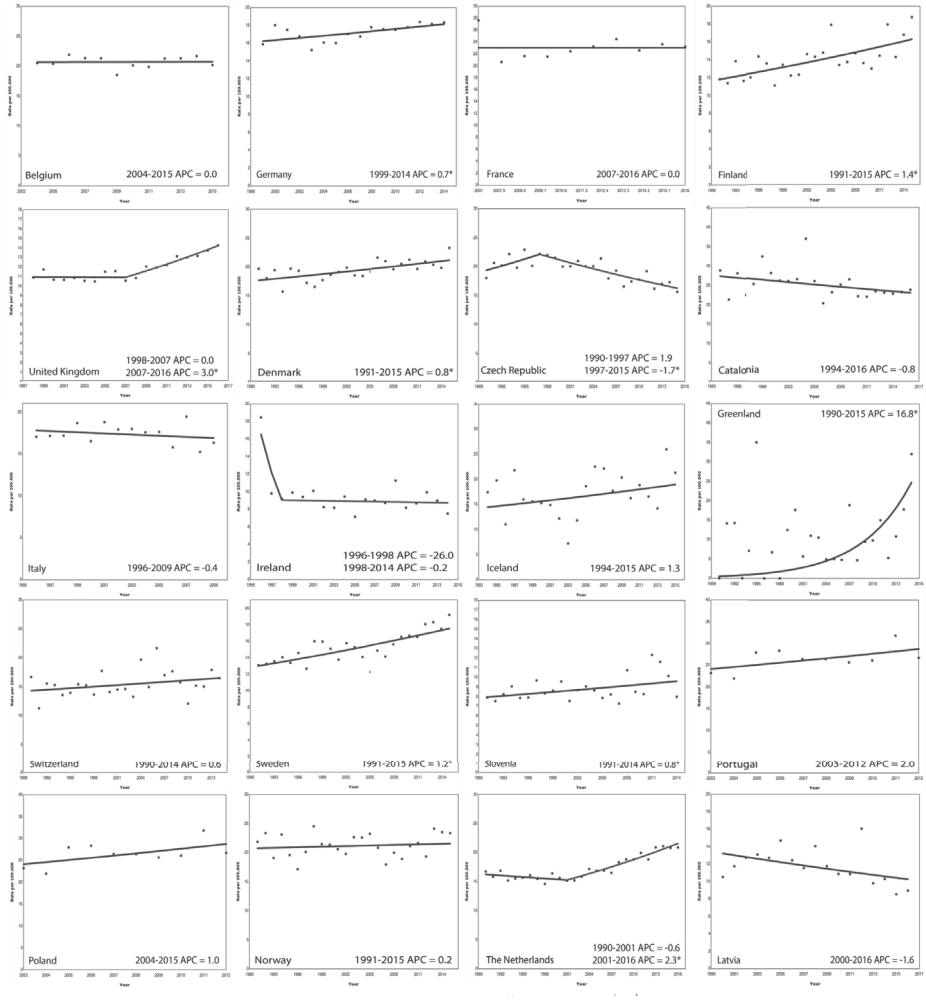
Country	Incidence	Mortality	Population	Microscopically verified (MV) (%)*	DCO (death certificate only) rate (%)*	Level of completeness°	National coverage°
Greenland	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	Association of the Nordic Cancer Registries. Danish Cancer Society. http://www-dep.iarc.fr/NORDCAN/english/frame.asp	United states CENSUS bureau https://www.census.gov/en.html	-	-	100%	All data available from 1968
Switzerland	The Swiss national dataset managed by the Foundation National Institute for Cancer Epidemiology and Registration (NICER). http://www.nicer.org/en/statistics-atlas/	The Swiss national dataset managed by the Foundation National Institute for Cancer Epidemiology and Registration (NICER). http://www.nicer.org/en/statistics-atlas/	United states CENSUS bureau https://www.census.gov/en.html	93.4	2.0		1990-1994: 53.5% of data available 1995-1999: 56.9% of data available 2000-2004: 57.9% of data available 2005-2009: 61.8% of data available 2010-2014: 74.1% of data available
France	Data from health insurance administrative database: Source Sniiram CNAMTS Source INSEE, estimation of the population as of 1st January, published on 17th January 2017.	NA	Source INSEE, estimation of the population as of 1st January, published on 17th January 2017	>93.7	-	100%	All data available from 2007

Country	Incidence	Mortality	Population	Microscopically verified (MV) (%)*	DCO (death certificate only) rate (%)*	Level of completeness°	National coverage°
Latvia	The Centre of Disease Prevention and Control, Republic of Latvia Register for Patients with Particular Diseases, Patients with Cancer.	Register of Causes of Death	United states CENSUS bureau https://www.census.gov/en.html	80.7	5.5	100%	-
Catalonia	El Càncer a Catalunya. Monografia 2016. Registre de cáncer de Catalunya. Pla Director d'Oncologia. http://cancer.gencat.cat/ca/professionals/estadistiques/ Registre del Càncer de Girona. Registre del càncer. Institut Català d'Oncologia http://ico.gencat.cat/ca/professionals/serveis_i_programes/registre_del_cancer/ Registre del Càncer de Tarragona. Funca - registre https://funca.cat/registre	El Càncer a Catalunya. Monografia 2016. Registre de cáncer de Catalunya. Pla Director d'Oncologia. http://cancer.gencat.cat/ca/professionals/estadistiques/ Registre de Mortalitat de Catalunya. Mortalitat. Departament de Salut http://salutweb.gencat.cat/ca/el_departament/estadistiques_sanitaries/dades_de_salut_i_serveis_sanitaris/mortalitat/	Institut d'Estadística de Catalunya. Idescat www.idescat.cat	91.7	2.2	100%	100%
Slovenia	Cancer Registry of Republic of Slovenia RS Data from: www.slora.si/en and information on: https://www.onko-i.si/eng/crs/	Cancer mortality data are collected by the National Institute of Public Health (NIPH)	United states CENSUS bureau https://www.census.gov/en.html	95.2	<0.5	100%	-
Portugal	Registo Oncológico Regional do Norte	Registo Oncológico Regional do Norte	Registo Oncológico Regional do Norte	98.5%	-	100%	100%

Country	Incidence	Mortality	Population	Microscopically verified (MV) (%)*	DCO (death certificate only) rate (%)*	Level of completeness°	National coverage°
Czech Republic	Czech National Cancer Registry	Czech National Cancer Registry and Czech Statistical Office (since 1994)	Czech Statistical Office	95.7	0.4	95%	100%
United Kingdom	Office for national statistics https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/cancerregistrationstatistics-cancerregistrationstatisticsengland	NA	United states CENSUS bureau https://www.census.gov/en.html	84.6	0.1	98.4%	-
Poland	Krajowy Rejestr Nowotworów, Centrum Onkologii - Instytut im. Marii Skłodowskiej - Curie http://onkologia.org.pl	Krajowy Rejestr Nowotworów, Centrum Onkologii - Instytut im. Marii Skłodowskiej - Curie http://onkologia.org.pl	Krajowy Rejestr Nowotworów, Centrum Onkologii - Instytut im. Marii Skłodowskiej - Curie http://onkologia.org.pl	>90.4	<2.9	100%	-



Supplementary figure 1 Incidence annual percent change (APC) per country in age group 20 to 39 year. *Statistical significant change in trend.



Supplementary figure 2 Incidence annual percent change (APC) per country in age group 40 to 49 year. * Statistical significant change in trend.

Chapter 5

Clinicopathological characteristics of early onset colorectal cancer

F.E.R. Vuik, S.A.V. Nieuwenburg, I.D. Nagtegaal, E.J. Kuipers, M.C.W. Spaander

Alimentary Pharmacology Therapeutics, October 2021

Abstract

Introduction The rising incidence of early onset colorectal cancer (EOCRC) might reflect a novel tumour entity The aim of this study is to evaluate clinicopathological characteristics of sporadic EOCRC (in patients < 50 years old) and investigate changes over time.

Methods All patients with sporadic EOCRC between 1989 and 2016 were included and divided by age: 20-29 years (group I), 30-39 years (group II) and 40-49 years (group III).

Results We included 6400 patients. The presence of signet-ring cells and more poorly differentiated tumours were more common in the younger age groups: 5.4% and 3.7% for signet-ring cells in group I and II vs 1.4% in group III ($P < 0.01$), and 28.5% and 20.3% for poorly differentiated in group I and II vs 16.6% in group III, ($P < 0.01$ group I; $P = 0.07$ group II). Positive lymph nodes were more frequently observed in the younger age groups: 16.2% in group I vs 9.3% in group II ($P = 0.01$) and 7.9% ($P < 0.01$) in group III. Over time, a greater proportion of CRCs were diagnosed in women in group I (34.5% < 2004 vs 54.9%>2005, $P = 0.09$), and a higher percentage of rectal cancer was found in age group III (34.3% < 2004 vs 40.7% > 2005, $P < 0.01$). Mean overall survival was 6.3 years and improved over time.

Conclusions EOCRC is not only characterised by age of onset but also by the more frequent presence of signet-ring cells, more poorly differentiated tumours, and higher risk of lymph node metastases. In the most recent years, a higher proportion of rectal cancer was found from the age of 30 years, and a higher proportion of CRCs were diagnosed in females below the age of 30 years.

Introduction

Colorectal cancer (CRC) incidence and mortality are decreasing in adults older than 50 years due to screening and improvements in CRC treatment in both the US and Europe (1, 2). Conversely, CRC incidence in young adults, early-onset CRC (EOCRC), is rising in several parts of the world (2, 3). It is known that individuals with Lynch syndrome (LS) or familial adenomatous polyposis (FAP) are more likely to develop CRC at a relatively young age. However, this group accounts for only 2%-3% of all CRC cases (4). Most of EOCRCs are sporadic cases. The underlying factors contributing to the increasing incidence of sporadic CRC in young adults are still incompletely understood but seem to include obesity, lack of physical activity, alcohol intake and cigarette smoking (5-7). Also, several drugs have been reported to be associated with CRC risk. The use of oral antibiotics is associated with an increased CRC risk, while the use of statin and aspirin might decrease this risk (8-10). Association studies on sporadic EOCRC show that male gender, being black or Asian, having inflammatory bowel disease (IBD) or a family history of CRC might be associated with an increased EOCRC risk (11). To fully elucidate causes and mechanisms of EOCRC, it is important to have more insight into both patient and tumour characteristics of these CRCs. Data on location, histology, and tumour stages of sporadic EOCRC compared to late-onset CRC are scarce and conflicting. Some studies indicate a higher prevalence of right-sided CRC in EOCRC while other studies showed a higher prevalence of a more distal location (12, 13). Signet-ring cells were described to be more prominent in EOCRC, while conflicting studies were published on KRAS, NRAS and BRAF mutations among EOCRC patients (14, 15). These conflicting data might be a result of differences between and within EOCRC cohorts. For example, the very young patients (below the age of 30 years) might have a different type of CRC than the slightly older EOCRC patients (30-50 years of age). The latter might resemble more the sporadic CRC in adults above the age of 50 years of age. Furthermore, it is questioned whether the rising incidence of sporadic EOCRC might reflect the rise of a novel tumour entity. Therefore, the aim of this study was to assess the clinicopathological characteristics of sporadic EOCRCs within different age categories (20-29 years vs 30-39 years vs 40-49 years) and investigate changes over time.

Methods

Study population

All CRC patients below the age of 50 years were identified from the Netherlands Cancer Registry (NCR) and the Dutch national pathology registry PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands between 1989

and 2016 with follow-up of each case until 31 January 2018. EOCRCs were defined as sporadic cancers of the colon or rectum in individuals under the age of 50 years that were tested for LS and showed an MSS phenotype. Patients were divided into three age groups: group I (20-29 years); group II (30-39 years) and group III (40-49 years). All patients with an adenocarcinoma located in the colon and/or rectum were included. Excluded from this study were patients with LS tumours, neuroendocrine tumours, neuroendocrine carcinomas and squamous cell carcinomas.

The study was conducted in accordance with the Declaration of Helsinki Principles and approved by the ethical committee of the Erasmus University Medical Center, Rotterdam (MEC-2020-0048).

Data source

Data on age-related histopathological features were retrieved from the NKR and the Dutch national pathology registry PALGA (16, 17). NKR complies clinical data of all newly diagnosed patients with cancer in the Netherlands since 1989. The PALGA database covers all pathology laboratories in the Netherlands. Summaries of all histopathology and cytopathology reports are generated automatically at the laboratories and transferred to the central databank of PALGA.

Data collection

Tumours on which molecular analyses were performed and were negative for a hereditary disorder, were defined as sporadic CRC. Clinical characteristics included gender, age at diagnosis, tumour location and tumour stage. Tumour location was grouped by primary site, where cecum to sigmoid (ICD-O-3 codes C180, C182-C187 and C199) was defined as colon and rectum (C209) was defined separately. Pathological characteristics included histopathology, degree of differentiation, presence of (lymph node) metastasis, lymphatic invasion and angioinvasion. For N stage the UICC 7th edition was used (18). Lymph node metastasis were categorised in two groups: patients with no or <7 lymph nodes (≤N2a) or patients with >7 lymph nodes (N2b)

TNM stage was based on histopathologic examination (pTNM). In case pTNM stage was not available, TNM stage before treatment (cTNM) was used. Data on the presence of lymphatic invasion and angioinvasion was only available for the years 2015 and 2016. Furthermore, the prevalence of the following genes was examined: BRAF, NRAS and KRAS. Overall survival (OS) was defined as the time between the date of diagnosis to the date of death from any cause or the end of follow-up.

Statistical analyses

The proportions between age categories were compared using chi-squared or Fishers exact tests when appropriate. Group-wise comparisons were performed when the overall P-value of a group was $P < 0.10$. To elucidate the clinical and histopathological characteristics of patients with sporadic EOCRC over time, the study period was divided into two time periods (period 1: 1989-2004 and period 2: 2005-2018) comparing the first 15 years of data to the second 15 years. Differences between the time periods were compared using the chi-squared test. Kaplan-Meier curves and log-rank tests were used to evaluate differences in survival. A two-sided P-value of less than 0.05 was considered statistically significant. Data analyses were performed using spss version 25.

Results

Baseline characteristics

In total, 15 925 CRC patients under the age of 50 years were identified between 1989 and 2016 (52% male, mean age 43 years, SD 5.8) (Figure 1). No molecular diagnostics were performed on 7.905 (49.6%) patients. Differences in characteristics between patients with and without molecular diagnostics are depicted in Table S1. Patients tested for MSI were slightly older 43.5 years vs 42.7 years ($P < 0.01$), were more often females 49.5% vs 46.5% ($P < 0.01$), had more often more than seven positive lymph nodes (8.1% vs 5.9%, $P < 0.01$) and had a well-differentiated tumour (80.1% vs 78.1%, $P < 0.01$). Of the other 8020 patients, 69 patients were excluded because the tumour was not an adenocarcinoma.

Of the remaining 7951 patients with an adenocarcinoma and MSI tested, 6400 (80.5%) was a sporadic EOCRC, 681 patients (8.6%) were diagnosed with LS, and of 870 patients (10.9%) the result of molecular diagnostics was unknown.

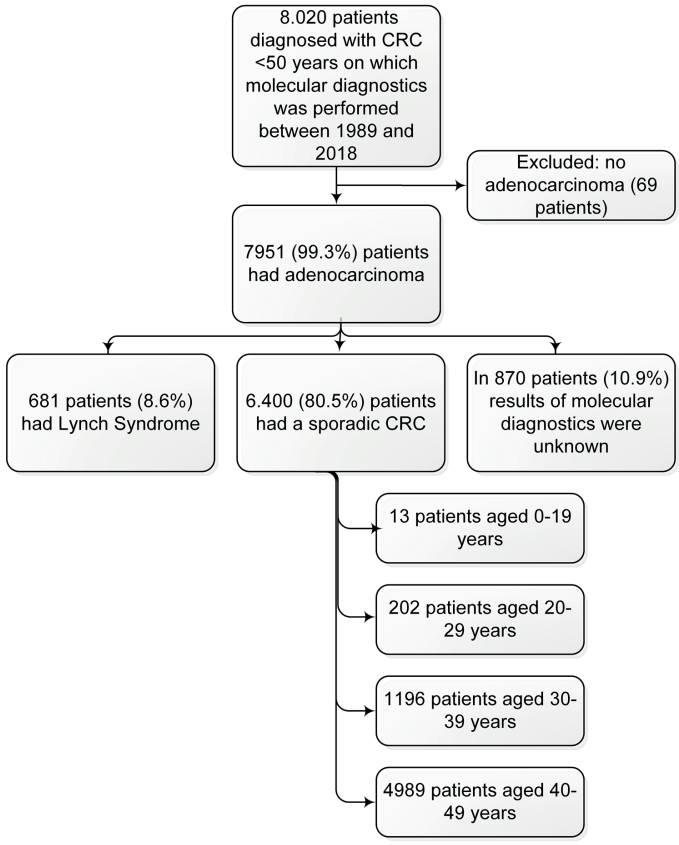


Figure 1 Flowchart

Sporadic EOCRC

When focusing on the 6400 sporadic EOCRC patients, 49.2% was male with a mean age of 43 years (SD 5.6). In total, 202 (3%) patients were diagnosed at the age of 20-29 years old (group I); 1196 (19%) patients at the age of 30-39 years old (group II) and 4.989 (78%) patients at the age of 40-49 years old (group III). Due to the low number of patients in age group 0-19 years of age (n = 13 [0.2%]), clinicopathological features were described and not included in the comparison analyses.

Clinical and pathological characteristics of patients with sporadic EOCRC

Characteristics per age group

In the youngest sporadic EOCRC age group (0-19 years) patients had a mean age of 16 years (SD 2·2), 61.5% was female, and in 38.5% the tumour was located in the rectum. CRC was poorly differentiated in 46.2% and in 38.5% signet-ring cell carcinoma was present.

Between age groups I, II and III no difference in gender (P = 0.43) and location (P = 0.10) was observed (Table 1). More often positive lymph nodes were diagnosed in group I, 16.2% vs 9.3% in group II (P = 0.01) and 7.9% (P < 0.01) in group III. Also, in group I more poorly differentiated tumours 28.5% were found, followed by 20.3% in group II and 16.6% in group III (P < 0.01). Both in groups I and II more signet-ring cell carcinomas 5.4% and 3.7% vs 1.4% in group III (P < 0.01) were present (Figure 2). The only differences between age groups and TNM stage, were more prevalent TNM stage I tumours in age group III compared to age group II (13.0% vs 11.1%, P = 0.04) and more frequently diagnosed TNM stage III tumours in age group II compared to age group III (9.9% vs 6.8%, P < 0.01). No differences in the number of metastases were observed between the age groups. Also, no difference in the number of mucinous carcinoma and presence of angioinvasion was observed. Lymphatic invasion was more commonly found in groups I and II compared to group III, 33.3% and 28.0% vs 20.3% (P = 0.09) respectively. No difference was observed in the number of KRAS, NRAS and BRAF mutations.

EOCRC characteristics over time

In age group I, 34.5% of the cancers were diagnosed in women in time period 1989-2004 compared to 54.9% in time period 2005-2018 (P = 0.01) (Figure 3 and Table S2). In age groups II and III no differences in gender were observed over time. For tumour location age group I showed the highest percent of cancers located in the colon in both men and women, and this did not change over time. In age group II the percent of rectal cancer was 33.8% in time period 1989-2004 and 41.6% in period 2005-2018 (P = 0.01) and in age group III the percent of rectal cancer was 34.3% in period 1989-2004 and 40.7% in period 2005-2018 (P < 0.01). The percent of poorly differentiated CRCs remained stable in age group I. In age groups II and III a decline over time was observed, 25.1% of the patients were diagnosed with a poorly differentiated CRC in age group II between 1989 and 2004 and declined to 17.4% between 2005 and 2018 (P = 0.05) and in age group III 20.3% had a poorly differentiated CRC between 1989 and 2004 and declined to 15.0% between 2005 and 2018 (P < 0.01). A higher proportion of patients had lymph nodes metastases after 2005 in all three age groups.

Table 1 Clinical and pathological features of sporadic EOCRC divided in three age groups. †Data of lymphatic invasion and angioinvasion was only available for years 2015 and 2016.

Characteristic of EOCRC patients	Group I 20-29 years	Group II 30-39 years	Group III 40-49 years	P-value	Group I vs group II	Group I vs group III	Group II vs group III
Total number	202	1196	4989				
Gender							
Male	103 (51.0)	569 (47.6)	2470 (49.5)	0.43			
Female	99 (49.0)	627 (52.4)	2519 (50.5)				
Location							
Colon	133 (68.6)	714 (61.0)	2977 (61.0)	0.10	.	.	.
Rectum	61 (31.4)	456 (39.0)	1905 (39.0)				
Mucinous histology							
Absent	188 (93.1)	1126 (94.1)	4741 (95.0)	0.25			
Present	14 (6.9)	70 (5.9)	248 (5.0)				
Signet-ring cell histology							
Absent	191 (94.6)	1152 (96.3)	4919 (98.6)	**<0.01**	0.23	<0.01	<0.01
Present	11 (5.4)	44 (3.7)	70 (1.4)				
Differentiation grade							
Well/moderate	108 (71.5)	721 (79.7)	3206(83.4)	**<0.01**	0.02	<0.01	<0.01
Poor	43 (28.5)	184 (20.3)	636 (16.6)				
TNM stage							
I	30 (14.9)	133 (11.1)	668 (13.0)	**0.08**	0.13	0.55	0.04
II	12 (5.9)	71 (5.9)	238 (4.8)	0.21			
III	13 (6.4)	118 (9.9)	340 (6.8)	**<0.01**	0.12	0.83	<0.01
IV	26 (12.9)	174(14.5)	633 (12.7)	0.23			
Number of metastasis							
0	146 (72.3)	886 (74.1)	3795 (76.1)	0.19			
1	35 (17.3)	204 (17.1)	745 (14.9)	0.14			
2	11 (5.4)	71 (5.9)	306 (6.1)	0.90			
3	9 (4.5)	31 (2.6)	130 (2.6)	0.27			
Number of positive lymph nodes							
<7 positive lymph nodes	129 (83.8)	816 (90.7)	3599 (92.1)	**<0.01**	0.01	<0.01	0.16
>7 positive lymph nodes	25 (16.2)	84 (9.3)	307 (7.9)				
Lymphatic invasion†							
No	16 (66.7)	67 (72.0)	468 (79.7)	**0.09**	0.61	0.12	0.09
yes	8 (33.3)	26 (28.0)	119 (20.3)				
Angioinvasion†							
No	14 (66.7)	41 (69.5)	331 (74.4)	0.56			
yes	7 (33.3)	18 (30.5)	114 (25.6)				

Table 1 Clinical and pathological features of sporadic EOCRC divided in three age groups. †Data of lymphatic invasion and angioinvasion was only available for years 2015 and 2016. (*continued*)

Characteristic of EOCRC patients		Group I 20-29 years	Group II 30-39 years	Group III 40-49 years	P-value	Group I vs group II	Group I vs group III	Group II vs group III
KRAS mutation								
	Absent	14 (58.3)	72(63.2)	261 (55.9)	0.37			
	Present	10 (41.7)	42 (36.8)	206 (44.1)				
NRAS mutation								
	Absent	13 (92.9)	64(98.5)	244 (94.6)	0.38			
	Present	1(7.1)	1 (1.5)	14 (5.4)				
BRAF mutation								
	Absent	18 (100)	73 (93.6)	299 (91.8)	0.42			
	Present	0 (0)	5 (6.4)	26 (8.0)				

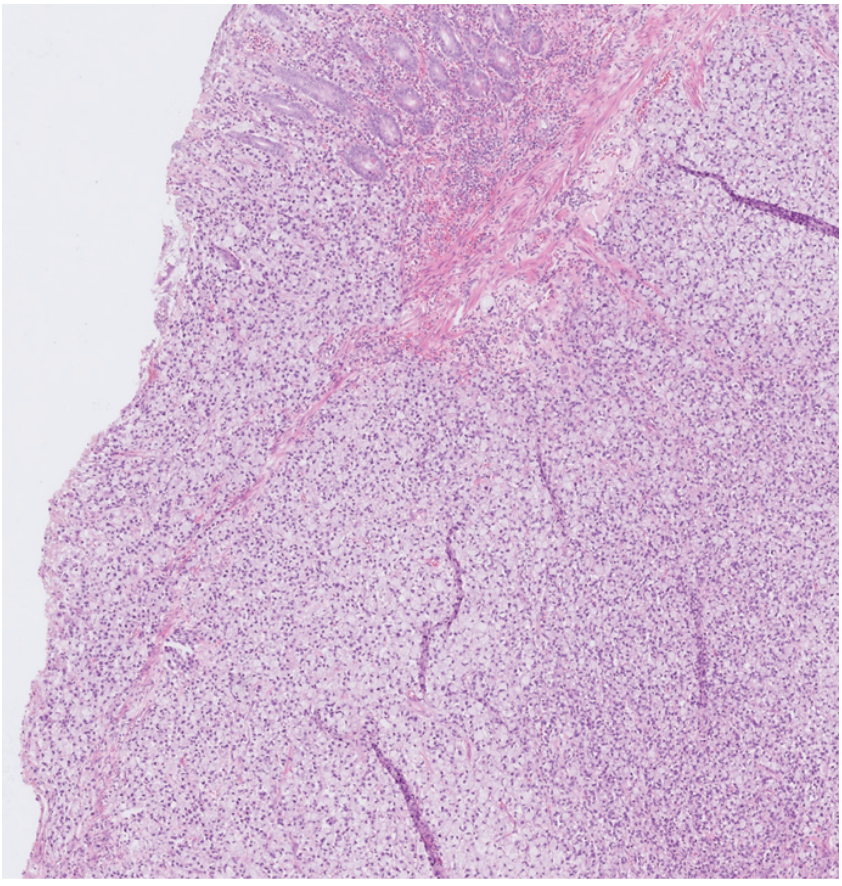


Figure 2 Microscopic image of a signet-ring cell carcinoma in the colon

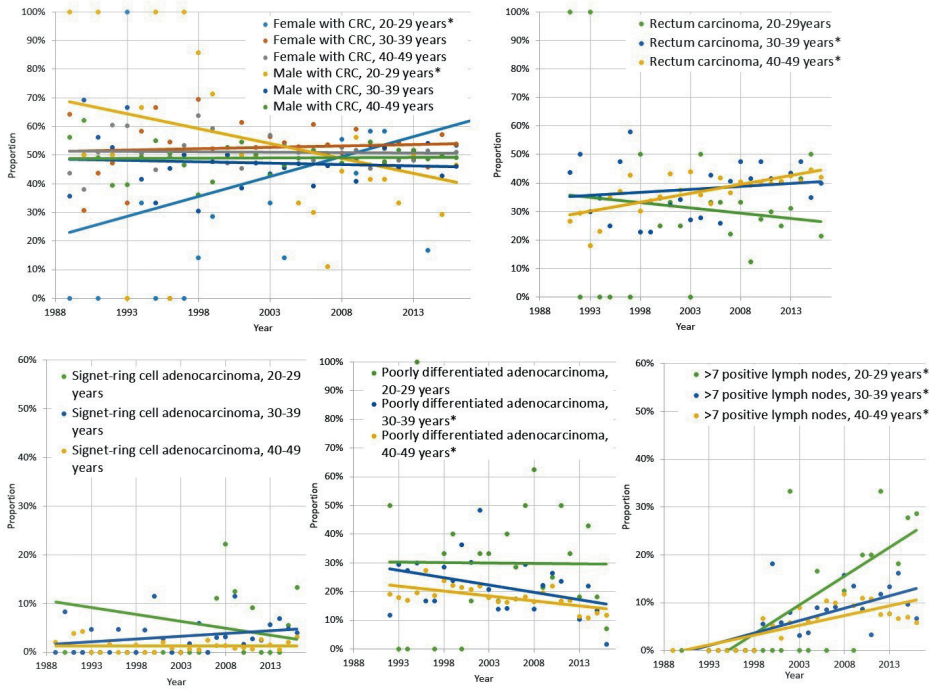


Figure 3 Proportion of female and male patients with colorectal cancer (CRC), rectum carcinomas, signet-ring cell adenocarcinomas, poorly differentiated CRC and CRC with more than 7 positive lymph nodes over time divided into three age groups. *Significant difference

Overall survival outcome

Mean OS time was 6.3 years (SD 6.2). Overall 5-year disease-free survival rates were 60.9% in group I, 62.7% in group II, and 64.2% in group III. OS did not significantly differ between the three groups (P = 0.72) (Figure 4). A better survival rate was found for patients diagnosed with CRC between 2005 and 2018, with an overall 5-year disease-free survival rate of 65.8% vs 58.4% for patients diagnosed between 1989 and 2004 (P < 0.01; Figure 4).

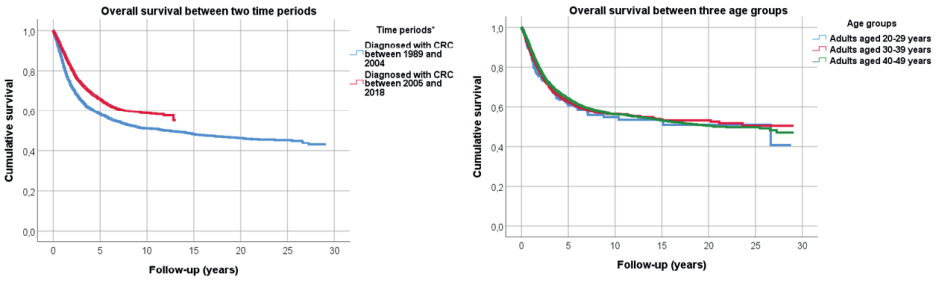


Figure 4 Overall disease-free survival analyses in sporadic early-onset colorectal cancer (EOCRC) patients per time period (1989-2004 vs 2005-2018) and per age group. *Significant difference

Discussion

This study presents a nationwide analysis of clinical and histopathological characteristics of CRC in patients <50 years of age over the past 30 years. Poorly differentiated tumours, presence of signet-ring cells, and higher number of lymph node metastasis were significantly more prevalent in 20-39 years old compared to the 40-49 years old. Over time, a higher proportion of EOCRCs were diagnosed in women below the age of 30 years, while a higher proportion of tumours were located in the rectum in the older group, 30-49 years old. OS was 6.3 years and improved over time.

This is the first study to assess clinicopathological features between different age groups of true sporadic EOCRC patients, without obscuration of patients with LS-CRC. Identification of EOCRC remains a major challenge and is expected to become more prevalent in the upcoming years. Insights about EOCRC both from a patient and tumour perspective may help to better recognise EORCC patients.

The results from our study confirm the observations of two other studies from the US. In one study 55 EOCRC patients below the age of 40 years were compared to sporadic CRC patients older than 40 years of age (15). In the other US study, more than 36 000 patients were included (19). Both studies showed a higher prevalence of signet-ring cell carcinomas and a higher proportion of tumours located in the left side of the colon or in the rectum in the youngest age group (15, 19). In addition, we found that sporadic EOCRC patients <40 years of age had more often lymph nodes metastases. Another study using the SEER 9 Registries concluded that EOCRC were more often found at an advanced stage and were more often mucinous carcinomas (20). However, in this study they were unable to exclude LS patients which may have biased the results.

A consistent finding is that the incidence of rectal cancer in EOCRC patients increased over time. In a previous study, it was shown that the incidence of rectal cancer in patients <40 years of age over two time periods (1992-1996 and 2010-2014) increased from 2.7 per 100 000 to 4.4 per 100 000 patients (21). The incidence rates, however, of carcinoid carcinomas located in the rectum increased more steeply than adenocarcinomas. This may partly explain the rapid rise of rectal carcinomas, especially for those studies that did not assess cancers by histological subtypes (22).

We found that a higher proportion of CRCs were diagnosed in women aged 20-29 years old in more recent years. A true increase in incidence could however not be calculated because of the missing population numbers of women per time period. It is known that men are at greater risk for late-onset CRC, but recent studies revealed that men also have a higher risk for EOCRC (10, 23). These studies however did not stratify by age or ethnicity. An American study for example found that rural Non-Hispanic black women had the highest incidence rate ratios, which was primarily driven by colon cancers (24). Differences may possibly explained by differences in genetic make-up and life style factors, such as obesity and red meat consumption, but does not fully explain the gender difference in EOCRC (25). More research is required, stratifying groups by age, ethnicity and tumour site (colon vs rectal cancer) to elucidate explanations that may better clarify gender differences in EOCRC. Furthermore, a remarkable finding was the decline of poorly differentiated EOCRC over time, while more positive lymph nodes were found over time. The latter could be explained by the fact that the evaluation of lymph nodes became a quality measure for colon cancer care, since the number of lymph nodes examined is positively associated with the survival of patients (26). Another explanation for the higher proportion of patients with positive lymph nodes could be the improved techniques to harvest lymph nodes, such as fat clearance (27).

Our study included data on KRAS, NRAS and BRAF genes. KRAS is a common gene in CRC patients and has the ability to promote tumour proliferation and suppress differentiation. As biomarker, KRAS predicts response to anti-EGFR therapies (28, 29). NRAS is less prevalent in CRC patients and are able to suppress apoptosis (28). BRAF genes are found in 7% of the tumours and is considered as a driver in the serrated pathway (30). Previous literature showed conflicting results regarding the prevalence of KRAS, NRAS and BRAF genes in EOCRC patients. A review from Italy included 46 articles, of which ten studies reported on prevalence of KRAS genes in EOCRC (14). Seven studies reported a lower prevalence of KRAS genes in EOCRC compared to older CRC patients, two studies showed a similar prevalence and one study had a higher prevalence. The prevalence of BRAF genes was reported to be similar among EOCRC compared to older patients (14). NRAS mutation prevalence in EOCRC patients was only reported in one study with a

small patient population, they reported three NRAS mutations in 69 patients (31). Our results showed no difference in KRAS, NRAS and BRAF genes between the different EOCRC age groups.

There is controversy around the prognosis of patients with sporadic EOCRC, varying from worse to better outcome compared to late-onset CRC patients (20, 32-35). The latter might be explained by the mixture with LS-CRC patients in these studies. Although OS increased over time, our study observed no difference in OS between the age groups in EOCRC. The increased OS over time may be explained by improved diagnostic modalities and treatment options (36). But also more early diagnosis of CRC in time may have contributed to the increased survival. Unfortunately, we were not able to analyse the CRC specific mortality due to the retrospective design of this study.

One could theorise that the low survival rate of EOCRC patients is the result of a patient- or doctor delay in diagnosing CRC, whereas for patients known with a hereditary disease awareness of CRC occurrence exists. Young patients seek medical attention at a later stage because they neglect their symptoms or delay seeking medical attention. Doctors may attribute the alarm symptoms of young patients with CRC to benign causes without further examination. However, some characteristics of sporadic EOCRC could not be subjected to patient or doctor delay, like gender, location of the tumour and type of histology. Therefore, it is reasonable that differences in tumour features suggestive of differences in tumourigenesis may play a role in clinical outcome. The question what is causing the histopathological changes is still unanswered.

Previous studies on EOCRC have pooled the data of all CRC patients under the age of 40 or 50 years (37, 38). This study provides a more in-depth clinical and histopathological characterisation of young adults with sporadic CRC aged 20-29 years, 30-39 years and 40-49 years. We found that poor prognosis features of EOCRC were more prevalent in 20- to 29-year-old adults, followed by 30- to 39-year-old and less prevalent in 40- to 49-year-old adults. This makes a period effect resulting from external factors that equally affect all age groups at a particular time period less likely. In literature, it is hypothesised that the increased trend of EOCRC follows the pattern of a cohort effect where the youngest generation is more susceptible for the development of a different, more aggressive type of CRC. While CRC detected in adults aged 40-49 years are more comparable to the CRC found in the general population with comparable clinical and pathological features. The cause of the cohort effect is still unknown. Possible risk factors may be the increasing prevalence of obese individuals in the last decades or alterations in gut microbiota due to a more frequent use of antibiotics (39). But also germline variants of multiple genes could be associated with increased EOCRC risk. One study revealed that EOCRC

patients have unique molecular features, with less BRAF V600 mutations compared to patients with late-onset CRC, and the presence of more subtypes of CMS1 and CMS2 (19). Another study showed a high prevalence (16%) of germline mutations in patients with EOCRC (40). Both studies however included LS patients. A recent published study showed that EOCRC exhibits a different genetic risk compared to late-onset CRC due to low-penetrance common genetic polymorphisms, with a stronger association in patients without a CRC family history (41). Though genetic factors probably play a role in the increased risk of EOCRC, most likely multiple (risk) factors are involved.

Strength of this study was the large nationwide database covering all patients diagnosed with CRC below the age of 50 years over the past 30 years in the Netherlands on which molecular analyses were performed. This study also has several limitations. First, the retrospective design of the study. This could have led to information and selection bias or misclassification of data. To ensure that LS patients were not included, we excluded all patients in who no molecular diagnostics was performed. Comparing the MSI tested group with the non-tested group, significantly more women were molecularly tested for LS. This may have been caused by the fact that women had more often features of LS. Although we identified significant differences between the tested and non-tested group, the clinical relevance of this selection bias is less clear than including all patients, including unidentified LS patients. Ideally, one would like to follow a cohort of young adults over a long period of time. Although prospective studies should be initiated, it takes time before conclusions can be drawn and recommendations are given. With the increase in EOCRC incidence in different parts of the world, it is important to gather information at this moment in order to understand this trend and attempt to reverse it. This large retrospective study will help to contribute to the understanding of EOCRC. Second, because of the retrospective design of this study, we had no access to data regarding risk factors (e.g. smoking status, obesity, use of antibiotics). Also, no information was available regarding family history and ethnicity. Third, no linear analyses overtime were possible due to the small sample size in the youngest age groups.

To conclude, this study revealed clinicopathological differences within the groups defined as EOCRC in the last 30 years. The proportion of rectal cancer increased from the age of 30 years in more recent years, while in patients below the age of 30 years a higher proportion of CRC was found in females and characterised by a more frequent presence of signet-ring cells and poor histological features. Clinicians should be aware of these differences in clinicopathological characteristics to optimise (early) detection and eventually targeted CRC treatment.

Acknowledgements

The authors thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry as well as IKNL staff for scientific advice. We also thank the Dutch pathology registry (PALGA) for the collection of data and their scientific advice.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68(1):7-30.
2. Vuik FE, Nieuwenburg SA, Bardou M, et al. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. Gut 2019;68(10):1820-1826.
3. Siegel RL, Fedewa SA, Anderson WF, et al. Colorectal Cancer Incidence Patterns in the United States, 1974-2013. J Natl Cancer Inst 2017;109(8):djw322.
4. Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481-7.
5. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA 2008;300(23):2765-78.
6. Wei EK, Giovannucci E, Wu K, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004;108(3):433-42.
7. Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr 2007;86(3):556-65.
8. Zhang J, Haines C, Watson AJM, et al. Oral antibiotic use and risk of colorectal cancer in the United Kingdom, 1989-2012: a matched case-control study. Gut 2019;68(11):1971-1978.
9. Cheung KS, Chen L, Chan EW, Seto WK, Wong ICK, Leung WK. Statins reduce the progression of non-advanced adenomas to colorectal cancer: a postcolonoscopy study in 187 897 patients. Gut 2019;68(11):1979-1985.
10. Low EE, Demb J, Liu L, et al. Risk Factors for Early-Onset Colorectal Cancer. Gastroenterology 2020.
11. Gausman V, Dornblaser D, Anand S, et al. Risk Factors Associated With Early-onset Colorectal Cancer. Clin Gastroenterol Hepatol 2019: S1542-3565.
12. Silla IO, Rueda D, Rodriguez Y, Garcia JL, de la Cruz Vigo F, Perea J. Early-onset colorectal cancer: a separate subset of colorectal cancer. World J Gastroenterol 2014;20(46):17288-96.
13. Savas N, Dagli U, Akbulut S, Yuksel O, Sahin B. Colorectal cancer localization in young patients: should we expand the screening program? Dig Dis Sci 2007;52(3):798-802.
14. Mauri G, Sartore-Bianchi A, Russo AG, Marsoni S, Bardelli A, Siena S. Early-onset colorectal cancer in young individuals. Mol Oncol 2019;13(2):109-131.
15. Chang DT, Pai RK, Rybicki LA, et al. Clinicopathologic and molecular features of sporadic early-onset colorectal adenocarcinoma: an adenocarcinoma with frequent signet ring cell differentiation, rectal and sigmoid involvement, and adverse morphologic features. Mod Pathol 2012;25(8):1128-39.
16. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol 2007;29(1):19-24.
17. Sanden GACvd, Coebergh JWW, Schouten LJ, Visser O, Leeuwen FEv. Cancer incidence in the Netherlands in 1989 and 1990: First results of the nationwide Netherlands cancer registry. European Journal of Cancer 1995;31(11):1822-1829.
18. Obrocea FL, Sajin M, Marinescu EC, Stoica D. Colorectal cancer and the 7th revision of the TNM staging system: review of changes and suggestions for uniform pathologic reporting. Rom J Morphol Embryol 2011;52(2):537-44.
19. Willauer AN, Liu Y, Pereira AAL, et al. Clinical and molecular characterization of early-onset colorectal cancer. Cancer 2019;125(12):2002-2010.

20. Wang R, Wang MJ, Ping J. Clinicopathological Features and Survival Outcomes of Colorectal Cancer in Young Versus Elderly: A Population-Based Cohort Study of SEER 9 Registries Data (1988-2011). Medicine (Baltimore) 2015;94(35):e1402.
21. Murphy CC, Wallace K, Sandler RS, Baron JA. Racial Disparities in Incidence of Young-Onset Colorectal Cancer and Patient Survival. Gastroenterology 2019;156(4):958-965.
22. Montminy EM, Zhou M, Maniscalco L, et al. Contributions of Adenocarcinoma and Carcinoid Tumors to Early-Onset Colorectal Cancer Incidence Rates in the United States. Ann Intern Med 2021;174(2):157-166.
23. Murphy G, Devesa SS, Cross AJ, Inskip PD, McGlynn KA, Cook MB. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. Int J Cancer 2011;128(7):1668-75.
24. Zahnd WE, Gomez SL, Steck SE, et al. Rural-urban and racial/ethnic trends and disparities in early-onset and average-onset colorectal cancer. Cancer 2021;127(2):239-248.
25. Kim S-E, Paik HY, Yoon H, Lee JE, Kim N, Sung M-K. Sex- and gender-specific disparities in colorectal cancer risk. World J Gastroenterol 2015;21(17):5167-5175.
26. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. J Natl Cancer Inst 2007;99(6):433-41.
27. Cianchi F, Palomba A, Boddi V, et al. Lymph node recovery from colorectal tumor specimens: recommendation for a minimum number of lymph nodes to be examined. World J Surg 2002;26(3):384-9.
28. Haigis KM, Kendall KR, Wang Y, et al. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. Nat Genet 2008;40(5):600-8.
29. Watson R, Liu TC, Ruzinova MB. High frequency of KRAS mutation in early onset colorectal adenocarcinoma: implications for pathogenesis. Hum Pathol 2016;56:163-70.
30. Sanz-Garcia E, Argiles G, Elez E, Tabernero J. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives. Ann Oncol 2017;28(11):2648-2657.
31. Perea J, Arriba M, Rodríguez Y, et al. Frequency and impact of KRAS mutation in early onset colorectal cancer. Hum Pathol 2017;61:221-222.
32. Kaplan MA, Ozaydin S, Yerlikaya H, et al. Clinicopathologic and Prognostic Differences between Three Different Age Groups (Child/Adolescent, Young Adults, and Adults) of Colorectal Cancer Patients: A Multicentre Study. Oncol Res Treat 2019;42(10):516-522.
33. Vatandoust S, Price TJ, Ullah S, et al. Metastatic Colorectal Cancer in Young Adults: A Study From the South Australian Population-Based Registry. Clin Colorectal Cancer 2016;15(1):32-6.
34. Rodriguez L, Brennan K, Karim S, Nanji S, Patel SV, Booth CM. Disease Characteristics, Clinical Management, and Outcomes of Young Patients With Colon Cancer: A Population-based Study. Clin Colorectal Cancer 2018;17(4):e651-e661.
35. Burnett-Hartman AN, Powers JD, Chubak J, et al. Treatment patterns and survival differ between early-onset and late-onset colorectal cancer patients: the patient outcomes to advance learning network. Cancer Causes Control 2019;30(7):747-755.
36. Johdi NA, Sukor NF. Colorectal Cancer Immunotherapy: Options and Strategies. Front Immunol 2020;11:1624.
37. Strum WB, Boland CR. Characterization and Identification of Colorectal Cancer in Persons Younger Than 50 Years. Clin Gastroenterol Hepatol 2019;17(12):2600-2602.
38. Yeo H, Betel D, Abelson JS, Zheng XE, Yantiss R, Shah MA. Early-onset Colorectal Cancer is Distinct From Traditional Colorectal Cancer. Clin Colorectal Cancer 2017;16(4):293-299 e6.
39. Murphy CC, Singal AG, Baron JA, Sandler RS. Decrease in Incidence of Young-Onset Colorectal Cancer Before Recent Increase. Gastroenterology 2018;155(6):1716-1719 e4.

40. Pearlman R, Frankel WL, Swanson B, et al. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. JAMA Oncol 2017;3(4):464-471.
41. Archambault AN, Su Y-R, Jeon J, et al. Cumulative Burden of Colorectal Cancer–Associated Genetic Variants Is More Strongly Associated With Early-Onset vs Late-Onset Cancer. Gastroenterology 2020;158(5):1274-1286.e12.

Supplementary files

Supplementary Table 1 Baseline characteristics of MSI tested versus no MSI tested patients. MSI = microsatellite instability, SD = standard deviation.

	MSI tested n = 7951	No MSI tested n = 7619	P-value
Age, mean (SD)	43.5 (5.9)	42.7 (5.8)	<0.01
Gender			<0.01
Male	4016 (50.5)	4078 (53.5)	
Female	3935 (49.5)	3541 (46.5)	
Location			0.06
Colon	4978 (64.1)	4759 (65.6)	
Rectum	2787 (35.9)	2500 (34.4)	
Lymphatic invasion			0.35
Absent	610 (77.9)	74 (82.2)	
Present	173 (22.1)	16 (17.8)	
Angioinvasion			0.22
Absent	436 (74.4)	49 (81.7)	
Present	150 (25.6)	11 (18.3)	
Number of positive lymph nodes			<0.01
<7 positive lymph nodes	5562 (91.9)	3272 (94.1)	
>7 positive lymph nodes	488 (8.1)	206 (5.9)	
Differentiation grade			<0.01
Well/moderate	4942 (80.1)	4663 (78.1)	
Poor	1230 (19.9)	1309 (21.9)	
Signet-ring cell differentiation			0.08
Absent	7790 (98.0)	7433 (97.6)	
Present	161 (2.0)	186 (2.4)	
TNM stage			<0.01
I	1040 (31.5)	1239 (21.3)	
II	551 (16.7)	1439 (24.7)	
III	654 (19.8)	1366 (23.5)	
IV	1056 (32.0)	1778 (30.5)	

Supplementary Table 2 Absolute numbers (percentages) of patients with sporadic EOCRC between 1989-2004 and 2005-2018 divided over three age groups. *Fisher's exact test.

	20-29 years old N=201			**30-39 years old N=1200**			**40-49 years old N=5025**		
Year	≤ 2004	≥ 2005	P-value	≤ 2004	≥ 2005	P-value	≤ 2004	≥2005	P-value
Gender , N (%)			<0.01			0.86			0.19
Male	38 (65.5)	65 (45.1)		187 (47.2)	382 (47.8)		620 (48.0)	1850 (50.1)	
Female	20 (34.5)	79 (54.9)		209 (52.8)	418 (52.3)		673 (52.0)	1846 (49.9)	
Location, N (%)			0.64			**0.01**			**<0.01**
Colon	37 (66.1)	96 (69.6)		257 (66.2)	457 (58.4)		835 (65.7)	2142 (59.3)	
Rectum	19 (33.9)	42 (30.4)		131 (33.8)	325 (41.6)		435 (34.3)	1470 (40.7)	
Signet-ring cell carcinoma, N (%)			0.73*			0.07			0.16
Absent	56 (96.6)	135 (93.8)		387 (97.7)	765 (95.6)		1280 (99.0)	3839 (98.5)	
Present	2 (3.4)	9 (6.3)		9 (2.3)	35 (4.4)		13 (1.0)	57 (1.5)	
Differentiation grade, N (%)			0.80			**<0.01**			**<0.01**
Well/moderate	35 (72.9)	73 (70.9)		260 (74.9)	461 (82.6)		900 (79.7)	2306 (85.0)	
Poor	13 (27.1)	30 29.1)		87 (25.1)	97 (17.4)		229 (20.3)	407 (15.0)	
Number of positive lymph nodes, N (%)			**0.05***			**0.04**			**0.06**
<7 positive lymph nodes	28 (96.6)	101 (80.8)		198 (94.3)	618 (89.6)		590 (94.9)	3009 (91.6)	
>7 positive lymph nodes	1 (3.4)	24 (19.2)		12 (5.7)	72 (10.4)		32 (5.1)	275 (8.4)	

Part IV

Screening methods of gastrointestinal disease - applicability of colon capsule

Chapter 6

Colon Capsule Endoscopy in colorectal cancer screening: a systematic review

F.E.R. Vuik, S.A.V. Nieuwenburg, S. Moen, C. Spada, C. Senore, C. Hassan, M. Pennazio, E. Rondonotti', S. Pecere, E.J. Kuipers, M.C.W. Spaander

Endoscopy, August 2021

Abstract

Introduction Primary colonoscopy and fecal immunochemical test (FIT) are the most commonly used colorectal cancer (CRC) screening modalities. Colon capsule endoscopy (CCE) might be an alternative. Data on the performance of CCE as a CRC screening tool in a screening population remain scarce. This is the first systematic review to provide an overview of the applicability of CCE as a CRC screening tool.

Methods A systematic search was conducted of literature published up to September 2020. Studies reporting on CRC screening by second-generation CCE in an average-risk screening population were included.

Results 582 studies were identified and 13 were included, comprising 2485 patients. Eight studies used CCE as a filter test after a positive FIT result and five studies used CCE for primary screening. The polyp detection rate of CCE was 24%–74%. For polyps >6mm, sensitivity of CCE was 79%–96% and specificity was 66%–97%. For polyps ≥10mm, sensitivity of CCE was 84%–97%, which was superior to computed tomographic colonography (CTC). The CRC detection rate for completed CCEs was 93% (25/27). Bowel preparation was adequate in 70%–92% of examinations, and completion rates varied from 57% to 92%, depending on the booster used. No CCE-related complications were described.

Conclusion CCE appeared to be a safe and effective tool for the detection of CRC and polyps in a screening setting. Accuracy was comparable to colonoscopy and superior to CTC, making CCE a good alternative to colonoscopy in CRC screening programs, although completion rates require improvement.

Introduction

Colorectal cancer (CRC) screening programs have been implemented in many countries to reduce CRC incidence and mortality by early detection of CRC and endoscopic removal of adenomas before their potential progression to adenocarcinomas. Several effective screening modalities are available (1). Most European countries use a fecal immunochemical test (FIT) followed by colonoscopy in individuals with a positive FIT result (2). However, the performance of this screening strategy seems to be hampered by low participation rates for colonoscopy (3). This could be due to the fact that colonoscopy is perceived as an invasive and painful procedure and the fact that it requires some form of sedation (4). Therefore, alternative strategies for CRC screening that result in higher participation rates would be desirable. To date, many CRC screening programs use computed tomographic colonography (CTC) as the primary alternative to colonoscopy. However, another promising alternative to colonoscopy is colon capsule endoscopy (CCE).

CCE provides a clear overview of the complete colon and has several advantages over colonoscopy: it is a noninvasive test, it carries minimal risks, no sedation is needed, and it can be performed at home. The performance of CCE was comparable to the gold standard (colonoscopy) in several trials (5). Sensitivity for the detection of polyps >6mm and >10mm increased markedly between the first-generation (CCE I) and second-generation (CCE II) colon capsules (6). The European Society for Gastrointestinal Endoscopy guidelines has already recommended CCE II as an option for average-risk CRC screening, and the US Food and Drug Administration has approved CCE II in patients with a previous incomplete colonoscopy and as a diagnostic tool in patients with suspected lower gastrointestinal bleeding (7, 8).

Even though the overall accuracy of CCE has been described in several trials, information on the performance of CCE in a screening population remains scarce. This is the first systematic review to provide an overview of the applicability of CCE as a CRC screening tool in an average-risk screening population, including information on participation, diagnostic value, bowel preparation, and completion rates.

Methods

We conducted a systematic search of published trials and abstracts following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Table 1s). In collaboration with the Medical School Library of the Erasmus

University in Rotterdam, The Netherlands, a systematic search was conducted of literature published up to 20 September 2020 to retrieve studies that reported on the use of CCE in a CRC screening program. Embase, Web of Science, Ovid MEDLINE, and Cochrane CENTRAL were used as potential sources. The search was conducted using controlled vocabulary supplemented with several key words (see supplement).

Two independent reviewers (F.E.R.V. and S.A.V.N.) screened the selected studies by title and abstract. Studies that focused on the use of CCE in patients participating in a CRC screening program were included in the review. Studies using CCE I were excluded because of low sensitivity for detection of polyps compared with CCE II. Studies including first-degree relatives of patients with CRC were also excluded. The full texts of the selected publications were examined by the same authors. The reference lists from the included studies were hand-searched to identify potentially relevant studies that were not retrieved in the original search. Study authors were contacted when additional information was needed.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CCE were calculated using the gold standard colonoscopy results as reference. Lesions included in the analyses were CRC and polyps of any size. Significant lesions were defined in this study as ≥3 polyps or one polyp >10mm. Non-significant lesions were defined as all remaining abnormalities and were not included in the analysis. Lesions observed by CCE but not seen at colonoscopy were defined as false-positive lesions. The polyp detection rate (PDR) was defined as the number of patients with ≥1 polyp detected by CCE. A meta-analysis could not be performed owing to the heterogeneity of the study designs.

Assessment of methodologic quality

Methodologic quality and risk of bias were assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 assessment tool (9). The two main categories evaluated were risk of bias and applicability. Two reviewers (F.E.R.V. and S.A.V.N.) independently assessed the methodologic quality.

Results

Literature search

After removal of duplicates, retrieved records were screened for eligibility based on their title and/or abstract. In total, 582 records were assessed for eligibility, after which 547 were excluded (Figure 1). The full text of the 35 remaining studies was reviewed, after

which 23 were excluded for various reasons. A total of 13 studies were included in the review, including one additional study, which was presented during Digestive Disease Week (18–21 May 2019, San Diego, California, USA) (10). Two of the included studies used the same study cohort but with different study aims (11, 12). Eight investigators were contacted to obtain further information on their studies.

Study characteristics

Baseline characteristics of the included studies are shown in Table1. A total of 2485 patients were included. Eleven studies were performed in Europe and two were conducted in the USA. Ten studies were full papers. All studies were performed within a CRC screening setting in an average-risk population. Eight studies used CCE as a filter test after a positive FIT result and five studies used CCE as the primary screening tool. The design of the studies differed: in eight studies both CCE and colonoscopy were performed to assess the diagnostic accuracy of CCE for CRC and polyps (11-18); in one study CTC or CCE was offered to FIT-positive patients who refused colonoscopy (19); in one study the diagnostic accuracy of both CCE and CTC was compared with colonoscopy (20); in two studies the diagnostic yield was evaluated in patients who were randomized to undergo CCE or CTC before colonoscopy (10, 21); and in one study CCE was offered to study the effect of a new examination method on the uptake of CRC screening (22).

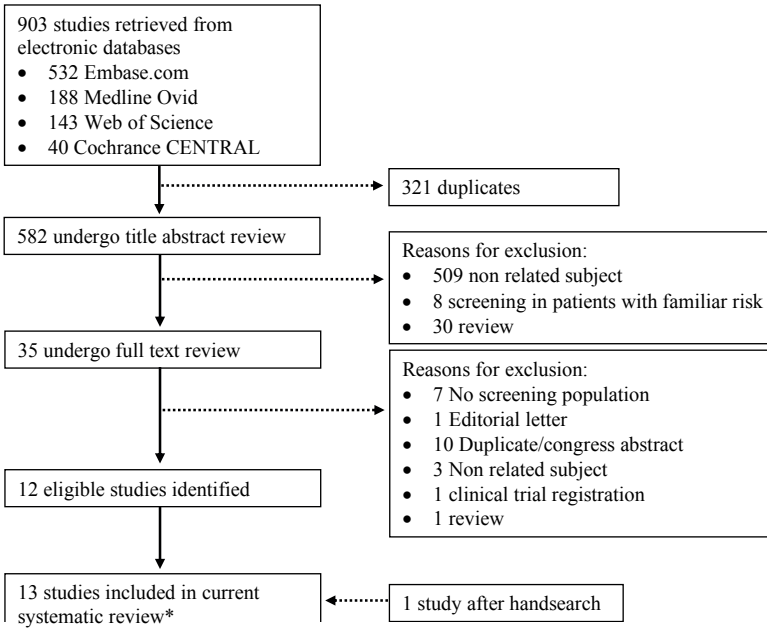


Figure 1 Flow chart of study selection. *Two studies used the same study cohort.

Table 1 Characteristics of the 13 included studies. *RCT,* randomized controlled trial.

Study	Year of publication	Type of article	Type of study	Centers	Patients enrolled, n	Patients included, n	Male sex, %	Mean age, y
Groth(22) Germany	2012	Full text	Cohort	Single center	154	90	64	62.7
Holleran(15) Ireland	2014	Full text	Cohort	Single center	62	62	55	62.5
Suchanek(14) Czech Republic	2014	Abstract	Cohort	Multicenter	225	225	-	59
Rondonotti(20) Italy	2014	Full text	Cohort	Single center	50	50	58	59.2
Romero(13) Spain	2015	Abstract	Cohort	Single center	67	53	58	61.3
Rex(16) US and Israel	2015	Full text	Cohort	Multicenter	884	695	44	57
Suarez(21) Spain	2016	Abstract	RCT	Single center	-	88	-	-
Kobaek-Larsen(11)* Denmark	2017	Full text	Cohort	Single center	306	253	58	64
Pecere(17) Italy, Spain	2018	Abstract	Cohort	Multicenter	222	203		
Voska(18) Czech Republic	2018	abstract	Cohort	Multicenter	200	105		
Pioche(19) France	2018	Full text	RCT	Multicentre	97	19	-	-
Thygesen(12)* Denmark	2019	Full text	Cohort	Single center	-	239	-	-
Cash (10) US	2019	Abstract	RCT	Multicenter	320	286	42.3	55.7
Total						2485		

* Both studies used the same Danish cohort–no information was available.

Quality of studies

The quality of included studies and risk of bias using the QUADAS-2 tool are presented in Table 2. Three studies did not assess the diagnostic accuracy of CCE compared with colonoscopy and therefore most domains were not applicable (12, 19, 22). None of the studies included had a high risk of bias.

Table 2 Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) analysis for the risk of bias in included studies

	Risk of bias				**Applicability concerns**		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Groth (22)	-	N/A	N/A	N/A	N/A	N/A	N/A
Holleran (15)	-	?	-	-	-	-	-
Suchanek (14)	-	?	?	?	?	-	-
Rondonotti (20)	-	-	-	-	-	-	-
Romero (13)	-	-	+	-	-	-	-
Rex (16)	-	-	-	-	-	-	-
Gonzalez-Suarez (21)	-	-	-	-	-	-	-
Kobaek-Larsen (11)	-	?	-	-	-	-	-
Pecere (17)	-	-	-	-	-	-	-
Voska (18)	-	-	-	-	-	-	-
Pioche (19)	-	N/A	N/A	N/A	N/A	N/A	N/A
Thygesen (12)	-	N/A	N/A	N/A	N/A	N/A	N/A
Cash (10)	-	-	-	-	-	-	-

–=low risk of bias;+=high risk of bias; ?=insufficient data; N/A, not applicable.

Participation rate

Only two studies reported the participation rate of CCE. CCE was used as the primary screening modality in one study and as a filter test in the other. The lowest participation rate of 4.2% was reported in a German opportunistic screening study where CCE was offered as an alternative to primary colonoscopy screening (22). The average screening uptake in that area was 1%, so offering CCE actually resulted in a fourfold increase in screening uptake. In another study, CCE was offered to patients who were unwilling to undergo colonoscopy after a positive FIT result, with a participation rate of 5% (19).

Three other studies reported on the enrollment rate of participants for their study. An enrollment rate of 8.2% was found in an Italian study in which FIT-positive patients were invited to undergo both CCE and CTC in addition to colonoscopy (20). In this study, patients had to take bowel preparation twice. A Danish study showed an enrollment rate of 17.4% in FIT-positive patients who were invited to undergo CCE in addition to colonoscopy (11). An enrollment rate of 52.7% was found in a Spanish study in which FIT-positive patients were randomized to either CCE or CTC in addition to colonoscopy (21).

Patient preferences

One study assessed patients' experiences of CCE at home compared with colonoscopy in an outpatient clinic in screening participants using the same bowel preparation. Nearly

90% of the patients undergoing colonoscopy experienced a medium to high degree of discomfort compared with only 10% of patients undergoing CCE. The advantages of CCE mentioned were no pain, no embarrassment, and a less invasive procedure. Disadvantages were the waiting time for results, extended duration of the CCE procedure if the capsule had a long transit time, and the need for an additional colonoscopy when significant lesions were found. Advantages of colonoscopy were the immediate availability of results and the possibility to remove tissue during the same procedure. Disadvantages were more pain, more embarrassment, and a more invasive procedure (12). The previously mentioned German study showed that the main reason for a final choice of CCE over colonoscopy was the fear of colonoscopy-related discomfort and complications (22). With regard to patient preferences, one study showed that more participants preferred colonoscopy as the primary screening tool (53%) compared with CCE (47%) (18).

Furthermore, it was shown that 78% of patients preferred to undergo CCE over CTC. In all cases this was due to the bloating and mild pain perceived during CTC (20). When CTC or colonoscopy was preferred over CCE, the main limitation for CCE seemed to be the need for rigorous bowel preparation (20).

Diagnostic yield

Detection rate of CRC

The CRC detection rate by CCE was reported in 9 out of 13 studies and varied from 64% to 100%. The CRC detection rate for completed CCEs was 93% (25/27). The lowest detection rate of 64% was caused by a low completion rate of 57%. In this study, CCE missed four CRCs, which were all located in the left colon, because the battery life expired before excretion of the capsule (11). In another study, one CRC was missed by CCE. Unblinded review of the capsule video determined that the cancer was photographed by the capsule in multiple frames, but overlooked by the reviewer (16). In one study, CRC was misjudged as a 5-mm polyp instead of a 10-mm malignant polyp (17). The detection rate of CRC in the remaining six studies was 100% (13-15,18, 19, 21).

Detection rate of polyps

Four CCE studies provided the PDR, two of which compared the PDR of CCE with that of colonoscopy. The CCE detection rates for polyps ranged between 24% and 74% (Table 3, Figure 2). In one study, CCE detected any type of polyp in 69% of participants compared with 58% for colonoscopy (15). When only significant lesions (defined in this study as ≥3 polyps or one polyp >10mm) were included, CCE found 18 polyps (detection rate of 29%), which was equal to the findings of colonoscopy. Another study also showed

that the PDR of CCE was significantly higher than the PDR of colonoscopy (74% vs. 64%, respectively) (11). The same study performed repeat colonoscopies to determine an explanation for the difference in PDR of CCE compared with colonoscopy. An additional 82 polyps were found during repeat colonoscopy, after which the PDR of colonoscopy increased to 85%. This suggests that the discrepancy between PDR of CCE and colonoscopy might be explained by a colonoscopy miss rate (11).

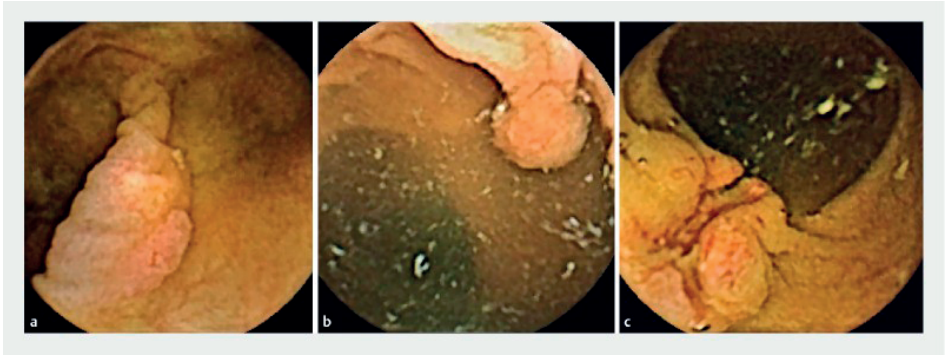


Figure 2 Lesions found during colon capsule endoscopy. a Sessile polyp. b Pedunculated polyp. c Colorectal cancer.

Diagnostic accuracy of CCE vs. colonoscopy

Sensitivity and specificity

Sensitivity and specificity of CCE are shown in (Table3). Sensitivity of CCE ranged between 79% and 96% for polyps >6mm and between 77% and 97% for polyps >9mm. Specificity of CCE varied between 66% and 97% for polyps >6mm and between 91% and 99% for polyps >9mm. Data from the study by Holleran et al. showed that specificity increased when only significant lesions were included. The authors reported a specificity of 65% for all polyps; however, when looking at significant lesions only, specificity increased to 96% (15).

Table 3 Overview of 13 studies reporting on diagnostic accuracy of colon capsule endoscpy and colonoscopy in a colorectal cancer screening population

CCE, colon capsule endoscopy; PDR, polyp detection rate; CRC, colorectal cancer; PPV, positive predictive value; NPV, negative predictive value; FIT, fecal immunochemical test; Sign. lesion, significant lesion (defined as ≥3 polyps or one polyp >10 mm). AN, Advanced neoplasia; –, Information not available. [1]Per-polyp sensitivity/specificity. [2]Per-patient sensitivity/specificity.[3]Both studies use the same (Danish) cohort.

Study	Type of screening	Participation, n (rate, %)	Completion rate CCE, %	PDR, % (95%CI)		CRC detection rate, %		Outcome, positivity threshold of CCE	CCE performance, % (95%CI)				Colon cleanliness, %
				CCE	Colonoscopy	CRC	Colonoscopy		Sensitivity	Specificity	PPV	NPV	
Groth [22]	Opportunistic, primary colonoscopy	90 (4.2)	82	–	–	–	–		–	–	–	–	–
Holleran [15]	FIT-based screening	62	73	69	58	100	100	Any polyp Sign. lesion, ≥3 polyps or 1 polyp >10 mm	95 89[1]	65 96[1]	79 89	90 96	92
Suchanek [14]	Opportunistic, primary colonoscopy or FIT	225	–	–	51	100	100	Polyp, 1 polyp ≥6 mm Polyp, 1 polyp ≥10 mm AN, 1 polyp ≥10 mm	79 (62–91) 88 (62–98)[1] 100 (72–100)	97 (94–99) 99 (97–100)[1] –	–	–	90
Rondonotti [20]	FIT-based screening	50	90	–	–	–	–	Polyp, 1 polyp ≥6 mm Polyp, 1 polyp ≥10 mm	88 (62–98) 93 (64–100)[2]	88 (78–99) 92 (76–98)[2]	–	–	70
Romero [13]	FIT-based screening	53	81	–	82	100	100	Polyp, 1 polyp >6 mm Polyp, 1 polyp >9 mm	87 88[2]	88 94[2]	–	94	–
Rex [16]	Opportunistic, primary colonoscopy	695	92	–	–	75	100	Polyp, 1 polyp ≥6 mm Polyp, 1 polyp ≥10 mm	87 (82–90) 85 (77–92)[1]	94 (92–96) 97 (96–99)[1]	–	–	80
Gonzalez-Suarez [21]	FIT-based screening	349	81		–	100	100	Polyp, polyp of any size Polyp, 1 polyp ≥6 mm Polyp, 1 polyp ≥10 mm	98 (94–99)[1] 96 (91–100) 97 (91–100)	77 (69–83)[1] 88 (80–95) 95 (91–99)	94 (88–97) 90 (84–96) 88 (77–97)	92 (86–95) 95 (89–100 99 (97–100	82
Kobaek-Larsen [11][3]	FIT-based screening	253	57	74 (69–79)	64 (58-70)	64	100	Polyp, 1 polyp >9 mm	87 (83–91)[2]	92 (89–95)[2]	–	–	85 (81–89)
Pecere [17]	FIT-based screening	178	88	–	70	91	100	AN, 1 polyp >6 mm AN, 1 polyp >9 mm	90 (79–96) 77 (67–86)[2]	66 (57–74) 91 (84–95)[2]	57 (47–68) 81 (68–90)	93 (85–97) 88 (81–93)	88
Voska [18]	Opportunistic, primary FIT	225	90	–	51	100	100	Polyp, 1 polyp >6 mm Polyp, 1 polyp >10 mm	79 88[1]	97 99[1]	–		90
Pioche [19]	FIT-based screening	19 (5.0)	68	63	–	100	100		–	–	–	–	74
Thygesen [12][3]	FIT-based screening	239	–	–	–	–	–		–	–	–	–	–
Cash [10]	Opportunistic, primary colonoscopy	286	84	24	–	–	–	Polyp, 1 polyp >6 mm Polyp, 1 polyp >10 mm	84 84[2]	93 97[2]	–	–	84

PPV and NPV

The PPV of CCE varied between 57% for polyps >6mm and 94% for any polyp (17, 21). The NPV varied between 88% for polyps >10mm and 99% (17, 21).

Diagnostic accuracy of CCE vs. CTC

Four studies compared the diagnostic accuracy of CCE with that of CTC. In general, the detection rate and sensitivity of polyps were higher for CCE than for CTC and the specificity was comparable.

In a randomized controlled trial, patients who were unwilling to undergo colonoscopy after a positive FIT result were randomized to CCE or CTC. Although more patients consented to participate in the CTC group than in the CCE group (7.4% vs 5.0%, respectively), the detection rate of polyps in the CCE group was 60% vs. 28.6% in the CTC group (19).

Another study comparing CCE with CTC in 50 FIT-positive patients reported a high accuracy of both CTC (sensitivity 88.2%, specificity 84.8%) and CCE (sensitivity 88.2%, specificity 87.8%) for polyps >6mm. When only polyps ≥10mm were included, a higher sensitivity for CCE (sensitivity 92.8%, specificity 91.6%) was found compared with CTC (sensitivity 78.6%, specificity 91.7%) [20]. Gonzalez-Suarez et al. randomized between CTC and CCE in FIT-positive patients and found a higher sensitivity for neoplastic lesions ≥6mm and neoplastic lesion ≥10mm for CCE vs. CTC (96.1% and 97.3 vs. 79.3 and 90.0%, respectively). Specificity for neoplastic lesions ≥6mm and neoplastic lesions ≥10mm was lower for CCE compared with CTC (88.2% and 95.3% vs. 96.3% and 99%, respectively). CCE was superior to CTC (100% vs. 93.1%) for the detection of advanced adenomas and for the detection of any neoplastic lesion (CCE 100% vs. CTC 81%) [21]. The study by Cash et al. showed a higher detection rate for CCE (32% for polyps >6mm and 14% for polyps >10mm) compared with CTC (9% for polyps >6mm and 6% for polyps >10mm). Sensitivity of CCE for polyps >6mm (84%) and polyps >10mm (84%) was higher than that for CTC (32% for polyps >6mm and 53% for polyps >10mm). Specificity was higher for CTC vs. CCE (99% vs. 93%, respectively) for polyps >6mm and comparable for polyps >10mm (99% vs. 97%, respectively) (10).

Quality scores

Bowel preparation

In this review, 10 studies reported adequate bowel preparation scores for CCE examinations (Table 3). One study (20) used a split-dose macrogol regimen of 2L, which resulted in the lowest adequate bowel preparation score of 70% (Table2s). Three studies used a split-dose polyethylene glycol regimen of 4L, which resulted in the highest scores,

between 88% and 92% (15, 17, 18). The bubbles effect scale was not reported in any of the studies.

Completion rate

One study used sulphate solution as a booster, which resulted in a completion rate of 92% (16). Sodium phosphate was used in five studies and was associated with completion rates of 68%–90% (17-20, 22). Two studies used polyethylene glycol as a booster, which resulted in the lowest completion rates of 57%–73% (11, 15).

Safety

No CCE-related adverse events occurred in any of the included studies. Furthermore, use of bowel preparation – especially the use of sodium phosphate – did not cause a serious adverse event in any of the studies. There was only one serious adverse event, which occurred after colonoscopy. This was a post-polypectomy bleed that required blood transfusion and colonoscopy to clip the visible vessel at the polypectomy base (15).

Experience of colon capsule readers

In 10 studies, the level of expertise of the CCE readers was provided. In seven studies, one or more gastroenterologists or endoscopists were trained in reading CCE videos (15-22). Two studies only mentioned that the videos were reviewed by centers that specialized in capsule endoscopy (14, 19). One study used the services of Corporate Health, a company of nurses and physicians trained in CCE reading (11). The remaining three studies did not mention the expertise of the viewers (10, 12, 13).

Discussion

This is the first review to provide an overview of the literature on the use of CCE as a CRC screening tool. Most of the studies included in this review investigated the use of CCE as a filter test after a positive FIT result in a CRC screening setting. CCE appeared to be a safe and effective method for finding polyps and CRC, with an accuracy comparable to that of colonoscopy and superior to that of CTC in a CRC screening setting. Its high yield and patient preference make it a suitable screening tool as an alternative to colonoscopy in CRC screening programs, although completion rates require improvement.

In a previous meta-analysis, the accuracy of the first- and second-generation colon capsules was evaluated (6). The analysis showed a sensitivity of 86% for polyps >6mm and 87% for polyps >10mm, with a specificity of 88.1% and 95.3%, respectively. These results are comparable to those in our study and confirm the good performance of CCE.

However, this previous study did not focus on the performance of CCE as a screening tool in a screening population. Participation rate is one of the key performance indicators in a population-based screening program (1, 3). The overall participation in 21 European countries was 49.5% in countries using FIT-based screening, while the desirable uptake according to the European guidelines is >65% (1). The German study by Groth et al. was the only trial that offered CCE as a primary screening method in an opportunistic screening setting and this study showed a fourfold increase in screening uptake (22). The participation rate in the French study by Pioche et al. was very low (5.0%) because the study population consisted only of FIT-positive patients who were unwilling to undergo colonoscopy; therefore, this study population was biased and does not reflect a real-life situation (19). Other studies included in the review showed the enrollment rate, which does not reflect the participation rate, as those studies offered CCE in addition to colonoscopy instead of offering CCE alone. However, the extensive bowel preparation required for CCE and the possibility that bowel preparation would need to be repeated if the CCE was positive could have a negative effect on the participation rate. However, when reviewing the questionnaires, patients still preferred CCE over colonoscopy and CTC.

The CRC detection rate by CCE was 100% in almost all studies, which is an important condition for using CCE in a CRC screening program. Low completion rate is the main cause for missing CRC. Eight included studies showed a completion rate below the threshold for colonoscopy screening (90% cecal intubation rate) (23). Completion rates were highly dependent on the type of booster that was used. With the use of sodium phosphate, completion rates of up to 90% were reached. As sodium phosphate draws plasma water into the bowel, significant volume and electrolyte shifts may occur. Therefore, in older patients with renal insufficiency, cardiovascular disease, and electrolyte imbalance, the use of sodium phosphate is contraindicated (8, 24).

Although the bubbles effect scale is an important grading scale for CCE bowel preparation, it was not reported in any of the included studies. Bubbles may affect the visualization of the colon and they are important because they represent a different problem from debris and require a different solution (25).

This systematic review provides the first overview of CCE performance in a CRC screening setting; however, it has some limitations. First, because of the heterogeneity of the studies, no meta-analysis could be performed. Second, sensitivity and specificity of CCE could not be compared directly between the different studies because some studies performed per-patient analyses and others performed per-polyp analyses. Third, no clear difference could be determined between the diagnostic accuracy of CCE as a

primary screening tool and CCE as a filter test because of the limited number of studies using CCE as a primary screening tool. Fourth, most videos from studies included in this systematic review were analyzed by experienced readers. It is known that diagnostic accuracy for small-bowel endoscopy increases with experience of the reader (26). Fifth, information about the variation of size, type, and location of polyps detected by CCE vs. colonoscopy was often lacking.

At this stage, the good diagnostic accuracy of CCE ensures that CCE could be used as a screening tool. This review shows that CCE is a noninvasive method, with almost no risk of adverse events. However, some questions remain unanswered. Information on the participation rate of CCE in a screening setting is scarce. The uptake of CCE vs. colonoscopy was studied in first-degree relatives with CRC and found that the uptake was similar between the groups (55.8% CCE vs. 52.2% colonoscopy), but the crossover rate was higher from the CCE group (57.4%) than from the colonoscopy group (30.2%). Unwillingness to undergo bowel preparation twice was the main reason that participants assigned to the CCE group crossed over to colonoscopy (27). However, first-degree relatives with CRC might have an increased risk of developing advanced neoplasia compared with the average-risk population and therefore their choice in screening modality might be biased. Furthermore, the completion rate is moderate in several studies, especially if sodium phosphate is not used. As the use of sodium phosphate should be avoided in patients with an increased risk of sodium phosphate toxicity, and is prohibited in several countries, alternatives are needed. With these moderate completion rates for CCE, it is expected that additional sigmoidoscopies would be performed to review the sigmoid and rectum. This will have a negative impact on patient preference, workload of gastroenterologists, and costs. Without a completion rate of ≥90% it will be difficult for CCE to match colonoscopy. Finally, the time required to review the colon is extensive and more studies should investigate the use of artificial intelligence for the recognition of polyps and CRC.

In conclusion, despite its good diagnostic accuracy and noninvasiveness, and despite the fact that patients often prefer CCE over colonoscopy and CTC, CCE is still not used as a standard screening method. Further larger trials are needed to determine the role of CCE in population-based screening programs. Based on our review of the currently available literature, we believe CCE is a suitable screening tool as an alternative to colonoscopy and CTC in CRC screening programs, although the completion rate requires improvement.

Acknowledgments

The authors of this systematic review would like to acknowledge the contribution of Wichor Bramer and Maarten Engel, biomedical information specialists of the Erasmus University Medical Center, for developing and updating the search strategies.

References

1 Senore C, Basu P, Anttila A. et al. Performance of colorectal cancer screening in the European Union Member States: data from the second European screening report. Gut 2019; 68: 1232-1244

2 Schreuders EH, Ruco A, Rabeneck L. et al. Colorectal cancer screening: a global overview of existing programmes. Gut 2015; 64: 1637-1649

3 Pasha SF. Applications of colon capsule endoscopy review. Curr Gastroenterol Rep 2018; 20: 22

4 Spada C, Barbaro F, Andrisani G. et al. Colon capsule endoscopy: what we know and what we would like to know. World J Gastroenterol 2014; 20: 16948-16955

5 Spada C, Hassan C, Marmo R. et al. Meta-analysis shows colon capsule endoscopy is effective in detecting colorectal polyps. Clin Gastroenterol Hepatol 2010; 8: 516-522

6 Spada C, Pasha SF, Gross SA. et al. Accuracy of first- and second-generation colon capsules in endoscopic detection of colorectal polyps: a systematic review and meta-analysis review. Clin Gastroenterol Hepatol 2016; 14: 1533-1543

7 FDA expands indication for PillCam Colon 2 capsule. FDA. Available at (Accessed 30 April 2020): https://www.healio.com/gastroenterology/interventional-endoscopy/news/online/%7Bbe015b5b-0375-413a-9c4f-abf60bc3ac71%7D/fda-expands-indication-for-pillcam-colon-2-capsule

8 Spada C, Hassan C, Galmiche JP. et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy 2012; 44: 527-535

9 Whiting PF, Rutjes AW, Westwood ME. et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011; 155: 529-536

10 Cash BD, Fleisher MR, Fern S. et al. A Multicenter, prospective, randomized study comparing the diagnostic yield of colon capsule endoscopy versus computed tomographic colonography in a screening population Results of the TOPAZ study. Gastrointest Endosc 2019; 89 : AB87-AB88

11 Kobaek-Larsen M, Kroijer R, Dyrvig AK. et al. Back-to-back colon capsule endoscopy and optical colonoscopy in colorectal cancer screening individuals. Colorectal Dis 2018; 20: 479-485

12 Thygesen MK, Baatrup G, Petersen C. et al. Screening individuals' experiences of colonoscopy and colon capsule endoscopy; a mixed methods study. Acta Oncol 2019; 58: S71-S76

13 Romero C, De Miguel CR, Serradesanferm A. et al. Pillcam© colon capsule for colorectal cancer screening: a prospective and comparative study with colonoscopy. Gastroenterology 2015; 148: S759

14 Suchanek S, Grega T, Voska M. et al. The efficiency of colonic capsule endoscopy in detection of colorectal polyps and cancers compared to colonoscopy – final results of multicenter, prospective, cross-over study. Gastrointest Endosc 2015; 81: AB265

15 Holleran G, Leen R, O'Morain C. et al. Colon capsule endoscopy as possible filter test for colonoscopy selection in a screening population with positive fecal immunology. Endoscopy 2014; 46: 473-478

16 Rex DK, Adler SN, Aisenberg J. et al. Accuracy of capsule colonoscopy in detecting colorectal polyps in a screening population. Gastroenterology 2015; 148: 948-967

17 Pecere S, Senore C, Hassan C. et al. Accuracy of colon capsule endoscopy for advanced neoplasia. Gastrointest Endosc 2020; 91: 406-414

18 Voska M, Zavoral M, Grega T. et al. Accuracy of colon capsule endoscopy for colorectal neoplasia detection in individuals referred for a screening colonoscopy. Gastroenterol Res Pract 2019; 2019: 5975438

19 Pioche M, Ganne C, Gincul R. et al. Colon capsule versus computed tomography colonography for colorectal cancer screening in patients with positive fecal occult blood test who refuse colonoscopy: a randomized trial. Endoscopy 2018; 50: 761-769

20 Rondonotti E, Borghi C, Mandelli G. et al. Accuracy of capsule colonoscopy and computed tomographic colonography in individuals with positive results from the fecal occult blood test. Clin Gastroenterol Hepatol 2014; 12: 1303-1310

21 Gonzalez-Suarez B, Pages M, Araujo IK. et al. Colon capsule endoscopy versus CT colonography in FIT-positive colorectal cancer screening subjects: a prospective randomised trial – the VICOCA study. BMC Med 2020; 18: 255

22 Groth S, Krause H, Behrendt R. et al. Capsule colonoscopy increases uptake of colorectal cancer screening. BMC Gastroenterol 2012; 12: 80

23 Rembacken B, Hassan C, Riemann JF. et al. Quality in screening colonoscopy: position statement of the European Society of Gastrointestinal Endoscopy (ESGE). Endoscopy 2012; 44: 957-968

24 Singhal S, Nigar S, Paleti V. et al. Bowel preparation regimens for colon capsule endoscopy: a review. Therap Adv Gastroenterol 2014; 7: 115-122

25 Leighton JA, Rex DK. A grading scale to evaluate colon cleansing for the PillCam COLON capsule: a reliability study. Endoscopy 2011; 43: 123-127

26 Lim YJ, Joo YS. Korean Gut Image Study Group. et al. Learning curve of capsule endoscopy. Clin Endosc 2013; 46: 633-636

27 Adrian-de-Ganzo Z, Alarcon-Fernandez O, Ramos L. et al. Uptake of colon capsule endoscopy vs colonoscopy for screening relatives of patients with colorectal cancer. Clin Gastroenterol Hepatol 2015; 13: 2293

Supplementary file

Systemic literature search

Embase

('colon capsule endoscopy'/de OR 'capsule colonoscopy'/de OR (('capsule endoscopy'/de OR 'capsule endoscope'/de OR microcapsule/de) AND ('colorectal cancer'/de OR colonoscopy/de OR colonoscope/de OR colon/exp)) OR ((colo* NEAR/6 (capsule* OR microcapsule*) NEAR/3 endoscop*) OR ((capsule* OR microcapsule*) NEAR/3 colonoscop*) OR PillCam*):ab,ti,kw) AND ('screening'/de OR 'cancer screening'/de OR 'early cancer diagnosis'/de OR 'screening test'/de OR (screening OR (positive NEAR/6 (fit OR Fecal-Immunochem*)) OR (early NEAR/3 cancer NEAR/3 (diagnos* OR detect*))):ab,ti,kw) AND [english]/lim NOT ([animals]/lim NOT [humans]/lim)

Medline Ovid

(((Capsule Endoscopy/ OR Capsule Endoscopes/ OR Capsules/) AND (exp Colorectal Neoplasms/ OR Colonoscopy/ OR Colonoscopes/ OR exp Colon/)) OR ((colo* ADJ6 (capsule* OR microcapsule*) ADJ3 endoscop*) OR ((capsule* OR microcapsule*) ADJ3 colonoscop*) OR PillCam*).ab,ti,kf.) AND (Mass Screening/ OR Early Detection of Cancer/ OR (screening OR (positive ADJ6 (fit OR Fecal-Immunochem*)) OR (early ADJ3 cancer ADJ3 (diagnos* OR detect*))).ab,ti,kf.) AND english.la. NOT (exp animals/ NOT humans/)

Web of science

TS=((((colo* NEAR/5 (capsule* OR microcapsule*) NEAR/2 endoscop*) OR ((capsule* OR microcapsule*) NEAR/2 colonoscop*) OR PillCam*)) AND ((screening OR (positive NEAR/5 (fit OR Fecal-Immunochem*))OR (early NEAR/2 cancer NEAR/2 (diagnos* OR detect*)))) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline OR rabbit OR cow OR cows OR bovine OR rodent* OR sheep OR ovine OR pig OR swine OR porcine OR veterinar* OR chick* OR zebrafish* OR baboon* OR nonhuman* OR primate* OR cattle* OR goose OR geese OR duck OR macaque* OR avian* OR bird* OR fish*) NOT (human* OR patient* OR women OR woman OR men OR man))) AND DT=(Article OR Review OR Letter OR Early Access) AND LA=(english)

Cochrane CENTRAL

(((colo* NEAR/6 (capsule* OR microcapsule*) NEAR/3 endoscop*) OR ((capsule* OR microcapsule*) NEAR/3 colonoscop*) OR PillCam*):ab,ti,kw) AND ((screening OR (positive NEAR/6 (fit OR Fecal NEXT Immunochem*)) OR (early NEAR/3 cancer NEAR/3 (diagnos* OR detect*))):ab,ti,kw)

Supplementary table 1 PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3, 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n.a.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4, 5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4, 5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4, 5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4, 5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4, 5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4, 5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	-
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

Supplementary table 1 PRISMA checklist. *(continued)*

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	12
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n.a.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5, 20
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	16
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6, 17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	18
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	n.a.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n.a.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10, 11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	13

Supplementary table 2 Overview of the bowel preparation and booster regimen, adequate bowel preparation score and completion rate of 9 out of the 13 studies included.

Study	Bowel preparation and booster regimen		Colon cleanliness	Completion rate
Holleran(1)	Day -2	4 senna tablets 10 glasses of water	92%	73%
	Day -1	Liquid diet 4:00 pm: 2L PEG		
	Day 0	8:00 am: 2L PEG 8:45 am: Swallow capsule Small bowel detection: 250 ml bowel preparation 3 hours later: 250 ml bowel preparation 10:00 pm: if capsule nog passed: rectal bisacodyl suppository		
Kobaek-Larsen(2)	Day -2	Morning: 1000mg oral magnesium-oxide and 2L water Evening: 1000mg oral magnesium-oxide	85%	57%
	Day -1	Clear fluids diet Evening: 1L moviprep and 2L water		
	Day 0	8:00 am: 1L moviprep and 1L water 08:45 am: Swallow capsule + 20 mg oral domperidon Small bowel detection: 0.75L moviprep and 1L water 3 hours later: 0.25L moviprep and 0.25L water and 10 mg rectal bisacodyl		
Pecere(3)	Day -2	At least 10 glasses of water Bedtime: 4 senna tablets	88%	88%
	Day -1	Clear liquid diet 07:00-09:00 pm: 2L PEG		
	Day 0	05:00-07:00 am: 2L PEG 8-9am: capsule ingestion Small bowel detection: 40ml NaP* & 1L water and 50ml of gastrografin 3 hours later: 20ml NaP & 0.5L water and 30ml of gastrografin 2hrs after 2nd boost: 10 mg bisacodyl suppository		
Rodonotti(4)	Day -3	Low fibre diet	70%	90%
	Day -2	Low fibre diet		
	Day -1:	Clear liquid diet 5:00pm: macrogol 3350, 100 g + ascorbid acid 10.6g in 1L water + 1L water		
	Day 0	7 am: 10:00 pm: bisacodyl 5mgl; 4 tablets macrogol 3350, 100g + ascorbid acid 10.6 g in 1L water + 1L water 8:45 am: capsule ingestion + metoclopramide 10 mg + saline 100ml iv in 30 min Small bowel detection: Booster of Nap 30 ml + 1L water 90 min after small bowel detection: NaP 15ml + 500ml of water 1:00pm: light lunch		

Supplementary table 2 Overview of the bowel preparation and booster regimen, adequate bowel preparation score and completion rate of 9 out of the 13 studies included. (*continued*)

Study	Bowel preparation and booster regimen		Colon cleanliness	Completion rate
Groth(5)	Day -2	Low-residue diet	-	82%
	Day -1	Clear liquids only 19:00-21:00: 2L PEG		
	Day 0	07:00-08:00: 1L PEG 08:15 am: 6mg Tegaserod 08:30 am: capsule ingestion 10.30 am: 30ml NaP + 1L water 13:00 pm: 6 mg Tegaserod 14:00 pm: 15ml NaP + 0.5L water 16:30 pm: bisacodyl rectal suppository		
Rex(6)	Day -2	Bedtime: 4 senna tablets	80	92
	Day -1	Clear liquids only 19:00-21:00: 2L PEG-ELS		
	Day 0	07:00-09:00 am: 2L PEG-ELS morning: capsule ingestion Small bowel detection: 0.5L sulfate solution + 1L water 3 hours later: 0.25L sulfate solution + 0.5L water 2 hours later: 10 mg bisacodyl suppository		
Pioche(7)	Day -2	10 glasses of water 4L PEG	74	68
	Day -1	Liquid diet 3L PEG		
	Day 0	Morning: 1L PEG Swallow capsule + 20 mg domperidon Booster 1: 30ml NaP + 1L water Booster 2: 25ml NaP + 0.5L water 1 bisacodyl suppository		
Gonzalez-Suarez(8)	Day -2	Pursenid 4 tablets (senosids A+B)	82	81
	Day -1	Clear liquid diet 7-9 pm: 1 L PEG based solution		
	Day 0	7-8 am: 1 L PEG based solution 9:30 am: Metoclopramide 10 mg 9:45 am: capsule ingestion (water + simethicone 80 mg) 1st Booster: 500 mL PEG based solution + Gastrografin (50 mL) 2nd Booster (3 h after 1st booster): 500 mL PEG based solution + Gastrografin (25 mL) 5 h after 1st booster: Bisacodyl suppository		

Supplementary table 2 Overview of the bowel preparation and booster regimen, adequate bowel preparation score and completion rate of 9 out of the 13 studies included. (*continued*)

Study	Bowel preparation and booster regimen		Colon cleanliness	Completion rate
Voska(9)	Day -2	Low-residue diet Abundant liquids	90	90
	Day -1	All day: clear liquids 07:00-09:00 pm: 3L PEG		
	Day 0	07:00-08:30 am: 1L PEG 9:30 am: swallow capsule If capsule in the stomach > 1hour: 10 mg metoclopramide Booster 1: 30ml NaP + 1L water Booster 2: 25ml NaP + 0.5L water Suppository: Gycerin suppository 2g		

References

1. Holleran G, Leen R, O'Morain C, McNamara D. Colon capsule endoscopy as possible filter test for colonoscopy selection in a screening population with positive fecal immunology. Endoscopy. 2014;46(6):473-8.
2. Kobaek-Larsen M, Kroijer R, Dyrvig AK, Buijs MM, Steele RJC, Qvist N, et al. Back-to-back colon capsule endoscopy and optical colonoscopy in colorectal cancer screening individuals. Colorectal Dis. 2018;20(6):479-85.
3. Pecere S, Senore C, Hassan C, Riggi E, Segnan N, Pennazio M, et al. Accuracy of colon capsule endoscopy for advanced neoplasia. Gastrointest Endosc. 2020;91(2):406-14.e1.
4. Rondonotti E, Borghi C, Mandelli G, Radaelli F, Paggi S, Amato A, et al. Accuracy of Capsule Colonoscopy and Computed Tomographic Colonography in Individuals With Positive Results From the Fecal Occult Blood Test. Clin Gastroenterol Hepatol. 2014;12(8):1303-10.
5. Groth S, Krause H, Behrendt R, Hill H, Börner M, Bastürk M, et al. Capsule colonoscopy increases uptake of colorectal cancer screening. BMC Gastroenterol. 2012;12.
6. Rex DK, Adler SN, Aisenberg J, Burch WC, Carretero C, Chowers Y, et al. Accuracy of capsule colonoscopy in detecting colorectal polyps in a screening population. Gastroenterology. 2015;148(5):948-67.e2.
7. Pioche M, Ganne C, Gincul R, De Leusse A, Marsot J, Balique J, et al. Colon capsule versus computed tomography colonography for colorectal cancer screening in patients with positive fecal occult blood test who refuse colonoscopy: A randomized trial. Endoscopy. 2018;50(8):761-9.
8. Gonzalez-Suarez B, Pages M, Araujo IK, Romero C, Rodriguez de Miguel C, Ayuso JR, et al. Colon capsule endoscopy versus CT colonography in FIT-positive colorectal cancer screening subjects: a prospective randomised trial-the VICOCA study. BMC Med. 2020;18(1):255.
9. Voska M, Zavoral M, Grega T, Majek O, Martinek J, Tacheci I, et al. Accuracy of colon capsule endoscopy for colorectal neoplasia detection in individuals referred for a screening colonoscopy. Gastroenterol Res Pract. 2019.

Chapter 7

Applicability of colon capsule endoscopy as pan-endoscopy: From bowel preparation, transit- and rating times to completion rate and patient acceptance

F.E.R. Vuik, S. Moen, S.A.V. Nieuwenburg, E.H. Schreuders, E.J. Kuipers, M.C.W. Spaander

Endoscopy International Open, April 2021

Abstract

Introduction Colon capsule endoscopy (CCE) has the potential to explore the entire gastrointestinal (GI) tract. The aim of this study is to assess the applicability of CCE as pan-endoscopy.

Methods Healthy participants received CCE with bowel preparation (bisacodyl, polyethylene electrolyte glycol (PEG)+ascorbic acid) and booster regimen (metoclopramide, oral sulphate solution (OSS)). For each segment of the GI tract, the following quality parameters were assessed: cleanliness, transit times, reading times, patient acceptance and safety of the procedure. When all GI segments had cleansing score good or excellent, cleanliness of the whole GI tract was assessed as good. Participants' expected and perceived burden was assessed by questionnaires and participants were asked to grade the procedure (scale 0-10). All serious adverse events (SAE) were documented.

Results A total of 451 CCE procedures were analysed. A good cleansing score was achieved in the stomach in 69.6%, in the SB in 99.1% and in the colon in 76.6%. Cleanliness of the whole GI tract was good in 52.8% of the participants. CCE median transit time of the whole GI tract was 583 minutes IQR 303-659). The capsule reached the descending colon in 94.7%. Median reading time per procedure was 70 minutes (IQR 57-83). Participants graded the procedure with a 7.8. There were no procedure-related SAEs.

Conclusion CCE as pan-endoscopy has shown to be a safe procedure with good patient acceptance. When cleanliness of all GI segments per patient, completion rate and reading time will be improved, CCE can be applied as a good non-invasive alternative to evaluate the GI tract.

Introduction

Colon Capsule Endoscopy (CCE) is a non-invasive technique to explore the colon mucosa using an ingestible, wireless and disposable capsule (1). Many studies showed that CCE has a good diagnostic value for abnormalities such as polyps and colorectal carcinomas (2, 3). Therefore, CCE could be used when colonoscopy is not possible or incomplete (4, 5). However, CCE provides images of the entire gastrointestinal (GI) tract and therefore has the potential to be used as a diagnostic tool for all GI mucosal pathology (6).

Despite its non-invasive character and its potential to explore the entire GI tract, implementation of CCE as pan-endoscopy has not yet been achieved. The diagnostic accuracy of CCE as pan-endoscopy is highly dependent on several quality parameters such as bowel preparation scores, transit times and capsule completion rate. Optimal stomach and bowel preparation is needed for high quality CCE images. However, current preparation protocols have led to contradictory results and there is no consensus on which bowel preparation schedule has the best results (7, 8). Moreover, in order to obtain images from the entire GI tract, the capsule needs to be excreted within the battery life (9). On the other hand, transit times should not be too fast, because lesions of the GI tract may then be missed.

The applicability of CCE is also highly dependent on other factors such as the workability for the staff, patient acceptance and safety of the procedure. Evaluation of the images can be time consuming and training is necessary to adequately review the images of the GI tract (10).

CCE provides a non-invasive alternative and is associated with significantly less discomfort compared to conventional endoscopy (11). However, the large volume of bowel preparation can be a challenge for patients and when CCE is positive patients still need to undergo an endoscopy (9). Finally, the implementation of a certain diagnostic tool can only expand when the procedure is safe. CCE has shown to be a safe procedure with few described serious events so far, although patients with obstructive symptoms should be treated with care (1).

In this study, different quality parameters of CCE for each GI segment and participants preferences about the CCE procedure were evaluated in order to investigate the applicability of CCE as pan-endoscopy.

Method

Participants

Asymptomatic participants 50-75 years of age who underwent CCE were included (12). People participating in the Rotterdam Study were eligible to participate in this study if aged between 50-75 years old and able to give informed consent. Participants were excluded when meeting one of the following conditions: 1) unable or unwilling to sign written informed consent, 2) severe or terminal disease with a life expectancy less than 5 years, 3) allergy or known contraindication to the medications used in this study, 4) chronic heart failure New York Heart Association III or IV, 5) severe kidney insufficiency (Glomerular filtration rate<30ml/min/1.73m3), 6) dysphagia or swallowing disorder, 7) increased risk for capsule retention (M. Crohn, prior abdominal surgery likely to cause bowel obstruction), 8) pacemaker or other implantable cardiac defibrillator, 9) Magnetic resonance imaging (MRI) scheduled within 14 days after ingestion of the capsule, 10) risk of congenital extended QT syndrome and/or medication known to extend the QT interval 11) diabetes mellitus with use of insulin.

The study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC-2015-453, date of approval: 26-04-2016). The protocol was registered in the Netherlands National Trial Register (NTR; NTR6321, registration date: 23-11-2016). All participants signed written informed consent before participation in the study.

Colon capsule endoscopy

The second generation colon capsule (PillCam™ COLON 2, Medtronic) was used. The ingestion of the capsule usually took place at 9 a.m. in the presence of a physician. A sensor belt was attached to the participant before ingesting the colon capsule. The sensor belt receives transmission data from the colon capsule. After ingesting the capsule, participants went home again. The belt was taken off by participants at 8 p.m. or earlier when the capsule had already left the body.

The participants received bowel preparation consisting of 5mg bisacodyl, 2L polyethylene electrolyte glycol (PEG+asc) (Moviprep; Norgine, Amsterdam, the Netherlands) and 2L water, both split-dose. They received a booster regimen with 10mg metoclopramide and 0,5L oral sulphate solution (OSS) (Eziclen, Zambon, the Netherlands) – in split dose 0,25L directly after small bowel recognition and 0,25L three hours after small bowel recognition (for detailed bowel preparation scheme see Supplementary Table 1).

Before starting with this trial, a pilot study was performed to compare two types of booster: PEG+asc or OSS. Cleansing scores were similar, but due to a higher completion rate for OSS, this booster was chosen for the conduct of this study (see supplementary section).

Quality parameters

For each part of the GI tract, the following quality parameters were assessed: cleanliness, transit times, reading times, patient acceptance and safety of the procedure.

Cleanliness

Cleansing of the stomach, small bowel and colon was graded according to three different grading scales (Table 1). Stomach cleansing was measured by the proportion of visualized mucosa (<70% poor, 70-90% fair, >90% good) (13). Small bowel cleansing was measured by the proportion of visualized mucosa (<25% poor, 25-50% fair, 50-75% good, >75% excellent) and degree of bubbles, debris and bile (>50% poor, 25-50% moderate, 5-25% good, <5% excellent) (14). Colon cleansing was measured by cleansing level (poor, fair, good, excellent) and the bubbles effect scale (interference of bubbles in examination defined as insignificant or significant) (15). The quality of colon cleanliness was evaluated for each segment of the colon: caecum, ascending colon, transverse colon, descending colon and rectum and an overall colon-cleansing grade was assessed using the same grading system. An overall score for cleanliness of the entire GI tract was defined "good" when both stomach cleansing was good and small bowel cleansing as well as colon cleansing were either good or excellent.

Transit times

For each CCE procedure the overall completion rate was evaluated and the transit times were calculated for the stomach, small bowel and colon separately by Rapid™ Software v7.0 (Medtronic, Minneapolis, MN, USA). Oesophageal transit time is usually so fast that only a few images of the oesophagus can be obtained. Therefore, for the oesophagus, Z-line objectification was evaluated, which is a commonly used marker for distal oesophageal mucosa visualization in capsule endoscopy (16).

Table 1 Definition of the cleansing grading scales of the stomach, small bowel and colon

Gastric grading scale	
Poor	<70% of the mucosa was observed
Fair	70-90% of the mucosa was observed
Good	>90% of the mucosa was observed
Small bowel grading scale	
Proportion of visualized mucosa	
Poor	<25%
Fair	25-50%
Good	50-75%
Excellent	>75%
The degree of bubbles, debris and bile	
Poor	>50%, severe obscuration
Fair	25-50%, moderate obscuration
Good	5-25%, mild obscuration
Excellent	<5%, no obscuration
Colon grading scale	
Cleansing level grading scale	
Poor	Large amount of faecal residue precluding a complete examination
Fair	Enough faeces or dark fluid present to prevent a reliable exam
Good	Small amount of faeces or dark fluid not interfering with examination
Excellent	No more than small bits of adherent faeces
Bubbles interfering effect scale	
Insignificant	No bubbles/content/blurry images or so that they do not interfere with the examination. Less than 10% of surface area is obscured
Significant	Bubbles/content/blurry images that interfere with the examination More than 10% of surface area is obscured

Reading times by the staff

CCE reading and evaluation was performed by one gastroenterologist, three medical doctors and one endoscopy nurse. The oesophagus was observed by scrolling manually through the images. To observe the mucosa of the stomach and small bowel, both sides of the colon capsule were used at the same time. The images were viewed at a rate that was comfortable for the reviewer, with an average speed of around 10 images per second. The detailed procedure of CCE reading for the colon has been described elsewhere (7). In short, reading the images of the colon was divided into 3 phases. A preview phase, in which both sides of the capsule were viewed simultaneously with a high speed to capture landmarks. A review phase which consisted of careful assessment and capture of all the relevant findings. And a report phase in which the findings were evaluated and described. For each part of the GI tract, the median reading time by the staff was evaluated. The reading time per procedure was also determined.

Patient acceptance

Participants were asked to fill in two questionnaires, one regarding their expectations (filled in prior to the CCE procedure) and one regarding their evaluation of CCE (filled in after the procedure). Participants were asked to grade the procedure on a scale from 0 to 10. They were also asked to grade their expected and perceived burden on a five point Likert scale (not at all, just a bit, a little, fairly, strongly). Questions on different aspects of burden (overall burden, pain and shame) of both the bowel preparation and CCE procedure itself were included in the questionnaires. Specific causes of burden were further evaluated, namely the swallowing of the capsule, more stomach ache than usual, hindrance in daily activities and trouble sleeping. Burden of swallowing the capsule and more stomach ache than usual were graded as either present or not present. Hindrance in daily activities was graded as present or not present, and was evaluated for both the day prior to the procedure, the whole procedure day and the day after the procedure. Finally, trouble sleeping was graded as present or not present, and was evaluated for both the night before the procedure and the night after the procedure.

Safety of the procedure

Safety of the procedure was measured by the number of (serious) adverse events.

Statistical analysis

Quality scores were presented as mean with standard deviation (SD) or medians with interquartile range (IQR). For differences between proportions of categorical variables the X^2-test was used. For all tests a significance level of 0.05 was used. Analyses were performed in IBM SPSS v.24.

Results

A total of 451 CCE procedures were included, 46.1% were performed in men with a mean age (SD) of 66.8 (4.8) years.

Cleanliness

Bisacodyl was taken in 99.3% and complete PEG+asc intake was achieved in 98.4% of the participants. Intake of OSS was reported in 373 participants (82.3%) and complete intake was achieved in 93.6% of the participants. Cleansing of the mucosal surface in the whole GI tract was adequate in 52.8% of the participants. When analysing the cleanliness of the mucosa per segment, the proportion of visualized stomach mucosa was good (>90%) in 69.6%. In the small bowel, both the proportion of visualized mucosa as the proportion of bubbles, debris and bile were good or excellent in 99.1%. The colon cleansing score

was good or excellent in 76.6% and the bubbles effect scale was insignificant in 74.6%. Cleansing scores per segment are listed in Table 2.

Table 2 Cleansing scores of stomach, small bowel and colon N = number of videos, SB = small bowel

Stomach cleansing – proportion of visualized mucosa (N=437)						
Poor					20 (4.6)	
Fair					113 (25.9)	
Good					304 (69.6)	
SB cleansing – proportion of visualized mucosa (N=446)						
Poor					0 (0)	
Fair					4 (0.9)	
Good					75 (16.8)	
Excellent					367 (82.3)	
SB cleansing – proportion of debris, bile and bubbles (N=446)						
Poor					0 (0)	
Fair					4 (0.9)	
Good					86 (19.3)	
Excellent					356 (79.8)	
Colon – cleansing level grading scale						
Cleansing	Cecum, n= 449	Ascending, N=443	Transverse, n= 434	Descending, n= 427	Rectum, n= 249	Overall, n=449
Poor	32 (7.1)	26 (5.9)	26 (6.0)	27 (6.3)	19 (7.6)	29 (6.5)
Fair	87 (19.4)	68 (15.3)	69 (15.9)	72 (16.9)	56 (22.5)	76 (16.9)
Good	231 (51.4)	238 (53.7)	236 (54.4)	245 (57.4)	146 (58.6)	257 (57.2)
Excellent	99 (22.0)	111 (25.1)	103 (23.7)	83 (19.4)	28 (11.2)	87 (19.4)
Colon – bubbles interfering effect scale						
	Cecum, n=449	Ascending, n=443	Transverse, n=434	Descending, n=427	Rectum, n=249	Overall, n=449
Insignificant	436 (97.1)	418 (94.4)	375 (86.4)	365 (85.5)	240 (96.4)	335 (74.6)
Significant	13 (2.9)	25 (5.6)	59 (13.6)	62 (14.5)	9 (3.6)	114 (25.4)

Transit times

The completion rate of the colon capsule was 51.9%. In 99.6% of the participants, the capsule reached the cecum, in 98% the ascending colon, in 96% the transverse colon, in 94.7% the descending colon and in 55.4% the rectum. Thirteen participants (2.8%) doubted if the capsule was excreted and an abdominal X-ray was performed. In all participants the capsule was excreted and therefore not visualized on X-ray. CCE median transit time of the whole GI tract was 583 minutes (IQR 303-659). Oesophageal visualization consisted of just a few images, and therefore a median transit time could not be adequately measured. Z-line objectification was achieved in 44.8%. CCE median transit

time was 55 minutes (IQR 40-92) in the stomach, 47 minutes (IQR 29-78) in the small bowel and 392 (IQR 191-528) minutes in the colon (Table 3).

Table 3 Completion rate, transit times and reading time of colon capsule endoscopy (CCE) N=number; IQR = interquartile range

Total number of procedures	**451**
Quality indicators	
Completion rate, n (%)	231 (51.9)
Transit times	
	Median time (min), (IQR)
Period of time CCE in whole GI tract	583 (303-659)
Period of time CCE in stomach	55 (40-92)
Period of time CCE in small bowel	47 (29-78)
Period of time CCE in colon	392 (191-528)
Reading times by the staff	
	Median time (min), (IQR)
Whole GI tract	70 (57-83)
Stomach	3 (2-5)
Small bowel	10 (8-15)
Colon	55 (43-65)

Reading times by the staff

Median time to review one complete CCE procedure was 70 minutes (IQR 57-83). When analysed per GI segment, median reading time needed was 3 minutes for the gastric mucosa (IQR 2-5)), 10 minutes (IQR 8-15) for the small bowel mucosa and 55 minutes (IQR 43-65) for the colonic mucosa.

Patient acceptance

From a total of 451 participants, 396 participants (87.8%) filled in the first questionnaire prior to the procedure regarding their expectations and 395 participants (87.6%) filled in the second questionnaire after completing the procedure regarding their experience with CCE.

Participants graded the overall CCE procedure with an average of 7.8. Of all participants, 91.1% would consider to undergo CCE again. Only 6.6% of the participants would advise others against CCE. Most participants (89.2%) experienced bowel preparation as the most burdensome part of the CCE procedure, the other participants considered the day of the CCE procedure (8%) or stomach complaints after the procedure (3%) to be the most burdensome part of the procedure.

Regarding the overall burden of the bowel preparation, participants described the bowel preparation as little burdensome in 22.6%, fairly burdensome in 19.8% and strongly burdensome in 6.4%, which was roughly similar to their expectations (Figure 1). Only 15.8% experienced no burden at all from the bowel preparation. Regarding the overall burden of the day of the CCE procedure itself, participants rated the day of the procedure as little burdensome in 21%, fairly burdensome in 12.2% and strongly burdensome in 2.8%. The experienced burden was higher than expected, since participants expected the day of the procedure to be little burdensome in 17.2%, fairly burdensome in 6.2% and strongly burdensome in 0.3%. Participants did not experience a lot of shame or pain from the bowel preparation and the CCE procedure, which was roughly similar to their expectations prior to the procedure.

For the specific causes of burden: swallowing of the capsule was not found burdensome in 89.3% of the participants. More stomach ache than usual was experienced in only 11.2% of the participants. The majority of participants (58.9%) experienced hindrance in daily activities the day of the CCE procedure itself, 40.4% of the participants had hindrance in daily activities in the day prior to the procedure and 12.4% experienced hindrance in the day after the procedure. Only a few participants had trouble sleeping: 28.2% of the participants the night before the procedure and 8.4% the night after the procedure.

Safety of the procedure

A procedure-related adverse event occurred in 19 participants (4.1%). The reported adverse events were: nausea (1.9%), abdominal pain (0.6%), general malaise (0.6%), headache (0.6%) and vomiting (0.4%). All adverse events were mild and were the result of ingestion of the bowel preparation.

One non-procedure-related serious adverse event occurred in a participant who already had melena a few days before ingesting the colon capsule. In the afternoon after ingestion of the colon capsule, the participant had melena again and was admitted to the hospital. Upper endoscopy was performed and a Mallory Weiss lesion was found as cause of the bleeding.

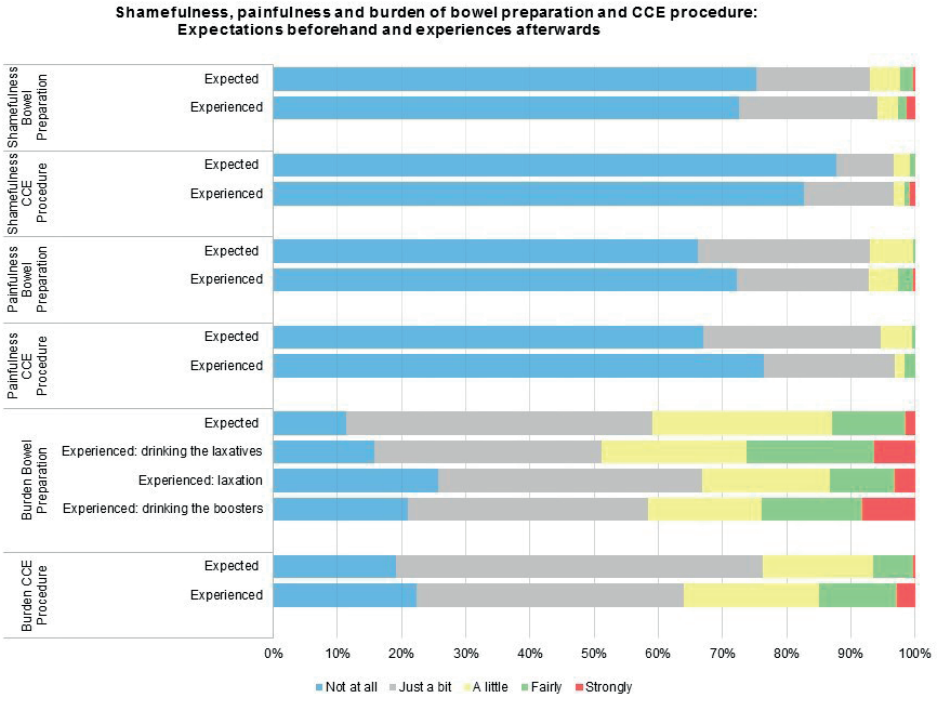


Figure 1: Shamefulness, painfulness and burden of bowel preparation and CCE procedure: expectation beforehand and experiences afterwards

Discussion

This study is the first to investigate the use of CCE as pan-endoscopy in a large population. We conclude that CCE is a safe method with good patient acceptance. Although cleanliness of each GI segment, stomach, small bowel and colon were good or excellent, the overall cleanliness score per patient was low. Only half of the patient had an overall cleanliness score of at least 'good'. In order to use CCE as pan-endoscopy for daily practice, improvement of cleanliness of all segments per patient, a higher completion rate and solutions to shorten the extensive reading time are warranted.

Using CCE to visualize the mucosa of the GI tract has many advantages: it is a non-invasive procedure, without subjection to radiation and sedation, the procedure can be done at home, it can avert endoscopy when no lesions are present and when a lesion is detected therapeutic endoscopy can directly focus on the lesion found (17). In patients with occult blood loss or unexplained complaints it is a good method to observe the entire GI tract without using multiple invasive methods such a upper endoscopy, double balloon endoscopy or colonoscopy. Therefore, it is a promising diagnostic instrument.

However, before introducing CCE as pan-endoscopy it is necessary to discuss the quality measures of CCE as pan-endoscopy.

First, the cleanliness of the whole GI tract was good in 52.8% of the participants, which means that all segments of the GI tract had cleansing score good or excellent. To the extent of our knowledge, this is the first cleanliness score developed to score the whole GI tract. The whole GI tract cleansing score 'good' was lower compared to each separate GI segment. This is caused by the alternately fair and poor cleansing scores of the stomach and colon and shows that the whole GI tract cleansing score gives an additional insight in the cleanliness of the GI tract when CCE is used as pan-endoscopy. The high adequate cleanliness score of the small bowel (99.1%) was notable, which could be explained by the large amount of bowel preparation. The European Society of Gastrointestinal Endoscopy recommended in their guideline to ingest 2L PEG before small bowel capsule endoscopy (18). Our bowel preparation consisted of a period of fasting from solid food, 2L PEG+asc and 2L water split dose. Colon cleansing score was comparable to other studies using the same bowel preparation (19).

Second, the median transit time showed a great variation between the different segments. The Z-line was only observed in 44.8% of the participants. The Z-line objectification is dependent on both cleanliness and transit time. Participants received extensive bowel preparation to facilitate colonic evaluation and in most participants only a few images of the oesophagus were retrieved, indicating transit time in the oesophagus was too fast. For the stomach it is well known that the fundus cannot be well observed when using a passive capsule that is propelled only by gastric motility. Therefore, a magnetically guided capsule endoscope has been designed to explore the stomach (20, 21). Furthermore, the small bowel transit time (47 minutes (IQR 29-78)) was faster than expected based on the literature. A recent study using the Pillcam SB3 (small bowel) capsule found a median small bowel transit time of 198.5 minutes (22). In another study CCE was used to evaluate the small bowel, and showed a small bowel transit time of 61 minutes (23). Yet, the optimal transit time is dependent on the purpose of the examination. For example, when the purpose is to specifically examine the small bowel only, a longer transit time may be warranted, while in case of screening for lesions in the GI tract e.g. to search for causes of anemia transit time may be accelerated. Though, to use CCE as pan-endoscopy, a fast small bowel transit contributes to a higher completion rate.

In our study the fast small bowel transit time did not result in an acceptable completion rate, which was only 51.9% and is lower compared to other studies (19, 24). The reason for the low completion rate was a long median colonic transit time of 392 minutes (IQR 191-528). In other studies the median colonic transit time was 6 and 244 minutes (24,

25). Those studies used a 4L PEG split dose regimen. It is likely that our bowel preparation or booster regimen was not sufficient enough to boost the capsule to the anal verge. Sodium phosphate (NaP) was a key component of the bowel preparation for colon capsule for a long time and is used in many trials as a booster (17). However, NaP can potentially lead to serious adverse events like acute renal failure and mineral imbalance and therefore its use is prohibited in some countries (25, 26). Even though sulphate solutions have shown to be a good alternative, we showed that in a large population study the completion rate is low (27). Alternatives are needed in order to make CCE an interesting instrument for pan-endoscopy. Besides achieving a higher completion rate, an alternative option for bowel preparation should also take into account that a colonic transit time below 40 minutes is defined as a technically inadequate study (24).

Third, our study showed that CCE was a safe procedure with good patient acceptance. Participants graded colon capsule with a 7.8 and 91.1% would consider to undergo CCE again in the future. Our results were comparable to a study comparing the experiences of screened individuals undergoing both colonoscopy and CCE (11). They found that 88.5% of the screened individuals had a low level of discomfort using CCE versus 35.2% when undergoing colonoscopy. A recent study assessed patient tolerance and acceptance of three colonic imaging modalities: colonoscopy, CCE and CTC (28). This study showed that the willingness to undergo the same test was high for all three types of colonic imaging: 93.6% for colonoscopy; 96.1% for CTC and 85.7% for CCE. Fourth, reviewing the images of the entire GI tract is time consuming. A solution for using CCE as pan-endoscopy in the most time efficient way is when artificial intelligence (AI) would review the images and highlight abnormalities. Multiple deep learning based approaches for CCE have been developed which resulted in a higher accuracy and sensitivity. More CCE video databases are needed to develop precise machine learning methods and prospective trials are needed to verify the accuracy of the developed software (29).

This study gives an overview of the applicability of CCE as pan-endoscopy. It was conducted in a large population of healthy participants. There was a high compliance with both the ingestion of the bowel preparation and boosters and filling in the questionnaires.

This study has several limitations to address. First, the included participants in this trial were from a relatively elderly population. Aging may slow down colonic transit time, which could have had an impact on the transit times of the colon capsule, resulting in a lower completion rate compared to earlier studies using the same bowel preparation (30). However, evidence on this matter is scarce. Several studies did not show a slower colonic transit time in the elderly but did show a delayed gastric emptying in this popula-

tion (31, 32). Second, the participants included in this study were from a selected group of participants that were willing to undergo CCE. Therefore, patient acceptance may be higher than when CCE is used for clinical purposes. Third, not all participants filled in the questionnaires, which may have influenced the outcomes. However, from a total of 451 participants, 396 participants filled in the first questionnaire and 395 participants filled in the second questionnaire, still resulting in 88% compliance, which is an acceptable response rate (over 75%) for surveys (33).

To conclude, the current advanced features of the colon capsule make it possible to use CCE as an instrument for pan-endoscopy. CCE has proven to be a safe procedure with good patient acceptance. When technical and procedural issues will be resolved and especially when AI technique advances, CCE as pan-endoscopy will be a good non-invasive alternative to the current (invasive) diagnostic methods to evaluate the GI tract.

References

1. Pasha SF. Applications of Colon Capsule Endoscopy. Curr Gastroenterol Rep 2018; 20: 22
2. Spada C, Pasha SF, Gross SA et al. Accuracy of First- and Second-Generation Colon Capsules in Endoscopic Detection of Colorectal Polyps: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2016; 14: 1533-1543 e1538
3. Kjølhede T, Ølholm AM, Kaalby L et al. Diagnostic accuracy of capsule endoscopy compared with colonoscopy for polyp detection: systematic review and meta-analyses. Endoscopy 2020.
4. Deding U, Kaalby L, Bøggild H et al. Colon Capsule Endoscopy vs. CT Colonography Following Incomplete Colonoscopy: A Systematic Review with Meta-Analysis. Cancers (Basel) 2020; 12:
5. Spada C, Hassan C, Bellini D et al. Imaging alternatives to colonoscopy: CT colonography and colon capsule. European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) Guideline - Update 2020. Endoscopy 2020; 52: 1127-1141
6. Cortegoso Valdivia P, Elosua A, Houdeville C et al. Clinical feasibility of panintestinal (or panenteric) capsule endoscopy: a systematic review. European Journal of Gastroenterology & Hepatology 2021; Publish Ahead of Print:
7. Spada C, Hassan C, Sturniolo GC et al. Literature review and recommendations for clinical application of Colon Capsule Endoscopy. Dig Liver Dis 2011; 43: 251-258
8. Singhal S, Nigar S, Paleti V et al. Bowel preparation regimens for colon capsule endoscopy: a review. Therap Adv Gastroenterol 2014; 7: 115-122
9. Hong SN, Kang SH, Jang HJ et al. Recent Advance in Colon Capsule Endoscopy: What's New? Clin Endosc 2018; 51: 334-343
10. Koulaouzidis A, Dabos K, Philipper M et al. How should we do colon capsule endoscopy reading: a practical guide. Ther Adv Gastrointest Endosc 2021; 14:
11. Thygesen MK, Baatrup G, Petersen C et al. Screening individuals' experiences of colonoscopy and colon capsule endoscopy; a mixed methods study. Acta Oncol 2019; 58: S71-S76
12. Vuik FER, Nieuwenburg SAV, Moen S et al. Population-Based Prevalence of Gastrointestinal Abnormalities at Colon Capsule Endoscopy. Clin Gastroenterol Hepatol 2020.
13. Liao Z, Duan XD, Xin L et al. Feasibility and safety of magnetic-controlled capsule endoscopy system in examination of human stomach: a pilot study in healthy volunteers. J Interv Gastroenterol 2012; 2: 155-160
14. Park SC, Keum B, Hyun JJ et al. A novel cleansing score system for capsule endoscopy. World J Gastroenterol 2010; 16: 875-880
15. Leighton JA, Rex DK. A grading scale to evaluate colon cleansing for the PillCam COLON capsule: a reliability study. Endoscopy 2011; 43: 123-127
16. Park J, Cho YK, Kim JH. Current and Future Use of Esophageal Capsule Endoscopy. Clin Endosc 2018; 51: 317-322
17. Spada C, Hassan C, Galmiche JP et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy 2012; 44: 527-536
18. Rondonotti E, Spada C, Adler S et al. Small-bowel capsule endoscopy and device-assisted enteroscopy for diagnosis and treatment of small-bowel disorders: European Society of Gastrointestinal Endoscopy (ESGE) Technical Review. Endoscopy 2018; 50: 423-446
19. Rondonotti E, Borghi C, Mandelli G et al. Accuracy of capsule colonoscopy and computed tomographic colonography in individuals with positive results from the fecal occult blood test. Clin Gastroenterol Hepatol 2014; 12: 1303-1310

20. Rey JF, Ogata H, Hosoe N et al. Blinded nonrandomized comparative study of gastric examination with a magnetically guided capsule endoscope and standard videoendoscope. Gastrointest Endosc 2012; 75: 373-381
21. Hosoe N, Naganuma M, Ogata H. Current status of capsule endoscopy through a whole digestive tract. Dig Endosc 2015; 27: 205-215
22. O'Grady J, Murphy CL, Barry L et al. Defining gastrointestinal transit time using video capsule endoscopy: a study of healthy subjects. Endosc Int Open 2020; 8: E396-E400
23. Sato J, Nakamura M, Watanabe O et al. Prospective study of factors important to achieve observation of the entire colon on colon capsule endoscopy. Therap Adv Gastroenterol 2017; 10: 20-31
24. Rex DK, Adler SN, Aisenberg J et al. Accuracy of capsule colonoscopy in detecting colorectal polyps in a screening population. Gastroenterology 2015; 148: 948-957 e942
25. Pecere S, Senore C, Hassan C et al. Accuracy of colon capsule endoscopy for advanced neoplasia. Gastrointest Endosc 2020; 91: 406-414 e401
26. Markowitz GS, Nasr SH, Klein P et al. Renal failure due to acute nephrocalcinosis following oral sodium phosphate bowel cleansing. Hum Pathol 2004; 35: 675-684
27. Kroijer R, Dyrvig AK, Kobaek-Larsen M et al. Booster medication to achieve capsule excretion in colon capsule endoscopy: a randomized controlled trial of three regimens. Endosc Int Open 2018; 6: E1363-E1368
28. Ojidu H, Palmer H, Lewandowski J et al. Patient tolerance and acceptance of different colonic imaging modalities: an observational cohort study. Eur J Gastroenterol Hepatol 2018; 30: 520-525
29. Hwang Y, Park J, Lim YJ et al. Application of Artificial Intelligence in Capsule Endoscopy: Where Are We Now? Clin Endosc 2018; 51: 547-551
30. Madsen JL, Graff J. Effects of ageing on gastrointestinal motor function. Age Ageing 2004; 33: 154-159
31. Brogna A, Ferrara R, Bucceri AM et al. Influence of aging on gastrointestinal transit time. An ultrasonographic and radiologic study. Invest Radiol 1999; 34: 357-359
32. O'Mahony D, O'Leary P, Quigley EM. Aging and intestinal motility: a review of factors that affect intestinal motility in the aged. Drugs Aging 2002; 19: 515-527
33. Draugalis JR, Coons SJ, Plaza CM. Best practices for survey research reports: a synopsis for authors and reviewers. Am J Pharm Educ 2008; 72: 11

Supplementary file

Supplementary methods

Two types of boosters were compared in a pilot study in 27 patients: polyethylene glycol solution (PEG) plus ascorbic acid and oral sulphate solution (OSS). No difference was found in the cleansing score of the colon, with an adequate cleansing score of 77.5% with PEG and 71.4% with OSS (p=0.438). However, the completion rate was 35.7% with PEG versus 50.0% with OSS (p=0.533).

Table

Supplementary Table 1 Bowel preparation schedule for colon capsule endoscopy

Day	Time	Bowel preparation and booster
Day -2	8 p.m.	1 bisacodyl 5 mg tablet
Day 1		Light breakfast + lunch
	13 p.m.	Clear liquid diet
	18 – 20 p.m.	1L PEG + 1L clear liquid diet
Day 0	06 – 08 a.m.	1L PEG + 1L clear liquid diet
	~ 9 a.m.	Ingestion capsule
	1 hour after ingestion capsule	1 metoclopramide 10mg tablet *(only if capsule is still in stomach)*
	Small bowel detection	250ml OSS + 0.5L clear liquid diet
	3 hours after small bowel detection	250ml OSS + 0.5L clear liquid diet
	8 p.m.	Sensor belt removed by participant

Chapter 8

Predictors of gastrointestinal transit times in colon capsule endoscopy

S. Moen**, F.E.R. Vuik**, T. Voortman, E.J. Kuipers, M.C.W. Spaander

Clinical and Translational Gastroenterology, June 2022

Abstract

Introduction Optimizing the accuracy of Colon Capsule Endoscopy (CCE) requires high completion rates. To prevent incomplete CCE, we aimed to identify predictors associated with slow CCE transit times.

Methods In this population-based study, participants received CCE with split-dose PEG bowel preparation and booster regimen (0.5L oral sulfate solution and 10mg metoclopramide if capsule remained in stomach > 1 hour). The following predictors were assessed: age, gender, body mass index (BMI), smoking, coffee and fiber intake, diet quality, physical activity, dyspeptic complaints, stool pattern, history of abdominal surgery, medication use and CCE findings. Multivariable logistic and linear regressions with backward elimination were performed.

Results 451 CCE procedures with a completion rate of 51.9% were analyzed. Completion rate was higher among older participants (OR 1.54, 95% CI 1.04-2.28, p=0.03) and participants with changed stool pattern (OR 2.27, 95% CI 1.20-4.30, p=0.01). Participants with history of abdominal surgery had a lower completion rate (OR 0.54, 95% CI 0.36-0.80, p=0.003). Participants with higher BMI had faster stomach, small bowel (SB) and total transit times (β=-0.10, p=0.01; β=-0.14, p=0.001; β=-0.12, p=0.01). A faster SB transit was found in participants with changed stool pattern (β=-0.08, p=0.049) and use of metoclopramide (β=-0.14, p=0.001). Participants with high fiber intake had a slower colonic transit (β=0.11, p=0.03).

Conclusion Younger age, unchanged stool pattern, history of abdominal surgery, low BMI and high fiber intake resulted in slower CCE transit times and lower completion rates. In future practice, these factors can be considered to adjust preparation protocols.

Introduction

Colon Capsule Endoscopy (CCE) provides a non-invasive technique that enables exploration of the colon without the need for sedation nor gas insufflation. Despite the framework for potential clinical indications that was provided by the European Society of Gastrointestinal Endoscopy (ESGE) and the U.S. Food and Drug Administration (FDA), standardized use of CCE in daily practice is still limited (1-3).

CCE accurately detects various colonic abnormalities such as colorectal polyps and colorectal cancer (4-6). However, its accuracy highly depends on optimal bowel preparation to allow adequate visualization of the colonic mucosa and on capsule transit time (1, 7). In order to obtain images of the entire colon, the optimal capsule transit time has to be fast enough to achieve completion within the battery time but not so fast that lesions may be missed. CCE has a flexible frame rate of 4-35 images per second that adapts automatically based on the capsule speed (4). However, since the capsule is not equipped to actively move forward, capsule progression needs to be stimulated to achieve excretion within the battery time. This requires booster medication on the day of the capsule endoscopy in addition to the bowel preparation. Many studies have been performed in order to determine the optimal boosters for CCE, but completion rates still vary widely (6, 8-10).

The wide variation in CCE completion rate and transit times is not completely understood. Several factors that are known to influence the physiological GI transit times might have an impact on CCE transit as well. Aging may delay gastric emptying or colonic transit time and men have a faster transit than women (11-14). Different lifestyle associated factors also affect GI transit times such as body mass index (BMI), exercise level, smoking and coffee intake (15-17). Literature on factors that specifically influence transit times in CCE is scarce. One study identified a BMI above twenty-five and the absence of constipation as CCE transit time accelerating factors (18). Another study concluded that coffee and chewing gum did not improve the CCE completion rate (19).

In order to optimize CCE transit times, more knowledge is needed on which factors can predict the CCE speed through the different segments of the GI tract. In future practice, such factors could be used to anticipate capsule transit times and possibly adapt the preparation protocol for certain patient groups. The aim of this study was to identify possible predictors for CCE transit times in a prospective population-based cohort.

Methods

Participants

This study was embedded in the Rotterdam Study, an ongoing prospective population-based cohort study in Rotterdam, the Netherlands (20). A subset of participants with ages ranging from 50-75 years underwent CCE, as described in more detail elsewhere (21). The study was approved by the Institutional Review Board of Erasmus MC (registration number MEC-2015-453). The protocol was registered in the Netherlands National Trial Register (NTR; NTR6321). All participants signed written informed consent before participation in the study.

Colon Capsule Endoscopy

The second- generation colon capsule (Medtronic, Minneapolis, MN, USA) was used. The ingestion of the capsule usually took place at 9 a.m. in the presence of a physician. A sensor belt was provided which received transmission data from the capsule and sent the images to the corresponding recorder. The belt was taken off by the participants at 8 p.m. or earlier if the capsule had left the body.

Prior to the ingestion of the capsules, the participants received bowel preparation consisting of 5mg bisacodyl, 2L poly-ethylene glycol with ascorbic acid (Moviprep; Norgine, Amsterdam, the Netherlands) and 2L water, both split-dose. After ingestion of the capsule, the participants received a booster regimen. When the capsule remained in the stomach for longer than 1 hour, an alarm went off and participants were instructed to take 10mg metoclopramide. After small bowel recognition another alarm went off and participants were instructed to take 0.25L oral sulfate solution (OSS; Eziclen, Zambon, the Netherlands) and 3 hours after small bowel recognition they had to take another 0.25L OSS.

Predictors of CCE transit times

For each CCE video, segmental transit times were calculated for the stomach, small bowel and colon by Rapid Software v8.0 (Medtronic, Minneapolis, MN, USA). The procedure was classified as "complete" when the capsule observed the anal verge. Possible transit time predictors were obtained through questionnaires and included patient characteristics, relevant symptoms, relevant medical history, relevant medication, CCE procedure-related factors and CCE findings.

Patient characteristics

Patient characteristics that were used as possible transit time predictors were age, gender, BMI, smoking status, habitual coffee and fiber intake, diet quality and physical activity. Smoking status was classified as either "ever smoked" or "never smoked".

Habitual coffee intake and fiber intake were both obtained through a food frequency questionnaire and expressed in grams per day. Both variables were adjusted for the total energy intake (22). Diet quality was defined as a score from 0-14 based on the adherence to fourteen items of the Dutch dietary guidelines (23). Physical activity was measured by the Longitudinal Aging Study Amsterdam (LASA) questionnaire and expressed in metabolic equivalent of task (MET)-hours per week. This value gives an indication of both the duration and the intensity by expressing the sum of the duration of all activities weighed with the MET-value of each activity (24).

Relevant symptoms, medical history & medication

Relevant symptoms, medical history and medication that were used as possible predictors for CCE transit times were presence of dyspeptic complaints, changes in stool pattern, history of abdominal surgery, general medication use and the use of gastro protectant drugs. Dyspeptic complaints included general dyspeptic complaints, heart burn, feeling of being full and belches. Stomach protectors included proton pomp inhibitors (PPI's), H2-antagonists, anti-emetics and gastric acid binders.

CCE procedure-related factors and CCE findings

CCE procedure-related factors and CCE findings that were used as possible predictors for CCE transit times were the intake of metoclopramide, the presence of diverticula in the small bowel found by CCE and the presence of diverticula in the colon found by CCE.

Statistical analysis

Baseline characteristics were presented as mean with standard deviation (SD) for the numerical data or as number with percentage for the categorical data. Transit times were presented as median with interquartile range (IQR). Completion rate was also presented as number with the corresponding percentage.

Due to missing values in some of the variables (Supplementary table 1), multiple imputation was performed to improve the validity of the results (25). The assumption was made that the missing values were missing at random (MAR). A total of 5 imputations were performed using all variables from each model and some additional variables including history of lung disease, the use of laxatives and the presence of diverticula in the medical history as predictors.

Univariable linear regression and multivariable linear regression with and without backward elimination were performed to predict CCE stomach, small bowel, colonic and total transit times. For each of these analyses, cases were excluded from the analysis when they did not have a complete transit of the investigated GI segment (e.g. when

predicting stomach transit, cases where the capsule did not reach the small bowel were excluded). Univariable- and multivariable logistic regression models were performed to predict CCE completion rate in all cases. The main conclusions were based on the multivariable analyses with backward elimination.

For all tests, a two-sided statistical significance level of 0.05 was used. Analyses were performed in IBM SPSS v.25 (IBM Corp., Armonk, NY).

Results

Baseline characteristics

Four hundred and fifty-one participants were included. They all underwent CCE. Participants had a mean (SD) age of 67.3 (4.8) years and 46.1% was male. All baseline characteristics after imputation are shown in Table 1. Baseline characteristics of the original data are included in Supplementary table 2. In total 450 videos had a complete transit of the stomach, 449 videos had a complete transit of the small bowel and 234 videos had a complete colonic transit. The entire GI tract was visualized in 234 videos (completion rate 51.9%)

Table 1 Baseline characteristics. n = number, SD = standard deviation, BMI = body mass index, CCE = colon capsule endoscopy, SB = small bowel, MET = metabolic equivalent of task.

	Total study cohort (n=451)
Patient characteristics	
Mean age (SD), years	67.3 (4.8)
Gender, male, n (%)	208 (46.1%)
Mean BMI (SD)	26.3 (3.8)
(History of) smoking, n (%)	306 (67.8%)
Mean coffee intake (SD), grams/day	418.6 (266.5)
Mean fiber intake (SD), grams/day	28.1 (8.1)
Mean diet quality score (SD)	7.3 (1.8)
Mean physical activity score (SD), METh/wk	57.7 (58.0)
Relevant symptoms	
Dyspeptic complaints, n (%)	33 (7.3%)
Changes in stool pattern, n (%)	51 (11.3%)
Relevant medical history	
Abdominal surgery, n (%)	171 (37.9%)
Relevant medication	
Medication use, n (%)	343 (76.1%)
Stomach protectors, n (%)	109 (24.2%)
Procedure CCE	
Intake metoclopramide, n (%)	151 (33.5%)
Findings CCE	
Presence diverticula SB, n (%)	15 (3.3%)
Presence diverticula colon, n (%)	392 (86.9%)

CCE transit times

The median transit times were 55 minutes (IQR=39-93) in the stomach, 47 minutes (IQR=29-78) in the small bowel and 391 minutes (IQR=191-528) in the colon (Figure 1). The median total transit time was 574 minutes (IQR 308-659).

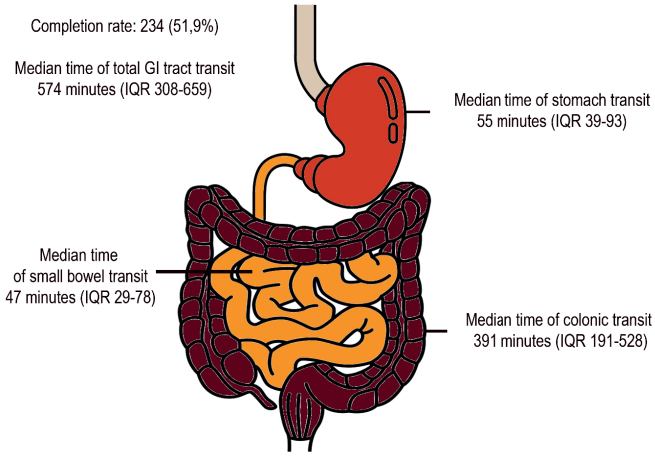


Figure 1 Heat map illustrating gastrointestinal transit times and completion rate
IQR = interquartile range, completion rate: the number of complete videos.

Predicting of CCE transit times

Stomach transit

Participants with a higher BMI had a faster stomach transit (0.10 SD faster transit per 1 SD higher BMI (standardized β=-0.10, 95% CI -0.19 – -0.02, p=0.01)), while those with higher physical activity had a slower stomach transit (β=0.10, 95% CI 0.02 – 0.18, p=0.02) (Table 2). A trend was shown for a slower stomach transit in men (β=0.08, 95% CI -0.01 – 0.16, p=0.07).

Small bowel transit

Participants with a higher BMI (β=-0.14, 95% CI -0.22 – -0.05, p=0.001), higher physical activity (β=-0.14, 95% CI -0.22 – -0.05, p=0.002) and changes in stool pattern (β=-0.08, 95% CI -0.167 – 0.000, p=0.049) had a faster small bowel transit, all independent of the other predictors (Table 3). Participants who took metoclopramide due to a long stomach transit also had a significantly faster small bowel transit (β=-0.14, 95% CI -0.23 – -0.05, p=0.001).

Colonic transit

Participants with higher fiber intake had a slower colonic transit (β=0.11, 95% CI 0.01 – 0.21, p=0.03). A trend was shown for a slower colonic transit in the presence of colonic diverticula (β=0.10, 95% CI -0.004 – 0.204, p=0.06) (Table 4).

Total transit

Participants with a higher BMI had a faster total transit (β=-0.12, 95% CI -0.22 – -0.03, p=0.01), while participants who took metoclopramide due to a long stomach transit had a slower total transit (β=0.15, 95% CI 0.04 – 0.25, p=0.01) (Table 5). A trend was shown for a slower total transit with higher fiber intake (β=0.08, 95% CI -0.01 – 0.18, p=0.09) and in the presence of diverticula (both small bowel- (β=0.08, 95% CI -0.004 – 0.156, p=0.06) and colonic diverticula (β=0.09, 95% CI -0.01 – 0.19, p=0.09)).

Predictors of CCE completion rate

Overall completion rate was higher among older participants (OR 1.54 per SD higher age, 95% CI 1.04-2.28, p=0.03) and among those with changes in stool pattern (OR 2.27, 95% CI 1.20-4.30, p=0.01) (Table 5). A trend was shown for a higher completion rate with the presence of small bowel diverticula (OR 2.94, 95% CI 0.91-9.49, p=0.07). A lower completion rate was seen in those participants with a history of abdominal surgery (OR 0.54, 95% CI 0.36-0.80, p=0.003) and in those who had to take metoclopramide due to a long stomach transit (OR 0.60, 95% CI 0.40-0.91, p=0.02).

Table 2 Predictors of stomach transit time (dependent variable) among participants with complete stomach transit (n=450)

	Univariable analysis			Multivariable analysis			Multivariable analysis with backward elimination		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
Patient characteristics									
Age	0.04	-0.05 – 0.12	0.40	0.04	-0.05 – 0.12	0.37			
Gender, male	0.06	-0.02 – 0.15	0.13	0.07	-0.02 – 0.16	0.11	0.08	-0.01 – 0.16	0.07
BMI	-0.11	-0.20 – -0.03	0.01	-0.11	-0.20 – -0.03	0.01	-0.10	-0.19 – -0.02	0.01
(History of) smoking	-0.05	-0.13 – 0.03	0.22	-0.06	-0.14 – 0.02	0.16			
Coffee intake	0.02	-0.08 – 0.11	0.70	0.02	-0.07 – 0.12	0.66			
Diet Quality	-0.02	-0.10 – 0.07	0.68	-0.03	-0.11 – 0.06	0.55			
Physical activity	0.11	0.03 – 0.20	0.01	0.09	0.004 – 0.173	0.04	0.10	0.02 – 0.18	0.02
Relevant symptoms									
Dyspeptic complaints	-0.02	-0.11 – 0.06	0.57	-0.01	-0.10 – 0.07	0.79			
Relevant medical history									
Abdominal surgery	-0.02	-0.11 – 0.06	0.57	-0.001	-0.09 – 0.09	0.99			
Relevant medication									
Medication use	-0.07	-0.15 – 0.02	0.12	-0.04	-0.12 – 0.05	0.43			
Stomach protectors	-0.04	-0.12 – 0.04	0.34	-0.01	-0.10 – 0.08	0.83			

β = standardized beta, BMI = body mass index

Linear regression analyses were performed. Univariable models (each predictor one by one), a multivariable model (including all predictors in the table) and a multivariable model after backward selection (subsequent removal of the predictor with the highest p-value until all p-values were <0.1) are included in this table. β values are standardized regression coefficients and here represent differences in stomach transit times per SD higher predictor variables.

Table 3 Predictors of small bowel transit time (dependent variable) among participants with complete small bowel transit (n=449)

	Univariable analysis			Multivariable analysis			Multivariable analysis with backward elimination		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
Patient characteristics									
Age	0.002	-0.08 – 0.09	0.96	0.03	-0.05 – 0.12	0.43			
Gender, male	0.04	-0.05 – 0.12	0.38	0.06	-0.03 – 0.15	0.17			
BMI	-0.10	-0.18 – -0.01	0.02	-0.14	-0.22 – -0.05	0.002	-0.14	-0.22 – -0.05	0.001
(History of) smoking	-0.04	-0.13 – 0.04	0.31	-0.06	-0.14 – 0.03	0.20			
Coffee intake	-0.05	-0.15 – 0.05	0.31	-0.05	-0.14 – 0.05	0.33			
Fiber intake	0.02	-0.08 – 0.12	0.73	-0.003	-0.10 – 0.09	0.96			
Diet Quality	0.04	-0.06 – 0.15	0.43	0.04	-0.06 – 0.15	0.42			
Physical activity	-0.11	-0.20 – -0.03	0.01	-0.15	-0.24 – -0.06	0.001	-0.14	-0.22 – -0.05	0.002
Relevant symptoms									
Changes in stool pattern	-0.06	-0.15 – 0.02	0.16	-0.08	-0.166 – 0.001	0.054	-0.08	-0.167 – 0.000	0.049
Relevant medical history									
Abdominal surgery	0.06	-0.02 – 0.15	0.16	0.09	0.000 – 0.174	0.049			
Relevant medication									
Medication use	-0.05	-0.13 – 0.04	0.25	-0.07	-0.16 – 0.02	0.12			
Procedure CCE									
Intake metoclopramide	-0.12	-0.20 – -0.03	0.01	-0.14	-0.22 – -0.05	0.002	-0.14	-0.23 – -0.05	0.001
Findings CCE									
Presence diverticula	-0.01	-0.10 – 0.07	0.75	-0.003	-0.09 – 0.08	0.94			

β = standardized beta, BMI = body mass index, CCE = colon capsule endoscopy

Linear regression analyses were performed. Univariable models (each predictor one by one), a multivariable model (including all predictors in the table) and a multivariable model after backward selection (subsequent removal of the predictor with the highest p-value until all p-values were <0.1) are included in this table. β values are standardized regression coefficients from linear regression models and here represent differences in small bowel transit times per SD higher predictor variables.

Table 4 Predictors of colonic transit time (dependent variable) among participants with complete colonic transit (n=234)

	Univariable analysis			Multivariable analysis			Multivariable analysis with backward elimination		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
Patient characteristics									
Age	0.02	-0.08 – 0.11	0.75	0.01	-0.09 – 0.11	0.84			
Gender, male	0.02	-0.08 – 0.12	0.67	0.04	-0.06 – 0.14	0.48			
BMI	-0.06	-0.11 – -0.01	0.22	-0.08	-0.18 – 0.03	0.14			
(History of) smoking	0.02	-0.07 – 0.12	0.62	0.02	-0.07 – 0.12	0.64			
Coffee intake	-0.02	-0.13 – 0.08	0.70	-0.01	-0.11 – 0.10	0.93			
Fiber intake	0.12	0.02 – 0.21	0.02	0.11	-0.004 – 0.215	0.06	0.11	0.01 – 0.21	0.03
Diet Quality	0.05	-0.04 – 0.15	0.29	0.01	-0.10 – 0.11	0.93			
Physical activity	-0.02	-0.12 – 0.07	0.63	-0.05	-0.15 – 0.06	0.37			
Relevant symptoms									
Changes in stool pattern	0.02	-0.03 – 0.06	0.67	-0.002	-0.09 – 0.09	0.96			
Relevant medical history									
Abdominal surgery	0.02	-0.08 – 0.13	0.64	-0.001	-0.11 – 0.11	0.99			
Relevant medication									
Medication use	0.06	0.01 – 0.11	0.79	0.06	-0.04 – 0.17	0.23			
Findings CCE									
Presence diverticula	0.11	0.002 – 0.208	0.045	0.12	0.01 – 0.24	0.03	0.10	-0.004 – 0.204	0.06

β = standardized beta, BMI = body mass index, CCE = colon capsule endoscopy

Linear regression analyses were performed. Univariable models (each predictor one by one), a multivariable model (including all predictors in the table) and a multivariable model after backward selection (subsequent removal of the predictor with the highest p-value until all p-values were <0.1) are included in this table. β values are standardized regression coefficients from linear regression models and here represent differences in colonic transit times per SD higher predictor variables.

Table 5 Predictors of total GI tract transit time (dependent variable) among participants with complete transit (n=234) and predictors of completion rate (dependent variable) among all participants (n=451)

Total GI tract transit	**Univariable analysis**			**Multivariable analysis**			**Multivariable analysis with backward elimination**		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Patient characteristics									
Age	0.04	-0.06 – 0.13	0.47	0.01	-0.09 – 0.11	0.78			
Gender, male	0.04	-0.06 – 0.14	0.40	0.07	-0.03 – 0.17	0.16			
BMI	-0.13	-0.22 – -0.03	0.01	-0.12	-0.22 – -0.02	0.02	-0.12	-0.22 – -0.03	0.01
(History of) smoking	-0.003	-0.10 – 0.09	0.96	0.004	-0.09 – 0.10	0.93			
Coffee intake	-0.04	-0.14 – 0.06	0.47	-0.03	-0.13 – 0.08	0.62			
Fiber intake	0.10	0.01 – 0.20	0.04	0.08	-0.03 – 0.19	0.14	0.08	-0.01 – 0.18	0.09
Diet Quality	0.05	-0.04 – 0.15	0.26	0.01	-0.10 – 0.12	0.87			
Physical activity	0.03	-0.07 – 0.13	0.59	0.004	-0.10 – 0.11	0.94			
Relevant symptoms									
Dyspeptic complaints	0.01	-0.10 – 0.12	0.85	-0.01	-0.13 – 0.10	0.80			
Changes in stool pattern	0.04	-0.05 – 0.12	0.42	0.03	-0.06 – 0.12	0.53			
Relevant medical history									
Abdominal surgery	0.02	-0.08 – 0.12	0.70	0.02	-0.08 – 0.13	0.66			
Relevant medication									
Medication use	0.04	-0.07 – 0.14	0.50	0.03	-0.07 – 0.14	0.56			
Stomach protectors	0.03	-0.07 – 0.13	0.57	0.02	-0.09 – 0.12	0.72			
Procedure CCE									
Intake metoclopramide	0.15	0.05 – 0.26	0.003	0.15	0.05 – 0.25	0.004	0.15	0.04 – 0.25	0.01
Findings CCE									
Presence diverticula SB	0.09	0.01 – 0.17	0.04	0.07	-0.01 – 0.16	0.10	0.08	-0.004 – 0.156	0.06
Presence diverticula colon	0.08	-0.02 – 0.19	0.13	0.09	-0.02 – 0.20	0.12	0.09	-0.01 – 0.19	0.09

Completion rate	Univariable analysis			Multivariable analysis			Multivariable analysis with backward elimination		
	Odds	**95% CI**	**P-value**	**Odds**	**95% CI**	**P-value**	**Odds**	**95% CI**	**P-value**
Patient characteristics									
Age	1.01	0.97 – 1.05	0.67	1.001	0.960 – 1.043	0.97	1.54	1.04 – 2.28	0.03
Gender, male	1.66	1.14 – 2.41	0.01	1.52	1.01 – 2.29	0.04			
BMI	1.05	1.00 – 1.10	0.08	1.04	0.98 – 1.10	0.21			
(History of) smoking	0.86	0.58 – 1.28	0.45	0.80	0.52 – 1.22	0.29			
Coffee intake	1.000	0.999 – 1.001	0.60	1.000	0.999 – 1.001	0.70			
Fiber intake	0.98	0.95 – 1.01	0.12	0.98	0.95 – 1.02	0.27			
Diet Quality	0.95	0.84 – 1.08	0.44	0.99	0.85 – 1.15	0.91			
Physical activity	1.000	0.997 – 1.003	0.91	1.001	0.997 – 1.004	0.62			
Relevant symptoms									
Dyspeptic complaints	0.66	0.32 – 1.36	0.26	0.81	0.36 – 1.78	0.59			
Changes in stool pattern	2.18	1.17 – 4.07	0.01	2.27	1.18 – 4.36	0.01	2.27	1.20 – 4.30	0.01
Relevant medical history									
Abdominal surgery	0.53	0.36 – 0.78	0.001	0.50	0.33 – 0.77	0.002	0.54	0.36 – 0.80	0.003
Relevant medication									
Medication use	1.28	0.83 – 1.97	0.27	1.43	0.87 – 2.36	0.16			
Stomach protectors	0.997	0.647 – 1.537	0.99	0.995	0.606 – 1.635	0.99			
Procedure CCE									
Intake metoclopramide	0.63	0.42 – 0.94	0.02	0.61	0.40 – 0.94	0.03	0.60	0.40 – 0.91	0.02
Findings CCE									
Presence diverticula SB	2.51	0.78 – 8.04	0.12	2.83	0.85 – 9.47	0.09	2.94	0.91 – 9.49	0.07
Presence diverticula colon	1.19	0.67 – 2.11	0.55	1.19	0.63 – 2.26	0.59			

β = standardized beta, BMI = body mass index, CCE = colon capsule endoscopy, SB = small bowel

For determining predictors of the total GI tract transit time, linear regression analyses were performed. Univariable models (each predictor one by one), a multivariable model (including all predictors in the table) and a multivariable model after backward selection (subsequent removal of the predictor with the highest p-value until all p-values were <0.1) are included in this table. β values are standardized regression coefficients from linear regression models and here represent differences in total GI tract transit times per SD higher predictor variables.

For determining predictors of GI tract completion rate, logistic regression analyses were performed. Univariable models (each predictor one by one), a multivariable model (including all predictors in the table) and a multivariable model after backward selection (subsequent removal of the predictor with the highest p-value until all p-values were <0.1) are included in this table. Odds represent the chances of completion per SD higher predictor variables.

Discussion

To our knowledge, this is the largest prospective population-based cohort study so far in identifying predictors of CCE gastrointestinal transit times. The low completion rate of 51.9% in this study emphasizes the need for entry points which can be used to anticipate and prevent incomplete CCE procedures. We observed that lower BMI, unchanged stool pattern, higher fiber intake, younger age and history of abdominal surgery were significant predictors for slower CCE transit times and lower completion rate. In future practice these factors can be used to anticipate a longer capsule transit time and possibly adjust the preparation protocol. The faster SB transit in participants who took metoclopramide due to a long stomach transit, suggests that it might be beneficial to use metoclopramide in all CCE procedures.

Some of the associations in our study can be explained according to what is already known about the etiology of differences in physiological gastrointestinal transit times. For example, participants with a higher BMI generally had a faster CCE stomach, small bowel and total transit time. Even though a higher BMI is associated with delay in physiological colonic transit, previous literature has shown it actually has an accelerating effect on gastric emptying and small bowel transit, which could have resulted in a faster CCE total transit time (15). Participants with higher physical activity had a faster CCE small bowel transit but a slower stomach transit, with no apparent effect on total transit time. In line with this, previous literature has shown that physical activity can accelerate small bowel transit, but with increasing intensity it can cause delayed gastric emptying

(16, 26). This delayed gastric emptying seen in heavy exercise might be due to the inhibitory effects of increased catecholamine on splanchnic blood flow and gastric motility (27). Further our data showed a lower CCE completion rate in participants with a history of abdominal surgery, which may be explained by the possible presence of abdominal adhesions and its associated bowel obstructing effects (28, 29). On top of that, our study revealed trends for a slower colonic and total transit in the presence of diverticula, which may be due to possible causes of these diverticula such as disordered intestinal motility and obstipation (30). Participants with changes in stool pattern had a faster CCE small bowel transit and a higher completion rate. Unfortunately, our data did not differentiate what type of changed stool pattern was present. A possible explanation for this result can be that these changes in stool pattern could have been mostly diarrhea instead of obstipation.

The intake of metoclopramide in those participants with a prolonged stomach transit, subsequently led to a significantly faster CCE small bowel transit. This can be explained by the known stimulating effect of metoclopramide on the peristalsis of the entire upper GI channel (31). Still, intake of metoclopramide in this study was associated with a slower total transit time and lower completion rate, likely due to the fact that the medication was only taken when participants had a long stomach transit of more than 1 hour.

Some of the observed associations in our study were opposite to what we expected based on human physiology. It has been reported that aging may delay gastric emptying or slow down colonic transit time; possibly due to nerve dysfunction (11-13), but our study population (with ages ranging from 50-75 years) showed a higher CCE completion rate with older age. Our study also observed a non-significant trend for a slower CCE stomach transit in men, while a previous study has shown that men have physiological faster gastric emptying (14). Perhaps these differences can be explained by possible differences in commitment to the CCE protocol in different age groups and genders. Also, it was expected that a higher fiber intake would lead to a faster colonic transit, but we found that a higher habitual fiber intake was associated with a slower colonic transit. A previous meta-analysis showed a faster transit with higher wheat dietary fiber intake, but only among those with an initial transit time greater than 48h. The effect was not shown for those with a faster initial transit time (32). If our participants had an overall faster initial transit time, this could partly explain our result, but unfortunately this parameter was unknown for our study population. On top of that, the fiber intake reported in our study included all types of dietary fiber. While insoluble fibers (such as wheat) can accelerate colonic transit, some soluble fibers can actually have a constipating effect (33), which may explain the slower colonic transit with higher fiber intake that we observed in our participants. Further, there was a non-significant trend for a higher

completion rate in those participants with small bowel diverticula which cannot be explained. Possibly the number of 15 participants with small bowel diverticula was too low to provide a reliable outcome. Finally, we did not observe any association between (history of) smoking, coffee intake, diet quality, dyspeptic complaints, medication use in general or stomach protectors with any of the transit times.

Previous literature on influential factors of transit times in CCE specifically is scarce. One study identified a high BMI and the absence of constipation as promoting factors for CCE transit time (18), which is in accordance with our results. Contrary to our current study, the previous study did not investigate the effect of possible predictors on stomach-, small bowel- and colonic transit separately.

Major strengths of our study are the prospective population-based cohort design and the examination of predictors for each GI segment transit separately. To our knowledge, this study with 451 participants is the largest study so far to investigate the possible predictors of CCE transit times. However, this study also has some limitations to address. First, in the analysis for stomach, small bowel, colonic and total transit times, cases were excluded from the analysis when they did not have a complete transit of the investigated GI segment. Since the excluded cases probably had relatively longer transit times compared to the included cases this might have affected the results. Second, in order to improve the validity of the results, multiple imputation was performed where the assumption was made that the missing values were missing at random. With this assumption there is always a small chance that the results might be biased. However, the imputed and original data showed almost no differences in its baseline characteristics (Supplementary Table 2). Therefore, we believe the current results based on the imputed dataset are reliable.

To conclude, lower BMI, unchanged stool pattern, higher fiber intake, younger age and history of abdominal surgery were significant predictors for slower CCE transit times and lower completion rate. Clinicians can use these factors to anticipate a longer capsule transit time and adapt the preparation protocol. On top of that, the faster SB transit in those participants who took metoclopramide due to a long stomach transit, suggests that it might be beneficial to use metoclopramide in all CCE procedures.

References

1. Spada C, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2012;44(5):527-36.
2. Spada C, Hassan C, Bellini D, Burling D, Cappello G, Carretero C, et al. Imaging alternatives to colonoscopy: CT colonography and colon capsule. European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) Guideline - Update 2020. Eur Radiol. 2021;31(5):2967-82.
3. Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, et al. Colorectal Cancer Screening: Recommendations for Physicians and Patients from the U.S. Multi-Society Task Force on Colorectal Cancer. Am J Gastroenterol. 2017;112(7):1016-30.
4. Spada C, Pasha SF, Gross SA, Leighton JA, Schnoll-Sussman F, Correale L, et al. Accuracy of First- and Second-Generation Colon Capsules in Endoscopic Detection of Colorectal Polyps: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2016;14(11):1533-43.e8.
5. Pecere S, Senore C, Hassan C, Riggi E, Segnan N, Pennazio M, et al. Accuracy of colon capsule endoscopy for advanced neoplasia. Gastrointest Endosc. 2020;91(2):406-14 e1.
6. Kjølhede T, Ølholm AM, Kaalby L, Kidholm K, Qvist N, Baatrup G. Diagnostic accuracy of capsule endoscopy compared with colonoscopy for polyp detection: systematic review and meta-analyses. Endoscopy. 2020.
7. Yamada K, Nakamura M, Yamamura T, Maeda K, Sawada T, Mizutani Y, et al. Clinical Factors Associated with Missing Colorectal Polyp on Colon Capsule Endoscopy. Digestion. 2020;101(3):316-22.
8. Kobaek-Larsen M, Kroijer R, Dyrvig AK, Buijs MM, Steele RJC, Qvist N, et al. Back-to-back colon capsule endoscopy and optical colonoscopy in colorectal cancer screening individuals. Colorectal Dis. 2018;20(6):479-85.
9. Kroijer R, Dyrvig AK, Kobaek-Larsen M, Stovring JO, Qvist N, Baatrup G. Booster medication to achieve capsule excretion in colon capsule endoscopy: a randomized controlled trial of three regimens. Endosc Int Open. 2018;6(11):E1363-E8.
10. Togashi K, Fujita T, Utano K, Waga E, Katsuki S, Isohata N, et al. Gastrografin as an alternative booster to sodium phosphate in colon capsule endoscopy: safety and efficacy pilot study. Endosc Int Open. 2015;3(6):E659-61.
11. Madsen JL, Graff J. Effects of ageing on gastrointestinal motor function. Age Ageing. 2004;33(2):154-9.
12. Brogna A, Ferrara R, Bucceri AM, Lanteri E, Catalano F. Influence of aging on gastrointestinal transit time. An ultrasonographic and radiologic study. Invest Radiol. 1999;34(5):357-9.
13. Nandhra GK, Mark EB, Di Tanna GL, Haase AM, Poulsen J, Christodoulides S, et al. Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: Influence of age, gender, and body mass index. Neurogastroenterol Motil. 2020;32(2):e13734.
14. Degen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. Gut. 1996;39(2):299-305.
15. Mushref MA, Srinivasan S. Effect of high fat-diet and obesity on gastrointestinal motility. Ann Transl Med. 2013;1(2):14.
16. Keeling WF, Martin BJ. Gastrointestinal transit during mild exercise. J Appl Physiol (1985). 1987;63(3):978-81.

17. Bohlin J, Dahlin E, Dreja J, Roth B, Ekberg O, Ohlsson B. Longer colonic transit time is associated with laxative and drug use, lifestyle factors, and symptoms of constipation. Acta Radiol Open. 2018;7(10):2058460118807232.
18. Sato J, Nakamura M, Watanabe O, Yamamura T, Funasaka K, Ohno E, et al. Prospective study of factors important to achieve observation of the entire colon on colon capsule endoscopy. Therap Adv Gastroenterol. 2017;10(1):20-31.
19. Buijs MM, Kobaek-Larsen M, Kaalby L, Baatrup G. Can coffee or chewing gum decrease transit times in Colon capsule endoscopy? A randomized controlled trial. BMC Gastroenterol. 2018;18(1):95.
20. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. Eur J Epidemiol. 2020;35(5):483-517.
21. Vuik FER, Nieuwenburg SAV, Moen S, Schreuders EH, Oudkerk Pool MD, Peterse EFP, et al. Population-Based Prevalence of Gastrointestinal Abnormalities at Colon Capsule Endoscopy. Clin Gastroenterol Hepatol. 2020.
22. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65(4 Suppl):1220S-8S; discussion 9S-31S.
23. Voortman T, Kiefte-de Jong JC, Ikram MA, Stricker BH, van Rooij FJA, Lahousse L, et al. Adherence to the 2015 Dutch dietary guidelines and risk of non-communicable diseases and mortality in the Rotterdam Study. Eur J Epidemiol. 2017;32(11):993-1005.
24. Stringa N, van Schoor NM, Milaneschi Y, Ikram MA, Del Panta V, Koolhaas CM, et al. Physical activity as moderator of the association between APOE and cognitive decline in older adults: Results from three longitudinal cohort studies. J Gerontol A Biol Sci Med Sci. 2020.
25. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009;338:b2393.
26. Neufer PD, Young AJ, Sawka MN. Gastric emptying during walking and running: effects of varied exercise intensity. Eur J Appl Physiol Occup Physiol. 1989;58(4):440-5.
27. Mittal R, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, et al. Neurotransmitters: The Critical Modulators Regulating Gut-Brain Axis. J Cell Physiol. 2017;232(9):2359-72.
28. Strik C, Stommel MW, Schipper LJ, van Goor H, Ten Broek RP. Long-term impact of adhesions on bowel obstruction. Surgery. 2016;159(5):1351-9.
29. ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. BMJ. 2013;347:f5588.
30. Matrana MR, Margolin DA. Epidemiology and pathophysiology of diverticular disease. Clin Colon Rectal Surg. 2009;22(3):141-6.
31. Schulze-Delrieu K. Metoclopramide. Gastroenterology. 1979;77(4 Pt 1):768-79.
32. de Vries J, Miller PE, Verbeke K. Effects of cereal fiber on bowel function: A systematic review of intervention trials. World J Gastroenterol. 2015;21(29):8952-63.
33. McRorie JW, Jr., McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. J Acad Nutr Diet. 2017;117(2):251-64.

Chapter 9

Artificial intelligence in colon capsule endoscopy

– a systematic review –

S. Moen, **F.E.R. Vuik**, E.J. Kuipers, M.C.W. Spaander

Diagnostics, Augustus 2022

Abstract

Introduction Applicability of Colon Capsule Endoscopy in daily practice is limited by the accompanying labor-intensive reviewing time and risk of inter-observer variability. Automated reviewing of colon Capsule Endoscopy images using artificial intelligence could be timesaving whilst providing an objective and reproducible outcome. This systematic review aims to provide an overview of the available literature on artificial intelligence for reviewing colonic mucosa by colon capsule endoscopy and assess necessary action points for its use in clinical practice.

Methods A systematic literature search was conducted of literature published up to January 2022 using Embase, Web of Science, OVID MEDLINE and Cochrane CENTRAL. Studies reporting on artificial intelligence for reviewing second generation colon capsule endoscopy colonic images were included.

Results 1017 studies were evaluated for eligibility of which nine were included. Two studies reported on computed bowel cleansing assessment,five studies reported on computed polyp- or colorectal neoplasia detection and two studies reported on other implications. Overall, sensitivity of proposed artificial intelligence models was 86.5%-95.5% for bowel cleansing and 47.4%-98.1% for detection of polyps and colorectal neoplasia. Two studies performed per-lesion analysis, in addition to per-frame analysis, which improved sensitivity of polyp- or colorectal neoplasia detection to 81.3%-98.1%. By applying a Convolutional Neural Network, the highest sensitivity of 98.1% for polyp detection was found.

Conclusion Artificial intelligence for reviewing second generation colon capsule endoscopy images is promising. Highest sensitivity of 98.1% for polyp detection was achieved by deep learning with Convolutional Neural Network. Convolutional Neural Network algorithms should be optimized and tested with more data, possibly requiring the set-up of a large international colon capsule endoscopy database. Finally, the accuracy of the optimized Convolutional Neural Network models need to be confirmed in a prospective setting.

Introduction

Colon Capsule Endoscopy (CCE) provides a promising non-invasive alternative to colonoscopy for exploration of the colonic mucosa (1, 2). It uses an ingestible, wireless, disposable capsule to explore the colon without the need for sedation or gas insufflation. The first generation CCE was introduced in 2006 and a second generation CCE was developed in 2009 (PillCam Colon 2, Medtronic, Minnesota, USA) (3). The second generation colon capsule endoscopy (CCE-2) has a high diagnostic accuracy for the detection of colorectal polyps, with a sensitivity of 85% and specificity of 85% for polyps of any size, sensitivity of 87% and specificity of 88% for polyps ≥ 6mm and a sensitivity of 87% and specificity of 95% for polyps ≥ 10mm (4).

An important limitation in the applicability of CCE in daily practice is the accompanying labor-intensive reviewing time of the CCE images. A recent study showed a median reading time of 70 minutes for the entire gastrointestinal tract and 55 minutes for review of the colon alone (5). On top of that, agreement in and between different readers may also be a topic of concern. Literature regarding intra- and inter-observer variability in reviewing CCE images is scarce, but one study demonstrated a poor level of agreement among both expert- and beginner readers in determining the indication for follow-up colonoscopy based on the number and size of detected polyps (6). There was also a poor agreement in determining the bowel cleansing quality.

Automated reviewing of CCE images using artificial intelligence (AI) could be timesaving for clinicians whilst providing an objective and reproducible outcome. AI is a very broad term that describes the computerized approach including machine and deep learning methods for interpreting data that normally requires human intelligence (7, 8). Basic AI methods can classify images by computing scores based on features such as texture and color. Machine-learning based on pre-defined features is a another AI method to classify images, where a classifying algorithm is created based on feature classification by experts. An important example of this method is the support vector machine (SVM). Deep-learning is a sub-class of machine-learning where features do not have to be pre-defined. It is based on a neural network structure that can learn discriminative features from data automatically, giving them the ability to solve very complex problems. Convolutional neural network (CNN) is the most common deep learning algorithm for classifying images. It uses many images to develop and train a classification model by learning rich features and repeating patterns from these images (9).

In colonoscopy, research investigating the use of AI as an aid for the detection of colorectal lesions is already rapidly evolving (10, 11). However, blindly applying the

same automated methods to CCE would be blunt due to the differences in the images provided by CCE and colonoscopy. For example, localizing polyps and determining their exact number is more difficult in CCE since the capsule spins around and moves back and forth while the lack of air insufflation causes the intestinal wall to protrude into the lumen sometimes mimicking polyps. Therefore, a reliable AI method specifically developed for reviewing CCE images is warranted. Some literature is available on automated methods to review small bowel capsule endoscopy (SBCE) (7), but literature on AI in CCE is scarce. This systematic review aims to give an overview of the available literature on AI methods for reviewing the colonic mucosa by CCE and assess the necessary action points to evolve AI technology for CCE in daily clinical practice.

Methods

A systematic search aimed to retrieve published trials and abstracts reporting on AI for CCE was conducted following the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) (12). A systematic search was conducted on literature databases from inception until the 4th of January 2022. Embase, Web of Science, OVID MEDLINE and Cochrane CENTRAL were used as potential sources. The search was conducted using controlled vocabulary supplemented with several key words (Table 1).

In 2006 the first- generation colon capsule (CCE-1) was developed and in 2011 the second- generation colon capsule (CCE-2) came to the market. New technology was implemented in the second- generation colon capsule: the capsule frame rate increased from 4 to 35 images per second; the angle of view increased from 156º to 172º for each lens and the data recorder was improved. The CCE-2 achieved a substantial higher sensitivity and specificity to detect polyps compared to the first- generation colon capsule. (3) Therefore, studies using CCE-1 were excluded. Two independent reviewers (S.M. and F.E.R.V.) first screened the selected studies by title and abstract. Studies reporting on AI for reviewing CCE-2 colonic images were selected. Included studies could report on AI for detection of abnormalities, determining the location of the capsule in the colon and assessment of bowel cleansing quality. Full-text examination of the selected publications was performed by the same reviewers independently. Reference lists of the included studies were hand-searched to identify potentially relevant studies that were not retrieved in the original search.

Details regarding the development of the proposed AI models and numbers on the performance of these models were extracted from the final set of included studies. A meta-analysis could not be performed due to the heterogeneity of the study designs.

Quality assessment of the included studies

The quality of the included studies in terms of risk of bias and concerns regarding applicability were independently assessed by two reviewers (S.M. and F.E.R.V.) using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) -2 assessment tool (13).

Table 1 Systematic literature search

Embase.com (1971-)
('capsule endoscopy'/exp OR 'capsule endoscope'/de OR ((capsule* OR videocapsule*) NEAR/3 (endoscop* OR colonoscop*)):ab,ti) AND ('large intestine'/exp OR 'large intestine disease'/exp OR 'large intestine tumor'/exp OR colonoscopy/exp OR (colon* OR colorectal* OR rectal OR rectum OR large-intestin*):ab,ti) AND ('artificial intelligence'/exp OR 'machine learning'/exp OR 'software'/exp OR 'algorithm'/exp OR automation/de OR 'computer analysis'/de OR 'computer assisted diagnosis'/de OR 'image processing'/de OR ((artificial* NEAR/3 intelligen*) OR (machine NEAR/3 learning) OR (compute* NEAR/3 (aided OR assist* OR technique*)) OR software* OR algorithm* OR automat* OR (image NEAR/3 (processing OR matching OR analy*)) OR support-vector* OR svm OR hybrid* OR neural-network* OR autonom* OR (unsupervis* NEAR/3 (learn* OR classif*))):Ab,ti) NOT ([animals]/lim NOT [humans]/lim)
Medline ALL Ovid (1946-)
(Capsule Endoscopy/ OR Capsule Endoscopes/ OR ((capsule* OR videocapsule*) ADJ3 (endoscop* OR colonoscop*)).ab,ti.) AND (Intestine, Large/ OR Colorectal Neoplasms/ OR exp Colonoscopy/ OR (colon* OR colorectal* OR rectal OR rectum OR large-intestin*).ab,ti.) AND (exp Artificial Intelligence/ OR exp Machine Learning/ OR Software/ OR Algorithms/ OR Automation/ OR Diagnosis, Computer-Assisted/ OR Image Processing, Computer-Assisted/ OR ((artificial* ADJ3 intelligen*) OR (machine ADJ3 learning) OR (compute* ADJ3 (aided OR assist* OR technique*)) OR software* OR algorithm* OR automat* OR (image ADJ3 (processing OR matching OR analy*)) OR support-vector* OR svm OR hybrid* OR neural-network* OR autonom* OR (unsupervis* ADJ3 (learn* OR classif*))).ab,ti.) NOT (exp animals/ NOT humans/)
Web of Science Core Collection (1975-)
TS=((((capsule* OR videocapsule*) NEAR/2 (endoscop* OR colonoscop*))) AND ((colon* OR colorectal* OR rectal OR rectum OR large-intestin*)) AND (((artificial* NEAR/2 intelligen*) OR (machine NEAR/2 learning) OR (compute* NEAR/2 (aided OR assist* OR technique*)) OR software* OR algorithm* OR automat* OR (image NEAR/2 (processing OR matching OR analy*)) OR support-vector* OR svm OR hybrid* OR neural-network* OR autonom* OR (unsupervis* NEAR/2 (learn* OR classif*)))))
Cochrane CENTRAL register of Trials (1992-)
(((capsule* OR videocapsule*) NEAR/3 (endoscop* OR colonoscop*)):ab,ti) AND ((colon* OR colorectal* OR rectal OR rectum OR large-intestin*):ab,ti) AND (((artificial* NEAR/3 intelligen*) OR (machine NEAR/3 learning) OR (compute* NEAR/3 (aided OR assist* OR technique*)) OR software* OR algorithm* OR automat* OR (image NEAR/3 (processing OR matching OR analy*)) OR support-vector* OR svm OR hybrid* OR neural-network* OR autonom* OR (unsupervis* NEAR/3 (learn* OR classif*))):Ab,ti)
Google scholar
"capsule|videocapsule endoscopy|colonoscopy" colon|colonoscopy|colorectal "artificial intelligence"|"machine learning"|"computer aided|assisted"|software|algorithm|automated|"image processing|matching|analysis"|"support vector"|"neural network"

Results

Literature Search

After removal of duplicates, retrieved articles were screened for eligibility based on their title and/or abstract (Figure 1). A total of 1017 articles were evaluated for eligibility, after which 903 were excluded. The remaining 114 studies underwent full-text review, after which 105 were excluded for various reasons. No additional studies were retrieved by hand-search. A total of nine studies were included in the final review.

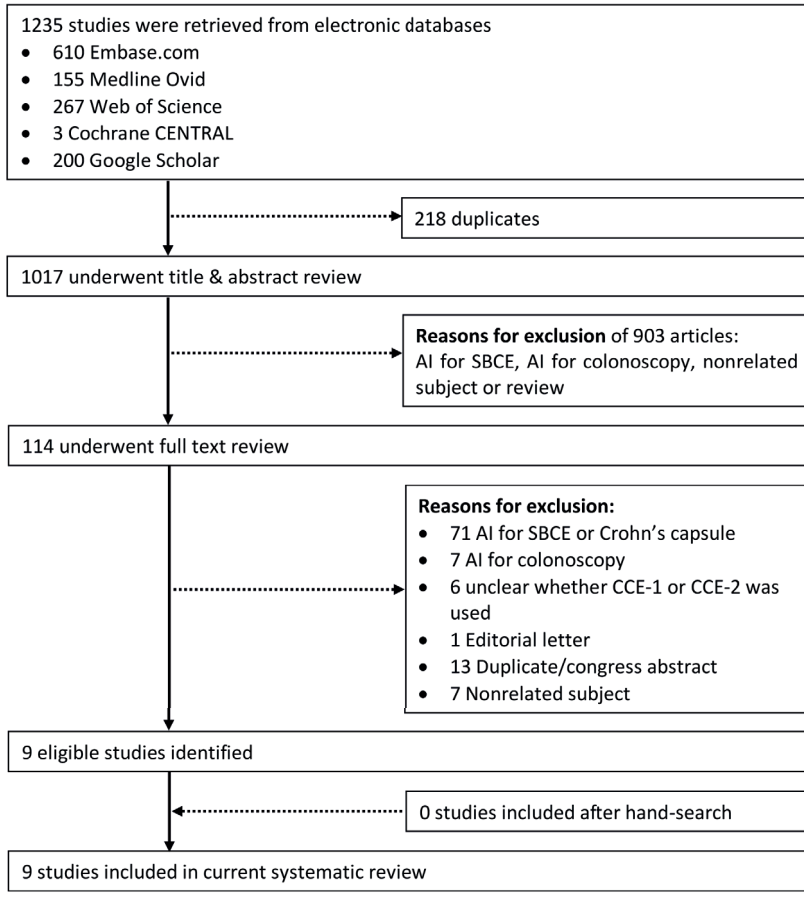


Figure 1 Flow chart of study selection

Study characteristics

Baseline characteristics of the included studies are shown in Table 2. All included studies were full-text papers presenting cohort studies reporting on AI for reviewing CCE-2 colonic images. Two studies reported on computed assessment of bowel cleansing, five studies reported on computed polyp- or colorectal neoplasia detection, one study reported on computed blood detection and one study reported on computed capsule localization. For the studies reporting on bowel cleansing, one study evaluated bowel cleansing for each video-frame while the other study evaluated this for the entire video. All other studies evaluated presence of polyps, presence of blood or capsule localization for each frame. Regarding the AI method, five studies developed a SVM- or CNN model, where a selection of frames is needed for the training of the model. To evaluate the performance of the proposed AI methods, all studies used a separate evaluation of the CCE images as a reference. Seven studies used the evaluation of CCE readers as a reference, one study used the known outcomes from a CCE database and one study used the findings from subsequent colonoscopy.

Quality of the included studies

The risk of bias and applicability concerns in the included studies determined by using the QUADAS-2 tool are presented in Table 3. All studies had a high risk of bias regarding patient selection, since they included CCE videos derived from previous trials or databases and information on the patient population behind the CCE videos was limited or lacking. One study regarding AI bowel cleansing assessment also raised applicability concerns regarding patient selection, since CCE videos were excluded when they were too poor in quality after the first lecture or when the CCE videos were incomplete (14). Two studies had a high risk of bias regarding their index test, since they determined their models' optimal cut-off values yielding in the highest diagnostic performance by using a ROC curve, which could have led to overoptimistic results which could likely be poorer when using the same threshold in an independent sample (14, 15). Three studies raised applicability concerns regarding their index test, since they did not report on the performance of their AI models in terms of sensitivity and specificity (16-18).

Table 2 Characteristics of the nine included studies

First author, year of publication, country	Application	Type of AI method	Evaluation for each frame or for each video	Included videos, n	Frames available from these videos	Frames available for training the model if applicable	Selected frames for testing the developed AI method	Reference group
Becq 2018 France (14)	Bowel cleansing assessment	1. Red over green (R/G ratio) 2. Red over brown (R/(R+G ratio)	Frame	12	79,497	N/A	216 (R/G set) 192 (R/(R+G) set)	2 CCE readers
Buijs 2018 Denmark (16)	Bowel cleansing assessment	1. Non- linear index model 2. SVM model	Video	41	Unknown	Unknown	N/A	4 CCE readers
Figueiredo 2011 Portugal (17)	Polyp detection	Protrusion based algorithm	Frame	5	Unknown	N/A	1700	Subsequent colonoscopy
Mamonov 2014 USA (15)	Polyp detection	Binary classification after pre-selection	Frame	5	18,968	N/A	18,968	Known reviewed CCE dataset
Nadimi 2020 Denmark (19)	Polyp detection	CNN	Frame	255	11,300	7910	1695	Unknown amount of CCE readers
Yamada 2020 Japan (20)	Colorectal neoplasia detection	CNN	Frame	184	20,717	15,933	4784	3 CCE readers
Saraiva 2021 Portugal (21)	Protruding lesion detection	CNN	Frame	24	1,017,472	2912	728	2 CCE readers
Saraiva 2021 Portugal (22)	Blood detection	CNN	Frame	24	3,387,259	4660	1165	2 CCE readers
Herp 2021 Denmark (18)	Capsule localization	T-T model	Frame	84	Unknown	N/A	Unknown	Unknown amount of CCE readers

AI = Artificial Inteligence, SVM = Support Vector Machine; CNN = Convolutional Neural Network, CCE = Colon Capsule Endoscopy, N/A = Not Applicable, R/G = Red over Green, R/(R+G) = Red over Brown

Table 3 QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) analysis for the assessment of the risk of bias in the included studies

	Risk of bias				Applicability concerns		
	Patient selection	**Index test**	**Reference standard**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
Becq (14)	+	+	-	-	+	-	-
Buijs (16)	+	-	-	-	-	+	-
Figueiredo (17)	+	-	-	-	-	+	-
Mamonov (15)	+	+	-	-	-	-	-
Nadimi (19)	+	-	-	-	-	-	-
Yamada (20)	+	-	-	-	-	-	-
Saraiva (21)	+	-	-	-	-	-	-
Saraiva (22)	+	-	-	-	-	-	-
Herp (18)	+	-	-	-	-	+	-

- = low risk of bias; + = high risk of bias

Artificial intelligence for the assessment of bowel cleansing quality in CCE-2

Two studies reported on computed assessment of bowel cleansing in CCE-2 (Table 4, Figure 2).

Development of the proposed AI models for computed assessment of bowel cleansing

The first study created two computed assessment of cleansing (CAC) scores using the ratio of color intensities red over green (R/G ratio) and red over brown (R/(R+G) ratio) (14). After sorting and random selection, for each ratio a set of frames representative of the range of these ratios were obtained. These sets of frames were also evaluated by two experienced CCE readers who were blinded to the CAC scores. The experienced readers classified the frames as having either poor, fair, good or excellent bowel cleansing. Frames with poor or fair quality were defined as inadequately cleansed and frames with good or excellent quality were defined as adequately cleansed. Using the assessment of the experienced reviewers as a reference, the optimal cut-off values yielding the highest diagnostic performance for cleansing assessment were determined for both ratios using a receiver operating characteristic (ROC) curve.

The second study developed two CAC models, a non-linear index model and a support vector machine (SVM) model (16). In both models, each pixel was defined as being clean or dirty after which cleanliness of each frame was determined based on the number of clean and dirty pixels it contained. The cleansing level of the complete video was determined by the median cleansing of all frames and weighted based on the number

of pixels in the frames. The non-linear index model classified pixels as either clean or dirty based on the distribution of the colors red, green and blue. The SVM model is based on machine-learning concepts. A medical doctor classified pixels as being either clean or dirty. Using these evaluated pixels, a SVM algorithm was created through machine-learning to assess the cleanliness of each pixel. For defining the cleanliness of each frame and subsequently for each video, thresholds for unacceptable, poor, fair and good cleansing were predicted and corrected using learning techniques within the algorithm. To be able to evaluate both models, bowel cleansing quality of each video was also classified by four CCE readers including two international experts and two medical doctors with short formal training.

Performance of the proposed AI models for computed assessment of bowel cleansing

The CAC scores developed in the first study resulted in a bowel cleansing evaluation for each CCE frame defined as either adequately or inadequately cleansed (14). The R/G ratio discriminated adequately cleansed frames from inadequately cleansed frames with a sensitivity of 86.5% and a specificity of 78.2%, whereas the R/(R+G) ratio did this with a higher sensitivity of 95.5% but a lower specificity of 63.0%.

The CAC models developed in the second study resulted in a bowel cleansing classification for each CCE video defined as either unacceptable, poor, fair or good (16). Evaluation of the performance of their models was not expressed in terms of sensitivity and specificity, but in levels of agreement with the CCE readers. The non-linear index model classified 32% of the videos in agreement with the CCE readers, while the SVM model reached a higher agreement level of 47%. The non-linear index model misclassified 32% of the videos with more than one level of cleanliness compared to 12% in the SVM model.

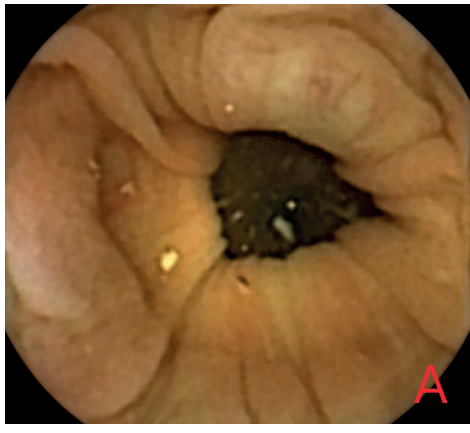


Figure 2A Adequately cleansed CCE frame

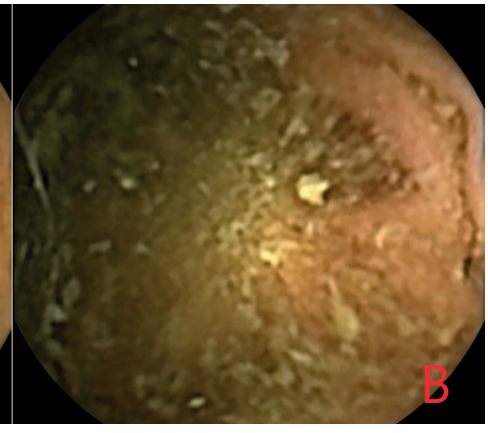


Figure 2B Inadequately cleansed CCE frame

Table 4 Results of the two included studies examining computed assessment of bowel cleansing in CCE

Study	Type of AI	Frames/videos analyzed, n	Adequately cleansed frames/ videos, %	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Level of agreement AI with readers, %	Videos misclassified more than one class
Becq* (14)	R/G ratio	216 frames	16.7%	86.5%	78.2%	45.1%	96.6%	-	-
	R/(R+G) ratio	192 frames	9.9%	95.5%	63.0%	25.0%	99.0%	-	-
Buijs** (16)	Non-linear index model	41 videos	Unknown	-	-	-	-	32%	32%
	SVM model	41 videos	Unknown	-	-	-	-	47%	12%

AI = Artificial Intelligence, PPV = Positive Predictive Value, NPV = Negative Predictive Value, R/G = Red over Green, R/(R+G) = Red over Brown, SVM = Support Vector Machine, CCE = Colon Capsule Endoscopy

The computed assessment of cleansing (CAC) scores developed by Becq et al resulted in a bowel cleansing evaluation for each frame defined as either adequately or inadequately cleansed. The CAC models developed by Buijs et al resulted in a bowel cleansing classification for each video defined as either unacceptable, poor, fair or good.

** The percentage of adequately cleansed frames/videos was based on the evaluation by the reference group.*

*** 31 adequately cleansed (fair or good) and 10 inadequately cleansed (unacceptable or poor) videos were selected from a previous trial. The videos were re-evaluated by the reference group in this study, however, numbers on the adequate cleansing levels from these evaluations were not reported.*

Artificial intelligence for polyp detection in CCE-2

Five studies reported on AI polyp detection in CCE-2 (Table 5, Figure 3).

Development of the proposed AI models for polyp detection

The first two studies developed algorithms for automated polyp detection based on the geometric characteristic of polyps that they have a roundish protrusion into the colonic lumen compared to the surrounding mucosal surface. In the first study the amount of protrusion was gauged into a special function called P, where the value of P is closely related to the size of the protrusion in the images (17). Findings from a subsequent colonoscopy were used as a reference to determine which frames contained polyps. In the second study a binary classification algorithm was developed that resulted in the output "polyp" or "normal" (15). Frames that potentially contained polyps were first pre-selected based on the texture content. The surface of polyps is often highly textured, however too much texture implies the presence of bubbles or trash liquid. Therefore, in the preselection procedure all frames with too little or too much texture were discarded. Subsequently, a measure of protrusion was created which was used as the decision parameter of the final binary classifier with pre-selection. From the used CCE dataset it was known which frames contained polyps. Based on the entire dataset, the optimal threshold of the created binary classifier with pre-selection used to classify a frame as containing a polyp was determined by using a ROC curve. To limit the number of frames that need to be manually re-assessed by an expert, a desired level of 90% specificity was used.

The other three studies on CCE polyp detection developed a convolutional neural network (CNN) that classified frames as either "normal" or "containing a polyp/colorectal neoplasia/protruding lesion" (19-21). CNN uses many images to develop and train a model by learning rich features from these images. Ideally, a large amount of data is needed to develop and train these models. However, available data in the form of CCE images is limited which makes it difficult to create a CNN for CCE polyp detection from scratch. To partially overcome this problem, all three studies used an existing CNN architecture and trained this model with CCE images to improve its performance. To test the performance of the proposed CNN models, all studies used separate images that were not used for the training of the models. The third study used manual analyses performed by trained nurses and gastro-enterologists as the reference group (19). The fourth study used manual analyses performed by three expert gastroenterologists (20). The fifth study used manual analyses performed by two expert gastroenterologists (21). The proposed CNN model in the fourth study was not only developed to detect polyps but also colorectal cancer (colorectal neoplasia) and the CNN model in the fifth study was developed to detect protruding lesions such as polyps, epithelial tumors, submu-

cosal tumors and nodes. These last two studies created a ROC curve to measure the performance of their CNN model.

Performance of the proposed AI models for polyp detection

The first study did not evaluate the accuracy of their developed algorithm in terms of sensitivity and specificity [17]. They only provided a description of the amount of protrusion into the lumen of CCE images expressed in p-values for different colonic anomalies. 80% of all polyps had a p-value higher than 500. All polyps that expressed a p-value higher than 2000 were polyps that were larger than 1 centimeter. The p-value was always under 500 in frames with cecal ulcer, diverticula, bubbles or trash liquid. However, some examples were shown that some folds mimicked polyps and were associated with a high p-value.

The other studies did provide numbers on the accuracy of their AI models for automated polyp detection in CCE. The binary classifier with pre-selection developed in the second study resulted in a sensitivity of 47.4% and a specificity of 90.2% on a per frame basis using a threshold value of 37 (15). Since in a clinical setting it is important that each polyp is detected in at least 1 frame, a ROC curve was also determined on a per polyp basis. At the same threshold value, this resulted in a sensitivity of 81.3% and a specificity of 90.2%. At a threshold of 40 a specificity of 93.5% was reached while maintaining the same per polyp sensitivity.

Even though the CNN model created in the third study was only evaluated on a per frame basis, their model resulted in an even better performance with a sensitivity of 98.1% and a specificity of 96.3% (19). The fourth study also evaluated performance on both per frame and per lesion basis, but again this did not result in a better performance than the CNN model in the third study. The model from the fourth study resulted in a sensitivity of 79.0% and a specificity of 87.0% for colorectal neoplasia on a per frame basis. Per lesion analysis increased the sensitivity to 96.2% (20). The CNN model in the fifth study was only evaluated on a per frame basis and resulted in a sensitivity of 90.7% and a specificity of 92.6% (21).

Other artificial intelligence for CCE-2

Besides the studies on artificial intelligence for the assessment of bowel cleansing and polyp detection in CCE-2, two other studies were included. One study reported on the detection of blood in the colonic lumen (22). They developed a convolutional neural network (CNN) that classified frames as either "normal" or "containing blood". The same strategy for CNN development was used as in the previously mentioned study on polyp detection conducted by the same research group (21). The CNN model only evaluated

the presence of blood on a per frame basis and resulted in a sensitivity of 99.8% and a specificity 93.2%.

Another study reported on artificial intelligence for the localization of CCE-2 (18). A model describing the shape of the intestine was created and feature points such as edges, corners, blobs or ridges were identified. Subsequently, capsule movement and speed were estimated by determining movement towards, away or rotated from these feature points, also taken the capsule's frames per second (Hz) into account. The model was run many times and resulted in similar colonic shaped paths. Points usually associated with the ascending colon, hepatic flexure, transverse colon, splenic flexure and descending colon were identified. The model's predictions of colonic sections were compared to expert labeled sections. The average accuracy of the model for frame colonic section classification was 86%.

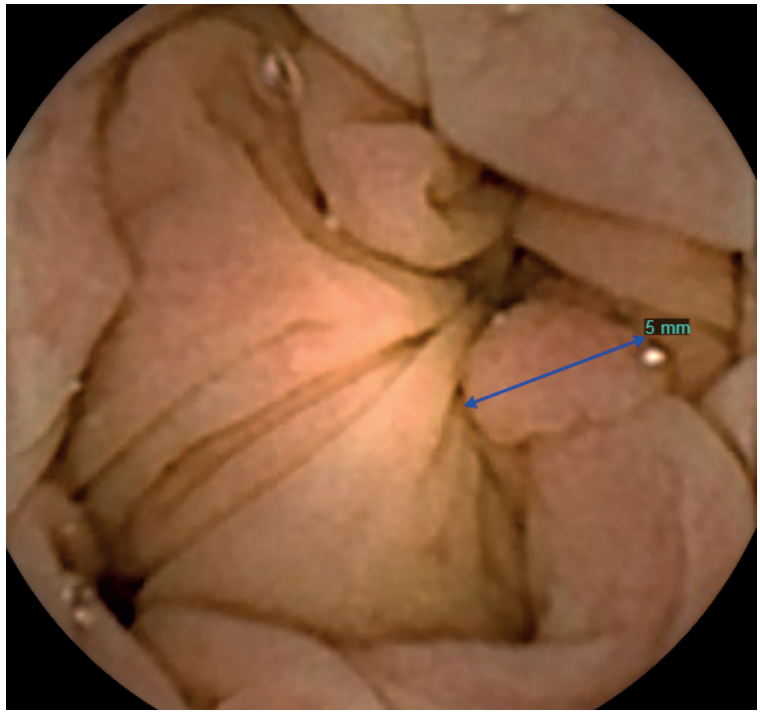


Figure 3 Polyp visualized in CCE

Table 5 Results of the five included studies examining computed polyp- or colorectal neoplasia detection in CCE

Study	Type of AI	Application	Frames analyzed, n	Amount of polyps or colorectal neoplasia, n	Amount of frames containing polyps, n	Cut-off value	Accuracy	Sensitivity on a per frame basis, %	Specificity on a per frame basis, %	Sensitivity on a per polyp basis, %	Specificity on a per polyp basis, %
Figueiredo (17)	Protrusion based algorithm	Polyp detection	1700	10	Unknown	-	-	-	-	-	-
Mamonov (15)	Binary classification after pre-selection	Polyp detection	18968	16	230	37	-	47.4%	90.2%	81.3%	90.2%
						40	-	-	-	81.3%	93.5%
Nadimi* (19)	CNN	Polyp detection	1695	Unknown	Unknown	-	98.0%	98.1%	96.3%	-	-
Yamada (20)**	CNN	Colorectal neoplasia detection	4784	105	Unknown	-	83.9%	79.0%	87.0%	96.2%	Unknown
Saraiva (21)	CNN	Protruding lesion detection	728	Unknown	172	-	92.2%	90.7%	92.6%	-	-

AI = Artificial Intelligence, CNN = Convolutional Neural Network

Unknown means the numbers were not described, - means the numbers were not part of the outcomes of the study.

** The entire dataset consisted of 11,300 CCE images of which 4800 contained colorectal polyps. Of the entire dataset, 15% was used to test the performance of the CNN. The amount of frames containing a polyp in this test dataset was not described.*

*** From the 105 observed colorectal neoplasia, 103 were polyps and 2 were colorectal cancers. 1850 images of patients with colorectal neoplasia were included. It was not described how many of the frames of the CCE-2 videos of these patients contained polyps or colorectal cance*

Discussion

To our knowledge, this is the first systematic review providing an overview on the use of AI methods for reviewing CCE-2 colonic images. CCE provides a non-invasive alternative to colonoscopy for exploration of the colonic mucosa, but its applicability is limited by the accompanying labor-intensive reviewing time and risk of inter observer variability. Automated reviewing of CCE images is an important step in the evolution of CCE. AI methods show promising results, with high sensitivity but lower specificity for the assessment of bowel cleansing and high sensitivity and specificity for polyp or colorectal neoplasia detection and blood detection.

Only one study reported the AI assessment of CCE-2 bowel cleansing in terms of sensitivity and specificity (14). However, this study shows promising results for its two developed CAC scores yielding in high sensitivities (86.5% and 95.5% respectively) but lower specificities (78.2% and 63.0% respectively) for discriminating adequately cleansed from inadequately cleansed images. Adequately cleansed frames were only observed in 16.7% and 9.9% respectively. CCE videos were excluded when they were identified as being too poor in quality after the first lecture and when they were incomplete, so actual overall adequate cleansing levels were even lower. In a previous meta-analysis on the accuracy of CCE compared to colonoscopy, the rate of adequate bowel preparation varied from 40-100%, where most studies reported adequate cleansing levels over 80% (4). The low number of adequately cleansed frames in the study included in this current review makes the risk of falsely identifying frames as adequately cleansed higher, which could explain the lower specificities of the CAC scores compared to its sensitivities. Since this was the only study reporting on AI for CCE bowel cleansing assessment in terms of sensitivity and specificity, the observed accuracy of bowel cleansing assessment by the CAC scores in this study cannot be compared to previous literature. However, optimal cut-off values yielding the highest diagnostic performance were determined for scores using a ROC curve, which could have led to overoptimistic results which could likely be poorer when using the same threshold in an independent sample (13).

The other study reporting on the AI assessment of CCE bowel cleansing did not report accuracy results of their proposed AI models in terms of sensitivity and specificity or the percentage of adequately cleansed videos (16). However, the low agreement levels of the non-linear index model (32%) and the SVM model (47%) with the reference group CCE readers are alarming. More studies on the AI assessment of CCE bowel cleansing in terms of sensitivity of specificity, with realistic adequate cleansing levels, are needed to be able to evaluate newly developed AI models accurately.

The proposed AI models for polyp or colorectal neoplasia detection resulted in high sensitivities of 47.4%-98.1% and high specificities of 87.0% to 96.3% in per-frame analysis (15, 19-21). Two studies performed per-lesion analysis, in addition to per-frame analysis, which improved sensitivity of polyp- or colorectal neoplasia detection to 81.3%-98.1% (15, 20). It should be noted that the abovementioned results from four included AI studies were all compared to CCE-2 readers, so the concluded sensitivities and specificities represent the ability of the AI models to reach the same performance levels as CCE-2 readers. The previously mentioned meta-analysis on the accuracy of per-lesion detection by CCE-2 readers compared to colonoscopy reported a sensitivity of 85% and a specificity of 85% for polyps of any size (4).

One study determined the optimal threshold of their binary classifier with pre-selection by using a ROC curve, which may have led to overoptimistic estimates of its performance (15). Still, highest sensitivities were reached in the other three studies that developed a CNN model for polyp or colorectal neoplasia detection (19-21). We believe future development of AI methods for reviewing CCE images should be focused on the creation of CNN models. While other AI methods fail to reach the same performance as humans, previous literature has shown that CNN is able to match human performance in different tasks (8, 23). However, optimal CNN requires training the algorithms with large amounts of data, which can be a challenge in the field of CCE for which the availability of data is limited.

Only one study reported on the computed detection of blood in the colonic lumen (22). Even so, their CNN model shows a promising result with a high sensitivity of 99.8%. Computed localization of the capsule within the colon was also only reported in one study. Accuracy for classifying frames to a specific colonic section was high (86%), but further studies are needed to validate this application in terms of sensitivity and specificity.

While conducting our literature search, it was remarkable how many articles did not specify whether they used small bowel capsule endoscopy (SB-CE) or colon capsule endoscopy (CCE). Even when the use of CCE was reported, it was not always reported whether the first generation (CCE-1) or second generation (CCE-2) capsule was used. CCE-1 is an outdated version of the colon capsule with low sensitivity for detection of polyps compared to CCE-2. Therefore, articles not specifying the use of CCE-2 were excluded from this review. Future studies on the AI assessment of reviewing CCE images should report on the type of capsule that was used.

Overall, literature on AI for reviewing CCE-2 colonic images is scarce. Two studies reported on the AI assessment of bowel cleansing and five studies reported on AI polyp

or colorectal neoplasia detection. Only one study reported on the detection of blood in the colonic lumen and only one study created a rough AI model for determining the location of the capsule within the colon. The used AI methods and study designs were heterogeneous. Therefore, we could not perform a formal meta-analysis. Most studies had a limited sample size to test the performance of their AI models. Especially for studies using machine or deep learning, a large proportion of CCE images is needed for training the model, limiting the amount of images left for testing the models. Three out of nine studies included in this review did not report on the performance of their AI models in terms of sensitivity and specificity, making it hard to determine their value (16-18).

Nevertheless, the studies presented in this systematic review show promising results for using AI in reviewing CCE-2 colonic images with high sensitivities for both bowel cleansing assessment as well as polyp or colorectal neoplasia detection and blood detection. Manual CCE review is time-consuming and faces problems regarding inter observer variability. Improvements in imaging recognition will improve the reading time and inter observer variability and may accelerate the use of CCE. This systematic review gives hope that AI can provide a timesaving, objective and reproducible method for reviewing CCE images.

Necessary action points to reach implementation of AI technology for CCE in daily practice

Actual implementation of AI for reviewing CCE-2 colonic images is a crucial step in the applicability of CCE in daily clinical practice. Future studies should preferably focus on CNN, because of its high potential in reaching human performance. In order to reach its implementation, several steps need to be taken. CNN algorithms need to be optimized and tested with more data, possibly requiring the set-up of a large international CCE database. To ensure adequate evaluation of the added value of the AI method, studies should always report the used capsule and accuracy of their models in terms of sensitivity and specificity. On top of that, studies should preferably only use the results from expert CCE readers to test the performance of their AI methods, since the concluded sensitivities and specificities represent the ability of the AI models to reach the same performance levels as these readers. Besides CNN, which requires an adequate number of coloscopy images, also synthetic samples can be used as artificial intelligence methods.(24, 25) Finally, when these gaps and barriers have been overcome, prospective clinical trials have to confirm the accuracy of the optimized CNN models

Acknowledgements

The authors wish to thank Wichor M. Bramer from the Erasmus MC Medical Library for developing and updating the search strategies..

References

1. Spada C, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2012;44(5):527-36.
2. Spada C, Hassan C, Bellini D, Burling D, Cappello G, Carretero C, et al. Imaging alternatives to colonoscopy: CT colonography and colon capsule. European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) Guideline - Update 2020. Eur Radiol. 2021;31(5):2967-82.
3. Spada C, Pasha SF, Gross SA, Leighton JA, Schnoll-Sussman F, Correale L, et al. Accuracy of First- and Second-Generation Colon Capsules in Endoscopic Detection of Colorectal Polyps: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2016;14(11):1533-43.e8.
4. Kjolhede T, Olholm AM, Kaalby L, Kidholm K, Qvist N, Baatrup G. Diagnostic accuracy of capsule endoscopy compared with colonoscopy for polyp detection: systematic review and meta-analyses. Endoscopy. 2020.
5. Vuik FER MS, Nieuwenburg SAV, Schreuders EH, Kuipers EJ, Spaander MCW. Applicability of Colon Capsule Endoscopy as Pan-endoscopy: From bowel preparation, transit- and rating times to completion rate and patient acceptance. Accepted for publication at Endoscopy International open.
6. Buijs MM, Kroijer R, Kobaek-Larsen M, Spada C, Fernandez-Urien I, Steele RJ, et al. Intra and inter-observer agreement on polyp detection in colon capsule endoscopy evaluations. United European Gastroenterol J. 2018;6(10):1563-8.
7. Soffer S, Klang E, Shimon O, Nachmias N, Eliakim R, Ben-Horin S, et al. Deep learning for wireless capsule endoscopy: a systematic review and meta-analysis. Gastrointest Endosc. 2020;92(4):831-9 e8.
8. Hosny A, Parmar C, Quackenbush J, Schwartz LH, Aerts H. Artificial intelligence in radiology. Nat Rev Cancer. 2018;18(8):500-10.
9. LeCun Y, Bengio Y, Hinton G. Deep learning. Nature. 2015;521(7553):436-44.
10. Hassan C, Spadaccini M, Iannone A, Maselli R, Jovani M, Chandrasekar VT, et al. Performance of artificial intelligence in colonoscopy for adenoma and polyp detection: a systematic review and meta-analysis. Gastrointest Endosc. 2021;93(1):77-85 e6.
11. Antonelli G, Badalamenti M, Hassan C, Repici A. Impact of artificial intelligence on colorectal polyp detection. Best Pract Res Clin Gastroenterol. 2021;52-53:101713.
12. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.
13. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529-36.
14. Becq A, Histace A, Camus M, Nion-Larmurier I, Abou Ali E, Pietri O, et al. Development of a computed cleansing score to assess quality of bowel preparation in colon capsule endoscopy. Endosc Int Open. 2018;6(7):E844-E50.
15. Mamonov AV, Figueiredo IN, Figueiredo PN, Tsai YH. Automated polyp detection in colon capsule endoscopy. IEEE Trans Med Imaging. 2014;33(7):1488-502.
16. Buijs MM, Ramezani MH, Herp J, Kroijer R, Kobaek-Larsen M, Baatrup G, et al. Assessment of bowel cleansing quality in colon capsule endoscopy using machine learning: a pilot study. Endosc Int Open. 2018;6(8):E1044-E50.

17. Figueiredo PN, Figueiredo IN, Prasath S, Tsai R. Automatic polyp detection in pillcam colon 2 capsule images and videos: preliminary feasibility report. Diagn Ther Endosc. 2011;2011:182435.
18. Herp J, Deding U, Buijs MM, Kroijer R, Baatrup G, Nadimi ES. Feature Point Tracking-Based Localization of Colon Capsule Endoscope. Diagnostics (Basel). 2021;11(2).
19. Nadimi ES, Buijs MM, Herp J, Kroijer R, Kobaek-Larsen M, Nielsen E, et al. Application of deep learning for autonomous detection and localization of colorectal polyps in wireless colon capsule endoscopy. Comput Electr Eng. 2020;81.
20. Yamada A, Niikura R, Otani K, Aoki T, Koike K. Automatic detection of colorectal neoplasia in wireless colon capsule endoscopic images using a deep convolutional neural network. Endoscopy. 2020.
21. Saraiva MM, Ferreira JPS, Cardoso H, Afonso J, Ribeiro T, Andrade P, et al. Artificial intelligence and colon capsule endoscopy: development of an automated diagnostic system of protruding lesions in colon capsule endoscopy. Tech Coloproctol. 2021;25(11):1243-8.
22. Mascarenhas Saraiva M, Ferreira JPS, Cardoso H, Afonso J, Ribeiro T, Andrade P, et al. Artificial intelligence and colon capsule endoscopy: automatic detection of blood in colon capsule endoscopy using a convolutional neural network. Endosc Int Open. 2021;9(8):E1264-E8.
23. Mnih V, Kavukcuoglu K, Silver D, Rusu AA, Veness J, Bellemare MG, et al. Human-level control through deep reinforcement learning. Nature. 2015;518(7540):529-33.
24. Adjei PE, Lonseko ZM, Du W, Zhang H, Rao N. Examining the effect of synthetic data augmentation in polyp detection and segmentation. Int J Comput Assist Radiol Surg. 2022;17(7):1289-302.
25. Ozyoruk KB, Gokceler GI, Bobrow TL, Coskun G, Incetan K, Almalioglu Y, et al. EndoSLAM dataset and an unsupervised monocular visual odometry and depth estimation approach for endoscopic videos. Med Image Anal. 2021;71:102058.

Part V

Screening methods of colorectal cancer – faecal immunochemical test

Chapter 11

Effect of anticoagulants and NSAIDs on accuracy of faecal immunochemical tests (FITs) in colorectal cancer screening: a systematic review and meta-analysis

S.A.V. Nieuwenburg, **F.E.R. Vuik**, M.J.H.A. Kruip, E.J. Kuipers, M.C.W. Spaander

Gut, May 2019

Abstract

Introduction Most colorectal cancer (CRC) screening programmes are nowadays based on faecal immunochemical testing (FIT). Eligible subjects often use oral anticoagulants (OACs) or non-steroidal anti-inflammatory drugs (NSAIDs), which could possibly stimulate bleeding from both benign and premalignant lesions in the colon. The aim of this meta-analysis was to study the effect of OACs and NSAIDs use on FIT performance.

Methods A systematic search was conducted until June 2017 to retrieve studies from PubMed, Embase, MEDLINE, Web of science, Cochrane Central and Google Scholar. Studies were included when reporting on FIT results in users versus non-users of OACs and/or NSAIDs in average risk CRC screening populations. Primary outcome was positive predictive value for advanced neoplasia (PPV_{AN}) of FIT in relation to OACs/NSAIDs use. Values were obtained by conducting random-effect forest plots.

Results Our literature search identified 2022 records, of which 8 studies were included. A total of 3563 participants with a positive FIT were included. Use of OACs was associated with a PPV_{AN} of 37.6% (95% CI 33.9 to 41.4) compared with 40.3% (95% CI 38.5 to 42.1) for non-users (p=0.75). Pooled PPV_{AN} in aspirin/NSAID users was 38.2% (95% CI 33.8 to 42.9) compared with 39.4% (95% CI 37.5 to 41.3) for non-users (p=0.59).

Conclusion FIT accuracy is not affected by OACs and aspirin/NSAIDs use. Based on the current literature, withdrawal of OACs or NSAIDs before FIT screening is not recommended. Future studies should focus on duration of use, dosage and classes of drugs in association with accuracy of FIT to conduct more specific guideline recommendations.

Introduction

Worldwide, most colorectal cancer (CRC) screening programmes are now based on faecal immunochemical testing (FIT) (1). In the European Union, FIT-based CRC screening programmes have an average FIT positivity rate (PR) around 6.2% and a positive predictive value for advanced neoplasia (PPV_{AN}) between 35% and 55% and are thereby more accurate than those for older, guaiac-based faecal occult. blood tests (gFOBT) (2–5). PPV of FIT depends on AN, gender, FIT cut-off and participation in previous screening rounds. It is affected by false-positive results from bleeding sources other than colorectal neoplasia (6, 7). Several studies suggest the use of oral anticoagulants (OACs) or non-steroidal anti-inflammatory drugs (NSAIDs) as a possible contributor to the false-PR of faecal blood tests. These studies hypothesise that OACs/NSAIDs could stimulate other, benign lesions to bleed and thereby decrease PPV_{AN} (8–10). In contrast, these drugs may in theory also increase the tendency of neoplastic lesions to bleed and thus increase PPV_{AN} (11, 12). Results of a previous meta-analysis and systematic review were inconclusive (13, 14). However, most studies at that time were performed with gFOBT and not with the currently practised FIT (1). Until today, clinicians lack clear recommendations. This is remarkable given the widespread use of CRC screening tests and the frequent use of OACs and NSAIDs in the target population of subjects aged 50 years and above (15, 16). Moreover, discontinuation of anticoagulant therapy is not without risk in terms of (re) occurrence of cardiovascular events, and discontinuation should thus be considered with care (17). Therefore, this meta-analysis aimed to evaluate the PPV_{AN} and positive predictive value for CRC (PPV_{CRC}) in OACs and NSAIDs users compared with non-users in an average risk FIT-based CRC screening population. Second, we assessed PRs, sensitivity/ specificity and negative predictive values (NPVs) when possible. Subgroup analyses were performed with respect to patient and drug characteristics when possible.

Method

Search strategy

We conducted a systematic review and meta-analysis of published trials and abstracts following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (18). Additionally, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist was used, containing specifications for the reporting of a meta-analysis of observational studies in epidemiology (19).

Data sources

In collaboration with the Medical School Library of the Erasmus University in Rotterdam, the Netherlands, a systematic search was conducted until June 2017 to retrieve studies that reported on FIT performance in OACs or NSAIDs users versus controls. PubMed, Embase, MEDLINE, Web of science, Cochrane Central and Google Scholar were used as potential sources. The search was conducted using controlled vocabulary supplemented with key words (supplementary S1). First, two independent reviewers (SAVN and FERV) screened the selected studies by title and abstract. Studies were excluded if they did not correspond with the inclusion and/or exclusion criteria that are stated below. Furthermore, full text of the selected publications were examined by the same authors. Discrepancies were discussed with a third party (MCWS). References of the retrieved studies were manually searched to locate any additional studies.

Study selection

Studies were included if they met the following criteria: (1) population-based one-sample FIT screening in an average risk population (>40 years old), (2) subjects were screened with FIT, while taking an OAC or NSAID, with subsequent colonoscopy in case of a positive faecal occult blood test; and (3) control group included patients who were screened by means of FIT, not taking OAC or NSAID, and also undergoing colonoscopy in case of a positive faecal occult blood test. The following studies were excluded: (1) those that used gFOBT instead of FIT; (2) systematic reviews and meta-analyses; and (3) editorials/letters.

Outcome parameters

Primary outcome was the pooled positive predictive value (PPV) of FIT for detecting advanced neoplasia (PPV_{AN}) in patients using any OACs and for aspirin/NSAIDs alone compared with non-users. Secondary outcomes were the pooled PR of FIT, the pooled NPV and sensitivity and specificity of FIT for advanced neoplasia (AN) and CRC during OACs/NSAIDs use versus no use.

Definitions

Advanced adenomas (AAs) were defined as adenomas >10 mm, or with villous histology, or high-grade dysplasia. CRC was considered to be the case when malignant cells were observed beyond the muscularis mucosa. AN comprised AA and CRC. Pooled OACs included use of vitamin K antagonists, platelet aggregation inhibitors and novel OACs. NSAIDs were not further specified. We converted units for FIT positivity cut-off into micrograms (μg) of haemoglobin (Hb) per gram of stool for each study when other units were practised.

Data extraction

Data were extracted by the same authors (SAVN and FERV) according to previously stated variables (supplementary S2). When data in the published studies were not conclusive for our analyses, authors were contacted by mail and/or telephone for additional data.

Data analyses

The sensitivity, specificity, PPV, NPV and PR with corresponding 95% CI were calculated for each study in case data were available. Pooled relative risks (RRs) were obtained by a random-effect forest plot using an inverse-variance estimator, in which an RR smaller than 1 reflects a higher PPV in users versus non-users. An RR greater than 1 implies a lower PPV in users versus non-users.20 Heterogeneity among studies was measured by calculating the inconsistency index (I^2). Heterogeneity levels can range from 0% to 100% (maximum heterogeneity), with greater than 25%, 50% and 75% being low, moderate and high heterogeneity, respectively (21).

Study quality

Publication bias was assessed by constructing funnel plots. Assessment of methodological quality of observational cohort studies and case–control studies was carried out using the Ottawa-New-castle Scale (22). This scale scores quality of design, content and ease of use directed to the task of performing and interpreting meta-analyses results. A star system has been developed in which a study is judged on (1) selection of study groups, (2) comparability of groups and (3) the ascertainment of either the exposure for case–control studies or the outcome of interest for observational studies. The outcome ranges from 0 (low) to 9 (high) stars. Assessment of quality of evidence was carried out using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (23). Two authors (SAVN and FERV) independently assessed study quality. Review Manager V.5.3 was used for all analyses. Forest plots were conducted in R V.3.4.2.

Results

Literature search

After removal of duplicates, we identified 2.022 studies through the electronic database search (figure 1). We excluded 1.970 studies after screening titles and abstracts. Of the remaining, 52 were examined by full-text review. Forty-four studies were excluded. We included six studies in full and two published abstracts in our meta-analysis (24–31).

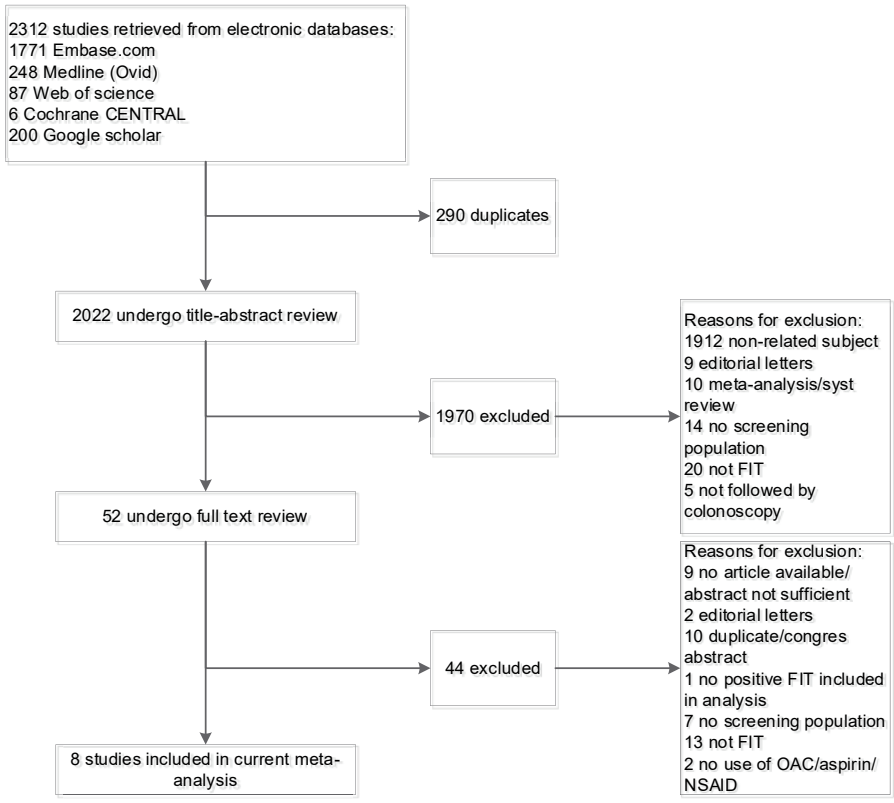


Figure 1 Flow chart: selection of studies for inclusion. FIT, faecal immunochemical testing; NSAID, non-steroidal anti-inflammatory drug; OAC, oral anticoagulant.

Study characteristics

Baseline characteristics of the included studies are shown in table 1. Eight observational cohort studies and one case–control study were included. Seven studies were performed in Europe and one in Asia. The cut-off for a positive FIT ranged between 2 µg and50 µg Hb/g faeces. Pooled analyses of different types of OACs were applied in the included studies (24, 27–29). Addition-ally, separate analyses were made for aspirin (24–26, 29–31). One study provided data on NSAIDs, and these users were pooled with aspirin users (31). All studies contained data to calculate PPVAN. Two studies additionally included data on sensitivity, specificity and NPV (30, 31). Another two studies contained data on PR of FIT (26, 27). Two studies comprised the same screening cohort, yet subgroups for medication use were defined differently in both studies (26, 27). For our analyses on pooled OACs, we used the most recent published data (27). For separate analysis for aspirin/NSAID use, we used the published data on the aspirin group (26). A summary of primary and secondary outcomes per study are presented in table 2. On methodological

quality, studies scored between six and eight stars (out of a maximum of nine) according to the Newcastle-Ottawa Scale (online supplementary S3). According to the GRADE guidelines, quality of evidence for our analyses scored 'low' (online supplementary S4). Heterogeneity between studies for pooled OAC analysis was scored as 'low'. Separate analysis on aspirin/ NSAIDs scored 'moderate' (figures 2 and 3). No publication bias was found when funnel plots were conducted (online supplementary S5).

Primary outcomes

Pooled OAC use versus no use

Positive predictive value for advanced neoplasia

Our meta-analysis composed pooled data on 633 OAC users and 2930 non-users, all FIT-positive patients. Users provided a PPVAN of 37.6% (95% CI 33.9 to 41.4) compared with a PPVAN of 40.3% (95% CI 38.5 to 42.1) for non-users. The forest plot shown in figure 2 showed no significant difference (p=0.75).

Table 1 Baseline characteristics of included studies. *OAC,* oral anticoagulants; *FIT,* fecal immunochemical test

Study	Type of article	Type of study	Country	Age interval (years)	Time period	Eligible participants (N)	FIT cut-off (µg Hb/g feces)	Type of FIT	Negative FIT + colonoscopy	Medication use
Wauters, 2017 (24)	Abstract	screening cohort	Belgium	55-75	2015	463	15	OC-sensor	0	OAC: 111 Aspirin: 75
Botteri, 2016 (25)	Full text	screening cohort	Italy	50-69	2007-2009	743	20	HM-Jack	0	Aspirin < 5yr: 49 Aspirin > 5 yr:52
Wong, 2015 (31)	Full text	screening cohort	Hongkong	50-70	2008-2012	505	50	Hemosure	4834	Aspirin / NSAID: 40
Bujanda, 2014 (27)	Full text	screening cohort	Spain	50-69	2008-2011	386	15	OC-sensor	0	OAC: 21
Bujanda, 2013 (26)	Full text	screening cohort	Spain	50-69	2008-2011	365	15	OC-sensor	0	Aspirin: 28
Denters, 2011 (28)	Abstract	screening cohort	Netherlands	50-75	2006-2008	510	10	OC-sensor	0	OAC: 88
Mandelli, 2011 (29)	Full text	screening case-control	Italy	50-69	2007-2009	675	20	OC-sensor	0	OAC: 225 Aspirin: 172
Brenner, 2010 (30)	Full text	screening cohort	Germany	> 55	2005-2009	281	2	RIDA-SCREEN Hb	1698	Aspirin: 47
Total	-		-	-	-	3928	-	-	6532	Pooled OAC: 445 Aspirin/NSAID: 463

Table 2 Summary of pooled data of oral anticoagulants users and non-users *PR*, positivity rate; *CI*, confidence interval; *PPV*, positive predictive value; *AN*, advanced neoplasia; *FIT,* fecal immunochemical test . * showed a significant result

Study		PR_{FIT} % (95%CI)	PPV_{AN} % [95%CI]	$Sensitivity_{AN}$ % [95%CI]	$Specificity_{AN}$ % [95%CI]
Wauters, 2017 (24)	Users	/	49.5 [40.0-59.1]	/	/
	Non-users	/	42.4 [37.1-47.7]	/	/
Botteri, 2016 (25)	Users	/	49.5 [39.5-59.6]*	/	/
	Non-users	/	54.2 [50.3-58.1]*	/	/
Wong, 2015 (31)	Users	/	7.5 [2.0-2.1]	15.8 [3.4-39.6]	89.1 [85.3-92.2]*
	Non-users	/	20.0 [16.5-24.0]	34.3 [28.7-40.3]	92.1 [91.3-92.3]*
Bujanda, 2014 (27)	Users	9.3 [6.0-14.2]	47.6 [26.4-69.7]	/	/
	Non-users	6.2 [5.7-6.9]	50.4 [45.2-55.6]	/	/
Denters, 2011 (28)	Users	/	43.2 [32.8-54.2]	/	/
	Non-users	/	46.9 [42.1-51.8]	/	/
Mandelli, 2011 (29)	Users	/	28.9 [23.2-35.4]	/	/
	Non-users	/	32.0 [27.8-36.6]	/	/
Brenner, 2010 (30)	Users	/	36.2 [23.1-51.5]	70.8 [48.9-87.4]*	85.7 [80.2-90.1]*
	Non-users	/	27.8 [22.2-34.1]	35.9 [28.9-43.4]*	89.2 [87.6-90.7]*

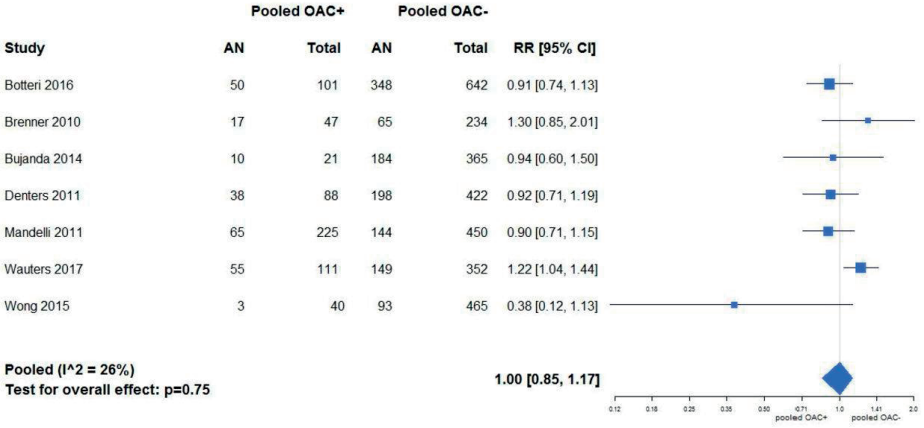


Figure 2 Forest plot on positive predictive value for advanced neoplasia (PPV_{AN}) of faecal immunochemical test (FIT) obtained with pooled oral anticoagulants (OAC) use versus no use. *AN*, advanced neoplasia; *RR*, relative risk.

Positive predictive value for CRC

Two studies provided data on CRC with pooled OAC use comprising 336 users and 802 non-users.24 29 Pooled OAC users provided a PPVCRC of 5.7% (95% CI 3.7 to 8.7) compared with 6.2% (95% CI 4.8 to 8.1) for non-users.

Pooled data for aspirin/NSAID use identified 463 users and 2438 non-users in FIT-positive patients. Users yielded a pooled PPVAN of 38.2% (95% CI 33.8 to 42.9) compared with 39.4% (95% CI 37.5 to 41.3) for non-users. The forest plot shown in **figure 3** revealed no significant difference (p=0.59).

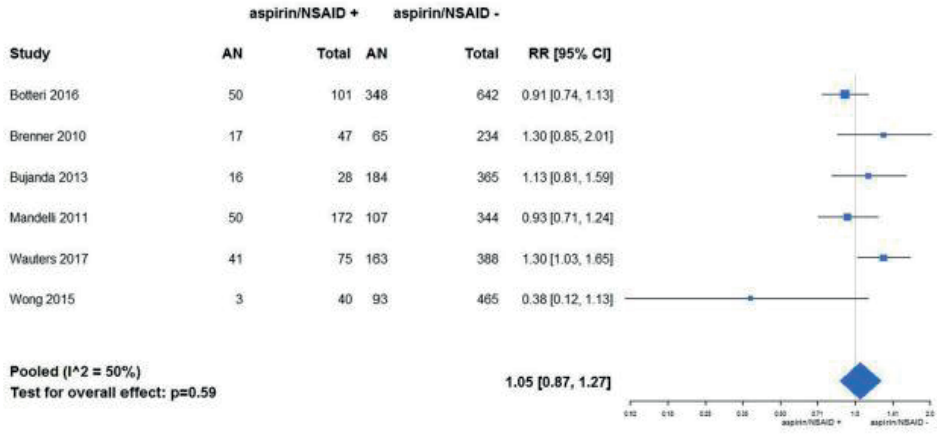


Figure 3 Forest plot on positive predictive value for advanced neoplasia (PPV_{AN}) of faecal immunochemical test (FIT) obtained with aspirin/non-steroidal anti-inflammatory drugs (NSAID) use versus no use. *AN*, advanced neoplasia; *RR*, relative risk

Secondary outcomes

Positivity rate

The PR of FIT was calculated in one cohort (27). An overall PR of 6.3% was observed. When acenocoumarol was used, PR of FIT was 9.3% versus 6.2% for non-users.

Subanalysis of aspirin alone was associated with a PR of 7.3%, compared with PR of 7.1% for non-aspirin antiplatelet agents.26 In patients undergoing dual antiplatelet therapy (DAPT), PR of FIT was 22.2% compared with 6.3% for non-users (OR 3.5; 95% CI 1.7 to 7.3). Also, the number of AN found in the DAPT subgroup was higher than in non-users (OR 2.8; 95% CI 1.1 to 7.2).

Sensitivity and specificity

No data were available on sensitivity and specificity of FIT in pooled OAC users.

One study assessed sensitivity and specificity in aspirin/NSAID users (31). Sensitivity for AN was 15.8% for users, compared with 34.2% for non-users (p=0.097). Specificity for AN was significantly lower for aspirin/NSAID users; 89.1% compared with 92.1% for non-

users (p=0.049). NPV showed no significant difference; 95.0% for users, compared with 96.1% for non-users (p=0.338).

Another study showed a sensitivity of 70.8% for aspirin users alone, compared with 35.9% for non-users (p=0.001). Specificity was 85.7% for aspirin users compared with 89.2% for non-users (p=0.13). NPV was 96.2% for aspirin users, compared with 92.3% for non-users (p=0.05) (30).

Subgroup analyses

Duration of drug use

One study made a distinction based on the median duration of aspirin use (25). Two categories were formed: a median use of ≤5 years and ≥5 years. A total of 49 patients using aspirin ≤5 years provided a PPV_{AN} of 61.2% (95% CI 47.2 to 73.6) compared with 52 aspirin users ≥5 years providing a PPV_{AN} of 38.5% (95% CI 26.5 to 52.0) (p=0.03) (25).

Type of FIT used

Seven studies used a quantitative FIT (24–30). One study used a qualitative FIT (31). When the study with a qualitative FIT was excluded, no changes in pooled results were seen (pooled PPV_{AN} in users of OAC: 39.6% vs 44.1% in non-users, RR: 0.99 (95% CI 0.89 to 1.11, p=0.44). Furthermore, five out of the eight studies included used the OC-sensor (24, 26–29). After excluding the three studies that used another FIT brand, no alterations in pooled results were seen (pooled PPV_{AN} in users of OAC: 37.8% vs 42.4% in non-users, RR: 1.00 (95% CI 0.87 to 1.14), p=0.99) (25, 30, 31).

FIT cut-off used

Different cut-offs were used; most studies vary between a cut-off level of 10–20 µg Hb/g faeces (24–29). Two studies used a cut-off of, respectively, 2 µg and 50 µg Hb/g faeces (30, 31). If these two outlier cut-offs were left out, no alterations in pooled results were seen (pooled PPV in users of OAC: 39.9% vs 45.8% in non-users, AN RR: 0.97 (95% CI 0.87 to 1.09), p=0.64).

Discussion

This is the first systematic review and meta-analysis to determine the PPV_{AN} of FIT in relation to OACs or NSAIDs use. Our results show that the use of OACs or aspirin/NSAIDs do not affect the PPVAN in FIT CRC screening. The PPV_{AN} of pooled OAC users was 37.6% versus 40.3% in non-users. For separate analyses on aspirin/NSAID users, the PPV_{AN} was

38.2%, whereas PPV_{AN} of non-users was 39.4%. Based on current literature, the withdrawal of OACs or aspirin/NSAIDs during FIT screening is not recommended. Our data are supported by previous work that pooled data on warfarin use during faecal occult blood test screening. Results showed no alterations in PPV of colorectal AN (13). However, included studies were performed on gFOBT and not on FIT. Another meta-analysis compared accuracy of FIT and gFOBT screening if OACs or NSAIDs were used (32). They showed a decrease in PPV_{AN} in gFOBT screening and no significant difference in PPV of FIT. Hence, only one study on FIT screening was included in this meta-analysis (29). FIT and gFOBT differ in their interaction with Hb. Guaiac-based tests interact with the haem part of Hb, and immunochemical tests detect the globin portion of Hb. The latter does not survive passage through the upper gastrointestinal tract, and therefore, FIT has a proven superior accuracy for colon or rectum bleeding compared with gFOBT (2, 3). For this reason, it is to assume that effects of OACs and NSAIDs could act differently in both tests. Growing literature on FIT screening helped to perform the current meta-analysis based on the today's practised FIT. Our results support the previous suggestion that OACs and aspirin/NSAIDs do not affect PPV_{AN} of FIT. Only one cohort provided data on PR of FIT in which a higher PR was seen in users compared with non-users (26, 27). As already hypothetically stated, this could be due to possible stimulation of bleeding from lesions in the colon (both benign and (pre) malignant). More so, the use of DAPT showed an even more strong effect on increased PR, supporting the literature on DAPT and its stimulating effect on lower gastrointestinal bleedings (33). Bearing in mind the similar PPV for users and non-users (or even a greater PPV in the case of DAPT users), this could presume the stimulation of premalignant lesions to bleed and causing a beneficial effect of OAC and aspirin/NSAID use by having more true FIT positives in users. One study used a qualitative test (ie, providing a positive or negative result without specific blood count) (Hemosure test kit) and calculated a PPV_{AN} of 20.0% for aspirin/NSAID users, compared with 7.5% for non-users.31 In our meta-analysis, these results act as an outlier compared with other study outcomes. When left out of our analysis, no evident effects on pooled PPV_{AN} of users versus non-users were seen. In our meta-analysis, all included studies applied a one-sample FIT. There is one study evaluating FIT performance and the use of antithrombotics in a two-sample FIT screening showing also that OAC use do not affect FIT performance (34). Globally, CRC screening guidelines focus mostly on age range of screening, time intervals, multiple test options and follow-up diagnostics. Although specific subgroups are discussed (eg, different ethnicities and individuals with a family history of CRC), OAC/NSAID users are left out (35, 36). Given the significant proportion of subjects using these drugs and the renewing scientific evidence on this topic, guideline adjustments should be considered. Although this has been an ongoing discussion (37), still no recommendations were made in the latest update of the US Multi-Society Task Force CRC screening guidelines (35).

Certain limitations have to be addressed in order to add specific recommendations. First, cut-off points of FIT were varying and overall relatively low. The use of different cut-off points of FIT affects accuracy of FIT. An increase in faecal Hb concentration cut-off is associated with higher PPV (6). Second, no subgroup analyses on age, gender, type of drugs or duration of drug use could be performed since the number of studies was too low. It was already pointed out that separate analysis on duration of drug use could play an important part in FIT performance (25).

In conclusion, OACs and aspirin/NSAID use do not affect the PPV of FIT in CRC screening. Based on current literature, withdrawal of OACs and/or NSAIDs before FIT sampling is not recommended. However, subgroup analyses on subject and drug characteristics should be performed in order to conduct specific guideline recommendations, and PR of FIT in relation to the PPV should be taken into account.

Acknowledgements

The authors of this systematic review and meta-analysis would like to acknowledge the contribution of Wichor Bramer, biomedical information specialist of the Erasmus University Medical Center, for performing the systematic literature search.

References

1. Schreuders EH , Ruco A , Rabeneck L , et al . Colorectal cancer screening: a global overview of existing programmes. Gut 2015;64:1637–49.
2. Hol L , van Leerdam ME , van Ballegooijen M , et al . Screening for colorectal cancer: randomised trial comparing guaiac-based and immunochemical faecal occult blood testing and flexible sigmoidoscopy. Gut 2010;59:62–8.
3. van Rossum LG , van Rijn AF , Laheij RJ , et al . Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. Gastroenterology 2008;135:82–90.
4. Toes-Zoutendijk E , van Leerdam ME , Dekker E , et al . Real-time monitoring of results during first year of dutch colorectal cancer screening program and optimization by altering fecal immunochemical test cut-off levels. Gastroenterology 2017;152:767–75.
5. Ponti AAA , Ronco G , Senore C , et al . Cancer screening in the European Union. J Eur Union 2015;327:34–8.
6. Wieten E , Schreuders EH , Nieuwenburg SA , et al . Effects of increasing screening age and fecal hemoglobin cutoff concentrations in a colorectal cancer screening program. Clin Gastroenterol Hepatol 2016;14:1771–7.
7. van Roon AH , Wilschut JA , Hol L , et al . Diagnostic yield improves with collection of 2 samples in fecal immunochemical test screening without affecting attendance. Clin Gastroenterol Hepatol 2011;9:333–9.
8. Sawhney MS , McDougall H , Nelson DB , et al . Fecal occult blood test in patients on low-dose aspirin, warfarin, clopidogrel, or non-steroidal anti-inflammatory drugs. Dig Dis Sci 2010;55:1637–42.
9. Clarke P , Jack F , Carey FA , et al . Medications with anticoagulant properties increase the likelihood of a negative colonoscopy in faecal occult blood test population screening. Colorectal Dis 2006;8:389–92.
10. Lee TJ , Hull MA , Rajasekhar PT , et al . Aspirin users attending for NHS bowel cancer screening have less colorectal neoplasia: chemoprevention or false-positive faecal occult blood testing? Digestion 2012;85:278–81.
11. Kershenbaum A , Lavi I , Rennert G , et al . Fecal occult blood test performance indicators in warfarin-treated patients. Dis Colon Rectum 2010;53:224–9.
12. Levi Z , Rozen P , Hazazi R , et al . Sensitivity, but not specificity, of a quantitative immunochemical fecal occult blood test for neoplasia is slightly increased by the use of low-dose aspirin, NSAIDs, and anticoagulants. Am J Gastroenterol 2009;104:933–8.
13. Ashraf I , Paracha S , Paracha SU , et al . Warfarin use during fecal occult blood testing: a meta-analysis. Gastroenterology Res 2012;5:45–51.
14. Konrad G , Katz A . Are medication restrictions before FOBT necessary?: Practical advice based on a systematic review of the literature. Can Fam Physician 2012;58:939–48.
15. Anderson FA , Wheeler HB , Goldberg RJ , et al . A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern Med 1991;151:933–8.
16. Kannel WB , Belanger AJ . Epidemiology of heart failure. Am Heart J 1991;121:951–7.
17. Rivera-Caravaca JM , Roldán V , Esteve-Pastor MA , et al . Cessation of oral anticoagulation is an important risk factor for stroke and mortality in atrial fibrillation patients. Thromb Haemost 2017;117:1448–54.

18. Moher D , Liberati A , Tetzlaff J , et al . Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 2010;8:336–41.
19. Stroup DF , Berlin JA , Morton SC , et al . Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008–12.
20. Borenstein M , Hedges LV , Higgins JP , et al . A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods 2010;1:97–111.
21. Higgins JP , Thompson SG , Deeks JJ , et al . Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
22. Wells GA , Bea S . The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. 2009 http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm Google Scholar
23. Guyatt G , Oxman AD , Akl EA , et al . GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64:383–94.
24. Wauters LI , Van der Voort V , Dobbels P , et al . 331 Antithrombotics Do Not Impact the Performance of Immunochemical Fecal Occult Blood Testing for Colorectal Cancer Screening. Gastrointest Endosc 2017;85:AB70.
25. Botteri E , Crosta C , Bagnardi V , et al . Predictors of advanced colorectal neoplasia at initial and surveillance colonoscopy after positive screening immunochemical faecal occult blood test. Dig Liver Dis 2016;48:321–6.
26. Bujanda L , Lanas Á , Quintero E , et al . Effect of aspirin and antiplatelet drugs on the outcome of the fecal immunochemical test. Mayo Clin Proc 2013;88:683–9.
27. Bujanda L , Sarasqueta C , Lanas Á , et al . Effect of oral anticoagulants on the outcome of faecal immunochemical test. Br J Cancer 2014;110:1334–7.
28. Denters M , Deutekom M , van Rijn AF , et al . Antithrombotic and/or Anticoagulant Use is Not Associated With a Higher False Positivity Rate in CRC Screening Using FIT. Gastroenterology 2011;140:S-413.
29. Mandelli G , Radaelli F , Paggi S , et al . Anticoagulant or aspirin treatment does not affect the positive predictive value of an immunological fecal occult blood test in patients undergoing colorectal cancer screening: results from a nested in a cohort case-control study. Eur J Gastroenterol Hepatol 2011;23:323–6.
30. Brenner H , Tao S , Haug U . Low-dose aspirin use and performance of immunochemical fecal occult blood tests. JAMA 2010;304:2513–20.
31. Wong MC , Ching JY , Chan VC , et al . Factors associated with false-positive and false-negative fecal immunochemical test results for colorectal cancer screening. Gastrointest Endosc 2015;81:596–607.
32. Gandhi S , Narula N , Gandhi S , et al . Does acetylsalicylic acid or warfarin affect the accuracy of fecal occult blood tests? J Gastroenterol Hepatol 2013;28:931–6.
33. Casado Arroyo R , Polo-Tomas M , Roncalés MP , et al . Lower GI bleeding is more common than upper among patients on dual antiplatelet therapy: long-term follow-up of a cohort of patients commonly using PPI co-therapy. Heart 2012;98:718–23.
34. Tsuji Y , Gunji T , Sato H , et al . Antithrombotic drug does not affect the positive predictive value of an immunochemical fecal occult blood test. Dig Endosc 2014;26:424–9.
35. Rex DK , Boland CR , Dominitz JA , et al . Colorectal Cancer Screening: Recommendations for Physicians and Patients from the U.S. Multi-Society Task Force on Colorectal Cancer. Am J Gastroenterol 2017;112:1016–30.

36. Cairns SR , Scholefield JH , Steele RJ , et al . Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). Gut 2010;59:666–89.
37. Baty V . Fecal immunochemical testing for colorectal cancer and medication restrictions. Gastrointest Endosc 2017;85:1310–1.

Supplementary Files

S1 Systematic Literature Search

Embase.com

('acetylsalicylic acid'/de OR 'anticoagulant agent'/exp OR 'anticoagulant therapy'/de OR 'anticoagulation'/de OR 'thrombocyte aggregation inhibition'/exp OR 'nonsteroid antiinflammatory agent'/exp OR (aspirin* OR (acetylsalicylic NEAR/3 acid*) OR acetylsalicylate* OR anticoagul* OR anti-coagul* OR antithromb* OR anti-thromb* OR (clotting NEAR/3 inhibitor*) OR heparin OR antifibrinolyt* OR anti-fibrinolyt* OR antiplatelet* OR anti-platelet* OR ((platelet* OR fibrinoly* OR vitamin-K OR Factor-Xa OR Factor-X OR thrombin OR thrombocyte*) NEAR/3 (inhibit* OR antagon* OR anti OR antiaggregat*)) OR warfarin* OR coumarin* OR aspirin* OR (acetylsalicylic NEAR/3 acid*) OR acetylsalicylate* OR ((nonsteroid* OR non-steroid*) NEAR/3 (antiinflamm* OR anti-inflamm*)) OR nsaid* OR ibuprofen*):ab,ti) AND ('occult blood'/exp OR 'feces analysis'/exp OR (('feces'/de OR defecation/de) AND ('immunochemistry'/exp)) OR (((faecal OR fecal OR faeces OR feces OR stool OR defecat*) NEAR/3 (immunohistochem* OR immunochem* OR fit)) OR ifobt OR fobt OR ifobts OR fobts OR (fit NEAR/3 (test*))):ab,ti) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim

Medline (Ovid)

("acetylsalicylic acid"/ OR exp "anticoagulants"/ OR exp Anti-Inflammatory Agents, Non-Steroidal/ OR (aspirin* OR (acetylsalicylic ADJ3 acid*) OR acetylsalicylate* OR anticoagul* OR anti-coagul* OR antithromb* OR anti-thromb* OR (clotting ADJ3 inhibitor*) OR heparin OR antifibrinolyt* OR anti-fibrinolyt* OR antiplatelet* OR anti-platelet* OR ((platelet* OR fibrinoly* OR vitamin-K OR Factor-Xa OR Factor-X OR thrombin OR thrombocyte*) ADJ3 (inhibit* OR antagon* OR anti OR antiaggregat*)) OR warfarin* OR coumarin* OR aspirin* OR (acetylsalicylic ADJ3 acid*) OR acetylsalicylate* OR ((nonsteroid* OR non-steroid*) ADJ3 (antiinflamm* OR anti-inflamm*)) OR nsaid* OR ibuprofen*).ab,ti.) AND ("occult blood"/ OR (("feces"/ OR defecation/) AND (exp "immunochemistry"/)) OR (((faecal OR fecal OR faeces OR feces OR stool OR defecat*) ADJ3 (immunohistochem* OR immunochem* OR fit)) OR ifobt OR fobt OR ifobts OR fobts OR (fit ADJ3 (test*))).ab,ti.) NOT (exp animals/ NOT humans/) AND english.la.

Cochrane CENTRAL

((aspirin* OR (acetylsalicylic NEAR/3 acid*) OR acetylsalicylate* OR anticoagul* OR anti-coagul* OR antithromb* OR anti-thromb* OR (clotting NEAR/3 inhibitor*) OR heparin OR antifibrinolyt* OR anti-fibrinolyt* OR antiplatelet* OR anti-platelet* OR ((platelet* OR fibrinoly* OR vitamin-K OR Factor-Xa OR Factor-X OR thrombin OR thrombocyte*)

NEAR/3 (inhibit* OR antagon* OR anti OR antiaggregat*)) OR warfarin* OR coumarin* OR aspirin* OR (acetylsalicylic NEAR/3 acid*) OR acetylsalicylate* OR ((nonsteroid* OR non-steroid*) NEAR/3 (antiinflamm* OR anti-inflamm*)) OR nsaid* OR ibuprofen*):ab,ti) AND ((((faecal OR fecal OR faeces OR feces OR stool OR defecat*) NEAR/3 (immunohistochem* OR immunochem* OR fit)) OR ifobt OR fobt OR ifobts OR fobts OR (fit NEAR/3 (test*))):ab,ti)

Web of science

TS=(((aspirin* OR (acetylsalicylic NEAR/2 acid*) OR acetylsalicylate* OR anticoagul* OR anti-coagul* OR antithromb* OR anti-thromb* OR (clotting NEAR/2 inhibitor*) OR heparin OR antifibrinolyt* OR anti-fibrinolyt* OR antiplatelet* OR anti-platelet* OR ((platelet* OR fibrinoly* OR vitamin-K OR Factor-Xa OR Factor-X OR thrombin OR thrombocyte*) NEAR/2 (inhibit* OR antagon* OR anti OR antiaggregat*)) OR warfarin* OR coumarin* OR aspirin* OR (acetylsalicylic NEAR/2 acid*) OR acetylsalicylate* OR ((nonsteroid* OR non-steroid*) NEAR/2 (antiinflamm* OR anti-inflamm*)) OR nsaid* OR ibuprofen*)) AND ((((faecal OR fecal OR faeces OR feces OR stool OR defecat*) NEAR/2 (immunohistochem* OR immunochem* OR fit)) OR ifobt OR fobt OR ifobts OR fobts OR (fit NEAR/2(test*))))) AND LA=(english)

Google scholar

anticoagulants|anticoagulation|"clottinginhibitor"|heparin|antifibrinolytics|antiplatelet "faecal|fecalblood|bleeding|analysis|test|immunochemical|sample"|"occult blood"|ifobt|fobt|gfobt|ifobts|fobts|gfobts

S2 Variables for data extraction

The following data was extracted when possible: (I) *Study characteristics* - first author, journal, year of publication, type of article, country of screening population, time period of patient inclusion; (II) *FIT characteristics* - number of samples per stool, FIT cut-off value, type of FIT; (III) *Study cohort characteristics* – total number of participants, total participants with a positive test or a negative test that underwent colonoscopy; (IV) *Medication use* – total number of participants on any OAC, total number of participants on any NSAID (incl. aspirin); (V) *Advanced neoplasia characteristics* – total number of AN/CRC after positive FIT in OAC and NSAID users and nonusers, total number of AN/CRC after negative FIT in OAC and NSAID users and nonusers.

S3 Newcastle-Ottawa Scale

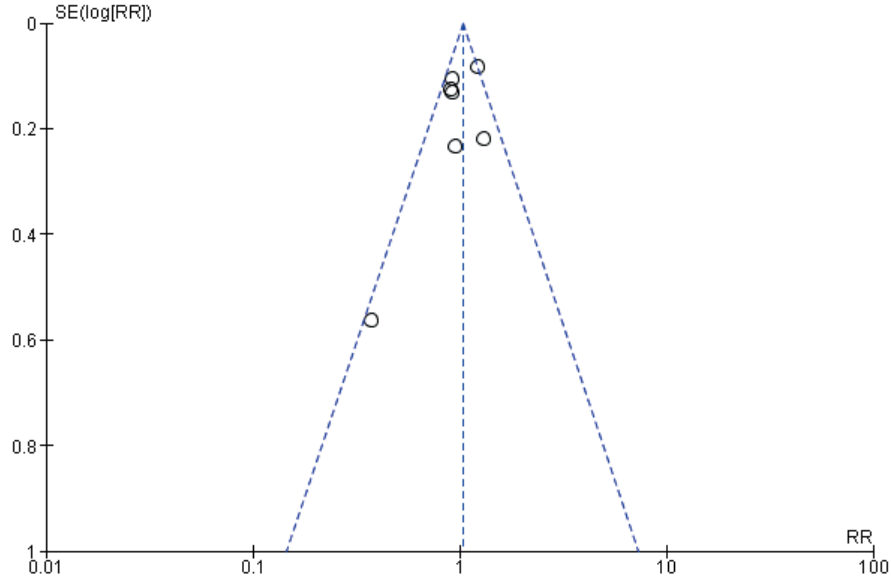
	Selection (max. 4)	Comparability (max. 2)	Outcome (max. 3)	Total (max. 9)
Wauters, 2017	***	*	**	******
Botteri, 2016	***	*	**	******
Wong, 2015	***	*	***	*******
Bujanda, 2014	***	*	**	******
Bujanda, 2013	***	*	**	******
Denters, 2011	***	*	**	******
Mandelli, 2011	****	*	***	********
Brenner, 2010	***	*	**	******

S4 GRADE score

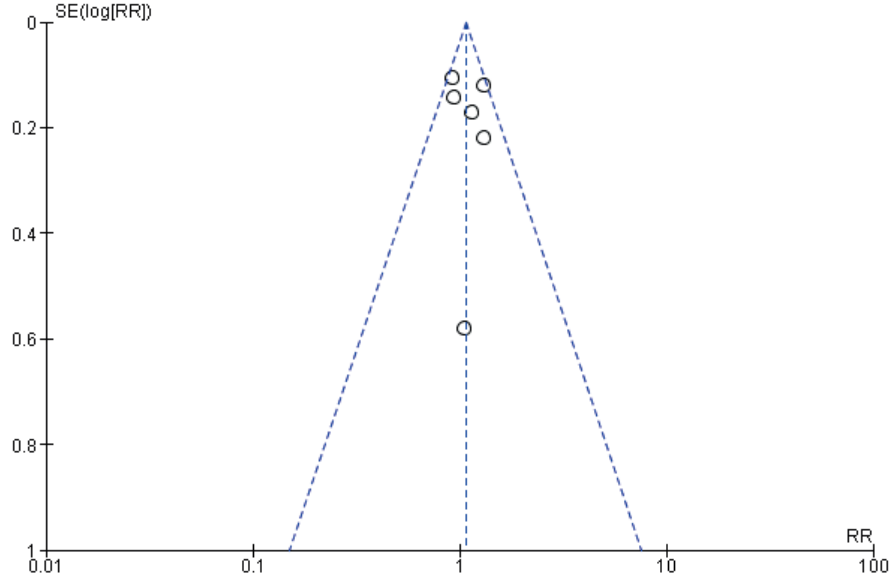
Study design	Quality of evidence	Lower if	Higher if
RCT	High (4 points)	*Risk of bias:* -1 serious -2 very serious	*Large effect:* +1 large +2 very large
	Moderate (3 points)	*Inconsistency:* -1 serious -2 very serious	*Dose response:* +1 evidence of gradient
Observational	Low (2 points)	*Indirectness:* -1 serious -2 very serious	*All plausible confounding:* +1 would reduce demonstrated effect +2 Would suggest spurious effect when results show no effect
	Very low (1 point)	*Imprecision:* -1 serious -2 very serious	
		Publication bias: -1 serious -2 very serious	

Comparison	Pooled PPV_{AN} *OR (95% CI)*	Quality of evidence	Lower	Higher	GRADE score
Pooled OAC Use vs no use	1.00 (0.85-1.17)	2 points	-	-	Low
Aspirin/NSAID Use vs no use	1.05 (0.87-1.27)	2 points	-	-	Low

S5 Funnel plots



S.5.1 Funnel plot for pooled oral anticoagulants (OAC) use and positive predictive value of advanced neoplasia (PPV_{AN}) of a fecal immunochemical test (FIT)



S5.2 Funnel plot for aspirin / NSAID use and positive predictive value of advance neoplasia (PPV_{AN}) of a fecal immunochemical test (FIT)

Part VI

Discussion and future perspectives

Chapter 12

Summary and discussion

Summary

This thesis aimed to explore the prevalence of gastrointestinal (GI) disease in a general asymptomatic population and investigate the diversity and composition of the microbiome in the entire GI tract in **Part II**. More insight in the trend of increasing incidence of early-onset colorectal cancer (EOCRC) and the pathophysiological characteristics of EOCRC were provided in **part III**. Next, the use and future applications of colon capsule endoscopy were discussed in **part IV**. In **part V** the accuracy of current CRC screening methods were discussed. In this final part, the findings of our research will be summarized and future perspectives will be discussed.

Prevalence of gastrointestinal disease

GI disease are usually detected when patients undergo a diagnostic procedure because of symptoms. A substantial proportion of patients with GI abnormalities remain undiagnosed since they do not always present with symptoms for which endoscopy is considered necessary. Therefore, prevalence rates of GI diseases in a general population are unknown. For this reason, we conducted a study to assess the prevalence of any GI lesion in a asymptomatic general population aged between 50-75 years, retrieved from the Rotterdam Study (1) **(Chapter 2)**. Colon capsule endoscopy (CCE) was used as instrument to detect GI lesions. The results showed that GI mucosal findings appeared to be very common. The prevalence of barrett oesophagus was 8.3%, esophagitis 5.8%, fundic gland polyps 18.1%, colon polyps 56.0%, and diverticula 81.6%. Furthermore, in 12% clinically relevant abnormalities were detected, of which most commonly clinically relevant lesions found were colon polyps >10 mm. This study provides a frame of reference on prevalence rates of GI mucosal findings in a largely asymptomatic general population. Incidental findings found during endoscopy can now be placed into perspective and helps clinicians to better inform patients about the (non-significant) lesions found. Nevertheless, findings should be interpreted with caution. Colon capsule endoscopy is a non-invasive method, but bowel preparation is mandatory. Because of this, the participation rate was low and could have led to selection bias. A restraining factor of colon capsule is the limited completion rate caused by the limited battery life. This may have led to an underestimation of the found prevalence rates. This clinical trial was embedded in the Rotterdam study, a prospective cohort study. All inhabitants of Ommoord, a region in Rotterdam, of 45 years and older were asked to participate. Though the overall response rate was 72%, selection bias could not be excluded (2). Considering only people pariticipating in the Rotterdam study within the age group 50-75 years were asked to participate, caution must be taken to extrapolate our findings to younger populations.

Chapter 3 focused on the composition of the microbiome in the entire GI tract, since most research investigated the colonic microbiome and often information was retrieved form fecal samples. Alterations in the microbiome have been linked to disease, such as CRC. To understand the significance of microbial dysbiosis observed in GI disease, it is important to map the microbial dysnamics in the healthy individuals for comparison. We aimed to characterize the mucosal microbiome along the entire GI tract within the same individuals. Patients undergoing doubleballoon enteroscopy (DBE) provided access to nine different GI sites for downstream molecular analysis. We found that the bacterial load of mucosal samples decreased form oesophagus to proximal ileum, but drastically increased again in the lower GI tract. The composition of the microbiota also changed markedly along the GI tract, with larger diversity in the lower GI tract compared to the upper GI tract. Though no pathophysiological diagnosis was found in the participants (except for one participant with a cecum tumor), all participants underwent a DBE because of complaints. The microbiome of the GI tract vary widely across healthy individuals and is dynamic. Large shifts in microbiome composition can take place in response to disease, environmental factors and change of diet (3). These factors need to be considered in future studies.

Early onset colorectal cancer

CRC is the third most common cancer and cause of death worldwide. CRC screening has been implemented across the world and is used to identify asymptomatic elderly individuals with advanced adenomas or (early stage) cancer (4). However, an increase in incidence of CRC among subjects aged 20-40 years has been observed in North America, Australia, and China (5, 6). The American Cancer Society therefore recently recommended to lower the age to start screening from 50 to 45 years (7). We analyzed the European trends in CRC incidence and mortality in subjects younger than 50 years **(Chapter 4)**. Data was collected on age-related CRC incidence and mortality between 1990 and 2016 form national and regional cancer registries across Europe. We found that the incidence of CRC increased in Europe among subjects aged 20-49 years. On average, CRC incidence increased with 7.9% per year among subjects aged 20-29 years. The increase in age group of 30-39 years was 4.9% per year, the increase in age group of 40-49 years 1.4% per year. The rise in incidence was not associated with a similar rise in mortality. Clinicians should be aware of the rising incidence of CRC in young adults. More research is necessary to monitor this trend in the coming years. At this moment, is not advisable to adjust the screening guidelines to start screening at the age of 45 years. The largest increase in incidence was observed in the youngest age group and the absolute numbers are still low compared to the elder patients.

To fully elucidate the cause of EOCRC, it is important to identify the clinical and pathological features of EOCRC. Though former studies found that clinicopathological features of EOCRC patients differed from late-onset CRC patients, data was scarce and conflicting (8). Moreover patients with Lynch syndrome (LS) were often not excluded, leading to obscuration of the clinical features of true sporadic EOCRCs. Consequently, we assessed the clinicopathological characteristics of sporadic EOCRC patients within different age categories and investigated changes over time (**Chapter 5**). We found that poorly differentiated tumours, presence of signet-ring cells, and a higher number of lymph node metastasis were significantly more prevalent in 20-39 years old compared to the 40-49 years old. Over time, EOCRC was more often diagnosed in women below the age of 30 years, while tumours were more often located in the rectum in the older group, 30-49 years old. We concluded that young patients had different clinicopathological factors within the age groups defined as EOCRC. Though these findings will give insights regarding EOCRC from a patient and tumour perspective, a true increase in incidence of women and rectal cancer over time could not be calculated because of missing population numbers per time period. To ensure that LS patients were not included in this study, we excluded all patients in who no molecular diagnostics was performed. This could have let to selection bias.

Screening methods of gastrointestinal disease - applicability of colon capsule

The overall accuracy of CCE has been described in several trials and showed that the performance of CCE was comparable to colonoscopy (9). CCE provides a clear overview of the complete colon and has several advantages over colonoscopy. However, information on the performance of CCE in a screening population remains scarce. **Chapter 6** comprises a systematic review which evaluated safety and accuracy of the colon capsule in detecting adenomas and CRC of the colon and rectum. When available literature was combined, the colon capsule appeared to be non-inferior to colonoscopy regarding the detection of adenomas and CRC. When colon capsule was compared with CT-colonography, colon capsule performance appeared to be better. Especially in patients where a colonoscopy would be too invasive the colon capsule might be a good alternative.

CCE is designed for imaging the colonic mucosa, but has the potential to explore the entire GI tract. However, the diagnostic accuracy of CCE as pan-endoscopy is dependent on several quality measures. Optimal stomach and bowel preparation is needed, capsule needs to be excreted within battery time, and transit times should not be too fast because of missed lesions. In **Chapter 7** we intended to investigate the quality measures when CCE is used as pan-endoscopy using the asymptomatic population cohort as mentioned in Chapter 2. The bowel preparation used consisted of bisacodyl, two liter

polyethylene electrolyte gycol (PEG) split dose and a booster regimen (metoclopramide, oral sulfate solution (OSS) after small bowel detection and three hours later). Participants were asked to fill in questionnaires. Furthermore, the workability for the staff and patient acceptance were explored. We found that of the 451 analyzed CCE procedure, cleanliness of the stomach was good in 69.6%, of the SB good or excellent in 99.1% and of the colon good or excellent in 76.6%. The completion rate of the colon capsule was 51.9% and the median transit time per procedure was 583 minutes. Participants graded the procedure with a 7.8. We concluded that CCE is a safe procedure and participants were content with the procedure. Due to a low completion rate, CCE is not yet feasible to be implemented on a large scale. Bowel preparation and booster regimens should be improved to achieve higher number of complete studies.

Though bowel preparation and booster regimens have an influence on the capsule transit time, the wide variation in CCE transit times and completion rates are not completely understood. Other factors might have an impact on CCE transit, like ageing and gender (10, 11). Also lifestyle factors are known to affect the GI transit times (12). Therefore we aimed to identify possible predictors for CCE transit times (**Chapter 8**). We found that younger age, unchanged stool pattern, history of abdominal surgery, low BMI and high fiber intake resulted in slower CCE transit times and lower completion rates. Participants who took metoclopramide due to a long stomach transit, had a faster SB transit. These factors can now be used to anticipate a longer capsule transit time and possibly adjust the preparation protocol. Also, this study showed that the use of metoclopramide might have a beneficial effect on the small bowel transit time. Especially when CCE is used to review the colon, use of metoclopramide could be recommended.

If CCE is going to be implemented on a large scale in general practices and hospitals, artificial intelligence (AI) should be designed and used to review images and highlight abnormalities to reduce the workload of the clinicians whilst providing an objective and reproducible outcome. Therefore, a reliable AI method specifically developed for reviewing CCE images is warranted. In **Chapter 9**, we performed a systematic review to provide an overview of the available literature on AI for reviewing colonic mucosa by CCE. Only studies reporting on AI for reviewing CCE-2 colonic images were included. In total, 1017 studies were evaluated of which nine were included. Two studies reported on computed bowel cleansing assessment and five studies reported on computed polyp- or colorectal neoplasia detection. Overall, sensitivity of proposed AI models was 86.5%-95.5% for bowel cleansing and 47.4%-98.1% for detection of polyps and CRC. The highest sensitivity of 98.1% for polyp detection was found by applying a Convolutional Neural Network (CNN). We concluded that AI for reviewing CCE-2 images is encouraging. However, CNN algorithms should be optimized and tested with more data, possibly

requiring estblishing of a large international CCE database. Finally, the accuracy of the optimized CNN models needs to be confirmed in a prospective setting.

Though CCE is approved by the FDA since 2014 to explore the colon and many trials proved its good diagnostic accuracy of polyps and CRC, the colon capsule is still not implemented in daily practice. This thesis provided further evidence of accuracy of CCE in a CRC screening setting and when used as pan-endoscopy. We were also able to uncover problems when CCE is used, like low completion rate and long transit time. Though abovementioned issues should be resolved, CCE deserves a more prominent role as diagnostic in certain conditions. For example for patients unable or unwilling to undergo colonoscopy or fragile older patients with complaints of abdominal pain or rectal blood loss and unwilling to undergo invasive diagnostics. Given the procedure could be performed at home, these patients could avoid the burden of travelling to the hospital. Furthermore, CCE could reduce the burden on endoscopy capacity. A trial has been set up (OCEAN trial) to evaluate the applicability of CCE in CRC screening in participants with a positive FIT who are unwilling or unable to undergo colonoscopy. However, future studies should also evaluate the accuracy of CCE as diagnostic tool in the outpatient clinic for patients with for example unexplained complaints of low hemoglobin level without visible blood loss. The general practicionar could send a referral for only those patients with observed abnormalities. Nonetheless, this is only feasible if the completion rate increases and AI is available to support clinicians in reviewing the images.

Screening methods of colorectal cancer – faecal immunochemical test

Finally, this thesis described the effect of FIT-based CRC screening program on CRC incidence and mortality. Most studies that evaluated the impact of stool-based CRC screening on CRC-related mortality used gFOBT (13). In the Netherlands, before implementation of a nationwide CRC screening program, a biennial FIT-based CRC screening program pilot was conducted between 2006 and 2014. In **Chapter 10**, we aimed to evaluate the impact of a FIT-based CRC screening program on CRC incidence and CRC-related mortality by comparing participants of the CRC screening program pilot to non-screened individuals. Over 13-years of follow-up, screenees had a significantly lower CRC incidence (hazard ratio (HR) 0.78) compared to the non-screened individuals. In the first five years of screening an initial increase in cumulative CRC risk was found, followed by a subsequently decrease after seven years. Screenees had a significantly lower CRC-related mortality (HR 0.39) compared to the non-screened individuals. These findings need to be interpreted with caution as healthy screenee bias may affaceted the results. Future studies are needed to confirm our found effect of FIT-based CRC screening on CRC incidence and CRC-related mortality.

Oral anticoagulants could have an effect on the efficacy of a screening program. FIT is based on finding occult blood in feces. The use of oral anticoagulants might increase the bleeding risk of lesions like a fissure or ulcer, which might negatively influence the accuracy of FIT. However, an increase of bleeding risk of a malignant lesions positively affects the accuracy of FIT. For this reason, we performed a meta-analysis in which eight studies were included comprising over 3,500 subjects in an average screening population that underwent FIT (**Chapter 11**). Users and non-users of oral anticoagulants were compared. The positive predictive value for the detection of advanced neoplasia of FIT was not different for users versus non-users (37.6% vs. 40.3%). Based on the current data, there is no reason to seize the use of anticoagulants prior to FIT sampling.

References

1. Ikram MA, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol. 2017;32(9):807-50.
2. [Available from: https://www.volksgezondheidenzorg.info/onderwerp/maag-darm-en-leveraandoeningen/overzicht.
3. Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR, et al. Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans. Cell Host & Microbe. 2019;25(6):789-802.e5.
4. Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJY, Young GP, et al. Colorectal cancer screening: a global overview of existing programmes. Gut. 2015;64(10):1637-49.
5. Siegel RL, Fedewa SA, Anderson WF, Miller KD, Ma J, Rosenberg PS, et al. Colorectal Cancer Incidence Patterns in the United States, 1974-2013. J Natl Cancer Inst. 2017;109(8).
6. Siegel RL, Torre LA, Soerjomataram I, Hayes RB, Bray F, Weber TK, et al. Global patterns and trends in colorectal cancer incidence in young adults. Gut. 2019;68(12):2179-85.
7. Wolf AMD, Fontham ETH, Church TR, Flowers CR, Guerra CE, LaMonte SJ, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin. 2018;68(4):250-81.
8. Willauer AN, Liu Y, Pereira AAL, Lam M, Morris JS, Raghav KPS, et al. Clinical and molecular characterization of early-onset colorectal cancer. Cancer. 2019;125(12):2002-10.
9. Spada C, Hassan C, Marmo R, Petruzziello L, Riccioni ME, Zullo A, et al. Meta-analysis shows colon capsule endoscopy is effective in detecting colorectal polyps. Clin Gastroenterol Hepatol. 2010;8(6):516-22.
10. Brogna A, Ferrara R, Bucceri AM, Lanteri E, Catalano F. Influence of aging on gastrointestinal transit time. An ultrasonographic and radiologic study. Invest Radiol. 1999;34(5):357-9.
11. Nandhra GK, Mark EB, Di Tanna GL, Haase AM, Poulsen J, Christodoulides S, et al. Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: Influence of age, gender, and body mass index. Neurogastroenterol Motil. 2020;32(2):e13734.
12. Mushref MA, Srinivasan S. Effect of high fat-diet and obesity on gastrointestinal motility. Ann Transl Med. 2013;1(2):14.
13. Gini A, Jansen EEL, Zielonke N, Meester RGS, Senore C, Anttila A, et al. Impact of colorectal cancer screening on cancer-specific mortality in Europe: A systematic review. Eur J Cancer. 2020;127:224-35.

Part VII

Appendices

Chapter 13
Dutch summary (Nederlandse samenvatting)

Chapter 14
Abbreviations

Chapter 15
Contributing authors

Chapter 16
List of Publications

Chapter 17
PhD portfolio

Chapter 18
Dankwoord

Chapter 19
About the author

Chapter 13

Dutch summary (Nederlandse samenvatting)

Nederlandse samenvatting

Maag- en darmaandoeningen zijn een veelvoorkomende reden voor een bezoek aan de huisarts of ziekenhuis. In 2014 bezochten er in de Verenigde Staten meer dan 40.7 miljoen mensen een dokter voor maag- en darmklachten. In 2017 waren er in Nederland 3.7 miljoen mensen met een maag- of darmaandoening, en de voorspelling is dat in 2030 meer dan 10% van de Nederlanders een maag- of darmprobleem zal hebben. Een derde van de mensen met een maag- of darmaandoening gaan naar de huisarts. Wanneer specifiek naar de prevalentie van maag- en darmaandoeningen wordt gekeken, dan is de prevalentie hoger in volwassenen ouder dan 65 jaar. Echter, gegevens over de prevalentie van maag- en darmaandoeningen in een asymptomatische populatie, is schaars.

Het microbioom speelt mogelijk een belangrijke rol in het ontstaan van verscheidene maag- en darmaandoeningen. Metagenoom en 16S rRNA amplicon sequencing hebben meer inzicht gegeven in de microbiële samenstelling van het maagdarmkanaal. Sinds het gebruik van deze nieuwe technieken, wordt de complexiteit, diversiteit en de interactie van het microbioom beter begrepen. Het microbioom is uniek in elk individu en wordt beïnvloed door interne en externe factoren. Per regio van het maagdarmkanaal heeft het microbioom een andere diversiteit en samenstelling. Daarnaast hebben ziekten invloed op de samenstelling van het microbioom. Zo is de aanwezigheid van bepaalde bacteriele soorten geassocieerd met darmkanker, zoals Streptococcus bovis en Fusobacterium nucleatum.

Deel II van dit proefschrift heeft betrekking op het onderzoek naar de prevalentie van maag- en darmaandoeningen en de diversiteit en samenstelling van het microbioom in het gehele maagdarmkanaal in asymptomatische patiënten.

Prevalentie van gastro-intestinale aandoeningen

Maag- en darmaandoeningen worden doorgaans opgespoord wanneer patiënten een diagnostische procedure ondergaan vanwege symptomen. Bij een aanzienlijk deel van de patiënten met maag- of darmklachten wordt geen diagnose gesteld, omdat zij zich niet altijd presenteren met symptomen waarvoor endoscopie noodzakelijk wordt geacht. Daarom zijn de prevalentiecijfers van maag- en darmaandoeningen in een algemene bevolking onbekend.

Hoofdstuk 2 betreft het onderzoek naar de prevalentie van mucosale lesies in het maag- en darmkanaal in een asymptomatische algemene populatie tussen 50-75 jaar. De studie is geïntegreerd in de Rotterdam studie, een grote prospectieve cohort studie

waarbij gezonde individuen van 45 jaar en ouder worden gevolgd in het leven. De colon capsule werd gebruik als diagnosticum. We toonden aan dat bepaalde bevindingen in het maagdarmkanaal vaak voorkomend zijn. Zo werd bij 8.3% van de deelnemers een Barrett slokdarm gezien, bij 18.1% fundic glands in de maag, en bij 81.6% divertikels. Poliepen in de dikke darm zijn bij 56% van de deelnemers gevonden en bij 12% van de deelnemers zijn klinisch relevante afwijkingen gezien waarvoor verder onderzoek werd geadviseerd. Incidentele bevindingen bij endoscopie kunnen nu in perspectief worden geplaatst en helpt clinici om patiënten beter te informeren over de gevonden (niet-significante) laesies.

In **hoofdstuk 3** onderzochten wij de bacteriële samenstelling en diversiteit tussen negen mucosale locaties van het maagdarmkanaal. Veertien individuen werden geïncludeerd die allen een dubbelballon enteroscopie ondergingen. Er is gebruik gemaakt van 16S rRNA amplicon sequencing van de verkregen biopten. Wij tonen aan dat in het onderste deel van het maag-darm kanaal zowel de bacteriële dichtheid als microbiële diversiteit per locatie hoger is dan in het bovenste deel, en dat de bacteriële compositie verschilt van het bovenste deel van het maagdarmkanaal.

Darmkanker bij jongeren

Darmkanker treft meestal mensen tussen de 50-75 jaar oud. Hoewel de incidentie van darmkanker de laatste decennia is gedaald in deze leeftijdsgroep mogelijk als gevolg van screening, is de incidentie van darmkanker bij jongeren (EOCRC) gestegen. EOCRC wordt over het algemeen gedefinieerd als darmkanker gediagnosticeerd vóór de leeftijd van 50 jaar. In de Verenigde Staten is de incidentie van darmkanker sinds de jaren tachtig jaarlijks met 1.0-3.4% gestegen bij volwassenen in de leeftijd van 20-39 jaar. De incidentie van endeldarmkanker neemt reeds langer en sneller toe: 3.2% per jaar sinds 1974-2013 bij patiënten van 20-29 jaar. Deze trend werd niet alleen waargenomen in de Verenigde Staten, maar ook in andere delen van de wereld. De Amerikaanse kanker vereniging (ACS) heeft daarom onlangs aanbevolen om de startleeftijd van darmkankerscreening te verlagen van 50 naar 45 jaar.

In **hoofdstuk 4** hebben wij de Europese trends in darmkankerincidentie en mortaliteit bij personen jonger dan 50 jaar geanalyseerd. We ontdekten dat de incidentie van CRC in Europa is toegenomen in de leeftijdsgroep 20-49 jarigen. Gemiddeld steeg de incidentie van darmkanker met 7,9% per jaar in 20-29 jarigen, bij 30-39 jarigen was de stijging 4,9% per jaar en bij 40-49 jarigen 1,4% per jaar. Artsen moeten zich meer bewust zijn van de stijgende incidentie van darmkanker in deze leeftijdsgroepen. Meer onderzoek is nodig om deze trend in de komende jaren te volgen. Op dit moment is het niet raadzaam de leeftijdsgrens van darmkanker screening te verlagen naar 45 jaar. De grootste stijging

van de incidentie werd namelijk waargenomen in de jongste leeftijdsgroep en de absolute aantallen zijn nog steeds laag in vergelijking met de ouderen.

Om te achterhalen wat de oorzaak is van de stijgende incidentie van darmkanker bij jongeren, is het belangrijk te weten welke klinische en pathologische kenmerken deze tumoren hebben. Mogelijk is de darmkanker die op jonge leeftijd ontstaat een ander type tumor dan de darmkanker die ontstaat op latere leeftijd. In **hoofdstuk 5** hebben wij de klinische en pathologische kenmerken van sporadische darmkanker bij jongeren onderzocht binnen verschillende leeftijdscategorieën en door de tijd heen. Wij vonden dat slecht gedifferentieerde tumoren, aanwezigheid van zegelringcellen, en een hoog aantal lymfekliermetastasen significant vaker voorkwamen bij 20-39 jarigen vergeleken met de 40-49 jarigen.

Screeningsmethoden voor maag- en darmaandoeningen – toepasbaarheid van de colon capsule

Er bestaan verschillende screeningmethoden om maag- en darmaandoeningen op te sporen. Colon capsule endoscopie (CCE) is een niet-invasieve techniek die het mogelijk maakt het gehele darmkanaal in beeld te brengen. Er is geen sedatie nodig en de procedure kan thuis worden uitgevoerd. De coloncapsule heeft twee camera's aan elke kant van de capsule en kan beelden maken met een snelheid van 4-35 beelden per seconde. De capsule zendt gegevens naar een recorder die de patiënt aan een riem draagt. De gegevens kunnen vervolgens op de computer worden gedownload in de vorm van een video. Een optimale voorbereiding van de darm is nodig om het slijmvlies van het darmkanaal goed in beeld te kunnen brengen.

Diverse onderzoeken hebben laten zien dat CCE een effectief diagnosticum is in het detecteren van poliepen en darmkanker in de dikke darm. Echter, informatie over de prestaties van CCE in een screeningspopulatie is schaars. In **Hoofdstuk 6** hebben we de literatuur op dit punt op een rij gezet door middel van een systematische review die de veiligheid en nauwkeurigheid van de colon capsule evalueert bij het opsporen van (pre) maligne lesies van de dikke darm. De colon capsule bleek niet-inferieur te zijn aan colonoscopie. Wanneer de colon capsule werd vergeleken met CT-colonografie, bleek de colon capsule superieur te zijn. CCE zou een goed alternatief kunnen zijn, met name voor patiënten bij wie colonoscopie te invasief zou zijn.

CCE heeft de potentie om het gehele maagdarmkanaal in beeld te brengen. Om CCE als pan-endoscopie van het maagdarmkanaal te gebruiken, moet het aan verschillende kwaliteitseisen voldoen. Zo moet het maagdarmkanaal goed gereinigd zijn, moet de capsule binnen de batterijduur het gehele kanaal gevisualiseerd hebben en mag de

passagetijd van de capsule door het maagdarmkanaal niet te snel zijn om te voorkomen dat het afwijkingen van het slijmvlies mist. In **hoofdstuk 7** onderzochten we de kwaliteitseisen wanneer CCE als pan-endoscopie wordt gebruikt. Wij vonden dat van de 451 geanalyseerde CCE procedure, de maag goed gereinigd was in 69,6% van de gevallen, de dunne darm goed of uitstekend was gereinigd in 99,1% van de gevallen en de dikke darm goed of uitstekend was gereinigd in 76,6% van de gevallen. Een complete procedure was het geval bij 51.9% van de deelnemers en de passagetijd van de capsule was gemiddeld 583 minuten. Deelnemers beoordeelden de procedure met een 7,8. We hebben geconcludeerd dat CCE een veilige procedure is en dat deelnemers tevreden zijn. Vanwege het lage percentage complete procedures, is CCE nog niet geschikt om op grote schaal te implementeren. Darmvoorbereiding en boosterschema's moeten worden verbeterd om een hoger aantal complete procedures te verkrijgen.

Hoewel darmvoorbereiding en boosterschema's een invloed hebben op de passagetijd van de capsule, spelen andere factoren waarschijnlijk ook een rol. Daarom hebben wij ons in **hoofdstuk 8** gericht op het identificeren van mogelijke voorspellers voor passagetijden van de colon capsule. Wij vonden dat een jongere leeftijd, onveranderd ontlastingspatroon, een geschiedenis van buik chirurgie, een laag BMI en hoge vezelinname resulteren in tragere CCE passagetijd en een lager aantal compleet gevisualiseerde video's. In de toekomst kunnen deze factoren in overweging worden genomen om de darmvoorbereidingsschema's aan te passen.

Om CCE op grote schaal te gebruiken als diagnosticum moet kunstmatige intelligentie worden ontworpen om de beelden te beoordelen en afwijkingen te markeren. Het beoordelen van een video kost een clinicus gemiddeld 70 minuten. Kunstmatige intelligentie zou de werklast kunnen verminderen en tevens kunnen zorgen voor een objectief en reproduceerbaar resultaat. In **hoofdstuk 9** hebben wij een systematische review uitgevoerd om een overzicht te geven van de beschikbare literatuur over kunstmatige intelligentie voor het beoordelen van het slijmvlies van de dikke darm door CCE. In totaal werden negen studies geïncludeerd. Twee studies rapporteren over hoe goed de dikke darm gereinigd was en vijf studies rapporteren over poliep of darmkanker detectie. In het algemeen is de sensitiviteit van de voorgestelde kunstmatige intelligentie modellen 86,5%-95,5% voor darmreiniging en 47,4%-98,1% voor detectie van poliepen en darmkanker. We hebben geconcludeerd dat kunstmatige intelligentie voor het beoordelen van CCE beelden veelbelovend is.

Screeningsmethoden voor darmkanker – fecale immunochemische test

Het opsporen van darmkanker is gunstig omdat darmkanker een vaak voorkomende ziekte is, het een lange fase kent met voorloperafwijkingen alvorens de afwijking zich

omvormt naar darmkanker, en wanneer de darmkanker vroeg ontdekt wordt de overleving verbeterd. In Nederland wordt sinds 2014 het bevolkingsonderzoek darmkanker georganiseerd waarbij om de twee jaar een ontlastingstest (fecale immunochemische test (FIT)) wordt aangeboden voor personen tussen de 55-75 jaar. Indien deze test positief is, wordt geadviseerd om een colonscopie te ondergaan.

Voorafgaand aan de implementatie van het landelijk bevolkingsonderzoek darmkanker, zijn Nederlandse wetenschappelijke onderzoeken gedaan. Dit zogenaamde proefbevolkingsonderzoek betrof een geselecteerde groep mensen die elke twee jaar voor darmkankerscreening met FIT werden uitgenodigd tussen 2006 en 2014. In **hoofdstuk 10** hebben wij het effect van een FIT-gebaseerd CRC screeningsprogramma op CRC incidentie en CRC-gerelateerde mortaliteit geëvalueerd door deelnemers aan het proefbevolkingsonderzoek te vergelijken met de niet gescreende personen. Wij vonden bij deelnemers aan het darmkanker bevolkingsonderzoek een lagere darmkanker incidenctie (Hazard ratio (HR) 0.78) en een lagere CRC-gerelateerde mortaliteit (HR 0.39) in vergelijking met de niet deelnemers aan het darmkanker bevolkingsonderzoek. Deelnemers aan het darmkanker bevolkingsonderzoek hadden in de eerste 5 jaar na deelname aan het bevolkingsonderzoek een hoger risico op darmkanker, maar na 7 jaar een lager risico.

Het gebruik van antistollende medicatie kan de effectiviteit van FIT screening beinvloeden. FIT detecteert namelijk occult bloed in de ontlasting. Gebruik van antistollende medicatie kan het bloedingsrisico van zowel een onschuldige als een kwaadaardige lesies verhogen, en daarmee de accuraatheid van FIT positief als negatief beinvloeden. Een meta-analyse werd verricht waarbij 8 studies werden geincludeerd met 3.500 deelnemers (**Hoofdstuk 11**). De positief voorspellende waarde van FIT voor de detectie van voorloperafwijkingen van darmkanker wordt niet beinvloed door het gebruik van antistolling.

Chapter 14

Abbreviations

Abbreviations

ADR	=	adenoma detection rate
AE	=	adverse event
AFR	=	adaptive frame rate
AI	=	artificial intelligence
APC	=	annual percent change
B	=	standardized beta
BMI	=	Body Mass Index
BO	=	Barrett's esophagus
CAC	=	computed assessment of cleansing
CCE	=	Colon Capsule Endoscopy
CCE I	=	first generation colon capsule
CCE II	=	second generation colon capsule
CNN	=	Convolutional Neural Network
CRC	=	Colorectal Cancer
CTC	=	CT colonography
cTNM	=	clinical tumor and node stage
DABT	=	undergoing dual antiplatelet therapy
DBE	=	double balloon entroscopy
DCO	=	death certificate only
DM	=	diabetes mellitus
EOCRC	=	early onset colorectal cancer
ESGE	=	European Society of Gastrointestinal Endoscopy
FAP	=	familial adenomatous polyposis
FDA	=	Food and Drug Administration
FDR	=	first degree relative
FGP	=	fundic gland polyps
FOBT	=	faecal occult blood test
GI	=	Gastrointestinal
gFOBT	=	guaiac fecal occult blood test
GRADE	=	Grading of Recommendations Assessment, Development, and Evaluation
Hb	=	hemoglobine
HGD	=	high grade dysplasia
HP	=	hyperplasia
I^2	=	inconsistency index
IBD	=	inflammatory bowel disease
IQR	=	inter quartile range
LASA	=	Longitudinal Aging Study Amsterdam

LGD	=	low grade dysplasia
LS	=	Lynch Syndrome
MAR	=	missing at random
MET	=	Metabolic Equivalent of Task
MMR	=	mismatch repair
MMR-d	=	MMR deficiency
MRI	=	Magnetic resonance imaging
MSI	=	microsatellite instability
MV	=	microscopically verified
N	=	number
NA	=	not applicable
NaP	=	sodium phosphate
NCR	=	Netherlands Cancer Registry
NPV	=	negative predictive value
NSAID	=	nonsteroidal anti-inflammatory drug
NTR	=	the Netherlands trial register
PALGA	=	the nationwide network and registry of histo- and cytopathology in the Netherlands
PCoA	=	Principal coordinate analysis
PDR	=	polyp detection rate
PEG	=	Polyethylene Glycol
PPV	=	positive predictive value
PPV_{AN}	=	positive predictive value for advanced adenoma
PPV_{CRC}	=	positive predictive value for colorectal cancer
PPI	=	proton pomp inhibitor
PR	=	positivity rate
PRISMA	=	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSE	=	polyp estimation tool
pTNM	=	pathological tumor and node stage
QUADAS	=	quality assessment of diagnostic accuracy studies
OAC	=	oral anticoagulants
OC	=	colonoscopy
OR	=	odds ratiod
OS	=	overall survival
OSS	=	Oral Sulfate Solutio
OUT	=	operational taxonomic unit
RCT	=	randomized controlled trial
R/G ratio	=	ratio of color intensities red over green
R/(R+G) ratio	=	red over brown ratio

ROC	=	receiver operating characteristic
RR	=	relative risks
SAE	=	serious adverse even
SB	=	Small Bowel
SBCE	=	small bowel capsule endoscopy
SD	=	standard deviation
SSA	=	sessile serrated adenoma
SVM	=	support vector machine
t	=	t-value
TA	=	tubular adenoma
TNM	=	Tumor Node Metastasis
TVA	=	tubulovillous adenoma
US	=	United States

Chapter 15

Contributing authors

Contributing authors

Albert Hofman
Department of Epidemiology
Harvard T.H. Chan School of Public Health
Boston, Massachusetts

Anouk van de Winkel
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Carlo Senore
Epidemiology and screening Unit – CPO
University Hospital Città della Salute e della Scienza
Turin, Italy

Cesare Hassan
Digestive Endoscopy Unit
Nuovo Regina Margherita Hospital
Rome, Italy

Cristiano Spada
Department of Digestive Endoscopy and Gastroenterology
Poliambulanza Foundation
Brescia, Italy
Department of Digestive Endoscopy
Fondazione Policlinico Universitario Agostino Gemelli - IRCCS, Catholic University Rome, Italy

Eline H. Schreuders
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Elisabeth F.P. Peterse
Department of Public Health
Erasmus University Medical Center
Rotterdam, the Netherlands

Emanuele Rondonotti
Gastroenterology Unit
Ospedale Valduce
Como, Italy

Ernst J. Kuipers
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Evelien Dekker
Department of Gastroenterology and Hepatology
Amsterdam University Medical Centers, Location Academic Medical Center
Amsterdam, The Netherlands

Gwenny M. Fuhler
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Ignacio Fernández-Urién
Department of Gastroenterology and Hepatology
Complejo Hospitalario de Navarra
Pamplona, Spain

Iris Lansdorp-Vogelaar
Department of Public Health
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Iris Nagtegaal
Department of Pathology
Radboud University Medical Center
Nijmegen, the Netherlands

Johan Dicksved
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences
Uppsala, Sweden

Lars Engstrand
Department of Microbiology, Tumor and Cell Biology
Karolinska institute
Stockholm, Sweden

Luc J.W. van der Laan
Department of Surgery
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Maikel P. Peppelenbosch
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Manon C.W. Spaander
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Marc Bardou
Department of Public Health
Erasmus MC University Medical Center
Rotterdam, the Netherlands
Centre d'investigations Clinique INSERM 1432
Dijon, France

Marcis Leja
Institute of Clinical and Preventive Medicine and Faculty of Medicine
University of Latvia
Riga, Latvia

Marco Pennazio
University Gastroenterology Unit
Città della Salute e della Scienza Universty Hospital
Turin, Italy

María Pellisé
Department of Gastroenterology
Hospital Clínic de Barcelona
Barcelona, Spain.

Marieke J.H.A. Kruip
Department of Hematology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Marinka D. Oudkerk Pool
Netherlands Heart Institute
Utrecht, the Netherlands

Mário Dinis-Ribeiro
Department of Gastroenterology
Portuguese Oncology Institute of Porto
Porto, Portugal
CINTESIS
Porto Faculty of Medicine, University of Porto
Porto, Portugal

Michal F. Kaminski
Cancer Prevention
The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology
Warsaw, Poland
Gastroenterology, Hepatology and Clinical Oncology
Medical Centre for Postgraduate Education
Warsaw, Poland
Department of Health Management and Health Economics
University of Oslo
Oslo, Norway

Nicole S. Erler
Department of Biostatistics
Department of Epidemiology
Erasmus MC University Medical Center,
Rotterdam, the Netherlands

Pieter H.A. Wisse
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Ondřej Májek
Faculty of Medicine
Masaryk University, Institute of Biostatistics and Analyses
Brno, Czech Republic
Institute of Health Information and Statistics of the Czech Republic
Prague, Czech Republic.

Ondřej Ngo
Faculty of Medicine
Masaryk University, Institute of Biostatistics and Analyses
Brno, Czech Republic
Institute of Health Information and Statistics of the Czech Republic
Prague, Czech Republic

Owen Epstein
Department of Gastroenterology
Royal Free Hospital
London, United Kingdom

Sarah Moen
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, the Netherlands

Serge R. Konstantinov
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Silvia Pecere
Digestive Endoscopy Unit
Fondazione Policlinico Universitario A. Gemelli IRCCS
Roma, Italia

Stella A.V. Nieuwenburg
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Stepan Suchanek
Department of Internal Medicine
Charles University, Military University Hospital
Prague, Czech Republic

Suk Yee Lam
Department of Gastroenterology and Hepatology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Trudy Voortman
Department of Epidemiology
Erasmus MC University Medical Center
Rotterdam, The Netherlands

Willemijn de Klaver
Department of Gastroenterology and Hepatology
Amsterdam University Medical Centers, Location Academic Medical Center
Amsterdam, The Netherlands

Chapter 16

List of Publications

Bibliography

This thesis

1. **Vuik FER**, Nieuwenburg SAV, Moen S, Schreuders EH, Oudkerk Pool MD, Peterse EFP, Spada C, Epstein O, Fernández-Urién I, Hofman A, Kuipers EJ, Spaander MCW. *Population-Based Prevalence of Gastrointestinal Abnormalities at Colon Capsule Endoscopy.* Clin Gastroenterol Hepatol. 2020 Oct 31:S1542-3565(20)31506-8.

2. **Vuik F***, Dicksved J*, Lam SY, Fuhler GM, van der Laan L, van de Winkel A, Konstantinov SR, Spaander M, Peppelenbosch MP, Engstrand L, Kuipers EJ. *Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals*. United European Gastroenterol J. 2019 Aug;7(7):897-907. *shared first authorship

3. **Vuik FE**, Nieuwenburg SAV, Bardou M, Lansdorp-Vogelaar I, Dinis-Ribeiro M, Bento MJ, Zadnik V, Pellisé M, Esteban L, Kaminski MF, Suchanek S, Ngo O, Májek O, Leja M, Kuipers EJ, Spaander MC. *Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years.* Gut. 2019 Oct;68(10):1820-182619 Sep 18;9(9):e032013.

4. **Vuik FER**, Nieuwenburg SAV, Nagtegaal ID, Kuipers EJ, Spaander MCW. *Clinicopathological characteristics of early onset colorectal cancer.* Aliment Pharmacol Ther. 2021 Dec;54(11-12):1463-1471.

5. **Vuik FER**, Nieuwenburg SAV, Moen S, Spada C, Senore C, Hassan C, Pennazio M, Rondonotti E, Pecere S, Kuipers EJ, Spaander MCW. *Colon capsule endoscopy in colorectal cancer screening: a systematic review.* Endoscopy. 2021 Aug;53(8):815-824.

6. **Vuik FER,** Moen S, Nieuwenburg SAV, Schreuders EH, Kuipers EJ, Spaander MCW. *Applicability of colon capsule endoscopy as pan-endoscopy: From bowel preparation, transit, and rating times to completion rate and patient acceptance.* Endosc Int Open. 2021 Dec 14;9(12):E1852-E1859.

7. Moen S, **Vuik FER**, Voortman T, Kuipers EJ, Spaander MCW. *Predictors of Gastrointestinal Transit Times in Colon Capsule Endoscopy*. Clin Transl Gastroenterol. 2022 Jan 1;13(6):e00498.

8. Moen S, **Vuik FER**, Kuipers EJ, Spaander MCW. *Artificial Intelligence in Colon Capsule Endoscopy. A Systematic Review.* Diagnostics (Basel). 2022 Aug 17;12(8):1994.

9. **F.E.R. Vuik***, P.H.A. Wisse*, W. de Klaver, S.A.V. Nieuwenburg, N.S. Erler, I. Lansdorp-Vogelaar, E.J. Kuipers, E. Dekker, M.C.W. Spaander. *Impact of fecal immunochemical test screening on colorectal cancer incidence and mortality.* Submitted *shared first authorship

10. Nieuwenburg SAV, **Vuik FER**, Kruip MJHA, Kuipers EJ, Spaander MCW. *Effect of anticoagulants and NSAIDs on accuracy of faecal immunochemical tests (FITs) in colorectal cancer screening: a systematic review and meta-analysis*. Gut. 2019 May;68(5):866-872

Not in this thesis

11. **Vuik FER,** Moen S, Spaander MCW. Colon capsule endoscopy as panendoscopy: Using current knowledge to enhance possibilities. Endosc Int Open. 2022 May 13;10(5):E584.

12. Spaander MCW, **Vuik F**, Nieuwenburg SAV. *When to stop colonoscopy surveillance in the elderly?* Neth J Med. 2018 Oct;76(8):350.

13. Nieuwenburg SAV, **Vuik FER**, Kruip MJHA, Kuipers EJ, Spaander MCW *Effect van antistolling en NSAIDs op de uitkomst van de fecale immunochemische test (FIT) bij screenen op darmkanker: een systematisch review en meta-analyse* Tijdschrift voor trombose en antistolling 2018; 46(2).

14. Berden FA, **Vuik FE**, Drenth JP, Kievit W. The gap between registration trials and real world in hepatitis C is closing. Dig Liver Dis. 2017 Jan 23. pii: S1590-8658(17)30160-3.

15. **Vuik FE**, Koehestanie P, Herbers AH, Terhaar Sive Droste JS. Chronic use of metamizole: not so safe after all? Neth J Med. 2017 Mar; 75(2): 81-83.

Chapter 17

PhD portfolio

PhD PORTFOLIO

Name PhD Candidate:	F.E.R. (Fanny) Vuik
PhD Period:	May 2016 – May 2022
Erasmus MC department:	Gastroenterology and Hepatology
Promotors:	Prof. dr. M.C.W. (Manon) Spaander

National Courses	**Year**	**Workload**
BROK (basiscursus regelgeving klinisch onderzoek), Erasmus MC, Rotterdam	2016	24 hours
Integrity in scientific research, Erasmus MC, Rotterdam	2016	16 hours
Basic Introduction Course on SPSS, Erasmus Postgraduate School for Molecular Medicine (Molmed), Erasmus MC, Rotterdam	2016	6 hours
Endnote workshop, , Erasmus Postgraduate School for Molecular Medicine (Molmed), Erasmus MC, Rotterdam	2016	6 hours
Systematic literature search Pubmed and other databases, Erasmus Postgraduate School for Molecular Medicine (Molmed), Rotterdam	2016	6 hours
OpenClinica, Erasmus MC	2017	6 hours
Biomedical English Writing Course, Erasmus Postgraduate School for Molecular Medicine (Molmed), Erasmus MC, Rotterdam	2017	24 hours
Biomedical English Writing and Communication, Erasmus MC, Rotterdam	2017	40 hours
Basiscursus Regelgeving Klinisch Onderzoek. Consultatiecentrum patientgebonden Onderzoek (CPO), Erasmus MC	2017	8 hours
Photoshop and Illustrator CS6, Erasmus Postgraduate School for Molecular Medicine (Molmed), Erasmus MC, Rotterdam	2017	8 hours
Microbiomics I course, Erasmus Postgraduate School for Molecular Medicine (Molmed), Erasmus MC, Rotterdam	2017	16 hours
Biostatistical Methods I: Basic principles Methology, Netherlands institute for Health Sciences (NIHES), Rotterdam	2018	56 hours
Management course, Erasmus MC, Rotterdam	2018	16 hours

International courses	**Year**	**Workload**
Masterclass colon capsule endoscopy, Rome, Italy	2016	12 hours
Three day course Capsule Endoscopy, London, United Kingdom	2016	21 hours
E-learning colon capsule endoscopy	2016	30 hours
Evidence bases guideline development, ESGE, Barcelona, Spain	2017	8 hours

Oral presentation	**Year**	**Workload**
Composition of mucosa-associated microbiome along the entire gastrointestinal tract in humans. Digestive Disease Days, Veldhoven, the Netherlands.	2017	12 hours
Composition of mucosa-associated microbiome along the entire gastrointestinal tract in humans. United European Gastroenterology Week (UEGW), Barcelona, Spain.	2017	12 hours
Incidence of colorectal cancer in young adults in Europe. World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Vienna, Austria	2018	12 hours

Incidence of colorectal cancer in young adults in Europe. Digestive Disease Days, Veldhoven, the Netherlands	2018	12 hours
Incidence of colorectal cancer in young adults in Europe. United European Gastroenterology Week (UEGW), Vienna, Austria.	2018	12 hours
Incidence of colorectal cancer in young adults in Europe. United European Gastroenterology Week (UEGW), Vienna, Austria. **Press conference**	2018	12 hours
Role of colon capsule endoscopy in a screening population. European society of gastrointestinal endoscopy (ESGE), Prague, Czech Republic	2019	12 hours
Impact of fecal immunochemical test screening on colorectal cancer incidence and mortality World Endoscopy Organisation: Colorectal Cancer Screeening Committee, San Diego, United States of America	2022	12 hours
Impact of fecal immunochemical test screening on colorectal cancer incidence and mortality Digestive Disease Days, Veldhoven, the Netherlands	2022	12 hours

Poster presentation	**Year**	**Work-load**
Comparison of polyethylene glycol and sulfate solution as cleansing regimen for colon capsule endoscopy. Digestive disease week, Chicago, United states of America	2017	12 hours
Composition microbiome along entire GI tract. Digestive disease week, Washington D.C. Unites States of America	2018	12 hours
Incidence of colorectal cancer in young adults in Europe. Digestive disease week, Washington D.C. Unites States of America	2018	12 hours
Applicability of colon capsule endoscopy as pan-endoscopy: from bowel-preparation, transit times and completion rate to rating times and patient acceptance. Digestive Disease Week, Chicago, United States of America	2020	12 hours
Impact of fecal immunochemical test screening on colorectal cancer incidence and mortality European Gastroenterology Week (UEGW), Vienna, Austria	2022	12 hours

Attended (inter) national conferences	Year	Work-load
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Barcelona, Spain	2017	8 hours
United European Gastroenterology Week (UEGW), Barcelona, Spain	2017	32 hours
Digestive Disease Days, Veldhoven, the Netherlands	2017	16 hours
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Chicago, United states	2017	8 hours
Digestive disease week, Chicago, United states =	2017	28 hours
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Vienna, Austria	2018	8 hours
United European Gastroenterology Week (UEGW), Vienna, Austria	2018	32 hours
Digestive Disease Days, Veldhoven, the Netherlands	2018	16 hours
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Washington D.C., United states	2018	8 hours
Digestive disease week, Washington D.C, United states	2018	16 hours
European society of gastrointestinal endoscopy (ESGE), Prague, Czech Republic	2019	32 hours
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, Barcelona, Spain	2019	8 hours
United European Gastroenterology Week (UEGW), Barcelona, Spain	2019	32 hours
World Endoscopy Organisation: Colorectal Cancer Screeening Committee, San Diego, United states	2019	8 hours
Digestive disease week, San Diego, United states	2019	28 hours
European society of gastrointestinal endoscopy (ESGE), Prague, Czech Republic	2019	28 hours

Awared grants and prices	Year
Digestive disease foundation grant- OCEAN trial	2018
United European gastroenterology week bursary	2018
Winner of best oral presentation, United European gastroenterology week	2018
Poster of distinction, Digestive disease week	2018

Extracurricular	Year
Board member Promeras, representing board of all PhD candidates, Erasmus MC, the Netherlands	2017-2018
Chair of Promeras, Erasmus MC, the Netherlands	2018
PhD Committee, Erasmus MC, Rotterdam	2017-2020
Working group PhD guidelines, Erasmus MC, Rotterdam	2019-2020
Board member Promovendi Netwerk Nederland (PNN), representing all PhD Candidates in the Netherlands	2018-2019
Board member, Vakcentrale voor Professionals (VCP) – Young professionals, Den Haag	2019-current
Sociaal Economische Raad (SER), Jongerenplatform, Den Haag	2019-current
Jongeren Denktank Corona (JDC), Den Haag	2020-2021
Editoral member MAGMA	2021-current

Chapter 18

Dankwoord

Dankwoord

Het verrichten van een promotieonderzoek heb ik ervaren als een bijzonder mooie periode. Traditiegetrouw gebruik ik graag het laatste hoofdstuk van mijn proefschrift om collega's, vrienden en familie te bedanken voor hun steun, hulp en vertrouwen.

Hooggeleerde prof. dr. Manon C.W. Spaander, lieve Manon, ik beschouw mezelf gelukkig om onder jouw vleugels te mogen promoveren. Ik kwam in een warm bad terecht. Jaren van hard werken waren reeds verricht voor mijn komst. Een vliegende start leek in het verschiet, maar helaas verliep de praktijk anders. We hebben veel hoogte- en dieptepunten meegemaakt en alle emoties hebben de revue gepasseerd. Maar soms ging het allemaal even aan ons voorbij, zoals een persconferentie waarbij het hele studieteam niet bereikbaar was vanwege een vliegreis van twaalf uur. Ik voelde me vrij om projecten uit te voeren naar eigen inzicht en nieuwe projecten te bedenken. Altijd gesteund door jouw scherpe blik en bescherming waar nodig. Ik waardeer jouw inzicht en pragmatisch aanpak. Jouw enthousiasme en oprechte interesse hebben voor vier fantastische jaren gezorgd. Onwijs veel dank! Ik kijk uit naar een mooie samenwerking in de kliniek.

Hooggeleerde prof. dr. Ernst J. Kuipers, beste Ernst, het was een eer om onder jouw hoede te mogen promoveren (helaas net niet tot het einde). Ik bewonder jouw kunde om te enthousiasmeren en te inspireren, het vermogen om op de hoogte te blijven van de meest recente ontwikkelingen in ons vakgebied ondanks andere drukke werkzaamheden, en hoe conflictsituaties op te lossen. Dat alles met een goed gevoel voor humor. Je gaf dit proefschrift richting, inzicht, en wees mij op het belang van de punten en komma's. Het feit dat ik na een bespreking altijd met meer werk weer wegging, nam ik maar voor lief. Ik heb geleerd stap voor stap te werk te gaan en lijntjes met mensen warm te houden, ook al is het lijntje flinterdun. Veel dank en succes met het ministerschap!

Graag wil ik de leden van de beoordeling- en promotiecommissie bedanken: prof. dr. J. van der Woude, prof. dr. M. van Leerdam en prof. dr. G. Meijer. Dank voor jullie interesse en tijd voor de beoordeling van dit proefschrift. Daarnaast wil ik de overige leden van de promotiecommissie bedanken voor de waardevolle samenwerking: dr. I. Lansdorp-Vogelaar, prof. dr. M. Peppelenbosch en prof. dr. I. Nagtegaal.

Ik wil mijn speciale dank uiten aan de medewerkers van het ERGO-centrum in Ommoord. Bedankt voor jullie interesse, betrokkenheid en hulp gedurende de inclusies voor de ORCA studie *'het videocapsule onderzoek'*. Jullie vertrouwen en steun heb ik enorm gewaardeerd. Bijzondere dank ben ik verschuldigd aan *Anneke Korving*, *Jolande Verkroost*

en *Paulien van Wijngaarden* voor het organiseren en coördineren van de studie. Jullie waren onmisbaar voor het project.

Daarnaast wil ik graag de medewerkers van het MDL-laboratorium bedanken voor hun inzet bij de microbioom- en ORCA studie. *Hanneke*, *Jan* en *Buddy*, bedankt voor het coördineren van al die ontlastingstesten.

Lieve *Sophia*, samen zijn we het grote ORCA-avontuur gestart met cursussen in Rome en London. De wilde taxirit door de straten van Rome kan ik mij nog als de dag van gisteren herinneren. Bedankt dat je altijd voor mij klaar stond. *Agnes*, jij weet als geen ander efficiënt te werken in het ietwat stroeve en ondoorzichtige wetenschappelijke systeem. Bedankt voor je hulp en succes met jouw laatste loodjes!

Al mijn co-auteurs wil ik bedanken voor de prettige samenwerking en de waardevolle inbreng voor de manuscripten van dit proefschrift. In het bijzonder wil ik *Nicole Erler* bedanken voor de tomeloze inzet en flexibiliteit. Ook wil ik graag *Gwenny Fuhler* bedanken. Met jouw geduld, uitleg en inzicht zijn de microbioom projecten goed van de grond gekomen.

Beste prof. dr. Janneke van der Woude en dr. Rob de Knegt, bedankt voor het in mij gestelde vertrouwen door mij op te leiden tot Maag-, Darm-, en Leverarts. Daarnaast wil ik dr. Felix de Jongh, dr. Marike Wabbijn en dr. Roel van de Laar als opleiders van de vooropleiding interne geneeskunde in het Ikazia ziekenhuis hartelijk bedanken voor het fijne leerklimaat en interesse in mij.

Tot slot wil ik graag de MDL-artsen in het Jeroen Bosch Ziekenhuis bedanken. Mijn eerste stappen als dokter bij de MDL-ziekten hebben ik daar gemaakt. Jullie hebben mij laten zien hoe mooi ons vak is!

Lieve mede-promovendi, mijn promotietijd zou niet half zo leuk zijn geweest zonder jullie. Gestart op het Dak, geëindigd op NA-6, het was een prachtige periode. *Eline*, zo hard gewerkt om de ORCA-studie van start te laten gaan. Ik ben zeer vereerd dat ik de studie heb mogen afronden. Bedankt voor je hulp en interesse gedurende het project. *Maren*, bedankt voor al je wijze adviezen gedurende mijn promotietraject. Ik waardeer jouw pragmatische en enthousiaste instelling. *Sophia, Joany en Louisa*, bedankt voor de gezelligheid, vele borrels en mooie congresreizen. Ik heb van jullie genoten. *Evelien en Renske*, ik hoor nog jullie lach door de muren op 't Dak. Bedankt voor alle vrolijkheid en op naar de volgende avonturen. *Jorn,* jij hebt het volgehouden als mijn kamergenoot/buurman en het eindeloos aanhoren van 'het videocapsule onderzoek'. Alleen al om die

reden heb ik veel respect voor je ;). *Arjan,* met jou is het altijd feest. *Loes*, begonnen op 't Dak, samen naar het Ikazia en nu opnieuw collega's in het Erasmus MC. Inmiddels zijn we bijna buren en ben je een goede vriendin. Bedankt dat je er bent, ook in moeilijke tijden. *Kasper*, wat was het een heerlijke tijd om samen met jou onderzoek te doen. Ik geniet van onze toeristisch activiteiten in eigen land en onze mooie gesprekken.

Natuurlijk moeten de 'zuurste' mensen van het zure kantje bedankt worden: *Luke, Judith, Sylvia, Lisette, Maria* en *Rozanne*. Ik heb genoten van jullie aan mijn zure zijde. *Rozanne*, ik wil jou graag speciaal bedanken voor jouw interesse, behulpzaamheid en vriendschap. Een ode aan de hardwerkende promovendi aan de darkside kan natuurlijk niet ontbreken. *Sarah*, bedankt voor al je hulp bij het videocapsule project, jouw gezelligheid en openheid. *Pieter*, als werken tijdens regulaire tijden geen optie meer is, dan maar op zondagochtend. Ik ben blij dat we er toch altijd een feestje van hebben kunnen maken. *Steffi*, ik bewonder jou om jouw bevlogenheid en doorzettingsvermogen. Ik kijk uit naar de koffiemomentjes in het Erasmus MC. *Ruben*, een dag zonder jouw ad-rem opmerkingen, is eigenlijk een verloren dag.

Dan mijn paranimfen, *Carlijn en Stella*. De afgelopen jaren waren onvergetelijk door jullie aanwezigheid. Ik kon jullie niet altijd bijbenen – met name bij de (foute) afkortingen, kabouter Wesley grappen, en de marathons –, maar wat is het een eer dat jullie aan mijn zijde staan vandaag. We hebben veel gelachen, mooie reizen gemaakt en (wetenschappelijke) discussies gevoerd. Lieve *Stella*, samen hebben wij veel studies opgepakt en afgerond. Helaas zijn er ook veel gesneuveld gedurende het traject. Samenwerken met jou is absoluut een feest. En als het even geen feest was, hadden we altijd nog jouw gevoel voor humor om ons er doorheen te slepen. Ik waardeer jouw pragmatische instelling, zelfverzekerdheid, jouw no-nonsense mentaliteit en onze mooie gesprekken. Bedankt voor jouw vriendschap! Lieve *Carlijn*, jij was mijn redder in nood wanneer het om statistische problematiek ging. Inmiddels ben jij mijn redder in nood omtrent de problematiek van het leven (zeker wanneer het insecten op slaapkamers betreft). Je staat altijd voor mij klaar, je bent attent en enthousiast. Ik waardeer jouw eerlijkheid en openheid. Ik kijk uit naar het volgende insectenslagveld.

Wat ben ik gezegend met mijn *lieve vriendinnen*. Jullie hebben elke stap van dit promotieonderzoek meegemaakt, moed ingesproken tijdens de tegenslagen, en met mij feest gevierd tijdens de hoogtepunten. Ik waardeer jullie geduld met mij en geniet enorm van alle momenten samen. Ik ben benieuwd naar de jaren die voor ons liggen.

Lieve schoonfamilie, *Hilde* en *Ronald*, *Tanne* en *Johan*, *Inger* en *Mark*, het is altijd fijn om even tot rust te komen in het mooie Maastricht. Bedankt voor jullie interesse, betrokkenheid en medeleven.

Graag wil ik mijn familie bedanken. Lieve *Katja,* ik ben trots op jou en jouw vasthoudendheid. Lieve *Pepijn* en *Kiekie*, wat geven jullie ons veel energie en vrolijkheid. Lieve *Niels*, zonder jou was dit proefschrift natuurlijk nooit tot een goed eind gekomen ;). Bedankt voor je steun en onze mooie gesprekken. Lieve *mama* en *papa*, er zijn geen woorden die mijn dankbaarheid voor jullie kunnen uitdrukken. De onvoorwaardelijke liefde, het geloof in mijn kunnen, de gekregen vrijheid en middelen om mij volledig te kunnen ontwikkelen. Bedankt dat jullie altijd voor mij klaar staan. Lieve *papa*, jij hebt het begrip 'doorzettingsvermogen' opnieuw gedefinieerd. Jij bent mijn voorbeeld. Lieve *mama*, als twee druppels water lijken we op elkaar. Ik heb diep respect hoe jij voor iedereen klaar staat, altijd met een vrolijk humeur en goed gevoel voor humor. Ik hou van jullie!

Tot slot, lieve *Etzel*. Ik heb de laatste woorden voor jou gereserveerd. Er ging geen dag, avond of weekend voorbij waar niet een van ons aan het proefschrift aan het werken was. Jij motiveert en stimuleert mij, geeft mij ruimte, zorgt voor mij en haalt het beste in mij naar boven. Wij zijn het beste team! Ik kijk uit naar de toekomst met jou, zonder proefschriften.

Chapter 19

About the author

About the author

Fanny Vuik was born on the 18th of February 1990, in Dordrecht, the Netherlands. After graduating from high school (Gymnasium, Valuascollege, Venlo) in 2008, she commenced medical school at the Radboud University, Nijmegen. She obtained her medical degree in 2015 and started working as resident not in training (ANIOS) in April 2015 at the department of Gatroenterology and Hepatology of Jeroen Bosch Hospital in 's-Hertogenbosc. In May 2016 she started her PhD trajectory under supervision of prof. dr. E.J. Kuipers and prof. dr. M.C.W. Spaander at the department of Gatroenterology and Hepatology of the Erasmus Medical Center in Rotterdam. During her PhD trajectory her interest in board functions was caught. She became board member and chair of Promeas, the representing body of all PhD candidates in the Erasmus MC and later board member of *Promovendi Netwerk Nederland (PNN)*, presenting all PhD candidates in the Netherlands. She is also active in *Sociaal Economische Raad (SER), het jongerenplatform* and *Jongeren Denktank Corona (JDC)* where she advocates for the interests of the youth.

In August 2020, she started with her Internal Medicine residency at the Ikazia Hospital under supervision of dr. M. Wabbijn and dr. R. van de Laar as part of her training in Gatroenterology and Hepatology at the Erasmus MC Unitversity Medical Center. In February 2022, she continued her training in Gastroenterology and Hepatology in the Erasmus MC (program director prof. dr. C.J. van der Woude and dr. R.J. de Knegt).