Faecal Haemoglobin level and mortality in colorectal cancer screening

Møller, Lasse Kaalby

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FAECAL HAEMOGLOBIN LEVEL AND MORTALITY IN COLORECTAL CANCER SCREENING

Ph.D. Dissertation

Lasse Kaalby Møller, MScPH

Department of Surgery, Odense University Hospital, Denmark

Department of Clinical Research, University of Southern Denmark, Denmark

CONTENT

SUPERVISORS

Main supervisor

Professor **Gunnar Baatrup,** Department of Surgery, Odense University Hospital, Denmark & Department of Clinical Research, University of Southern Denmark, Denmark

Co-supervisors

Professor **Aasma Shaukat,** Division of Gastroenterology, NYU Langone, New York, United States of America

Professor **Gabriele Berg-Beckhoff,** Unit for Health Promotion Research, Department of Public Health, University of Southern Denmark, Denmark & Unit for Health Research, Hospital South West Jutland, Denmark

Mr. **Issam Al-Najami,** Ph.D., Department of Surgery, Odense University Hospital, Denmark

Mr. **Morten Rasmussen**, Ph.D., Digestive Disease Center, Bispebjerg University Hospital, Denmark

ASSESSMENT COMMITTEE

Chair

Professor **Torben Knudsen**, Department of Regional Health Research, Hospital South West Jutland, Denmark

Opponents

Associate Professor **Katrine Emmertsen**, Institute for Clinical Medicine, Aarhus University, Denmark

Associate Professor **Thomas De Lange**, Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden

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PREFACE

This dissertation is made up of three original articles. The work has been completed during my time as a Ph.D. student at the Department of Surgery, Odense University Hospital and the Department of Clinical Research, University of Southern Denmark.

First, I would like to thank all my colleagues at the Surgical Research Unit, Odense University hospital for their enormous role in making the last 3½ years joyous, interesting and motivating. Especially thank you Ulrik for your companionship, humorous statements, motivating teamwork and significant amount of patience. Thank you Charlotte for always smiling in the face of great frustrations and for your ever-positive attitude. Thank you Lene for being a continuous source of joy and camaraderie. Thank you Jette for your good spirit and very significant improvement of the cheese-drawer. Thank you Thomas for your inclusive attitude and for the patient, and sometimes involuntary, approach to my myriad of questions about the world of medicine. Thank you Morten for your cheery, dad-joke-based attitude and for some much needed teaching in the fundamentals of biochemistry. Thank you Tassos for comprehensive discussions that has improved the quality of several papers and for securing a consistent supply of pastries that keeps malnourishment at bay. Thank you Marleen for years of humoristic provocations, fun times and recurring defeats in various board games. Thank you Benedicte for interesting discussions, pleasant commutes and entertaining perspectives on life. Thank you Niels for never failing to put a smile on my face. Thank you Marianne for valuable lessons about how to conduct research. Last, but not least, a thank you to Erik Zimmermann-Nielsen for always being a caring and selfless colleague.

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This work would not have been possible without the continuous support of my principal supervisor prof. Gunnar Baatrup. His inclusive and multidisciplinary approach has thought me many important things about the world research. Thank you for the numerous exciting projects, the many interesting talks and everything in between.

This dissertation is dedicated to my family, friends and wife. Thank you for unwavering love, support and patience.

ABBREVIATIONS

Term	Abbreviation
CRC	Colorectal cancer
f-HB	Faecal Haemoglobin
FIT	Faecal Immunochemical test
gFOBT	Guaiac faecal occult blood test
Hb	Haemoglobin
FOBT	Faecal Occult Blood Test
GI	Gastrointestinal
CVD	Cardiovascular disease
NSAID	Nonsteroidal anti-inflammatory drug
COX	Cyclooxygenase
RR	Relative Risk
RD	Risk Difference
aHR	Adjusted Hazard Ratio
AI	Artificial Intelligence
mtsDNA	Multitarget stool DNA

LIST OF PAPERS

Study 1

Shaukat A, Kaalby L, Baatrup G, Kronborg O, Duval S, Shyne M, Mandel JS, Church TR. Effects of Screening Compliance on Long-term Reductions in All-Cause and Colorectal Cancer Mortality. Clin Gastroenterol Hepatol. 2021 May;19(5):967-975.e2. doi: 10.1016/j.cgh.2020.06.019. Epub 2020 Jul 4. PMID: 32634624.

Study 2

Kaalby L, Al-Najami I, Deding U, Berg-Beckhoff G, Steele RJC, Kobaek-Larsen M, Shaukat A, Rasmussen M, Baatrup G. Cause of Death, Mortality and Occult Blood in Colorectal Cancer Screening. Cancers (Basel). 2022 Jan 4;14(1):246. doi: 10.3390/cancers14010246. PMID: 35008412; PMCID: PMC8750981.

Study 3

Kaalby L, Deding U, Al-Najami I, Berg-Beckhoff G, Bjorsum-Meyer T, Laurberg T, Shaukat A, Steele RJC, Koulaouzidis A, Rasmussen M, Kobaek-Larsen M, Baatrup G. Faecal haemoglobin levels are associated with all-cause mortality and cause of death in colorectal cancer screening. (In review, BMC Medicine).

ENGLISH SUMMARY

Screening for early detection of colorectal cancer (CRC) have become an integrated part of health care systems in many developed countries in recent years. Screening is conducted with the aim of reducing incidence and mortality by allowing for early diagnosis and treatment of cancers or by preventing onset by removing precursor lesions. Most programs invites participants to submit a stool sample that are checked for the presence of faecal haemoglobin (f-Hb) - an established biomarker for CRC. Previously, the guaiac faecal occult blood test (gFOBT) was the primary method for detecting f-Hb in screening. The test produced a positive or negative result. Today, the gFOBT has been replaced by the faecal Immunochemical Test (FIT) which produces a quantifiably interpretable result from which a positivity threshold can be established.

In the 1970s-1990s, three separate clinical trials created the scientific foundation of modern day CRC screening. The three trials, conducted in Minnesota (USA), Nottingham (UK) and on Funen (Denmark), all randomized a sizeable part of an adult population to undergo several rounds of biennial CRC screening. All three studies concluded that gFOBT-based screening have a preventive effect on CRC mortality. Years later, research groups in the US and in the UK, each presented findings suggesting a slightly reduced, but persistent, protective effect of screening on CRC mortality after 20 and 30 years of follow-up respectively.

When we started the work presented in this dissertation, the Danish CRC screening program had just completed its first round. The program appeared successful on several important parameters such as participation and detection of early cancers and high-risk adenomas. However, some problems did appear, one being the approximately 30% of participants with a negative colonoscopy. This large group of participants had faecal haemoglobin (f-Hb) levels above the positivity threshold (100 ng/mL) and no findings to explain the bleeding. This pattern appear to not be unique for screening in Denmark and researchers have begun considering other explanations than colorectal neoplasia as the source of the bleeding. Recently, a British study by Libby et al. presented findings that suggest an association between f-Hb and all-cause mortality (both including and excluding CRC deaths) in gFOBT-tested screening participants. Results also showed an association between f-Hb and a number of seemingly unrelated causes of death, such as cardiovascular disease and neuropsychological disease. Authors speculated that f-Hb could be used to indicate the presence of other serious and/or chronic conditions. While succeeding in creating initial support for their hypothesis, the study did, however, have some methodological limitations that needs to be addressed. This warrants additional studies on the subject.

Aim

The overall aim of this dissertation was to investigate the effects of screening for CRC and to investigate the proposed association between f-Hb and mortality outcomes. This divided into three studies where we wanted:

1. To investigate the role of CRC screening on both overall and cause-specific mortality in a pooled study of Danish and American data.
2. To investigate the association between f-Hb and both overall mortality and seemingly unrelated causes of death in a Danish gFOBT-tested screening population with more than 30 years of follow-up
3. To investigate the association between incrementally increasing f-Hb and mortality outcomes in a modern-day FIT-based Danish screening population.

Study 1. The long-term effect of colorectal cancer screening

In study 1, we pooled the data from the Danish and the US screening trials and enriched it with follow-up data. We then conducted an individual participant data meta-analysis comparing screening participants to the control population in terms of mortality. We found that CRC screening provides a sustained reduction in CRC mortality and a significant reduction in all-cause mortality (adjusted for compliance). We also observed no benefits from screening in the youngest group of women aged 50-59.

Study 2. Faecal haemoglobin and mortality outcomes after >30 years of follow-up

In study 2, we used enriched the Danish trial data from study 1 with register-data on education, income, cause of death and comorbidity and conducted a 33-year follow-up. We compared gFOBT positive to negative participants on a number of outcomes including all-cause mortality, CRC mortality and causes of death. We found an association between positive gFOBT result and increased risk of both all-cause mortality and several seemingly unrelated causes of death such as cardiovascular disease. The study did have a number of limitations as we were not able to adjust for the effects of prescription medication nor could we quantify the f-Hb levels to investigate potential dose-response relationships.

Study 3. Quantified faecal haemoglobin and mortality outcomes in the Danish colorectal cancer screening population

In study 3, we collected data on current screening participants in Denmark from several national registers. We introduced prescription medication as a covariate and FIT-levels as the exposure to address limitations of study 2. Participants were divided by FIT-level and compared in terms of mortality outcomes. Our results showed that even an incremental increase in FIT increased the risk of all-cause mortality – even after excluding CRC deaths. The same is true for different and seemingly CRC-unrelated causes of death. Interestingly, we observed a clear dose-response relationship between FIT and several of our outcomes underlining the association.

Conclusions & future perspectives

In conclusion, our results suggests that CRC screening as a concept appear viable for sustained reductions in mortality, but the preventive benefit varies significantly by age and gender – something that is worth considering when designing future initiatives. Our results also suggest that elevated f-Hb levels does appear to be associated with an increased both all-cause mortality and several causes of death not usually connected to CRC. This supports the notion that f-Hb could indicate the presence of non-communicable, chronic conditions. We believe that f-Hb may one day become an established biomarker for non-CRC diseases, which could add important perspectives to CRC screening and create a more nuanced understanding of the complicated relationship between gut health and health outcomes. Due to the complex and multifactorial nature of this topic, more work is needed for it to have a clinical impact.

DANISH SUMMARY

Tarmkræftscreening er blevet en integreret del af det danske sundhedsvæsen og tilbydes til alle borgere mellem 50 og 74. Screening er en samlet betegnelse for en proces der har fokus på tidlig opsporing af en sygdom. Screeningsprogrammer som det danske eksisterer i mange, særligt vestlige, lande og har til formål at nedbringe både forekomst og dødelighed af tarmkræft. De fleste benytter en afføringsprøve som inklusionsværktøj, hvor en borgers afføring undersøges for spor af blod. Tidligere brugte man en guaiac-baseret prøve (gFOBT) som kunne være enten positiv eller negative. I dag er denne erstattet af den immunokemiske FIT, der kan vise den eksakte mængde blod i en afføringsprøve. Herudfra kan man bestemme om der er nok blod til at denne bliver positiv. Hvis dette er tilfældet henvises borgeren til kikkertundersøgelse af tyk-og endetarm hvor forstadier fjernes in situ. Ved fund af cancer henvises til videre udredning. En stor del af det videnskabelige fundamentet for tarmkræftscreening blev lagt mellem 1970'erne og 1990'erne, hvor tre kliniske forsøg blev gennemført. Et blev gennemført i Minnesota, et i Nottingham og et på Fyn. Alle tre studier undersøgte en tilfældigt udvalgt gruppe af borgere og tilbød dem gentagne runder af gFOBT-baseret screening. Borgerne blev fulgt i mange år, og deres risiko for både at få og for at dø af tarmkræft blev sammenlignet med en kontrolgruppe. Konklusionerne fra de tre studer var enslydende: screening har en tydelig forebyggende effekt på risikoen for at dø af tarmkræft. Opfølgningsstudier i både USA og i UK har vist at denne forebyggende effekt er dalende som tiden går, men dog vedvarende. I nyere tid er forskere begyndt at spekulere på om blod i afføringen kan have bredere anvendelse end påvisning af tarmkræft. Ideen er opstået på baggrund af de mange "negative" kikkertundersøgelser foretaget efter positiv afføringsprøve, hvor der ikke kan påvises en årsag til blødningen. Forskere fra Skotland har for nyligt vist at der blandt deltagere i et screeningsforsøg med positiv afføringsprøve var en øget risiko for at dø i studieperioden. Der var også en øget risiko for at dø af årsager der ikke normalt relateres til tarmkræft såsom hjertekarsygdomme og diabetes. Disse fund har ført til spekulationer om muligheden for at bruge blod i afføringen som en biomarkør for andre sygdomme end tarmkræft. De få studier der er på områder har dog flere metodemæssige mangler og der er brug for større og anderledes studier.

Formål

Derfor er det formålet med denne afhandling at undersøge sammenhængen mellem blod i afføringen og både overordnet dødelighed og forskellige dødsårsager. Herunder ønskede vi at undersøge:

1. Hvilken indvirkning screening har på både den overordnede og den årsagsspecifikke dødelighed i både danske og amerikanske forsøgspersoner.
2. Sammenhængen mellem blod i afføringen og både overordnet og årsagsspecifik dødelighed i en population af danske forsøgspersoner med mere end 30 års opfølgning.
3. Om en inkrementel stigning i niveauet af blod er associeret med en øget risiko for både overordnet og årsagsspecifik dødelighed blandt danske FIT-testede screeningsdeltagere.

Studie 1. Tarmkræftscreening og dets langsigtede effekt på dødelighed

I studie 1 indhentede vi information om forsøgspersonerne fra både det danske og det amerikanske screeningsforsøg. Vi fulgte forsøgspersonerne i mere end 30 år og sammenlignede dem med en kontrolgruppe. Vores resultater viste at screeningsdeltagere har en lavere risiko for både at dø og for at dø af tarmkræft end personer i kontrolgruppen. Denne beskyttende effekt varierede med køn og alder. Det var særligt tydeligt blandt kvinder mellem 50 og 59, der ikke havde nogen påviselig gevinst af screening på dødelighed op til 30 år efter.

Studie 2. Blod i afføring og dødelighedsmål blandt forsøgsdeltagere efter 30 års opfølgning

I studie 2 brugte vi data fra de danske forsøgspersoner beriget med registerdata på til at undersøge sammenhængen mellem blod i afføringen og dødelighed efter 33 års opfølgning. Deltagere med en positiv afføringsprøve blev sammenlignet med dem der var negative ift. dødelighed og dødsårsag. Vores resultater viste at gFOBT positive deltagere havde en større risiko for at dø i studieperioden. De samme deltagere havde også en større risiko for at dø af tilsyneladende ikke-relaterede årsager, såsom hjertekarsygdomme.

Studie 3. Mængder af blod i afføring og dødelighedsmål blandt deltagere i det danske tarmkræftscreeningsprogram

I studie 3 ekstraherede vi data fra den danske tarmkræftscreeningsdatabase på alle deltagere fra 1. runde og berigede det med data fra nationale registre. Vi opdelte deltagere i grupper ud fra deres FIT-værdi og sammenlignede dem. Overordnet set viste vores resultater at selv en mindre stigning i FIT-niveau øger risikoen for at dø i studieperioden. Det samme var tilfældet for risikoen for at dø af bl.a. respiratoriske sygdomme, andre cancere og hjertekarsygdomme. Vores resultater viser en dosis-respons-lignende sammenhæng mellem øget FIT-værdi og øget risiko for at dø i studieperioden.

Konklusioner

Vores resultater viser samlet set at screening har en aftagende men vedvarende beskyttende effekt på risikoen for at dø af tarmkræft, en effekt der dog varierer markant med age og køn. Særligt ved den yngste gruppe af kvinder ser vi ingen indikation på at screening har nogen effekt på dødelighed. Vores resultater viser at screeningsdeltagere med blod i afføringen har en større risiko for at dø i studieperioderne generelt. Det samme er tilfældet for risikoen for at dø af en række, ikke-tarmkræft relaterede, sygdomme. Det understøtter hypotesen om at blod i afføringen kan indikere tilstedeværelsen af andre alvorlige sygdomme. I fremtiden er det derfor muligt at blod i afføringen kan se bredere anvendelse i sygdomsopsporingsøjemed som biomarkør for andre sygdomme end tarmkræft.

INTRODUCTION

DISEASE PREVENTION THROUGH EARLY DETECTION – THE CONCEPT OF SCREENING

In 1968, Wilson and Jungner published their framework on *Principles and Practice of Screening For Disease* encompassing a set of 10 principles for determining if, when, and how to initiate early detection efforts for any given disease - also referred to as screening. (1) Essentially, the principles facilitate considerations about the importance of a given disease, how the disease progress and if there are targetable stages in that process, if there are screening tests and treatments available, and organizational concerns about a given solution. (2) Since Wilson and Jungner published their principles in 1968, vast quantities of research have been conducted and recommendations published on screening. This includes publications from the World Health Organization, which employs the 10 principles as a foundation for modern day screening guidelines and recommendations. (3, 4) Today, this process has led to the introduction of screening programs for several different diseases in countries worldwide. One example of a widely implemented program is prenatal screening for a range of congenital conditions, where early treatment is important. Another is antenatal, or pregnancy, screening focusing on identifying hepatitis B, HIV and syphilis in pregnant women and initiating treatment to improve the chances of a successful pregnancy. Another common target for screening initiatives is cancers – a disease often characterized by significant improvements to the prognosis if detected early. In Denmark, screening for cervical cancer and breast cancer are good examples of screening programs where screening have had an impact on patient survival. (5, 6) For some cancers, the ability to detect and remove pre-cancerous lesions adds additional dimensions and possibilities for screening initiatives. The most common example is CRC. (7)

COLORECTAL CANCER

In 2020, 2.7 million people were diagnosed with, and 1.3 million died from, cancer within the European Union, representing a high (and increasing) burden for both patients and health care systems. (8, 9) CRC is the third most prominent cause of cancer death on a global scale, with an incidence that has more than doubled from 842,098 cases in 1990 to 2.17 million cases in 2019. This increase is especially pronounced in developing regions such as East Asia, where the age-standardized CRC incidence rate has increased by 143.3% and the mortality rate by 37.1% from 1990 to 2019. In comparison, a developed region such as Western Europe has seen an increase in the age-standardized incidence rate of 7.2%, but a decrease of 22.1% in mortality. (10)

Approximately 75% of CRCs are sporadically occurring cancers often associated with an array of modifiable, and often co-occurring and interacting, risk factors. The other 25% are linked to non-modifiable factors such as genetic disposition, inflammatory bowel disease, familial history of CRC or hereditary risk (i.e. lynch syndrome or familial adenomatous polyposis). A major part of the modifiable risks is lifestyle factors, especially so-called western lifestyle. Examples include obesity, smoking, high consumption of red meat and

processed foods, sedentary behaviour, low intake of fruit and vegetables, and excessive alcohol consumption. (11, 12) Another, and often interacting, factor to lifestyle is social determinants, where studies indicate that poverty, lack of education, immigration status, lack of social support, and social isolation all impacts CRC incidence and survival. (13) The prevalence of the modifiable risk factors have increased worldwide over the last decades, which has been named the primary driver of increased CRC incidence – especially in developing countries where elements of western lifestyle have become increasingly common. (10) A third category is medical factors, comprising conditions or medications, such as diabetes or *helicobacter pylori* infections, that influence the risk of developing CRC. Some medical factors, including the use of aspirin or hormone replacement therapy, have a preventive effect on CRC. (14)

Another important aspect for understanding the development in CRC incidence and mortality is advances in both medical and surgical treatments. The surgical management of CRC has moved towards less invasive techniques, such as laparoscopy or robot-assisted surgery, as an alternative to open surgery, which may reduce complication rates. (15, 16) For rectal cancer, examples include the introduction of Total Mesorectal Excision that has significantly reduced local recurrence rates or the "*Watch and Wait*"-approach which has been found to reduce the need for radical rectal resection. (17-20) Neo-adjuvant therapy is often used together with surgical treatment to further decrease the risk of recurrence. For later-stage tumours, adjuvant chemotherapy is normally needed, often accompanied by a colectomy. (21) There have also been advances in the medical treatment of cancer, where especially immunotherapy is expected to become impactful. (22) The success of treatment and the post-operative survival depends on the stage of the tumour, making early detection imperative. (14) Timely diagnosis only by symptoms can be challenging since CRC often presents with non-specific abdominal symptoms such as constipation, pain and bloating that can easily be confused with other conditions. The most recognizable is rectal bleeding which has been found to be a stronger indicator of CRC than other symptoms. (23)

COLORECTAL CANCER SCREENING – A HISTORICAL OUTLINE

The foundation of modern day CRC screening dates back to the 1920s, where an association between adenomatous tissue and CRC was discovered. This led to the notion that CRC develop from a pre-existing lesion and not directly from the mucosa. (24) Later, in the 1930s, Dukes et al. created the first CRC staging system to describe the tumour and then proceeded to show that early diagnosis of tumours followed by surgical treatment improved survival. (25) This contributed to the notion that CRC is curable if detected in time and maybe even completely preventable by the removal of polyps. The CRC transformation process was coined the "*polyp-cancer*"-sequence by Morson. (26) Despite a lack of knowledge about CRC, the first large-scale trial of CRC screening was initiated in 1948 by researchers from the University of Minnesota, using rigid sigmoidoscopy as their primary method on 21,500 people. The results showed an improved 5-year survival rate of 64% and an 85% reduction in CRC incidence in participants with rectal cancer. (27) In 1960, Hertz and

Debbish conducted a sigmoidoscopy feasibility trial in 26,000 asymptomatic people and found that the 58 patients with CRC had a 90% 15-year survival rate. However, the rigid sigmoidoscopy proved clinically inadequate. (28) In following years, several technological advances created new possibilities for screening. This included the introduction of testing the stool for occult blood by guaiac faecal occult blood test (gFOBT). Findings from the first gFOBT trial was presented in 1967 by Greegor, who, prior to the study, had observed a tendency of significant rectal bleeding in his primary care CRC patients and therefore speculated whether it was possible to detect cancers and their bleeding early. Greegor collected >2,000 gFOBTs from asymptomatic patients in his primary care facility and found seven cancers, all in gFOBT positive patients. He suggested including gFOBT as a routine examination in asymptomatic patients. (29)

One major issue at this time was the lack of a feasible method for accurate endoscopic follow-up. This problem was solved with the introduction of colonoscopy in clinical practice during the early 1970s. In one important study, Wolff and Shinaya presented findings showing the feasibility of conducting polypectomy via the colonoscope. (30) Building on these advances, four trials were launched in the 1970s and 1980s, one in Nottingham, UK, one in Minnesota, US, one in Gothenburg, Sweden, and one on Funen, Denmark. (31-33) All trials introduced repeated gFOBT-based testing to a large randomized population with colonoscopic intervention after positive test.

The first trial was initiated in Minnesota, recruiting 46,551 healthy volunteers aged 50-80 from 1976 to 1982 and from 1986 to 1992. Participants were randomized to either annual screening (15,570 people), biennial screening (15,587 people) or to a control group (15,396 people). After 13 years of follow-up, vital status was ascertained and causes of death obtained. Authors found that annual screening reduced CRC cumulative mortality by 33% and by 6% for the biennial group. (33) Later, Shaukat el al. conducted a follow-up study of the trial investigating survival and risk of CRC death. Authors followed participants from date of trial inclusion until death or end-of-study in 2008, using the National Death Index to achieve a follow-up of up to 30 years. In the underlying period, 33,020 (70.9%) participants had died, 732 (2.2%) of those from CRC. Among those dying from CRC, 200 (27.3%) were in the annual-screening group, 237 (32.4%) in the biennial-screening group and 295 (40.3%) in the control group. When comparing the two screening-groups to the controls, authors observed a relative reduction in CRC mortality of 32% in the annual screening group and of 22% for the biennial screening group. No association between screening and a reduction in all-cause mortality was observed. The authors concluded that the protective effect of CRC screening presented by Mandel et al., appeared to persist over a long period of time, supporting the effect of stool-based screening and polypectomy for managing CRC. (34)

In Nottingham, a pilot study was conducted from 1981-1982 and the main trial from 1985-1991. Potential participants between 50 and 74 years of age were identified through local general practice registers and randomized to either biennial screening (76,466 people) or control (76,384 people). When follow-up ended in

1995, 360 (0.47%) people in the screening group and 420 (0.55%) people in the control group had died from CRC, representing a 15% reduction in CRC mortality in those screened. Out of the 893 cancers detected in the screening group, 400 (44.8%) were found in non-responders. (31) Scholefield et al. later investigated the impact of screening on CRC incidence and mortality after up to 20 years. In the intervention arm, 40,681 (53.5%) had died, 1,176 (2.9%) from verified CRC. In the control arm, 40,550 (53.4%) participants had died, 1,300 (3.2%) of those from CRC. Those in the intervention group had a 13% reduction in CRC mortality, which increased to 18% when adjusted for non-compliance compared to the controls. The authors observed no differences in all-cause mortality. (35)

In Gothenburg, 68,308 inhabitants were randomized 1:1 to either a screening (34,144 people) or a control group (34,164 people). Those in the intervention group were invited to submit two gFOBTs – one at inclusion (prevalence) and one 16-24 months after (rescreening). The study began in 1982 and follow-up ended in 1992. At this time, results showed that among those invited to undergo screening, 63% submitted a gFOBT in the prevalence screening and 60% in the rescreening. A total of 175 (0.51%) subjects were diagnosed with CRC in the intervention group, 43 of those were gFOBT non-responders. In the control group, 191 (0.56%) subjects were diagnosed with CRC. In terms of diagnostic output, the Swedish trial thereby appear similar to the other trials, but unfortunately no mortality estimates were provided for further comparisons. (36)

In the Danish HM-II trial on the island of Funen, public registers were used to identify all 137,485 eligible participants in the area (after exclusion criteria were introduced). From this population, 61,933 people were randomly divided equally into an intervention and a control group. The intervention group were invited to participate in biennial CRC screening. The control group was not informed about the trial and used health care facilities as normal. After five rounds of screening, results showed that participants of the intervention and control group had the same number of detected cancers, 481 and 483 respectively. The number of CRC-related deaths in the intervention group, however, was only 205 compared to the 249 deaths in the control group. Authors conclude that biennial CRC screening reduces CRC morality by 18%. (32) The HM-II trial was the only one to complete nine consecutive rounds of screening with no interruptions. In their concluding study, authors included findings from all nine rounds and compared participants in the screening group to the control group. Follow-up was limited to end-of-study in august 2002. At this time, 12,205 (39.41%) of participants in the intervention group and 12,250 (39.55%) of participants in the control group had died, whereof 362 and 431 died from CRC respectively. Authors conclude that, with a 67% first round compliance, CRC screening leads to an 11% reduction in CRC mortality, which is lower than the 18% reported above, but proceeds to discuss the impact of faltering participation rate in the last four rounds as a likely explanation. Authors proceeds to conclude that their results support the introduction of CRC screening in Denmark. (37)

In the same period, researchers also investigated the use of sigmoidoscopy as a screening modality. The Norwegian Colorectal Cancer Prevention (NORCCAP) trial invited a random sample of 20,780 people from

the City of Oslo and the Telemark County to one of two screening interventions, either sigmoidoscopy-only or a combination of stool testing (using the immunochemical FlexSure® test) and sigmoidoscopy. The results show that sigmoidoscopy-based screening, for both designs, were feasible with a 65% participation rate, a 0.3% CRC detection rate and a 17% adenoma detection rate. In a 17-year follow-up study, authors report a 34% reduction in incidence and a 37% reduction in mortality among men, but no reduction among women. Authors discuss that this may be due to women having different CRC risk profiles or fewer adenoma findings at sigmoidoscopy than men. (38) Another potential explanation is that women may have a higher proportion of tumours located in the proximal bowel – an area that is not visible during sigmoidoscopy. (39-41) This could lead to more missed cancers among women, thereby limiting the effects of the screening initiative. Around the same time as the NORCCAP trial, the UK Flexible Sigmoidoscopy Screening Trial was completed. Here, a large randomized group of participants were offered once-only sigmoidoscopy screening. In the intervention arm, 40,621 (71%) participants underwent the procedure. After 17 years of follow-up, Atkin et al. showed a reduction in CRC incidence of 26% and mortality of 30% compared to the control group (unscreened). After adjusting for compliance, the reduction in CRC incidence was 35% and the reduction in CRC mortality was 41% for those screening. (42)

After the results of the gFOBT trials were presented in the 1990s and early 2000s, guidelines and recommendations for CRC screening began appearing, paving the way for future work. (43, 44) A Cochrane review was published in 2000 comparing available literature on whether stool-based CRC screening reduce CRC mortality. The authors concluded that across the included studies, a 16% reduction in CRC mortality was observed for those undergoing screening, which increased to a 23% reduction when adjusting for compliance. This corresponds to 8.5 CRC deaths prevented per 10,000 people over a 10-year period. Authors also flags the added risk of colonoscopy complications arising from the increasing number of investigations that influences the overall ratio between benefits and harms. Despite this, authors conclude that the benefits of screening appear to outweigh the harms. (45) From this consensus, the concept of CRC screening was adopted by researchers and became subject to a large quantity of future studies. (25) Many countries began adapting, testing and evaluating the concept of CRC screening. One example is colonoscopy-based screening. In the early 1990s results from the American National Polyp Study showed a reduction in CRC incidence after colonoscopic polypectomy. (46) In the US, this ultimately led to colonoscopy, without any pre-procedure risk assessment of participants, being approved for routine clinical use as a primary screening modality to be conducted in 10-year intervals. (47) Another such process took place in the UK, where a pilot study was launched in 2000, offering gFOBT-based screening to a total of 486,355 people. Overall, researchers were able to reproduce the findings of the Nottingham trial on important parameters such as participation, complication and detection rates. They conclude that population-based gFOBT screening is feasible and should be able to provide reductions in CRC mortality matching those of different trial populations. (48)

COLORECTAL CANCER SCREENING TODAY – METHODS AND STRATEGIES

The concept of CRC screening resonated with policymakers in the European Union. This lead a series of recommendations in 2003 and in 2008 on the implementation of CRC screening programs. (49) A large-scale report was published in 2017 showing that the process presented above from the UK are just one in many countries conducting similar both national and regional activities through the 2000s and early 2010s often resulting in the implementation of a CRC screening program. One conclusion of the report, however, was that the strategy and methods of implemented screening programs varied significantly between countries and regions. (50) On a strategic level, programs can utilize either an organized or an opportunistic approach. The International Agency for Research on Cancer (IARC) defines an organized screening program as "*an explicit policy with specified age categories, method, and interval for screening; a defined target population; a management team responsible for implementation; a health-care team for decisions and care; a quality assurance structure; and a method for identifying cancer occurrence in the target population"*. (51) In contrast, opportunistic screening refers to a process where the examination is requested by a patient or offered by a health care professional during usually unrelated care – often by a general practitioner. (51) Overall, the organized program uses a population-based centralized approach and is considered to be the most effective and consistent strategy of the two for achieving the desired reductions in CRC mortality and incidence. Because all members of an eligible population is invited, the organized approach is also better designed for addressing disparities. The opportunistic approach was common in the early years of screening, but over time the organized approach has become the dominating strategy for both new and existing programs. (47)

In terms of methods, most screening programs today utilize a stool-based test to identify participants with a higher than average risk of CRC. Different types of tests with different properties are available. The gFOBT detects the pseudo-peroxidase activity of heme, either in free form or as intact haemoglobin, which catalyse the transfer of oxygen from hydrogen-peroxide to aminophenzaone. When aminophenazone is oxidized, i.e. if sufficient traces of haemoglobin is detected, a blue colour is produced and the test is interpreted as positive. This process does have some weaknesses that increases the risk of creating a false-positive test result, where no discernible cause of the positive gFOBT can be identified during follow-up investigation. A false-positive gFOBT result may have several causes and derived definitions. (52) One of the ways a false-positive result can occur is if the collected faeces contains peroxidase-like substances, which can alter the produced colour and affect the interpretation. These substances be found in different kinds of food such as radishes, cabbage, cucumbers, melons and horseradish and may cause a positive gFOBT results even though no blood is detected. A similar scenario is caused by excess consumption of red meat around the time of stool collection, which may trigger a false-positive gFOBT result as the test is unable to differentiate between human and animal haemoglobin. In extension, the test may become false-positive if a bleeding occurs in other parts of the gastrointestinal tract (GI). (53) Finally, occult bleeding in the colon or rectum detected by gFOBT without the

presence of any polyps or cancers is also considered a false-positive result. A false-positive gFOBT result may therefore have several explanations and for several of them it is debatable whether the gFOBT is actually false-positive. This could be the case when considering a positive gFOBT with no neoplastic findings and detection of another obvious source (i.e. vascular malformations) of the bleeding. In this scenario, the gFOBT is only considered false-positive because it is applied in a screening-setting, where the only target of interest is polyps/cancer. Had it been applied as a diagnostic modality for i.e. vascular malformations, the result would be true-positive instead. Therefore, it is debatable whether it would be more appropriate to divide the term "false-positive" by what causes the results in the future. This is however beyond the scope of this thesis and we will use the term "false-positive" as a joint term for all causes.

To mitigate the risk of a false-positive result, the gFOBT had a number of dietary restrictions as interfering factors could affect the result. One example are the participants in the Funen trial, who were asked to avoid red meat, fresh fruit, iron preparations, vitamin C, aspirin and other NSAIDs up to three days before taking the sample. (32)

The approach requires participants to submit a total of six faecal samples – two from each of three consecutive stools – on a test card (Figure 1). (52)

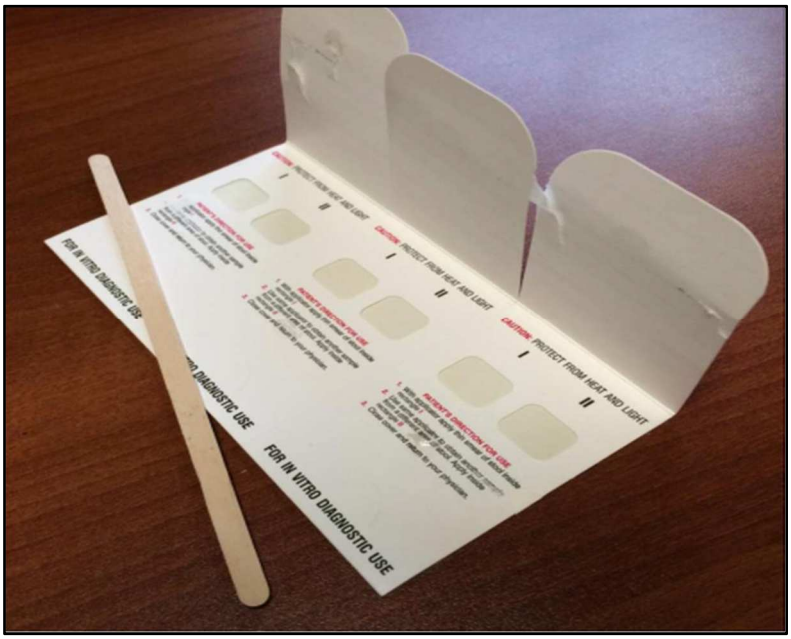

Figure 1. gFOBT test card with six windows for placing faecal samples from three consecutive stools (52)

This so-called "dry" approach is characterized by a good clinical specificity, but a low analytical (for lower f-Hb levels) and clinical sensitivity (high-false negative rates for CRC detection). (54) The stool samples can be rehydrated, which may improve sensitivity (false-negative rate) and reduces specificity (false-positive rate). In the Minnesota trial both dry (17%) and rehydrated (83%) samples were used. In the Funen trial only dry samples were used. (32, 33) The extensive nature of the test preparation requirements and the impractical need for three consecutive stools during collection of test material, was a challenge for uptake among those invited. This limited the clinical applicability of the gFOBT. Another issue was a mediocre test performance that was hindered by a high false-negative rate, translating into a high number of interval cancers (cancers detected after a completed round of screening but before the next). While modifications have been made to the test over the years, such

as the introduction of a high sensitivity gFOBT with greater analytical sensitivity, the applicability remains challenged. (52)

The use of the gFOBT has declined severely and most screening programs today have switched or are switching to a FIT-based approach. The test uses antibodies to detect human haemoglobin and produce a quantitative result for interpretation. The FIT is a one-sample test without any dietary restrictions that is easier to use than the gFOBT. The quantitative FIT uses immunoturbidimetric methods to assess the concentration of haemoglobin, typically using a "wet" approach where the stool is collected using a device that contains a buffer. For both methods, there are some factors that can cause performance variations between brands such as globin degrade in absence of stabilizers, the choice and capacity of a chosen buffer, analytical technique and collection-device reliability. (55, 56) Because of the quantitative nature of the test result, a positivity-threshold can be established – a useful tool in CRC screening. In Denmark, the CRC screening program uses the OC-Sensor (Eiken Chemical, Japan) test and considers all results above 100ng haemoglobin/mL buffer (corresponding to 20µg haemoglobin/g faeces) as positive. It should be noted that some FIT brands report a qualitative result similar to the gFOBT based on a manufacturer-decided threshold that varies from company to company. (52)

Figure 2. Faecal Immunochemical Test (FIT) collection kit

A study that compared gFOBT with FIT found that both participation and detection rates were significantly higher in those randomized to the FIT-group. (57) One meta-analysis assessing findings from 19 different studies reported a pooled sensitivity for CRC detection of 79% and a pooled specificity of 94%. Authors conclude that FIT is moderately sensitive, has a high specificity, and has a high overall diagnostic performance that all varies by positivity threshold. (58) When comparing this to the gFOBT performance, the FIT provides a relative increase in sensitivity of 31.7%-61.5%. (52) Because test performance is affected by the positivity threshold, modifying it to meet the local needs of different screening programs is possible. In general, lowering a threshold results in a higher sensitivity for CRC detection but more positive tests and thereby more colonoscopies. Increasing the threshold on the other hand, lowers the number of positive tests and reduces the number of colonoscopies, but lowers both specificity and sensitivity resulting in more missed cancers. (52) Moreover, the associated cost-effectiveness of screening is considerably affected by such

changes – an important consideration for the long-term survival of a screening program. Economic/political factors, clinical problems, colonoscopy capacity and the number of missed cancers (and associated deaths) is often considered when discussing a positivity threshold. One said process has occurred in the Netherlands, where an organized screening program was implemented in 2014 with a positivity threshold of 88 ng/mL using the FOB-Gold test (Sentinel CH. SpA, Milano, Italy) corresponding to OC-Sensor value 75ng/mL. The program was expected to have a 60% participation to FIT and a subsequent referral rate (positive FIT) of 6.4%. Based on these numbers, 79% of the screening colonoscopy capacity were expected to be used. One year into the program, an evaluation was conducted showing a referral rate of 13.1% - more than double the expected number. As a direct consequence, the screening program used 173% of the capacity allocated, resulting in long waiting times and/or reduction in capacity from other indications. A commissioned report recommended a rapid adjustment of the program, where positivity threshold should be raised from 88ng/mL to 275 ng/mL and accompanied by a full deployment of additional colonoscopy capacity. This later implemented solution, was expected to reduce the colonoscopy referral rate to 7.9% and subsequently the number of annually detected cancers and advanced adenomas from 133 to 117 and 404 to 290 respectively (using year 1 numbers for illustration). (59)

Another new method, which in recent years has become increasingly popular in the US, is multitarget stool DNA (mtsDNA) testing. Here, both methylated DNA markers and haemoglobin is detected and the results combined into one interpretable figure for determining positivity. One study has demonstrated a 92% CRC sensitivity and a 42% sensitivity for advanced adenomas for the mtsDNA test. In comparison, authors report a FIT sensitivity of 74% for CRC and of 42% for advanced adenomas. For specificity the FIT was superior to the mtsDNA (95% vs. 87%). (60) The Cologuard test (Exact Sciences, Wisconsin, USA) was approved in the US in 2014 for routine clinical use. Experiences hint at some barriers to implementation. For one, the mtsDNA costs 600$ per test, which is approximately 24 times the cost of a FIT (25$). Also, both the stool collection process and the subsequent lab analysis is more complex. Here, it has been suggested that 6% of participants failed to collect or send an adequate mtsDNA sample compared to 0.6% for the FIT. (47) While mtsDNA may be a promising tool for CRC screening, it has some barriers for implementation and is currently only approved for use in the US.

An alternative to stool-based screening is the use of either colonoscopy or flexible sigmoidoscopy without any preliminary testing. Supporting studies have reported a reduction in CRC mortality of 29%-68% in those who undergo the procedure - a better effect than those reported in stool-based studies. However, achieving these benefits on a population level requires a high level of participation. (47) A study compared participation rates between the stool-based approach and the colonoscopy-only approach, and found that only 38% of those invited for colonoscopy completed the screening process compared to 67% in the stool-based group. (61) During the procedure, all identified polyps are removed requiring more polypectomies and thereby a greater

risk of complications. Related disadvantages includes the extensive bowel preparation regimen, associated costs and a significant burden on existing endoscopy capacity. Flexible sigmoidoscopy as the primary screening modality is an alternative to the colonoscopy approach. The disadvantages and barriers of the procedure mimics those of colonoscopy, with the added issue of requiring follow-up colonoscopy for any polyp findings. (47) Several large-scale trials have been conducted, reporting a reduction in CRC mortality of 22%-31% and in CRC incidence of 18%-23% among participants undergoing screening. (62-65) Recently, Norwegian researchers presented findings from a large-scale trial comparing the effectiveness of FIT-based screening and sigmoidoscopy-based screening. Participants were randomly invited from 2012 to 2019 for either once-only sigmoidoscopy or biennial FIT screening. Outcome measures for the FIT group were reported by screening round. 139,291 individuals distributed equally between the groups were invited. Results showed a 52% sigmoidoscopy, a 58% round 1 FIT, and a 68% cumulative FIT participation rate. CRC detection rate was comparable for sigmoidoscopy screening (0.27%) and FIT screening after considering only the first round (0.25%). When including all 3 rounds of screening, the FIT based screening had a 0.49% CRC detection rate. For adenomas, once-only sigmoidoscopy (2.4%) had a higher detection rate than the first round of FIT (1.4%), but not cumulative FIT screening (2.7%). Also, no significant difference was observed in adverse events. (66) Overall, the findings by Randall et al. succeeds in highlighting flexible sigmoidoscopy as a feasible screening tool.

Colon capsule endoscopy (CCE) is a newer visualization tool that may be used for screening in the future. During the procedure, the patient orally ingest a small camera capsule and the entire GI system is recorded on video. This is followed by a diagnostic workup by a trained reader who reviews the video and highlights any significant findings. Its minimally invasive nature and ability to be performed at out-hospital clinics, combined with a very low complication rate and better patient satisfaction, makes it a potential alternative to colonoscopy. (67) In terms of clinical performance, CCE has been reported to have a sensitivity (85%) and a specificity (85%) for polyp detection (any size) that is comparable to colonoscopy. However, CCE still has some limitations (including costs and number of incomplete investigations) that creates a barrier for broader implementation. (68) Currently, the Danish CareForColon 2.015 trial, where screening participants are randomized to either a modified CCE-based screening course or to regular screening, are including patients and is expected to provide more in-depth knowledge about the use of CCE in CRC screening. (69)

Although the direct visualization approaches have some advantages, the range of downsides makes it less attractive than FIT-based screening. Consequently, many countries conducting CRC screening utilize or are switching to the FIT-based strategy. Overall, many screening programs appear successful in managing increasing mortality and incidence rates. However, the impact of CRC screening vary significantly between countries, heavily influenced by factors such as uptake, coverage and approach. (70)

COLORECTAL CANCER SCREENING IN DENMARK

In Denmark, a 1-year feasibility study was conducted from 2005 to 2006 in two counties inviting 176,782 residents to a single-round of gFOBT-based CRC screening. The conclusion was that organized CRC screening was doable in Denmark with reasonable waiting times and acceptable detection-and complication rates. (71, 72) The Danish Health Authority subsequently commissioned a Medical Technology Assessment (MTA) on CRC screening with a special focus on the consequences of low participation rates. In the MTA, available literature, feasibility study findings and clinical data were evaluated. (73) The following conclusions were presented:

1. Participation rates appear to decline over time and few mitigating efforts had been tested at the time.
2. The proportion of participants with cancer or adenomas does not appear to differ by participation rate.
3. The risk of complications during colonoscopy are very low.
4. A lack of knowledge about CRC and screening in general could explain low participation rates in the feasibility study.
5. Cost-efficiency is similar to those of cervical and breast cancer screening.

In addition, the MTA stated that alternatives to the gFOBT could drastically affect screening performance. (73) A set of recommendations was published by the Danish Health Authority in 2010 and 2012 in extension of the MTA, recommending the implementation of a FIT-based biennial CRC screening program for all Danish residents. (74)

On March 3rd 2014, an organized CRC screening program was implemented in Denmark, with a gradual roll-out over 46 months to ensure sufficient colonoscopy capacity. During the roll-out period all Danish residents between 50 and 74 were invited to submit a FIT once. Invitations are, after completion of the roll-out, sent out biennially and contains an invitation letter alongside an information pamphlet and the stool collection kit. A positive FIT is followed by a referral to colonoscopy. After a complete investigation, participants are allocated to different follow-up protocols based on their outcome. The possibilities are; CRC, high-risk polyps, medium-risk polyps, low-risk polyps and clean colon (negative colonoscopy). When CRC is detected, the patient immediately proceeds to further diagnostic and therapeutic procedures. In the case of high-medium risk adenomas, participants initially undergo the appropriate therapeutic intervention to remove the polyp, followed by either a 1 year or 3 year follow-up colonoscopy before returning to screening. In the case of low-risk polyps, no follow-up will be conducted and participants will be reinvited in the next screening round. Those with a negative colonoscopy will receive an 8-year quarantine from the screening program before being reinvited. (75, 76) The screening program is monitored using registrations from the Danish Colorectal Cancer Screening Database (DCCSD) that collects and stores data on all participants from a number of different sources. One is the Invitation & Administration Module (IAM) that contains all participation-related data incl. FIT results.

Another is the Danish National Patients Register (NPR) that contain data on all the procedures conducted in hospitals and their related diagnoses. Third is the Danish National Pathology Register, which contains the pathological conclusions of all removed cancers and polyps. (77)

After the first three years, the FIT participation rate was 62.6%, close to the desired level of 65% and significantly higher than the acceptable level of 45%. Among all analysed FIT tests, 6.9% were positive and 89.1% of these participants complied with the subsequent colonoscopy. Both of these were considered acceptable. In terms of clinical performance, the CRC detection rate among all FIT-tested participants was 3.5‰, representing 1,250 and 1,933 CRCs women and men respectively, which was within the expected range. In addition, 9,399 and 14,973 adenomas were detected in women and men respectively, with a total adenoma detection rate among those tested of 27.1‰ – significantly higher than the expected 13.3‰-22.3‰. The authors state that all performance indicators were above the acceptable level and close to the desirable level given by European guidelines. This leads them to conclude that the implementation of the Danish CRC screening program was a success. (75)

As a result, CRC screening in Denmark appear to be able to achieve a reduction in both CRC mortality and incidence through detection of medium-and high-risk adenomas and cancers. In the first round of screening, these significant findings were detected in 37.3% of screening colonoscopies. Another 18.6% of investigations revealed low-risk polyps and 9.77% had an unclear conclusion. Of interest to us is the 33.7% of FIT positive participants that has a negative colonoscopy, i.e. a colonoscopy with no discernible cause of the bleeding that caused the FIT to become (false)-positive. (78)

GASTROINTESTINAL BLEEDING

Bleedings can occur in the entire length of the GI tract from a number of different sources and they range from discrete occult bleedings to acute haemorrhages. They can be categorized by location as either upper (proximal to the ligament of Treitz) or lower (distal to the ligament of Treitz) GI bleeding.

Upper gastrointestinal bleeding

In the upper GI tract, the most common causes are peptic ulcer, erosive gastritis/esophagitis, ruptures of varices caused by portal hypertension and malignancies. Risk factors of upper GI bleeding include age, lifestyle, portal hypertension and certain medications. One noteworthy mechanism is the disruption of the gastric mucus production by cyclooxygenase (COX) inhibitors, which comprises nonsteroidal anti-inflammatory drug (NSAIDs) such as aspirin. COX enzymes are a part of the prostaglandin production, which are lipid compounds involved in inflammatory responses (COX-2) or vasodilation (COX-1). When inhibiting the prostaglandins, the submucosal flow of blood is decreased thereby lowering the mucus production leaving the mucosa vulnerable to injury that can cause bleedings. (79)

Lower gastrointestinal bleedings

Lower GI bleedings annually occurs in 20-30 per 100.000 adults (increasing 200-fold from age 20 to age 80) and often presents with another, and less acute, profile than the upper bleedings. Lower GI bleedings typically divides into three activity types; occult, slow, and rapid. The occult bleeding is very slow and often not visible. It is rarely associated with other symptoms and can have a long duration. If the occult bleeding becomes chronic it may lead to a substantial loss of iron that may lead to iron-deficiency anaemia. The slow bleeding can present with its own lesion or build on an existing chronic occult bleeding, and can lead to hemodynamic instability that may be dangerous to some, often fragile, patients. The rapid bleedings are a blood loss of 100 ml/hour that, if untreated, may lead to hypovolemic shock. (80)

The source of lower GI bleedings are mostly found in the colon. One is diverticulosis coli, a condition that becomes more common with age – 75% of people above age 75 are affected. The condition can result in bleedings from ruptured vasa recta or an eroded vessel in the diverticulum. The bleeding occurs in about 10-15% of patients, is often asymptomatic, may continue for some days and usually stops spontaneously. Diverticula can become inflamed and cause diverticulitis. Diverticular bleeding is suggested to be the most common cause of acute lower GI bleeding. (81, 82) Haemorrhoids are also known to cause bleeding, mostly intermittently in smaller amounts, but severe haemorrhage do occasionally happen. The condition is very common, with a population prevalence of 4.4%-12.8%. (82) Other non-neoplastic colonic sources include inflammatory bowel disease, angiodysplasia, vascular malformations, colonic ischemia and radiation colitis. Some bleedings (5-10%) originate from the small bowel, where common causes are inflammatory bowel

disease, diverticula, vasculitis, aortoenteric fistulas and endometriosis. Some lower GI bleedings are in fact upper GI bleedings, where the blood is passed to the lower GI tract (10-15%). (80, 82)

Colorectal cancer

The last frequent source is cancer or polyps (precursor lesions). Most of the tumours arise from one of two pre-cancerous polyps subtypes; either the sessile serrated lesions or the adenomatous polyps (adenomas). Each of the subtypes follow a different neoplastic pathway. The most common is the adenoma-carcinoma pathway (60-70% of all CRCs) initiated by mutations in the *APC* genes, which causes a slow transformation from benign polyp to carcinoma. The serrated neoplasia pathway is still not fully understood, but believed to occur predominantly via *BRAF* and the CpG-island methylator phenotype. An additional pathway, the MSI pathway, has also been suggested. (83, 84) During the transformation, minor bleedings occur in the early phases from the fragile blood vessels on the surface of the tumour. As the tumour invades surrounding tissue, it may grow into nearby blood vessels and cause additional bleedings. In these phases, bleedings are often occult and only detectable through testing. Because almost all CRCs arise from polyps that progress slowly, there is an opportunity to detect the bleeding by a stool sample and subsequently remove any polyps before a malignant transformation can occur. (85)

FAECAL HAEMOGLOBIN – AN INDICATOR FOR NON-COMMUNICABLE DISEASE?

The phenomenon of a false-positive FIT can have several explanations. One could be other GI diseases causes bleeding thereby turning the FIT positive. Some of the bleeding may come from the upper GI tract. However, since the FIT reacts to the haemoglobin proteins and since the proteins from upper GI sources are normally digested from proteins to amino acid before reaching the colon, the immunochemical response of the FIT should not normally detect it. However, it has been suggested that the FIT may be able to detect upper GI malignancies, indicating that there could be situations where the upper GI bleeding is rapid enough to exceed the capacity of what can be digested, allowing for non-deteriorated haemoglobin to be found in the lower GI tract. (86) However, due to both the rarity of these malignancies and the often acute nature of many rapid upper GI bleedings in general, we do not believe that they can explain our false-positive FIT rate.

Diseases in the lower GI tract such as diverticulosis and haemorrhoids may also explain some of the false-positive FITs, as they may cause bleeding. The association between FIT result and GI diseases have been investigated in several studies. Here, authors find no association between false-positive FIT result and neither peptic ulcers, diverticulosis nor haemorrhoids, reducing the likelihood of these GI conditions being able to explain the high rate of negative colonoscopies after positive FIT. Worth noting is the relationship between FIT and both inflammatory bowel disease and other non-neoplastic findings (including anal fissures). Here, both categories of conditions were found to increase the risk of a positive FIT. (87, 88) The use of NSAIDs and oral anticoagulants incl. aspirin, are another suggested reason for a false-positive FIT. Here, it has been

proposed that NSAID compounds enables the immunochemical reaction by stimulating benign lesions and making them bleed. However, one meta-analysis has concluded that FIT accuracy is not affected by the use of either medications, demoting this as a separate explanation. (89) These conclusions have been questioned by another, more recent, meta-analysis that showed a reduced Positive Predictive Value (PPV), which means that the probability of a patient with a positive test actually having the disease in question, for advanced colorectal neoplasia of the FIT. The study showed an 18% lower PPV among FIT positive participants taking either aspirin or antiplatelet agents. Results also showed a 34% lower PPV among FIT positives take oral anti-coagulants. (90) While consensus on the topic is not achieved, it seems possible that some types of medication may have some effect on a FIT result. However, while both the GI conditions and medications may influence a FIT result, we believe it unlikely that these alone can explain the many negative colonoscopies without any signs of colorectal neoplasia.

The quality of the colonoscopy may affect the adenoma detection rate, which could also explain some of the negative colonoscopies mentioned above. Here, factors such as bowel preparation quality, withdrawal time and caecal intubation rate are all considered important factors for obtaining a detailed mucosal evaluation and thereby better procedural quality. These, together with adenoma-detection rate, have all been linked to post-colonoscopy CRC (interval cancer). Common for these intra-procedural factors are the dependency on the endoscopists performance, which has been found to vary significantly between endoscopers. (91, 92) Also, pre- and post-procedural factors such as local endoscopy guidelines, documentation standards, educational standards, availability of supporting technology, reporting tools and surveillance protocols all impact the quality of the endoscopy and how the procedure is evaluated. (92) In relation to the negative colonoscopies, it is possible that variations in endoscoper performance will influence the adenoma-detection rate. This in turn could mean that some of the negative colonoscopies were in fact false-negative because of missed cancers or adenomas. Screening colonoscopies in Denmark is considered a specialized task that is normally conducted by experienced endoscopers, which should, to some degree, remedy this problem. (74) Despite this, we do not believe that endoscoper performance variations alone can explain the high false-positive FIT rate.

Overall, all of the mechanisms presented above may influence the risk of having a false-positive FIT and may explain some of the negative colonoscopies. However, we still find it unlikely that even a combined effect of these factors can result in false-positive FIT rate of 33.7%.

An alternative explanation, which is attracting increased scientific attention, is that the presence of detectable f-Hb without discernible neoplasia may indicate the presence of underlying chronic and/or non-communicable diseases not otherwise known to cause GI bleeding. Some studies have been published on the topic, but with differing outcomes, approach, size and quality. Some use cause of death as an indicator for the presence of underlying conditions. (93) In one study, all NHS Tayside participants from both the Scottish arm of the UK CRC screening pilot and the Scottish screening program were followed from submission of the gFOBT till

death or end of study. Among the 133,921 participants, 2,714 (2.03%) were gFOBT positive. The study revealed significant differences between the gFOBT positive and negative groups in terms of mortality. Those with a positive gFOBT had a 1.76 times higher all-cause mortality, which reduced to 1.58 when excluding CRC deaths. Authors also found that gFOBT positive participants were more likely to die from seemingly unrelated causes, including circulatory disease, respiratory disease, digestive disease, neuropsychological conditions, haematological-and endocrine diseases, and non-colorectal cancers. The risk of dying from CRC in participants with a positive gFOBT were almost eightfold compared to the negative participants. The associations persisted after authors adjusted for age, sex, social deprivation and prescription medication. Authors conclude that a 2-3 fold increased risk of dying from non-CRC conditions after a positive test is significant, but that more studies are needed to explore the association. The study were to some degree limited by the use of the gFOBT and the lack of more, individual-level confounder-adjustment. (94) A Taiwanese study proceeded to show an association between increasing f-Hb levels measured by FIT and increasing risks of developing and dying from cardiovascular disease (CVD). (95) In a similar study from South Korea, authors found an increased risk of ischemic stroke, myocardial infarction and all-cause mortality in those with a positive FIT compared to the negative. (96) Two studies have also been published that show an association between increasing f-Hb levels and an elevated risk of having diabetes mellitus. (97, 98) Also, Libby et al. mapped the use of prescription medication of participants in the aforementioned Scottish population and considered these as proxy-indicators for diseases. When comparing participants, results show that gFOBT positive participants were more likely to have a prescription for cardiovascular disease, depression and diabetes. (99)

In a recent review, authors propose that the evidence surrounding elevated f-Hb and its association with both all-cause mortality, cause-specific mortality and several non-communicable conditions is becoming increasingly convincing. Authors hypothesize that this association is caused by conditions having a systemic inflammatory component, which may present as a subclinical colonic inflammation that can lead to an occult bleeding producing detectable levels of f-Hb. Some studies have been published in support of this, showing an association between f-Hb and several inflammatory diseases such as psoriasis and rheumatoid arthritis. (100, 101) Dysbiosis of the gut microbiota, which may lead to a loss of intestinal mucosal integrity, has been suggested as a possible cause of the inflammation. (102) This inflammation and changes to the microbiota has also been linked to colorectal tumorigenesis. (103) The presence of f-Hb with an absence of disease appears to have a major influence on the composition of the microbiota. (104)

Moreover, the dysbiotic state has been linked to many chronic non-communicable diseases such as CVD, neurological disorders, respiratory diseases and metabolic illnesses. (105) In turn, the composition of the gut microbiota appears to be modified by many of the same lifestyle factors known to influence the risk of CRC. Many of these are components of western lifestyle and include an unbalanced diet, sedentary behaviour, lack

of sleep, intake of drugs and elevated stress-levels (Figure 3). (106) This complex, multifactorial relationship between the microbiota and health outcomes needs further elucidation, but it appears likely that inflammation is a revolving factor.

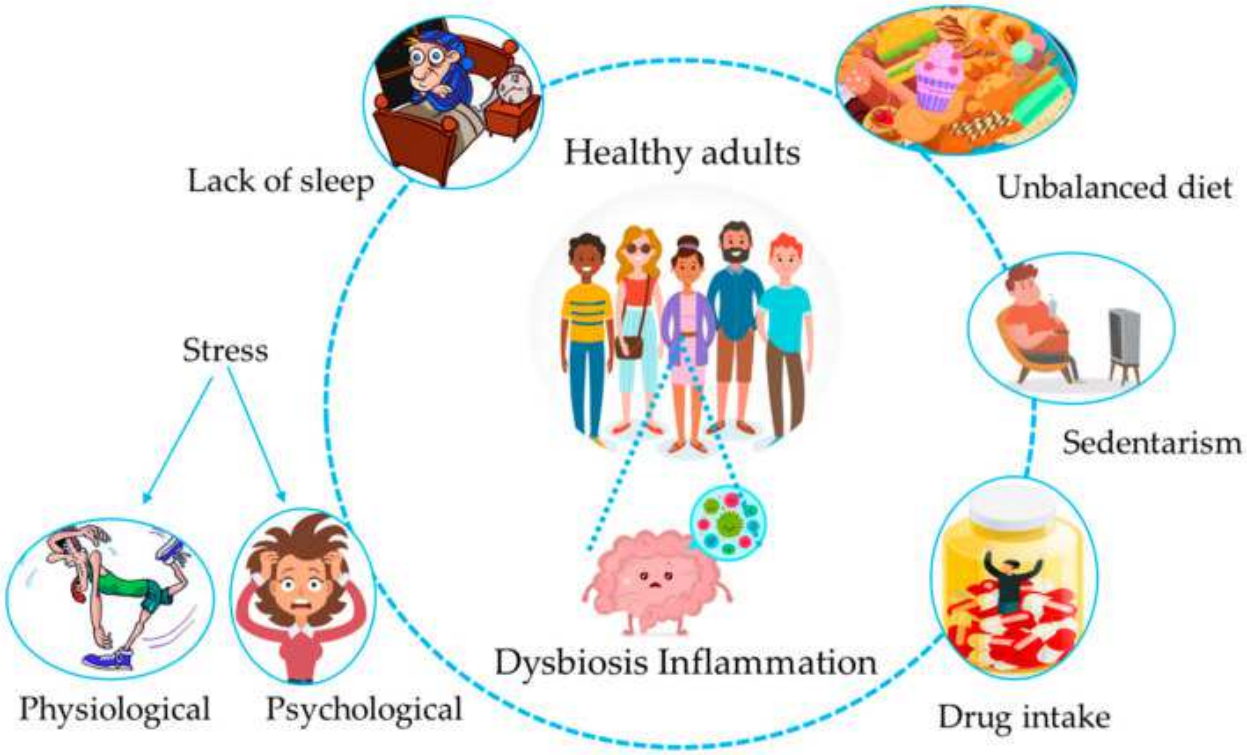

Figure 3. Lifestyle habits and the gut microbiota (106)

Moreover, we know that inflammation is associated with initiation, progression and treatment outcomes of CRC (Figure 4). Of special interest to this project is the inflammation-associated tumorigeneses that initiates tumour development. Here, chronic inflammation from different sources may initiate and promote tumorigenesis by inducing epigenetic changes or DNA damage. (84) Knowing this, and assuming the abovementioned hypothesis to be true, it is possible that the presence of a colonic inflammation caused by conditions elsewhere in the body can lead to an increased rate of polyp development by causing an inflammation-associated tumorigenesis. This could help explain why some polyps does not bleed when viewed endoscopically. (93)

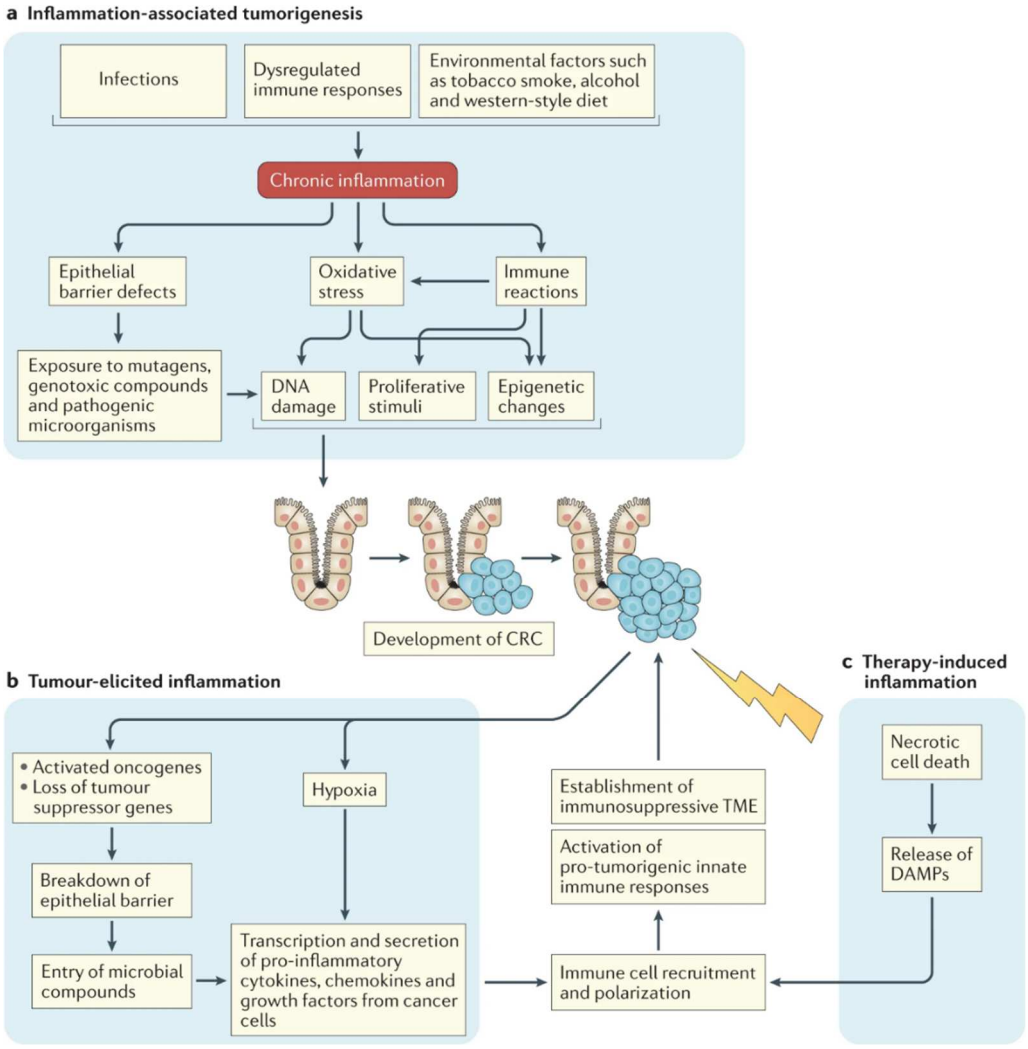

Figure 4. The Inflammatory Environment of Colorectal Cancer (84)

Diabetes may be one such condition, where inflammation has been found to be have a significant role in the pathophysiology and where the risk of colorectal adenomas is approximately 50% higher than among non-diabetics. (107, 108) Although these are two separate observations, they indirectly support the notion that an inflammatory state and colorectal polyps could be linked in the future.

We know that some polyps do not bleed and that these polyps produce no clinical symptoms and should therefore not be identified by gFOBT/FIT. (109) However, few studies have investigated the prevalence of polyps in people with no traces of occult blood, potentially biasing the perception on which polyps actually bleed. One of those studies found that polyps detected in gFOBT negative patients had a smaller surface area and were more often sessile serrated lesions. (109) Combining this, the moderate sensitivity of the FIT and the many false-positive findings in screening colonoscopies, leads to speculations about how many of the detected

polyps are de facto incidental findings that cannot explain the bleeding. Then, consider the 18.6% of colonoscopies in the Danish CRC screening program that reveals only small, low-risk polyps. It is possible that the polyps are incidental findings and that other causes has led to an occult bleeding that in turn results in a positive FIT. Consequently, a better and more nuanced understanding of the mechanisms behind colorectal bleedings may redefine how, who and why we screen people in the future.

In extension, it has been suggested that a FIT result could be used as a future biomarker for systematic inflammation and therefore many chronic non-communicable conditions, that is cheap, effective and reliable. (93) Before this is realistically possible, more research needs to be undertaken. For now, the published studies are sufficient for supporting the notion of an association between f-Hb and non-CRC diseases. However, if f-Hb is to transcend into a biomarker for non-CRC conditions, there is a need for studies that addresses the limitations of the previous studies, cements the presented associations and outlines the predictive value.

AIM

The overall aim of this dissertation was to investigate the association between f-Hb and mortality outcomes in different populations of CRC screening participants using a register-based approach. Specifically, we wanted to investigate:

1. The long-term effect of screening on both all-cause and CRC mortality in a pooled study of two screening trials.
2. The association between f-Hb and cause of death in participants of the gFOBT-based HM-II trial population with >30 years of follow-up.
3. The association between incrementally increasing f-Hb levels and mortality outcomes in a current FIT-tested Danish screening population.

NATIONAL REGISTERS AND THE REGISTER-BASED APPROACH

All studies in this dissertation are register-based and uses data from a number of Danish national registers to enrich data from either the HM-II trial or the DCCSD, both presented above. The national registers are presented below.

The Civil Registration System (CRS)

The CRS is an important tool for epidemiological research that contains nationwide administrative information from 1968 and onwards. In the register, all residents are assigned a unique 10-digit identifier, the Civil Personal Register (CPR)-number, that is used across all Danish registries. The CPR-number can therefore be used to link data from many different sources of data. The CRS also monitors and updates migration and vital status that allows for long-term follow-up with accurate censoring. (110)

The National Patients Registry (NPR)

The NPR is one of the oldest nationwide hospital registers in the world. It was initialized in 1977 and collects data from all Danish hospitals. The registry contains several types of data. One is administrative data, such as CPR-number and area of residence. Another is admission data including hospital codes, type of admission (acute or non-acute), patient contact, referrals, and dates of admission and discharge. More importantly, it contains all diagnoses related to each hospital contract registered using International Classification of Disease (ICD) codes. ICD-8 coding were used until 1993 after which it was replaced by ICD-10. Diagnoses are registered as primary (the reason for the hospital contact) or secondary (optional supplementary diagnoses). All contacts has a primary diagnosis. It is also possible to register referral diagnosis, temporary diagnosis, complications, and supplementary codes. The register also collects information on all treatments and examinations. The NPR is commonly used in research, where it allows researchers to include the entire population as a sample, thus enabling studies that are otherwise not doable, such as studies on rare diseases or longitudinal studies with very long follow-up. In this case, it is important to remember that the NPR only cover disease episodes associated with contacts to a hospital and therefore not all patients with a given disease. For some conditions, such as CRC, this probably has little effect on study results, but for others it may cause confounding by indication since only those hospitalized will be entered into a given study. It is also important to consider temporal trends in health care, such as changes in diagnostic criteria, classification systems, and diagnostic methods (diagnostic drift), that can affect the incidence of a given disease. In terms of overall validity, the data entered into the NPR is generally considered to be of high quality and completeness making it a valuable tool for epidemiological research. (111, 112)

The Danish Register of Cause of Death (DRCD)

The DRCD can be traced back to the introduction of the death certificate in 1871 and contains information on all deaths among Danish residents. The data in the DCRD has several types of information about the deceased, including CPR-number, time and place of death, manner of death (natural, accident etc.), post-mortem examination (autopsies), surgical interventions, and cause of death. Until 2007, the entries to the DRCD were entered by coders, without medical experience, working the Danish National Board of Health using medical information from the death certificates. The underlying cause of death was chosen by the coders based on their interpretation of the death certificate information. From 2007 onward, the task of submitting a death certificate became electronic and the task of registering cause of death shifted to the medical doctor who verified the death and issued the certificate. The central validation were abolished. Thus, the entries made to the register today relies entirely on the reporting of the responsible physician. The medical foundation of the death certificate has also changed. The autopsy rate has declined sharply from 75% in the 1970s to below 10% today, and since autopsies lead to a change in the underlying cause of death in approximately 30% of cases this somewhat limits the data quality. As a result, the validity of DRCD is debatable. Mediating actions include considering both the underlying and contributing causes of death or using other proxy measurements. (113) In our studies, we have chosen to consider CRC as a cause of death if it is registered as either contributing or underlying.

The Danish National Prescription Registry (DNPR)

The DNPR was established in 1994 and contains information on all prescription medication dispensed at Danish pharmacies on an individual-level basis – an approach that is unique compared to other countries. The DNPR collects data about the drug user (CPR-number, age, gender etc.), dispensing (such as dates, product code & name, dose, Anatomical Therapeutic Chemical (ATC) classification code), prescriber, and pharmacy. Only prescriptions dispensed at outpatient pharmacies are registered. Here, the data from the DNPR are a part of the reimbursement process, thus creating a strong economic incentive for thorough reporting by the pharmacies. As a result, the validity of the register is generally considered to be very high. (114)

The Income Statistics Register (ISR)

The ISR contains a broad range of information on personal income and transfer payments in Denmark from 1970 onwards. The register builds on a variety of smaller, specialized registers. In our work, we have collected data on personal income for all participants. For study 2, we considered personal income as equated per household to better account for the different workforce patterns at the time of inclusion in the 1980s. In study 3, we considered personal income strictly per person. For both studies, we created inflation-adjusted measures. Since the data from the ISR is a direct representation of the real income registered by the Danish Tax Administration, the validity of the data is generally considered very high. (115)

The Population's Education Register (PER)

The PER is one of several national education registers, and collects data on the education history of all Danish residents from 1980 onwards. In our work, we used the PER to obtain the highest completed level of education attained by each individual. The PER also collects data from before 1980, but based on self-reported information. In 2007, a validation showed a complete, non-missing registration of 97% for the ethnic Danish population born after 1945. The validity of the data is overall considered very high, but inconsistencies occur – especially for the very early registrations. (116)

Strengths and weaknesses

The register-based approach has several strengths. One is the availability, which often means that researchers will have easy, cheap and fast access to very large samples, and thereby higher statistical power, enabling studies on rare exposure or outcomes that would otherwise be impossible to do. Another is the high level of registration completeness, improving the representativeness of results and reducing the risk of selection bias and loss to follow-up. A third is the independently collected nature of the data reducing the risk of recall and other influencing biases. A fourth is the element of time, where many registers allow for very long follow-up that can be imperative for diseases with a long latency between exposure and manifestation. Our studies 1 and 2 are good examples of the value of this aspect, as CRC develops slowly. A fifth strength is the ability to adjust for confounding effects on a population-wide scale that are known to affect many different outcomes, such as income and education. Especially studies using Danish registers benefit from this since the CPR-number allows for linkage to a long list of data sources not limited to national registers. (117) The approach also has a number of limitations, several of which intertwine with the strengths. The central issue is that researchers are limited to the variables supplied by the register. One limitation here is that data are pre-collected for non-research purposes potentially resulting in a lack of important information. Another is the lack of confounders, where registers often contain limited and unspecific information, which may lead to inadequate adjustment of confounding effects. Missing data is another limitation, which, despite a high completeness of many registers, may significantly affect results since it often will be unclear why it occurs. This may lead to underreporting of some effects. In our study 2, the missing data in education levels are a good example. Mediating actions include sensitivity analyses or imputation where applicable. (117)

STATISTICS

The survival analysis approach is employed for longitudinal studies because it allows rates to vary over periods of time. This typically becomes relevant when dealing with events where risk varies over time. A typical example is the risk of death after heart surgery. Here, the risk is extremely high immediately after the procedure, then declines as the patient recovers and then increases again as life continues and time passes. (118) The survival analysis covers several tools and has been applied in all three studies, allowing us to consider the effects of screening on mortality outcomes over time. We use the term "person-time" to show how much time each person has contributed to the analysis before being censored because of either death, emigration or end-of-study.

One of the tools was the Kaplan-Meier estimates of the survival curve. This estimate is calculated using the risk sets of individuals each time an event occurs. The product is an estimate of the event probability at a given time. When creating a Kaplan-Meier curve to depict the estimates, the probability will change every time an event occurs. This is also called a "step function". (118) The final product is a curve that allows a graphical interpretation of the temporary of a group of people on the probability of a given event.

Another tool used in all three studies is the (Cox) proportional hazards regression which enables regression analysis of survival data. The model compares the exposed to the unexposed over time and produces a hazard ratio between groups for all given exposure variables. This model can be employed as either a univariate analysis with only one exposure variable or as a multivariate analysis considering multiple exposure variables. (118)

Both the proportional hazards regression model and the Kaplan-Meier estimates assume that the ratio between exposure groups remains constant over time. This is called the "proportional hazards assumption". This assumption is investigated by examining the cumulative hazard against a logarithmic scale of time. This can be done graphically by using a Log-Log plot, which allows interpretation of proportionality over time. (118)

Cuzick's method was employed in study 1. The method is developed for use in clinical trials, and estimates the magnitude of a treatment effect among compliers of a trial. This allows results of a given trial to be presented as compliance-adjusted estimates. This enables a more realistic interpretation of the effect of a given treatment. (119) The method is well-established and used in similar studies. (42, 120)

Also in study 1, we conducted an individual participant data meta-analysis. This approach to a meta-analysis uses data from each individual patient in all included studies. Using this method, it is possible to analyse the effect of a given treatment across trials increasing the statistical power, allowing for the presence of differences in trial designs and allowing for investigation on the influence of covariates. (121)

OUTLINE OF STUDIES

All figures below are replicated from the respective articles. See appendix for full articles.

Study 1. Effects of screening compliance on long-term reductions in all-cause and colorectal cancer mortality

Objective

The aim of this study was to evaluate the long-term reduction in both all-cause and CRC-specific mortality in two large-scale screening trials.

Methods

Data on all participants from the Minnesota Colon Cancer Control Study and the Funen HM-II trial were included. Investigators from both locations provided updated data with 30 years of follow-up. In Denmark, a combination of national registers, including the DRCD, was used to achieve this. In the US, the National Death Index was searched for updated information for follow-up. Both studies are described in detail elsewhere. (33, 122)

From Denmark, all 61,933 trial participants and controls aged 45-75 in 1985 were included. Results from all nine rounds of biennial gFOBT screening conducted between 1982 and 2002 were included. From the US, 46,551 trial participants and controls aged 50-80 years were available, but only participants from the biennial screening arm were included for the analyses due to comparability with the Danish data. This resulted in 15,587 participants in the intervention arm and 15,394 controls. The study ran from 1975 to 1992, with a 4-year hiatus from 1982-1986. Follow-up was completed in 2011. Participants were followed until December 31st 2018.

Kaplan-Meier analyses were used to estimate cumulative mortality for both CRC and all causes. The biennial groups from both trials were compared to the control groups by first intention-to-treat and then complier-restricted. To account for the effects of compliance, Cuzick's method was applied to the mortality estimates. An Individual Participant Data meta-analysis was conducted. This is an approach that takes study-specific effects and difference into account to provide more consistent and reliable results. In our case, this include number of completed screening rounds, age and sex. Pooled Relative Risks (RR) and Risk Differences (RD) were calculated using fixed effect models and I^2 tests were used for evaluate heterogeneity. Studies were evaluated both separately and combined.

Results

In the Funen trial population, 45,009 (72.7%) died before the end of follow-up, 1,637 (2.6%) of those from CRC. Slightly less of these were in the screening group (48.1%). In the Minnesota trial population, 21,948 (70.8%) had died at follow-up after 30 years, 532 (2.4%) of those from CRC. Again fewer CRC deaths occurred in the screening group (44.5%).

In Funen trial, we observed a small reduction in CRC mortality among those in the screening group compared to the control group after 30 years, however the result wasn't statistically significant (RR: 0.94, 95% C: 0.85 to 1.04; RD: -0.27%, 95% CI: -0.72% to 0.19%). For all-cause mortality, when adjusting for compliance, we observed small, but significant, reduction in RR (RR 0.98, 95% CI: 0.96 to 0.99) and RD of -1.49% (95% CI: -2.47 to -0.52) in those undergoing screening. In the Minnesota trial, those in the screening group had a significantly lower CRC mortality (RR: 0.78, 95% CI: 0.65 to 0.93; RD: -0.66%, 95% CI: -1.13% to -0.18%) after 30 years, but no statically significant reduction was observed for all-cause mortality. When pooling the data and adjusting for compliance, those in undergoing screening had a 16% lower risk of dying from CRC (RR: 0.84, 95% CI: 0.74 to 0.96) and a RD of -0.55% (95% CI: -0.96 to -0.15) compared to the control group. For all-cause mortality, we found a significant 2% reduction in mortality (RR: 0.98, 95% CI: 0.97 to 0.99) and a RD of -1.27% (95%CI: -2.00 to -0.54) (Figure 4 and 5).

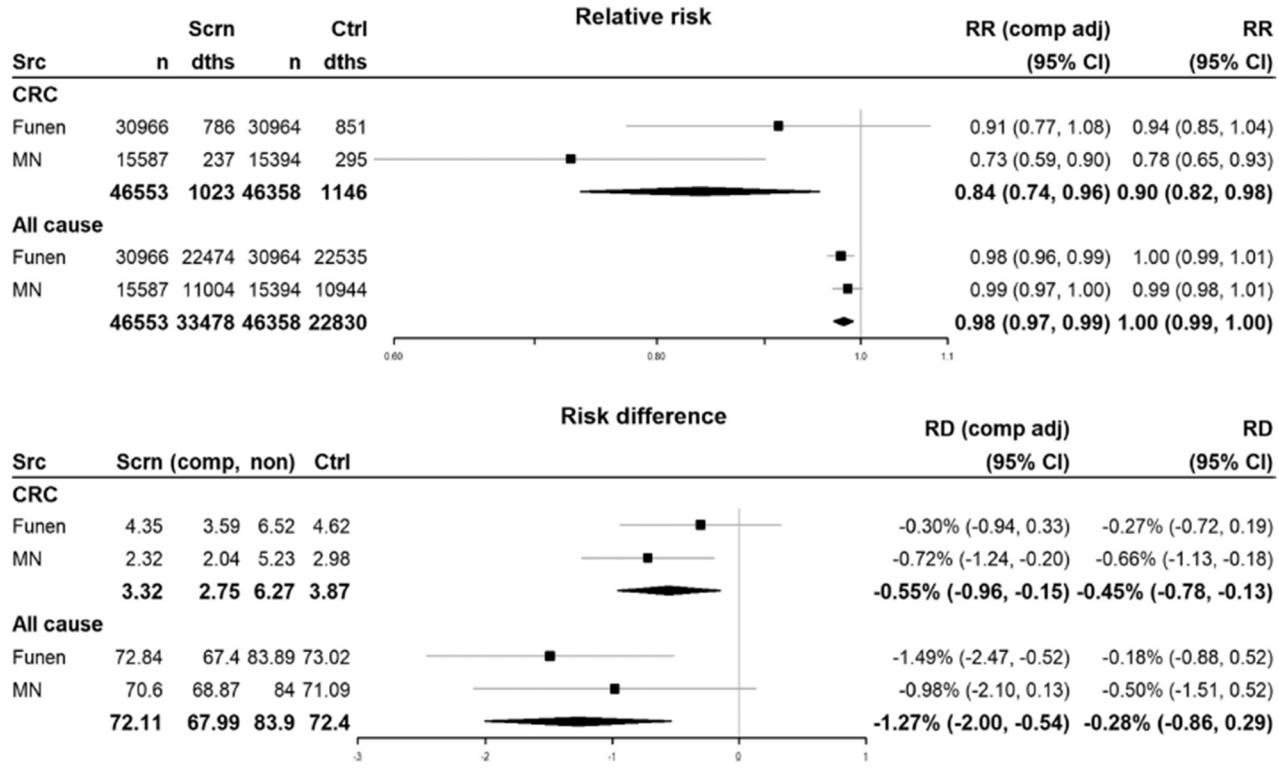

Figure 5. Forest plots of RR and RD for CRC and all-cause mortality

We repeated the analyses on different subgroups, investigating the impact of age and sex on CRC mortality. These showed a significant reduction in CRC mortality for males (RR: 0.75, 95% CI: 0.62 to 0.90) but not for females (RR: 0.91, 95% CI: 0.75 to 1.09). A significant reduction in CRC mortality that increased with age, was observed among screening participants with the greatest effect observed in those >70 (RR: 0.60, 95% CI: 0.37 to 0.99). No statistically significant gain was observed in participants aged 50-59 (RR: 0.89, 95% CI: 0.72 to 1.09). For females aged 50 to 59, the combined RR were 1.08 (95% CI: 0.80 to 1.46), the opposite direction of the rest of our results. This result was not statically significant.

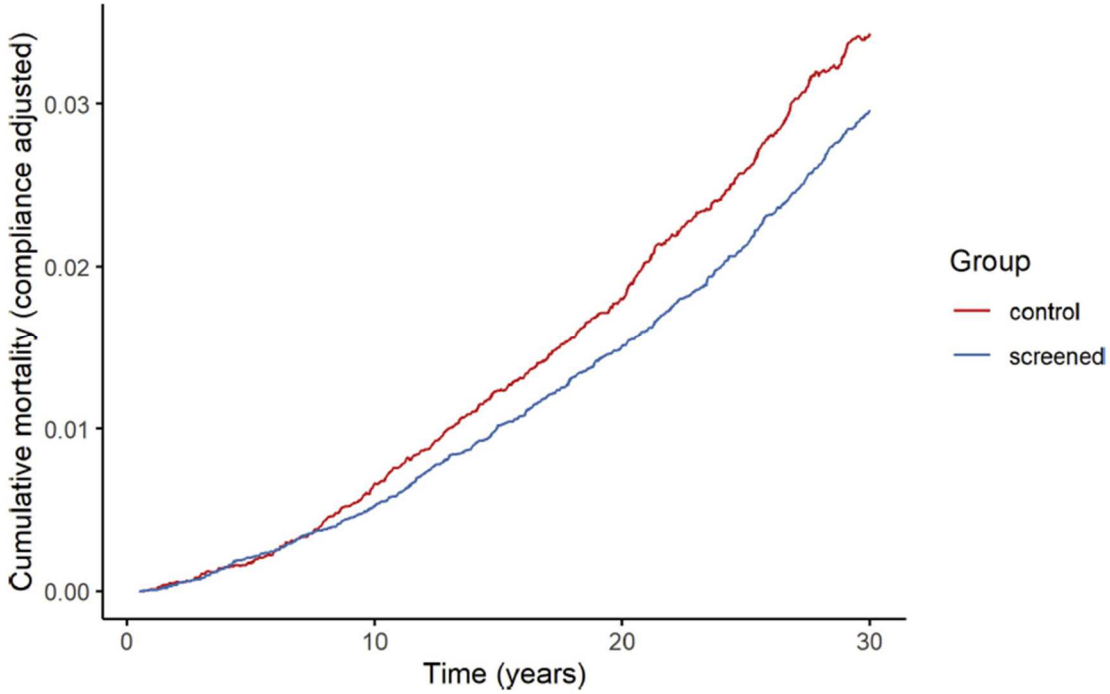

Figure 6. Pooled and compliance-adjusted cumulative CRC mortality

Discussion

Our results indicate that CRC screening does provide a reduction in CRC mortality despite differences in trial design. In addition, our results are the first to show a reduction in all-cause mortality in those undergoing screening. This is also the first study to employ a pooled approach, thereby increasing the sample size and adding statistical power. Worth highlighting is that females undergoing screening generally displayed a lower reduction in CRC mortality compared to males. Especially interesting is the group of females aged 50-59 who had no benefit from screening in neither CRC nor all-cause mortality. In these two trials, this group of females comprise 10,918 people, which is almost 25% of all those invited to screening.

Adjusting for compliance had a significant effect on our results, underling the importance of participation when evaluating screening efforts. It is possible that we somewhat underestimated the actual effect of screening

by considering compliance as a yes or no for one single procedure. This is supported by the results from the Funen trial, where a strength was the uncompromised repeated rounds of screening, which appear significantly impacted by adjusting for participation in repeated rounds of screening.

These differences between the two trials represent a general limitation of the study. The Funen and the Minnesota trial had several similarities, but did also diverge on some aspects. One example is the study sample, where the Funen trial population consisted of a random population-based sample of all residents on Funen, which included an unknowing controls. In contrast, the Minnesota trial used healthy volunteers for both intervention and control groups. By employing the individual patient data meta-analytic approach, we were able to address these differences to some degree. Because of the differences in study design, we could not account for other potentially confounding effects such as lifestyle/socio-economic factors, comorbidity, and factors influencing the risk of having a false-positive gFOBT (i.e. certain medications and conditions).

Conclusion

Our results show that biennial CRC screening leads to a sustained reduction in CRC mortality and a modest reduction in all-cause mortality when adjusting for participation. Importantly, we present clear differences between sexes showing that females benefitted less than males from CRC screening. Females aged 50-59 did not have any observed benefit from CRC screening.

Study 2. Cause of death, mortality and occult blood in colorectal cancer screening

Objective

The aim of this study was to investigate the association between elevated f-Hb and both all-cause and cause-specific mortality in a population of Danish CRC screening participants after 33 years of follow-up.

Methods

We included all participants from the intervention arm of the HM-II trial who submitted at least one gFOBT during the nine rounds of screening. Participants were identified from the original trial data obtained from the Danish National Archives and followed using national registers for up to 33 years from their date of trial inclusion and until death or end-of-study in December 2018. Data sources are presented in Figure 6. The trial is described in detail elsewhere. (122)

Participants were divided by gFOBT result, either positive or negative, and compared on separate mortality outcomes. We considered all gFOBT results in all rounds of screening a time-varying exposure, allowing for a more accurate representation of the different levels of accumulated exposure in each participant. Besides all-cause mortality, we considered death due the following diseases as outcomes: CRC, non-CRC cancers, cardiovascular disease, respiratory disease, digestive disease, neuro-psychological disease, haematological- and endocrine disorders, and external causes. Several baseline covariates were introduced, including age, sex, income, educational level, comorbidity, and diseases suspected of causing GI bleedings.

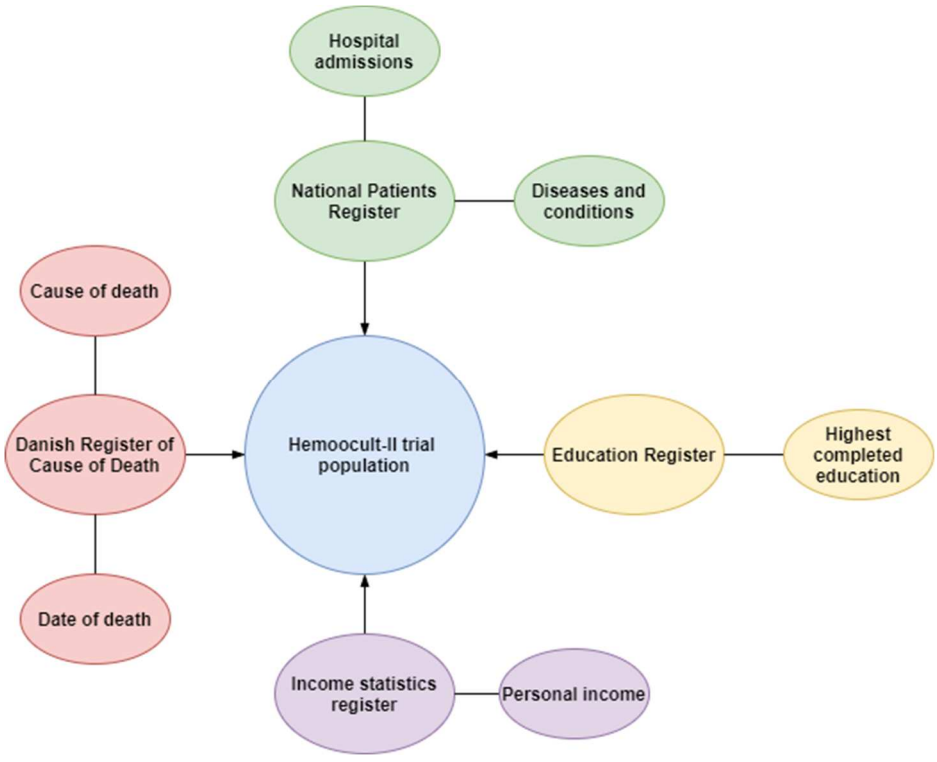

Figure 7. Data sources

Kaplan-Meier curves were used to present overall survival for both exposure groups. Cox proportional hazards regression models considering gFOBT as a time-varying exposure, were conducted on all outcomes to investigate the association between gFOBT result and mortality. Both univariate and multivariate analyses were conducted for all outcomes on all participants with no missing data on any covariates. Sensitivity analyses were conducted to investigate the impact of this decision.

Results

In the intervention arm of the HM-II trial, 20,694 (66.8%) submitted a gFOBT in the first round of screening and were included in this study. In this group, 1,766 people had one or more positive gFOBTs (8.5%). At the end of the study period, 15,542 (75.1%) of these participants had died, with a mean age of 80 (IQR, 74-87) and a median follow-up of 23 years (IQR: 13.8-32.6). Overall survival in the two groups are presented in the Kaplan-Meier curve in Figure 7, indicating that those with a negative gFOBT have an improved long-term survival.

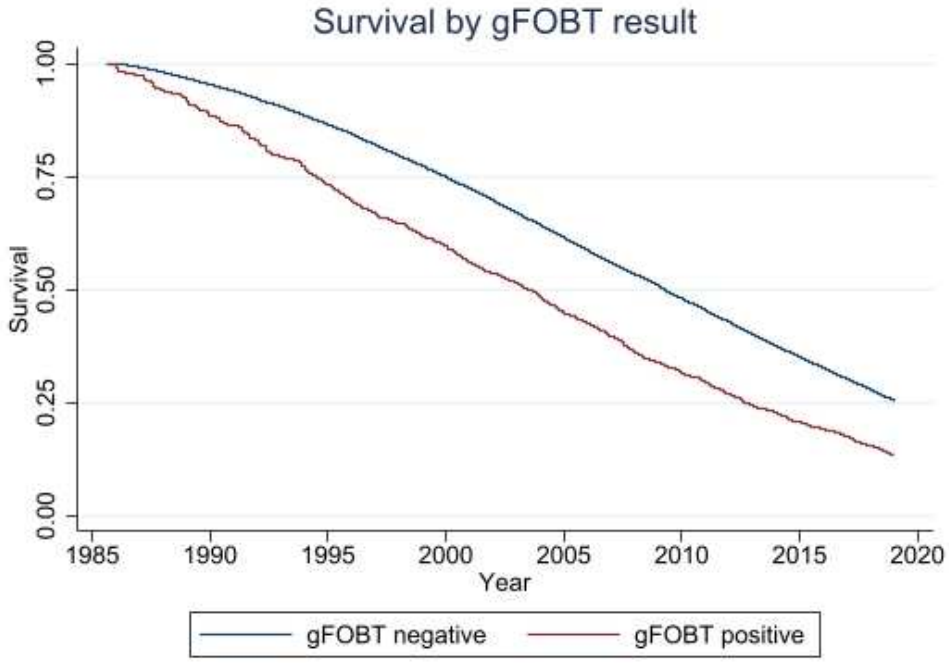

Figure 8. Survival curve for gFOBT positive and negative participants

A total 15,052 (72.7%) participants, of whom 10,070 (66.9%) had died, with no missing values in any covariates were included for the multivariate analyses. In those with a positive gFOBT, 882 (93.23%) died from other sources than CRC. The most common causes of death were cardiovascular disease (35.94%), non-CRC cancers (29.28%), and respiratory disease (24.95%). All causes of death are presented in Table 1.

Table 1. Cause of death by gFOBT result for participants with non-missing data.

	Positive gFOBT (*n* = 946) (%)	Negative gFOBT (*n* = 9122) (%)
All-cause excl. CRC	882 (93.23)	8813 (96.59)
CRC	64 (6.45)	311 (3.41)
Non-CRC cancer	277 (29.28)	2751 (30.17)
Cardiovascular disease	340 (35.94)	3328 (36.48)
Respiratory disease	236 (24.95)	2160 (23.67)
Digestive disease	57 (6.03)	444 (4.87)
Endocrine and haematological disease	73 (8.35)	595 (6.52)
External conditions	31 (3.28)	335 (3.67)

Abbreviations**:** gFOBT, guaiac faecal occult blood test; CRC, colorectal cancer.

Results from the multivariate analyses are presented in Figure 8. They show that compared to those with a negative test, gFOBT positive participants had a 1.28 (95% CI: 1.18-1.38) times higher risk of dying in the study period, an association that persisted after excluding CRC deaths (aHR: 1.20, 95% CI: 1.10-1.30). They also had a higher risk of dying from CRC (aHR: 4.07, 95% CI: 3.00-5.56), CVD (aHR: 1.22, 95% CI: 1.07-1.39), non-colorectal cancer (aHR: 1.30, 95% CI: 1.12-1.51), respiratory disease (aHR: 1.19, 95% CI: 1.01-1.40), digestive disease (aHR: 1.50, 95% CI: 1.07-2.10), and endocrine-and haematological disease (aHR: 1.58, 95% CI: 1.19-2.10). Sensitivity analyses where all participants with missing were included resulted in only minor changes to the results, with the exception of respiratory disease where the association to gFOBT was no longer statistically significant.

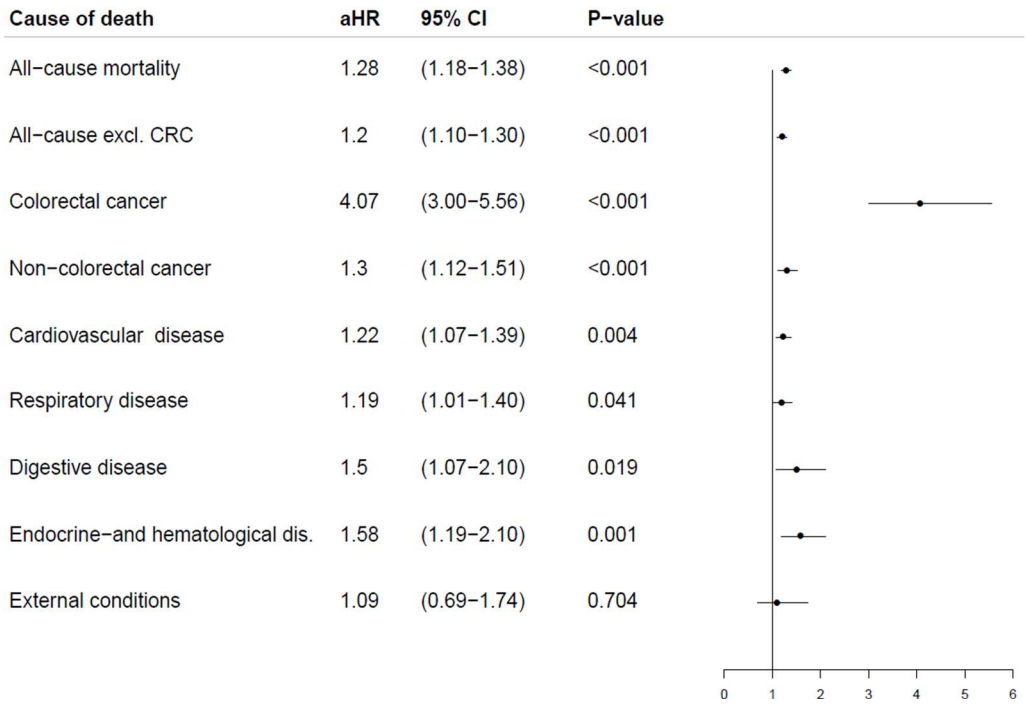

Figure 9. Multivariate analyses on gFOBT result and mortality outcomes

Discussion

For study 2, we set off to investigate the association between f-Hb and mortality outcomes as proposed by Libby et al. with a another approach and population focusing on achieving a longer follow-up, investigating if cumulative exposures affects the association and included more confounding effects. (94) We succeeded in doing so, and observed an association between gFOBT positivity and several mortality outcomes. Interestingly, we observed a difference in all-cause mortality that persisted after excluding CRC deaths from the analysis, hinting at a potential broader perspective of f-Hb. The highly elevated risk of dying from CRC in those with a positive gFOBT are in line with what we expected, since we used a cohort of screening participants knowing that f-Hb is an established biomarker for the disease. Had this been a strictly observational cohort only with no follow-up intervention after positive gFOBT, we would expect the risk of dying from CRC to be significantly higher.

Both the presence and magnitude of the increased risk of dying from CVD in gFOBT positive participants are noteworthy, since they confirm the findings from a similar, short-term FIT-based study by Moon et al. from Korea on the risk of experiencing myocardial infarction and ischemic stroke and from the study by Libby et al. who report similar risks of dying from CVD (94, 96) We also observed a 1.5 times higher risk of dying from (non-malignant) digestive disease in those with a positive gFOBT, which should be interpreted carefully. Partly because of the wide confidence intervals and partly because this group include all the upper GI conditions that are known for causing bleeding on their own. Despite having adjusted for the effect, we cannot rule out that an upper GI bleeding may have caused some of the gFOBTs to become positive.For endocrine- and haematological disorders, which includes diabetes, we did observe a more clear result that supports the findings by Libby et al. (94) This result match the conclusions by both Nakajima et al. and Kim et al. proposing an association between f-Hb measured by FIT and diabetes incidence. (97, 98)

While our findings all align with those of study by Libby et al., the magnitude of the reported risks vary significantly. The Scottish researchers presenting significantly bigger differences between the gFOBT positive and negative groups for almost all outcomes when compared to our, more modest associations. One example is CRC mortality, where we report an aHR of 4.07 compared to the Scottish 7.79. This could be caused differences in confounder-adjustment between the studies. However, the differences in magnitude is visible in the univariate analyses which somewhat rules this explanation out. The study participants and designs are also somewhat different. For instance, only the first screening result was used in Scotland versus all nine in Denmark. We were able to do a longer follow-up and include screening rounds as a time-varying exposure, which could be the reason why our results are more modest. This logic seem supported by both study 1 and results from other follow-up studies, where the impact of CRC screening persists but decreases with time. (34, 35)

Our study also addresses some of the limitations from the study by Libby et al. by including more and individual-level confounders including comorbidity, GI conditions causing bleeding and education and income. Unfortunately, we did not have any data on prescription medication suspected of causing GI bleedings because the Danish National Prescription Register did not exist before 1994. Including these medications might have had some impact on our results, especially because we used the now outdated gFOBT – the use of which is a weakness on its own. The large portion of missing data on education can theoretically have impacted our results since we do not know if the missing is differential or not. However, results from the sensitivity analyses did not give reason for any concerns on this subject. We used data from the National Patients Register collected from Danish hospitals for determining baseline comorbidity. This may lead to some underestimation of the effect of comorbidity on both the gFOBT result and the mortality outcomes, because only conditions leading to hospital contacts could be included.

Conclusion

Our study is the first of its kind to combine a long-term follow-up, time-varying exposure, extensive confounder-adjustment and a large sample size. The association between f-Hb and mortality appear plausible based on our findings that, although modest, were in coherence with the few related studies available, although the magnitude of the results were different.

Study 3. Cause of death, mortality and faecal occult blood in FIT-based colorectal cancer screening

Objective

The aim of this study was to investigate the association between incrementally increasing f-Hb levels and mortality outcomes in a FIT-tested population of Danish screening participants.

Methods

We conducted a register-based study, extracting data from the DCCSD on all participants from the first round of screening who submitted an eligible FIT between 2014 and 2017. Participants were followed until death, migration or end-of-follow-up on the 31st of December 2018, whichever came first. The data from the DCCSD was enriched with data from all data sources mentioned in the section above.

We considered mortality and causes of death as a number of separate outcomes. These were all-cause mortality (both in-and excluding CRC deaths) and death due to; CRC, respiratory disease, diabetes, CVD, and other (non-colorectal) cancers. We considered FIT-level as the exposure and grouped it into categories based on reported value; <36 ng/mL, 36-59 ng/mL, 60-99 ng/mL, 100-299 ng/mL and >299 ng/mL. We included several covariates including; age, sex, educational level, income, conditions and medication known to cause GI bleeding, and comorbidity. Comorbidity was included as both individual diseases and by Charlson Comorbidity Index to properly account for the effect on the risk of dying from the respective causes of death. Kaplan-Meier curves were used to depict developments in mortality. Cox proportional hazard regressions models were introduced to investigate the association between FIT and mortality. Participants with missing data were excluded. Participants with the lowest FIT-values, <36 ng/mL, were designated as the control group.

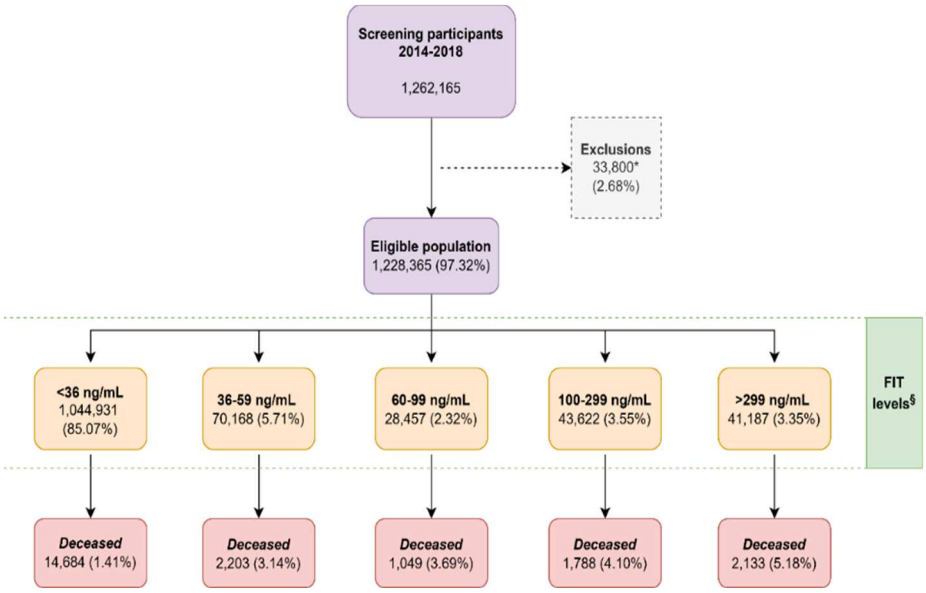

Figure 10. Flow of participants

Results

We considered all 1,262,165 Danish residents who participated in screening in the first round. After excluding 33,800 due to missing data, we had 1,228,365 participants eligible for analyses. At the end of follow-up, 21,857 (1.78%) of participants had died, of these 630 (2.88%) died from CRC. The proportion of deceased participants increased steadily with increasing FIT-levels from 1.41% in those with FIT <36 ng/mL to 5.18% in those with >299 ng/mL (Figure 9). Overall, the tendency was that participants with higher FIT-levels more often; were male, of higher age, had lower educational level, had lower income, had a higher comorbidity score, had registered conditions causing GI bleeding and collected prescription medication known to cause GI bleeding.

When comparing FIT-level groups by post-FIT mortality, the Kaplan-Meier curves show that the probability of dying in increased with FIT-level. The curves clearly show that even an incremental increase in FIT has a noticeable impact on all-cause mortality – both in-and excluding CRC deaths (Figure 10). Similar trends were observed when considering cause of death as the outcome.

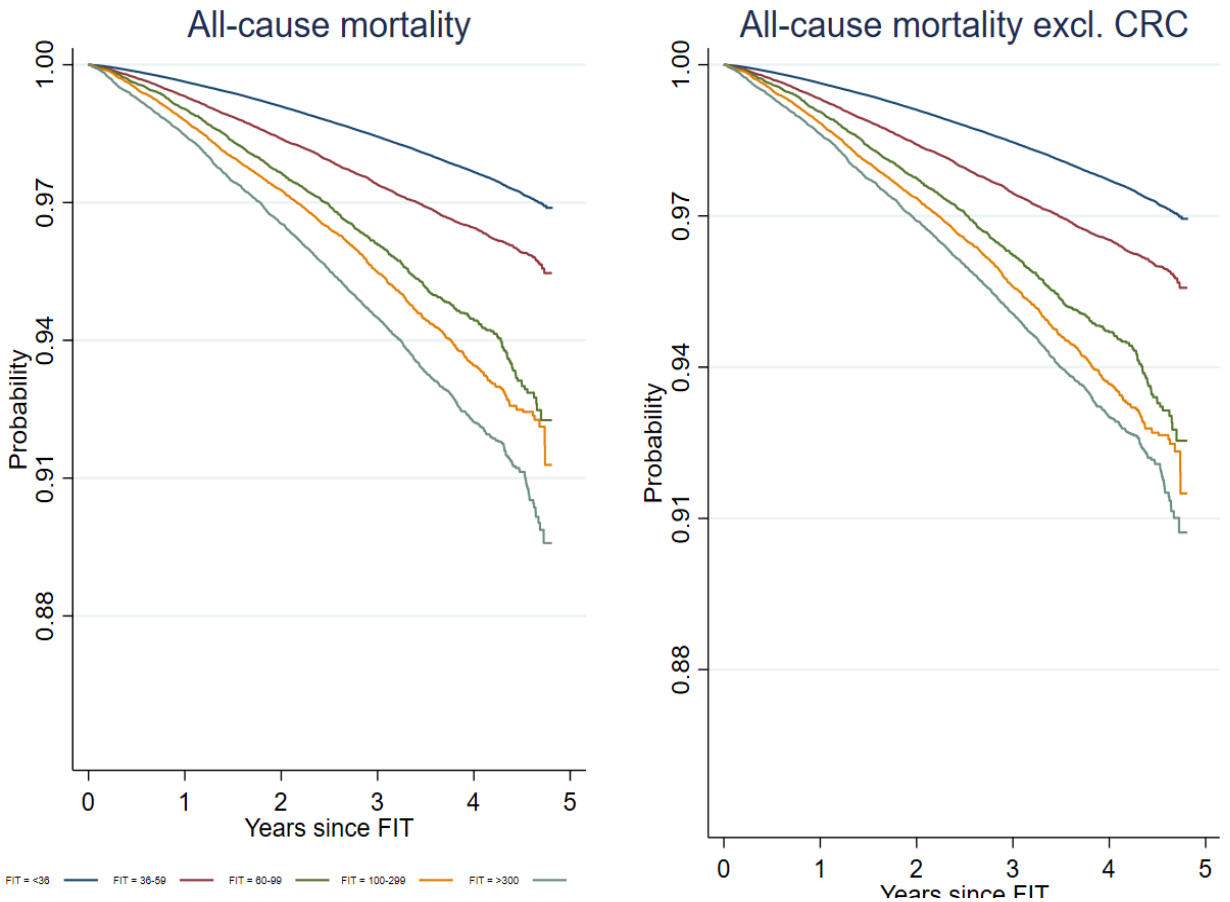

Figure 11. Kaplan-Meier curves by FIT-levels

These patterns persisted in the multivariate analyses, but the associations were more obvious for some outcomes. For all-cause mortality, we saw an increase in risk of dying with increasing FIT-levels. The pattern persisted after excluding CRC deaths. We saw a similar trend for death due to respiratory disease and CVD. Considering CRC death as the outcome, we observed a sharp increase in risk as FIT levels increased. For death due to other cancers, the risk of dying increased with FIT-levels, but appeared to reach a plateau at the highest levels. The risk of dying from diabetes appeared to increase with FIT-level, but confidence intervals were wide and overlapping (Figure 11).

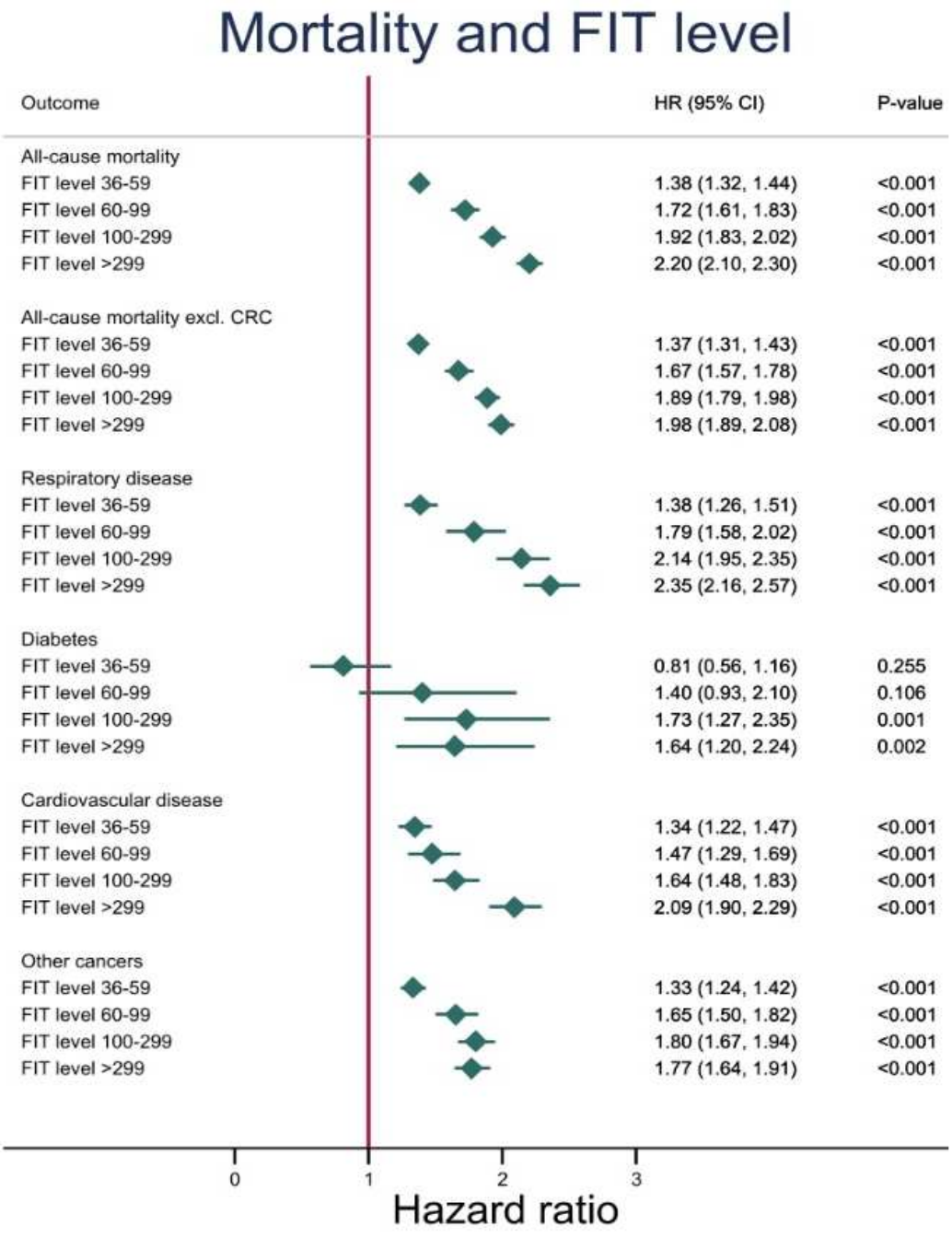

Figure 12. Multivariate analyses on FIT and mortality

Discussion

We investigated the association between f-Hb level and mortality in a FIT-tested population-based sample. Results showed that f-Hb measured by FIT is associated with several mortality outcomes. Especially the clear, trending increase in all-cause mortality with increasing FIT is of interest. Removing CRC deaths from this equation did little to change the interpretation and our results support the argument that f-Hb could indicate the presence of underlying conditions. The observed relationship between f-Hb and death due to CVD, other cancers and respiratory disease contributes to this understanding. The same is true for the risk of dying from diabetes, but our results on this outcome were hampered by a small number of events. The relationship between increasing f-Hb levels and an elevated risk of CRC death is less surprising as f-Hb is an established biomarker. Because we considered f-Hb as a quantitative variable, we were also able to discern that even an incremental increase results in an added risk of both dying in the study period and dying from certain diseases. This is important when evaluating the hypothesis presented in the background section – if f-Hb is indeed to be considered a reliable biomarker, proving a dose-response relationship is an important step. Prior studies primarily considered f-Hb as either a qualitative positive/negative (for both gFOBT and FIT studies) or as FIT-values in only the positive population. We were not able to include exact FIT-values <36 ng/mL since these are all coded as "35 ng/mL" in the screening database. Having these results could have provided some interesting information on whether the impact of an incremental increase is also present in the very low FIT levels. The primary limitation of our study is the lack of a long follow-up which is an important factors when considering mortality outcomes. We addressed this by including a very large sample and by conducting a time-sensitive analysis that allows for each individual to contribute person-time. Another potential limitation is the use of participants from the first round of screening. As presented earlier, the frequency of neoplastic findings in initial years of screening are evidently higher which could have a minor influence on mortality outcomes. (70) As in study 2, using the National Patients Register as the primary source of comorbidity may have led to some underestimation of the effect it has on either the mortality outcomes or the FIT result. This underestimation would be more elaborate in regards to conditions, such as haemorrhoids and anal fissures, managed outside the hospital. Future studies describing this relationship in detail is needed. We do not expect any of these to have significantly influenced our results, but future studies to rule this out would be prudent.

Conclusion

We observed an association between incrementally increasing f-Hb levels and both all-cause mortality and several causes of death. This appear to support the hypothesis that f-Hb may indicate the presence of chronic, non-communicable diseases leading to further speculations about its potential as a future biomarker for health and disease. Future studies are needed to clearly describe the relationship between f-Hb, individual health outcomes, and influencing factors before exploitation of these results can commence.

CONCLUSIONS AND FUTURE PERSPECTIVES

In study 1, we confirm that CRC screening utilizing stool-sample testing have an effect on both all-cause and CRC mortality after more than 30 years of follow-up, thereby providing important evidence on the long-term efficacy of CRC screening. In study 2, we investigated the hypothesis that f-Hb could indicate non-communicable diseases and found several associations between elevated f-Hb levels and higher all-cause and cause-specific mortality after 33 years of follow-up. These results were confirmed in study 3, where we repeated the study in a FIT-tested CRC screening population. Results confirmed the suggested association between f-Hb and both all-cause and cause-specific mortality and showed that an incremental increase in f-Hb values had a visible effect on mortality. We were able to adjust for several important confounding effects in all studies. However, while we are confident in our findings, we would have liked to adjust for the effects of prescription medication on f-Hb positivity in study 2. We were also not able to account for factors not registered in the registers, which may have led to an underestimation of the effect GI conditions, such as haemorrhoids, have on f-Hb positivity. Overall, our findings suggest that f-Hb is associated with mortality outcomes seemingly unrelated to CRC, adding support to the hypothesis that f-Hb may be able to indicate the presence non-communicable, chronic diseases.

Recent meta-analysis disagree about the relationship between medication that causes GI bleedings and FIT positivity. The magnitude and direction of this association needs to be clearly ascertained. Then, if a clear association is found between medication and FIT, the clinical relevance of this needs to be evaluated. Depending on the conclusion, it is possible that guidelines for FIT screening should be updated with restrictions to consumption of certain medications prior to stool collection. Moreover, if medications are found to heavily impact a FIT result, it could impact the future use of f-Hb as an indicator for non-CRC conditions. Since medications also indicate the presence of disease, some effect modification may occur if a potential association is not properly addressed. Prospective studies that investigates the predictive value of f-Hb for non-CRC conditions with extensive reporting on medications (both prescription and over-the-counter) is needed to clearly describe the association. A significant clinical impact can be achieved if f-Hb proves to be an effective biomarker for the non-communicable and/or chronic diseases. Estimating the magnitude of this impact is difficult as it depends on a large number of unknown factors and is beyond the scope of our work. However, if we consider the fact that 62.5% of all adult Danes have one or more chronic condition of any kind and that the average number of chronic conditions per Dane aged 45-74 is 2.7, it is fair to assume that future interventions could encompass and hopefully benefit large numbers of people. (123) In studies 2 and 3, we considered the prevalence of comorbidity by a Charlson Comorbidity Index which focuses on a pre-determined range of mostly serious conditions. In study 3, we say that 18.9% of the FIT-positive screening population were registered with one or more diseases included in the Charlson Index, further hinting at the prevalence of serious comorbidity in the screening cohort. The quantitative approach of the FIT creates opportunities for

reducing the burden of several of these diseases by allowing for people with a FIT-value over a certain threshold to undergo interventions targeting known risk factors or disease components. Examples could be interventions targeting lifestyle factors in participants prone to diabetes or hypertension for preventing disease progression or early initiation of drug therapies. A FIT-result could also be used for monitoring the same conditions and guide pre-emptive initiatives. Simple awareness could also provide some clinical impact. In patients presenting with a positive FIT and no discernible cause of bleeding, physicians who are aware of the association between f-Hb and disease could be prompted to conduct additional examinations.

Before interventions such as these can be designed and tested in a clinical setting, more work is needed. For one, the extent of the predictive value of f-Hb on diseases in general needs to be elaborated and, more importantly, the association between f-Hb and different stages of disease progression needs clarification. In extension, the relationship between f-Hb and disease severity is an important factor that must be described. From here, a threshold for f-Hb represents a significant risk for diseases must be established. Prospective studies can then be conducted to assess the clinical impact of using f-Hb to identify people who could benefit from early intervention or preventive initiatives seeking to either treat or manage the development of diseases. The association has primarily been tested in screening populations and patient populations with different indications should be investigated. It is important to note that the scientific interest in this field is still fairly new. We can give some examples for future exploitation of our results, but we lack the evidence to give any concrete suggestions and feasibility estimations on future interventions or initiatives. Neither do we have the evidence to speculate in the magnitude of the potential economic consequences.

The rise of Artificial Intelligence (AI) opens up several new avenues for utilizing patient data for preventive or diagnostic purposes, especially for multifactorial conditions. One example is prediction models for identifying high-risk individuals for any given disease or for allocating individuals to the optimal treatment or diagnostic modality. Depending on actual potential of f-Hb, it could be used in these models as either a predictor of multi-morbidity or for specific conditions in conjunction with other predictive factors. One such prediction model can be an important tool for allocating the right patients to the right pathway in future interventions. The future use of f-Hb will come with dilemmas regardless of the approach. Should the use of f-Hb as a biomarker for multi-morbidity in false-positive screening participants only, should the intervention include all screening participants or should completely new initiatives be launched for non-CRC conditions? It would also be necessary to have clear interventions available that are both cost-effective and secure for all included diseases. Today, it appears that the hypothesis of f-Hb as an indicator for systemic inflammation is gaining support, with several studies linking f-Hb and inflammatory diseases. Especially the increasing scientific focus on the role of microbiota dysbiosis as a driver for inflammation, provides much needed explanations for some of the underlying mechanisms. However, the complexity and many unknown dimensions of the association creates a need for more studies before any clear conclusions can be made.

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APPENDIX

Effects of Screening Compliance on Long-term Reductions in All-Cause and Colorectal Cancer Mortality

Aasma Shaukat,[*,‡,§] Lasse Kaalby,[||,¶,#] Gunnar Baatrup,[||,¶,#] Ole Kronborg,[||,¶,#] Sue Duval,[**] Michael Shyne,[‡‡] Jack S. Mandel,[§] and Timothy R. Church[§]

*Division of Gastroenterology, Minneapolis Veterans Affairs Healthcare System Minneapolis, Minnesota; ‡Department of Medicine, University of Minnesota School of Medicine, Minneapolis, Minnesota; §Division of Environmental Health Sciences, University of Minnesota School of Public Health, Minneapolis, Minnesota; ||Open Patient Data Explorative Network, Odense University Hospital and University of Southern Denmark, Odense, Denmark; ¶Department of Clinical Research, University of Southern Denmark, Odense, Denmark; #Department of Surgery, Odense University Hospital, Odense, Denmark; **Cardiovascular Division University of Minnesota Medical School, Minneapolis, Minnesota; and ‡‡Division of Biostatistics, University of Minnesota School of Public Health, Minneapolis, Minnesota

This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e52. Learning Objective–Upon completion of this activity, successful learners will be able to recall the options for colorectal cancer screening in average risk men and women.

BACKGROUND & AIMS: **Randomized trials have shown that biennial fecal occult blood test (FOBT) screening reduces mortality from colorectal cancer (CRC), but not overall mortality. Differences in benefit for men vs women, and by age, are unknown. We sought to evaluate long-term reduction in all-cause and CRC-specific mortality in men and women who comply with offered screening, and in different age groups, using individual participant data from 2 large randomized trials of biennial FOBT screening, compared with an intention to treat analysis.**

METHODS: **We updated the CRC and all-cause mortality from the Danish CRC screening trial (n = 61,933) through 30 years of follow up and pooled individual participant data with individual 30-year follow-up data from the Minnesota Colon Cancer Control trial (n = 46,551). We compared the biennial screening groups to usual care (controls) in individuals 50–80 years old using Kaplan Meier estimates of relative risks and risk differences, adjusted for study differences in age, sex, and compliance.**

RESULTS: **Through 30 years of follow up, there were 33,478 (71.9%) and 33,479 (72.2%) total deaths and 1023 (2.2%) and 1146 (2.5%) CRC deaths in the biennial screening (n = 46,553) and control groups (n = 46,358), respectively. Among compliers, biennial FOBT screening significantly reduced CRC mortality by 16% (relative risk [RR], 0.84; 95% CI, 0.74–0.96) and all-cause mortality by 2% (RR, 0.98; 95% CI, 0.97–0.99). Among compliers, the reduction in CRC mortality was larger for men (RR, 0.75; 95% CI, 0.62–0.90) than women (RR, 0.91; 95% CI, 0.75–1.09). The largest reduction in CRC mortality was in compliant men 60–69 years old (RR, 0.59; 95% CI, 0.42–0.81) and women 70 years and older (RR, 0.53; 95% CI, 0.30–0.94).**

CONCLUSIONS: **Long-term CRC mortality outcomes of screening among compliers using biennial FOBT are sustained, with a statistically significant reduction in all-cause mortality. The reduction in CRC mortality is greater in men than women—the benefit in women lags that of men by about 10 years.**

Key words: Colon Cancer; Survival; Early Detection; Compliance.

See editorial on page 892.

Several modalities are available for colorectal cancer (CRC) screening, including fecal occult blood test (FOBT) at intervals of 1 or 2 years. While annual testing is employed in the United States, biennial screening is practiced in many European countries and Canada.[1,2] Biennial screening reduced CRC mortality by 13%, 18%, and 21% in 3 large trials with follow-up of 18–19.5 years.[3–5] However none of the trials were sufficiently powered to study all-cause mortality, the effect of compliance adjustment, or whether the screening effects vary by age and sex. Given that these trials were initiated in the mid-1970s and early 1980s, long-term follow-up provides additional events and person-years of follow-

Abbreviations used in this paper: **CI, confidence interval; CRC, colorectal cancer; FOBT, fecal occult blood test; RD, risk difference; RR, relative risk.**

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up for meaningful comparisons. Previously, the Minnesota fecal occult blood trial updated the follow-up of the participants of the Minnesota trial (annual and biennial screening vs controls) through 30 years and reported a sustained reduction in CRC mortality of 18% with biennial screening.[6] They also observed nonsignificant differences in screening effects for men and women and by age. Since then, others have published updated follow-up and pooled analyses of the flexible sigmoidoscopy trials for CRC screening and reported significant differences in benefits of CRC screening between men and women.[7,8] None of the trials reported a reduction in all-cause mortality, while 1 meta-analysis of CRC screening trials reported a significant increase in non-CRC mortality.[9] Our aims were to assess the long-term effects of biennial screening on all-cause and CRC mortality using intention to treat and compliance adjustment, and evaluate age- and sex-specific effects by pooling individual participant data from the available randomized controlled trials of biennial FOBT screening, updated through 30 years of follow-up.

What You Need to Know

Background

Studies have shown that biennial fecal occult blood test (FOBT) screening reduces mortality from colorectal cancer (CRC) but not overall mortality. Differences in benefit for men vs women, and by age, are unknown.

Findings

Compliance biennial FOBT screening reduces CRC mortality over 30 years, with a statistically significant reduction in all-cause mortality. The reduction in CRC mortality is greater in men than women—the benefit in women lags that of men by about 10 years.

Implications for patient care

Screening programs for CRC should ensure compliance with biennial FOBT to reduce CRC and overall mortality over the long term.

Materials and Methods

We performed a systematic literature search for randomized trials evaluating annual or biennial FOBT screening for reduction in CRC mortality. We identified 5 trials. We excluded 2 trials for lack of follow-up colonoscopy in FOBT positive participants[10] and lack of individual randomization design.[11] The investigators of the remaining 3 trials: the Minnesota trial (United States), the Funen trial (Denmark), and the Nottingham trial (United Kingdom) were asked to contribute data. The UK investigators elected not to participate. A collaborative agreement was reached between the Minnesota and Danish trial investigators. The study was approved by the institutional review board at University of Minnesota and by the Danish Data Protection Agency. The University of Minnesota and the University of Southern Denmark executed data sharing agreements to allow the meta-analysis.

Minnesota Colon Cancer Control Study

The Minnesota Colon Cancer Control Study[5,12–14] randomized healthy volunteers 50–80 years of age to annual screening, biennial screening, or usual care (control) (N = 46,551). The primary endpoint was CRC mortality. Individuals were recruited and randomized from 1975 to 1992 with a 4-year hiatus from 1982 to 1986. In total, 6 rounds of screening were offered to the biennial group. Adherence to 1 or more rounds of screening was 90%. Those with a positive test were invited to the University of Minnesota for a cost-free diagnostic workup that included colonoscopy. Polyps found during colonoscopy were removed during the procedure. If the colonoscopy was incomplete, an air-contrast barium enema was performed. Compliance with a follow-up diagnostic examination after a positive screen was 83%. Annual follow-up using written questionnaires and telephone calls took place between 1976 and 1999 with response to these annual follow-ups over 99% in all 3 groups. The role of CRC in deaths was determined by the deaths review committee for approximately the first 15 years, and thereafter based on coded death certificates through 2001. Death certificates were coded according to International Classification of Diseases–Eighth Revision (ICD-8a), 1CD-9, or ICD-10, depending on the date of death. In 2011 the study updated the cause of death through 30 years of follow-up by conducting an National death Index-plus search for vital status and cause of death for participants alive at last follow-up, using identifiers including name, sex, date of birth, Social Security number, and state of residence to obtain the best possible match. Updated results of the 30-year follow-up of the Minnesota Colon Cancer Control Study have been published previously[6] and showed a sustained reduction in CRC mortality of 33% and 22% in the annual and biennial screening arms, respectively. For the present study, we only used the biennial screening and control arms.

Funen Fecal Occult Blood Trial

The Funen fecal occult blood trial[4,15–17] randomized individuals 45–75 years of age to biennial screening or usual care (control) (N = 61,933). The primary endpoint was CRC mortality. In 1985, individuals residing in Funen were randomized and underwent 9 rounds of biennial FOBT screening. Individuals with known colorectal cancer, colorectal adenomas, or distant spread from any malignant disease were excluded before randomization. Individuals with positive results underwent a colonoscopy. Polyps found during colonoscopy were removed during the procedure. Adherence to the first round of

screening was 67%, and only those that adhered to screening in the previous round and without colorectal neoplasia were invited to the next round of screening. Adherence to diagnostic colonoscopy for those with a positive screen was 83%. Information on CRC was obtained through manual review of medical records, the Funen County database, and the Danish National Registration and Danish Cancer Registry through the first 13 years of follow-up. CRC as a cause of death was based on manual chart review and coded death certificates. Through 13 years of follow-up, biennial screening reduced CRC mortality by 11%.[4] For the current study in 2018, the study updated the cause of death of all trial participants through 30 years of follow-up using the Danish Civil Registration number as the unique identifier via the Danish National Patient Register, the Central Person Register, and the Danish Register of Cause of Death. All diagnoses and death certificates were coded using ICD-8 (until 1994) or ICD-10 (after 1994).

Statistical Analysis

Cumulative mortality from CRC or all causes was estimated by Kaplan-Meier survival analysis[18] through 30 years following randomization, and biennial screening and usual care (control) groups were compared at multiple follow-up times, first by intention to treat and then restricted to compliers. Cuzick's method[19] estimates the effect of screening among compliers in the group assigned to screening by comparing the outcomes with those in a corresponding group among control outcomes. Compliance was defined as undergoing at least 1 screening round. This method was applied to cumulative Kaplan-Meier estimates of all-cause and CRC mortality to compliance-adjust absolute mortality relative risk (RR) and risk difference (RD). Unadjusted and adjusted RR and RD were computed for each study and by sex, age, and their combination. Standard errors for the RR were derived from Greenwood's variance approximation. Standard errors for RD were based on normal approximations to the binomial distribution.

We used a 2-step approach to the individual participant data meta-analysis for biennial screening vs control groups, in which study-specific effects were first estimated and then combined into a single estimate for each outcome.[20] Pooled RR and RD were calculated with fixed effect meta-analytic models that were adjusted to the combined age-sex distribution. Proportional hazards models were used to conduct overall tests of interaction between demographic subgroups (age, sex, and age by sex) and screening or control groups. Owing to small numbers, compliance-adjusted analysis of the men 70 years of age or older in the Funen trial could not be calculated. The I^2 test was used to measure heterogeneity in effect estimates.

Results

The demographic characteristics of participants, person-years and events from the 2 trials are presented in Table 1. In the Funen trial, there were 45,009 (72.7%) deaths in the biennial screening and control groups combined through 30 years of follow-up. There were 1637 (2.6%) deaths from CRC: 786 (2.5%) in the screening group and 851 (2.7%) in the control group. In the Minnesota trial, there were 21,948 (70.8%) deaths through 30 years of follow-up, with 532 deaths from CRC: 237 (1.5%) in the screening group and 295 (1.9%) in the control group. Combined, there were 33,478 (71.9%) and 33,479 (72.2%) total deaths and 1023 (2.2%) and 1146 (2.5%) CRC deaths in the biennial screening group (n = 46,553) and control group (n = 46,358), respectively.

CRC Mortality

In the Funen trial, there was a small but not statistically significant difference in 30-year CRC mortality between the screening and control groups (RR, 0.94; 95% CI, 0.85 to 1.04; RD, −0.27%; 95% CI, −0.72% to 0.19%). In the Minnesota trial, there was a significant reduction in 30-year CRC mortality between the screening and control groups (RR, 0.78; 95% CI, 0.65, 0.93; and RD, −0.66%; 95% CI, −1.13% to −0.18%). Combined, biennial fecal occult blood screening reduced deaths from CRC by 10% (RR, 0.90, 95% CI: 0.82 to 0.98; and RD, −0.45%; 95% CI, −0.78% to −0.13%; I^2 = 63.5%; 95% CI, 0% to 91.6%). However, among compliers, the RR for CRC mortality was 0.84 (95% CI, 0.74 to 0.96) and the RD was −0.55% (95% CI, −0.96%, −0.15%) (Figures 1 and 2 and Supplementary Figure 1).

All-Cause Mortality

In neither the Funen nor the Minnesota trial was either RR or RD statistically significant in 30-year all-cause mortality using intention to treat. When the datasets were combined, the relative and absolute reduction in all-cause mortality were not statistically significant (RR, 1.00; 95% CI, 0.99 to 1.00; and RD, −0.28%; 95% CI, −0.86% to 0.29%), but among compliers, all-cause mortality was statistically significantly reduced (RR, 0.98; 98% CI, 0.97 to 0.99; and RD, −0.55%; 95% CI, −0.96% to −0.15%; I^2 = 0%) (Figures 2 and 3 and Supplementary Figure 2).

Subgroup Analyses

Figure 4 shows a forest plot with the numbers of participants who were randomized, CRC deaths, and compliance-adjusted RRs for age and sex subgroups, for the screening, control, and combined groups.

Table 1. Characteristics of the Included Trials

Characteristic	Trial	Screening	Control
Participants	Funen	30,966	30,964
	Minnesota	15,587	15,394
	Pooled	46,553	46,358
Female	Funen	16,103 (52.0)	16,111 (52.0)
	Minnesota	8143 (52.2)	7960 (51.7)
	Pooled	24,246 (52.1)	24,071 (51.9)
Age, y	Funen	59.4 ± 8.50	59.4 ± 8.50
	Minnesota	62.3 ± 7.80	62.3 ± 7.70
	Pooled	60.4 ± 8.40	60.3 ± 8.40
Follow-up, person-years	Funen	605,023	603,953
	Minnesota	328,287	323,993
	Pooled	933,310	927,946
Deaths at 30 y (all cause)	Funen	22,474 (72.6)	22,535 (72.8)
	Minnesota	11,004 (70.6)	10,944 (71.1)
	Pooled	33,478 (71.9)	33,479 (72.2)
Colorectal cancer	Funen	786 (2.5)	851 (2.7)
	Minnesota	237 (1.5)	295 (1.9)
	Pooled	1023 (2.2)	1146 (2.5)
Compliers	Funen	20,694 (66.8)	
	Minnesota	13,806 (88.6)	
	Pooled	34,500 (74.1)	

Values are n, n (%), or mean ± SD.

The RR for CRC mortality in the screening vs the control group varied noticeably by age, with the largest benefit in those 60–69 years of age. The compliance-adjusted RRs for individuals 50–59, 60–69, and ≥70 years of age were 0.89 (95% CI, 0.72 to 1.09), 0.71 (95% CI, 0.58 to 0.88), and 0.60 (95% CI, 0.37 to 0.99), respectively (p for trend = 0.46).

The RD among compliers increased with age and was –0.15% (95% CI, –0.61% to 0.31%), –1.45% (95% CI, –2.38% to–0.52%), and –2.48% (95% CI, –5.85% to 0.89%) for individuals 50–59, 60–69, and ≥70 years of age, respectively (Figure 5).

The reduction in CRC mortality among compliers was larger for men but was not statistically significant compared with women (RR, 0.75; 95% CI, 0.62 to 0.90; and RR, 0.91; 95% CI, 0.75 to 1.09, respectively; p for trend = 0.25). The RD was larger for men (–1.06%; 95% CI, –1.71% to –0.41%) compared with women (–0.20%; 95% CI, –0.71% to 0.31%). Screening men 60–69 years of age showed a strong effect on CRC mortality (RR, 0.59; 95% CI, 0.42 to 0.81; p for trend = 0.19).

Among women, the largest benefit of screening was seen in the 70 years of age and older age group for reduction in CRC mortality (RR, 0.53; 95% CI, 0.30 to 0.94). No statistical or numerical benefit was seen in the women 50–59 years of age (RR, 1.08; 95% CI, 0.80 to 1.46; p for trend = 0.21).

Discussion

In the 30-year follow-up of all participants randomized to biennial FOBT screening vs control screening, we found a statistically significant 10% relative reduction in CRC mortality and no difference in all-cause mortality in intention-to-treat analysis with long-term follow-up, similar to prior studies. There are several potential explanations for the inability to demonstrate a decrease in all-cause mortality. First, it is possible that the benefits gained in reduced deaths from CRC in the screened group is balanced by an increased death rate from non–CRC related deaths. The second possibility is that there is simply too much noise in the various published studies due to noncompliance and crossover to allow for the demonstration of a small expected difference in all-cause mortality. These 2 explanations have major differing clinical implications. If the former were correct, there truly is no benefit to all-cause mortality from

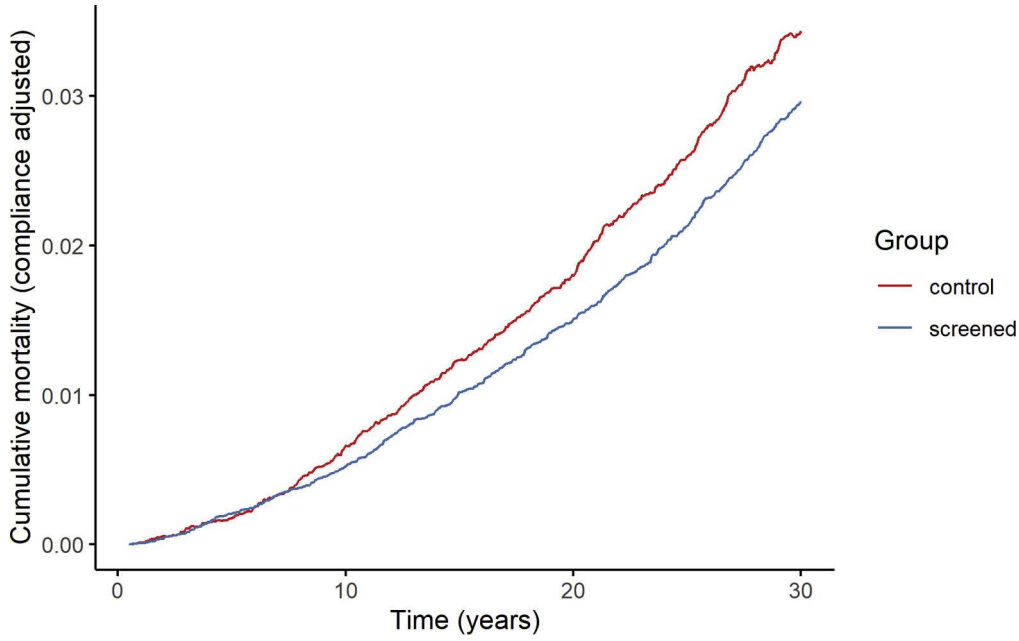

Figure 1. Compliance-adjusted plot of combined cumulative CRC mortality.

screening, which causes as many deaths as it prevents. One could make the case that such information should be included as part of the informed consent. The latter explanation assumes that there truly is a difference in all-cause mortality that has yet to be demonstrated because of the low fraction of all deaths accounted for by CRC. Differentiating between these 2 possible explanation clearly would be of interest to both health professionals as well as subjects undergoing screening.

One source of "noise" in randomized CRC screening studies is noncompliance in the screened group, which we have defined as failure to undergo a single screening procedure. While no possible benefit from screening can be expected for such individuals, they are included in the intention-to-treat analysis as screened, to keep randomization intact. To this end, we applied Cuzick's method,[19] which takes focuses on the differences between compliant and noncompliant subjects and makes

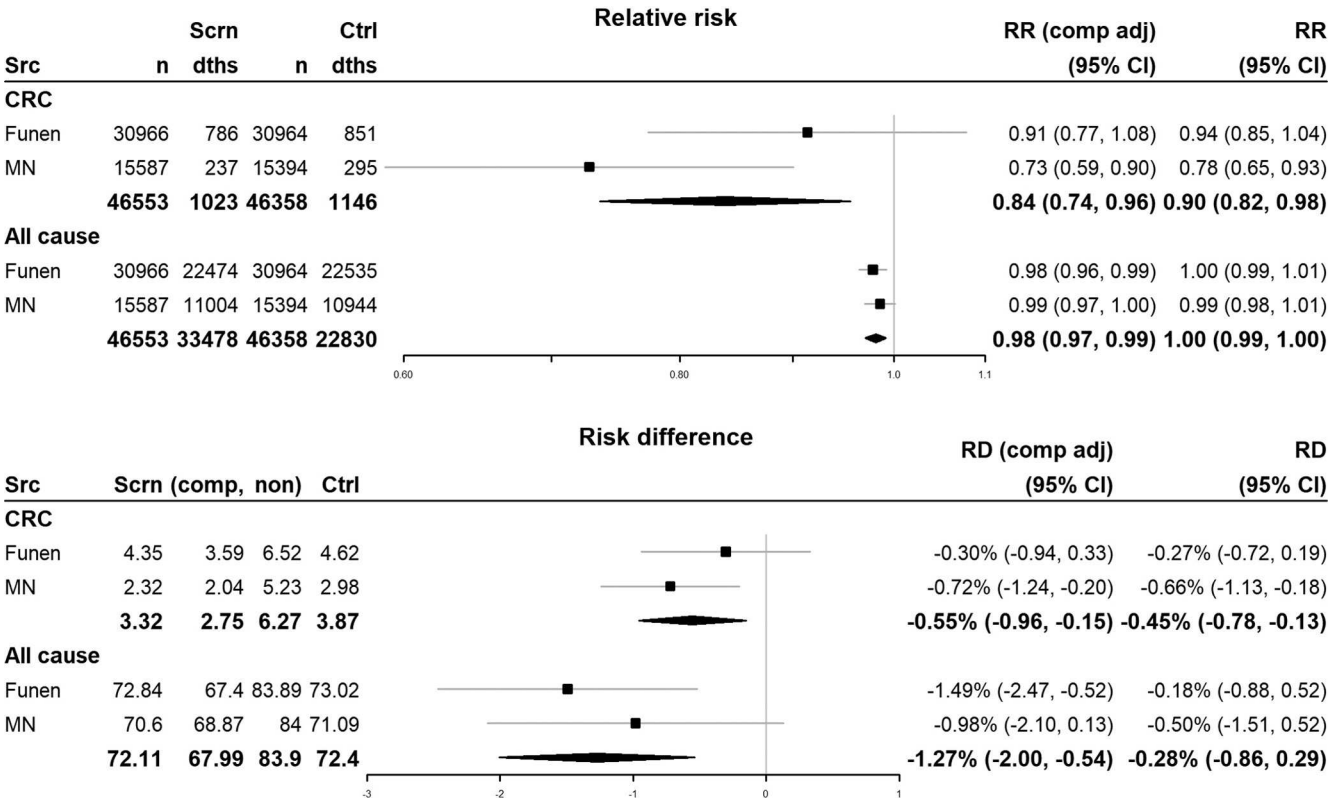

Figure 2. Forest plot of RRs (top) and absolute RDs (bottom) for CRC and all-cause mortality.

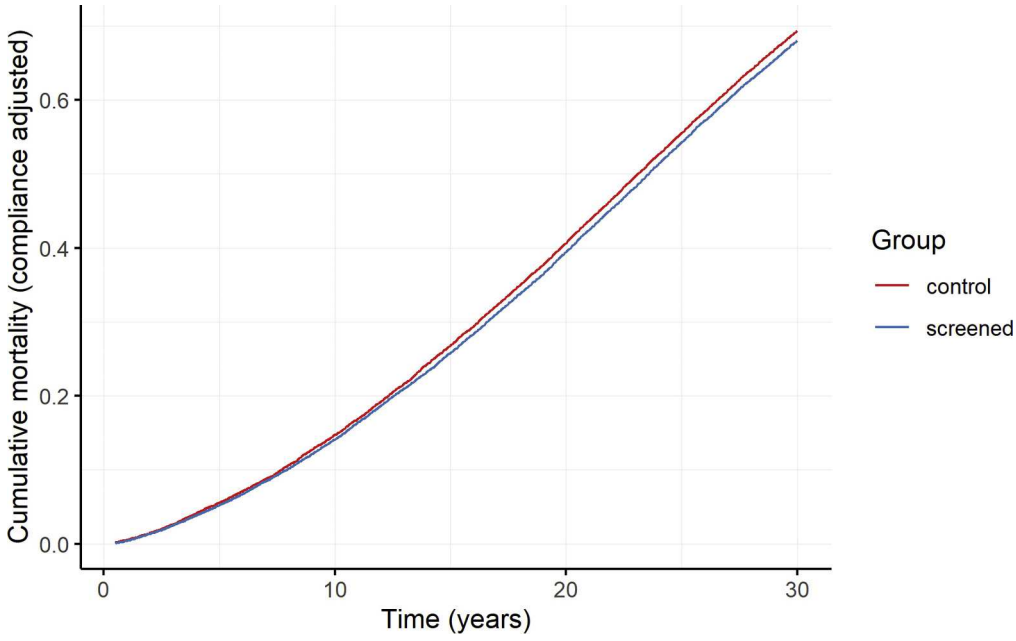

Figure 3. Compliance-adjusted plot of cumulative combined all-cause mortality.

possible an accurate estimate of the effect of screening on just the compliers in the group randomized to screening. Among compliers, there was a 16% reduction in CRC mortality rate in the screened group vs the 10% reduction observed in the nonadjusted (intention to treat) analysis. More important, a statistically significant 2% relative reduction in all-cause mortality was observed, the first such reduction reported in occult blood screening studies. The benefit in those that comply may be due to benefit of undergoing the screening, as well as other healthy behaviors that may contribute to lower risk of dying, such as not smoking, healthy eating, and lifestyle choices. The benefit could also be explained by indirect effects of accessing the healthcare system as a consequence of screening. While we do not have information on what may have led to the compliant group having lower all-cause mortality, the results reinforce that trying to achieve 100% compliance to screening is an important public health goal. If confirmed by other studies, the reduction in all-cause mortality in addition to reduction in CRC mortality is an important finding, laying to rest the concern that reductions in CRC mortality due to screening are being offset by increases in other causes.

Analysis by sex showed that reduction in CRC mortality was statistically significant only for men, despite comparable number of deaths from CRC in men (n = 585) and women (n = 561) in the control groups. The observed effect of screening on reducing deaths from CRC was greatest in men 60–69 years of age and in women over 70 years of age. The sample size of individuals 70 years of age and over was too small to draw meaningful conclusions. For 50- to 59-year-old women, screening was associated with a small but not statistically significant increase in CRC mortality.

Lack of a significant reduction in CRC mortality for women has also been evaluated in the pooled flexible sigmoidoscopy trials[21] with no reduction in CRC mortality. The explanation for the lack of a significant benefit of biennial FOBT in women is not clear. One possibility is that women may have proportionally more right-sided adenomas and CRC.[22–24] There may be important differences in the underlying biological pathways for women, including tumors that are less sensitive to detection with FOBT, or tumors that rapidly grow, such that biennial interval is not effective in detecting these at early stages. Independent of the explanation of the gender difference, it could be argued that biennial FOBT screening is not an effective screening modality in women.

Our findings are also consistent with the updated 15-year follow-up of the Norwegian flexible sigmoidoscopy screening trial, which reported no significant reduction in CRC mortality from screening in women 50–64 years of age but a 37% reduction in CRC mortality in men 50–64 years of age, despite similar or higher compliance with screening among women.[8] These findings are consistent with differences in incidence and mortality rates of CRC between men and women. Women's age-specific cumulative incidence rates lag behind that of men, as illustrated by Brenner et al.[25] At 50, 55, and 60 years of age, women achieved comparable 10-year cumulative incidence rates 4–6 years later than men.

This finding also needs to be factored into recent recommendations for reducing the age at which CRC screening should commence. Our data suggest that greater screening efficiency could be achieved by starting women at a later age then men and perhaps going longer compared with men.

While reduction in CRC mortality is a laudatory goal, the overall 10% reduction in CRC deaths observed with biennial screening in the present study is at the lower end of the effectiveness claims for various other

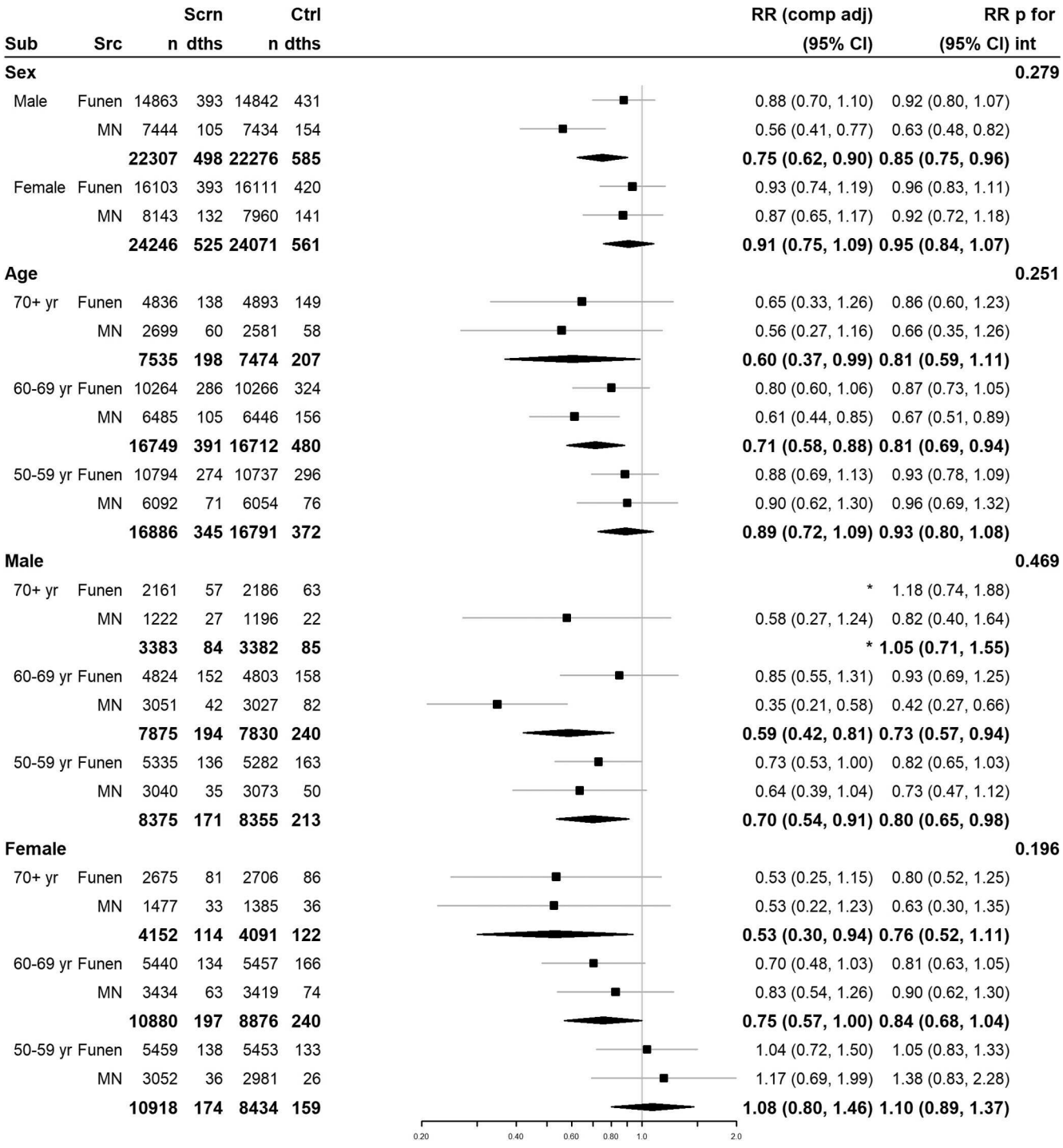

Figure 4. Forest plot of relative risks of CRC mortality for age and sex subgroups (Minnesota [MN], Funen, and combined trials).

screening modalities and well below the 33% reduction seen in annual screening with rehydrated FOBT.[13] The newer generation of more accurate and user-friendly occult blood tests methods such as fecal immunochemical testing or endoscopic methods like colonoscopy may be more effective, and their impact on CRC mortality and all-cause mortality remains an area of active research.

Our study has several limitations. First, the compliance was different in the 2 trials. To avoid this bias, to the extent possible, we use Cuzick's method, which focuses on compliers and their control group counterparts, similar to other long-term clinical trials for CRC screening.[6–28] Second, we were unable to pool or compare our findings with the individual data from the Nottingham trial, which has double the number of participants of our combined trials. Third, we do not have information on screening history of trial participants after the 2 original trials ended. CRC screening started to become widespread in the late 1990s in the United States and after

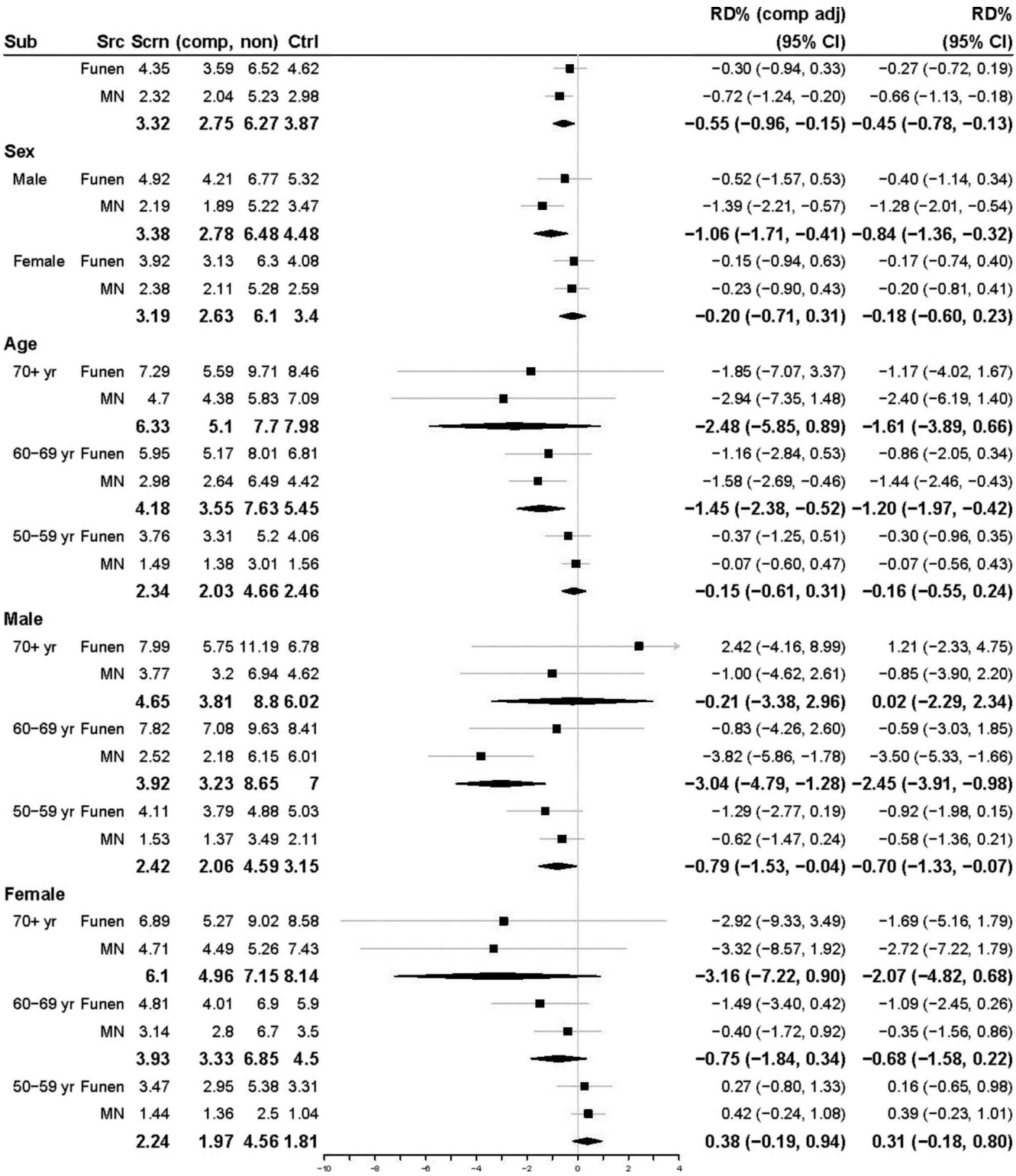

Figure 5. RD for CRC mortality by age and sex subgroups (Minnesota [MN], Funen, and combined trials).

2000 in Denmark. It is possible that since then many participants in the control group may have undergone screening and many individuals that were screened may not have undergone subsequent screening. Thus, our results show the combined 30-year effect of 9 and 6 rounds of biennial screening for the Funen and Minnesota FOBT trials, respectively, plus whatever screening and surveillance behaviors persisted after the trials ended.

In conclusion, our study shows that screening using biennial FOBT results in sustained reductions in CRC mortality and a statistically significant reduction in compliance-adjusted all-cause mortality. The reduction in CRC mortality is greater in men compared with

women, and the benefit in women lags that for men by about 10 years. We did not observe a benefit due to screening in women 50–59 years of age.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.06.019.

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Reprint Requests
Address requests for reprints to Aasma Shaukat, MD, MPH, University of Minnesota, One-Veterans Drive, 111-D, Minneapolis, Minnesota 55417. e-mail: shaukat@umn.edu; fax: (612) 725-2248.

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Conflicts of Interest
The authors disclose no conflicts.

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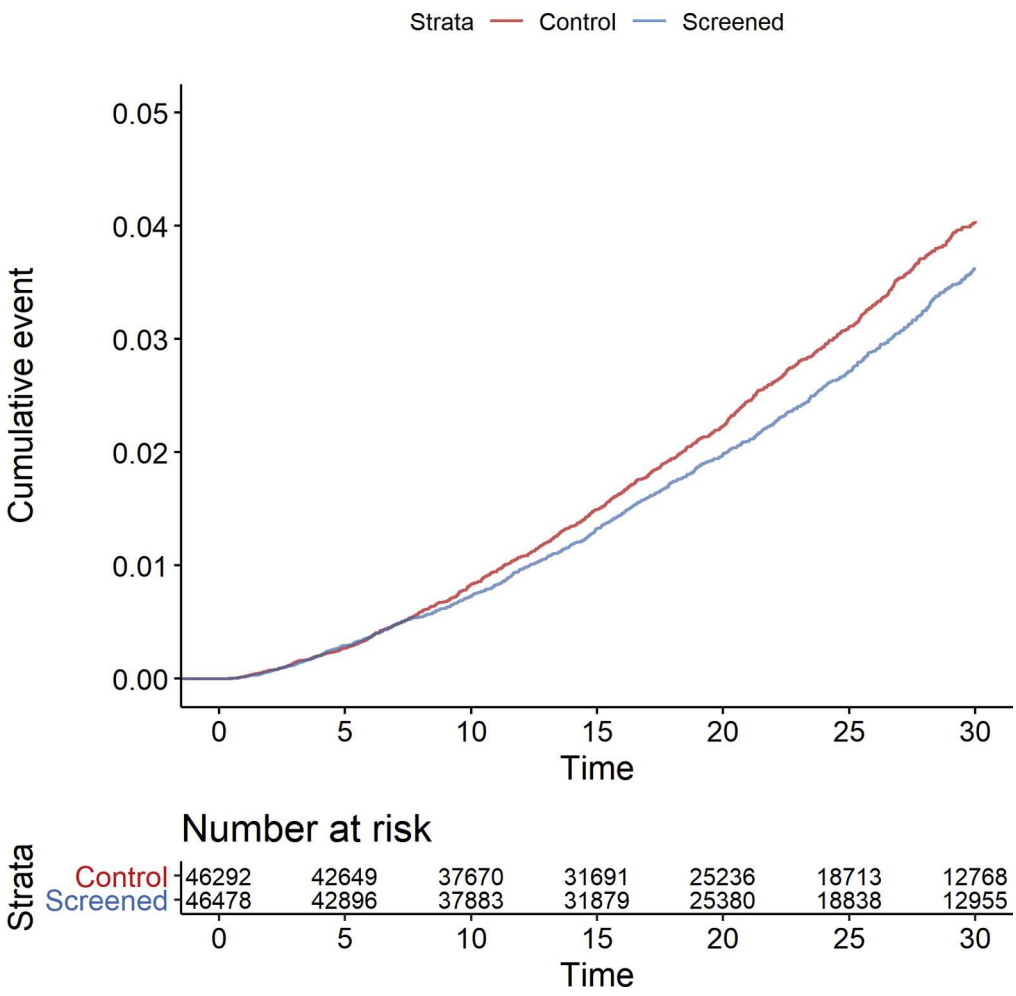
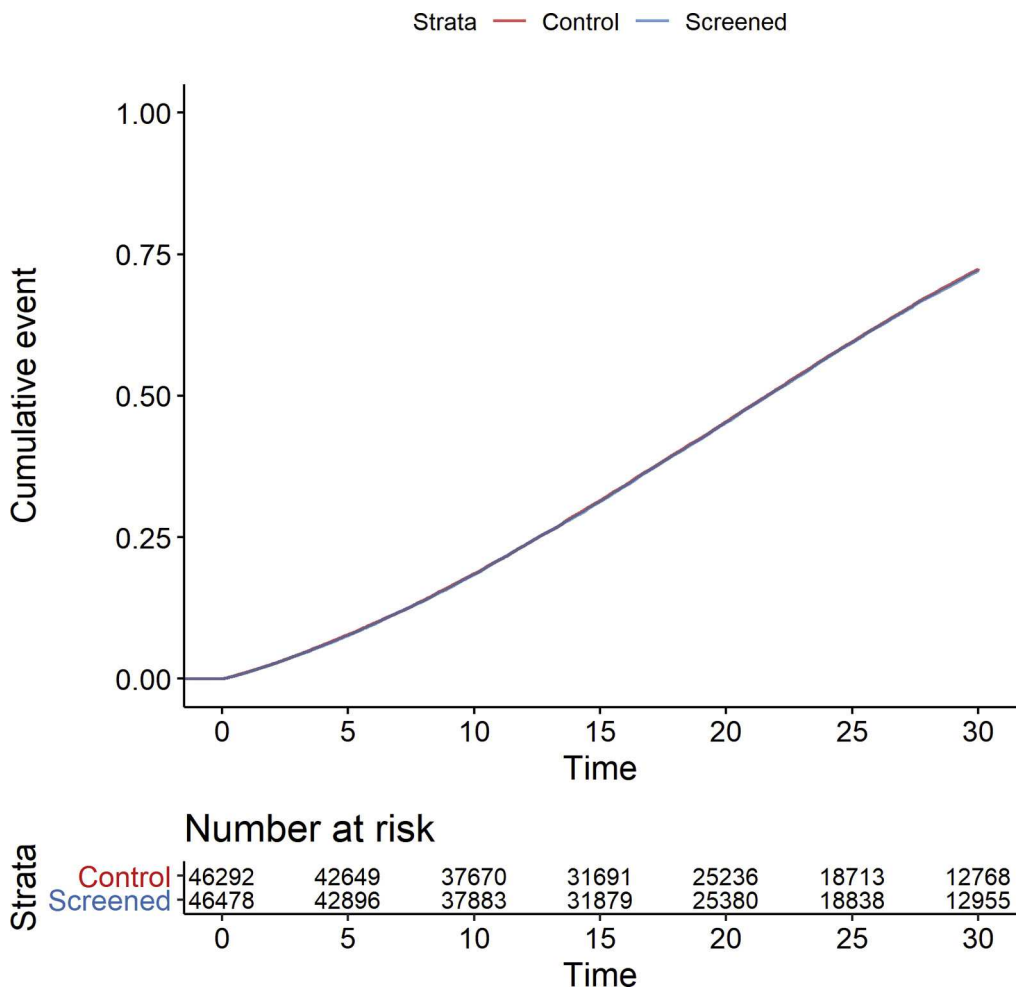


Supplementary Figure 1. Kaplan-Meier plot of combined cumulative colorectal cancer mortality (unadjusted) ($P = .03$).



Supplementary Figure 2. Kaplan-Meier plot of cumulative combined all-cause (unadjusted) ($P = .1$).

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Article

Cause of Death, Mortality and Occult Blood in Colorectal Cancer Screening

Lasse Kaalby [1,2,*], Issam Al-Najami [1], Ulrik Deding [1,2], Gabriele Berg-Beckhoff [3,4], Robert J. C. Steele [5], Morten Kobaek-Larsen [1,2], Aasma Shaukat [6,7], Morten Rasmussen [8] and Gunnar Baatrup [1,2]

1 Department of Surgery, Odense University Hospital, 5000 Odense, Denmark; Issam.Al-Najami@rsyd.dk (I.A.-N.); Ulrik.Deding@rsyd.dk (U.D.); Morten.Kobaek.Larsen@rsyd.dk (M.K.-L.); Gunnar.baatrup@rsyd.dk (G.B.)
2 Department of Clinical Research, University of Southern Denmark, 5000 Odense, Denmark
3 Unit for Health Promotion Research, Department of Public Health, University of Southern Denmark, 6700 Esbjerg, Denmark; gbergbeckhoff@health.sdu.dk
4 Unit for Health Research, Hospital South West Jutland, 6700 Esbjerg, Denmark
5 Centre for Research into Cancer Prevention and Screening, University of Dundee School of Medicine, Dundee DD1 9SY, UK; r.j.c.steele@dundee.ac.uk
6 GI Section, Minneapolis VA Medical Center and University of Minnesota, Minneapolis, MN 55417, USA; aasma.shaukat@va.gov
7 Division of Gastroenterology NYU Langone, New York, NY 10016, USA
8 Digestive Disease Center, Bispebjerg University Hospital, 2400 Copenhagen, Denmark; morten.rasmussen@regionh.dk
* Correspondence: LKM@rsyd.dk

Simple Summary: Colorectal cancer (CRC) screening participants with significant traces of hemoglobin in their stool have been reported to have higher mortality and different causes of death (other than CRC) compared to those without. We aimed to investigate these differences among screening participants after 33 years of follow-up. We confirmed that participants with detectable fecal hemoglobin were more likely to die in the study period and to die from different causes, such as cardiovascular and endocrine and hematological diseases, compared to those without detectable fecal hemoglobin. This confirms that fecal hemoglobin may have potential as a marker for diseases not directly related to the colon and rectum and may represent a target for future preventive measures.

Abstract: Fecal hemoglobin (f-Hb) detected by the guaiac fecal occult blood test (gFOBT) may be associated with mortality and cause of death in colorectal cancer (CRC) screening participants. We investigated this association in a randomly selected population of 20,694 participants followed for 33 years. We followed participants from the start of the Hemoccult-II CRC trial in 1985–1986 until December 2018. Data on mortality, cause of death and covariates were retrieved using Danish national registers. We conducted multivariable Cox regressions with time-varying exposure, reporting results as crude and adjusted hazard ratios (aHRs). We identified 1766 patients with at least one positive gFOBT, 946 of whom died in the study period. Most gFOBT-positive participants (93.23%) died of diseases unrelated to CRC and showed higher non-CRC mortality than gFOBT-negative participants (aHR: 1.20, 95% CI 1.10–1.30). Positive gFOBT participants displayed a modest increase in all-cause (aHR: 1.28, 95% CI: 1.18–1.38), CRC (aHR: 4.07, 95% CI: 3.00–5.56), cardiovascular (aHR: 1.22, 95% CI: 1.07–1.39) and endocrine and hematological mortality (aHR: 1.58, 95% CI: 1.19–2.10). In conclusion, we observed an association between positive gFOBT, cause of death and mortality. The presence of f-Hb in the gFOBT might indicate the presence of systemic diseases.

Keywords: colorectal cancer; screening; fecal hemoglobin; cause of death; mortality

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1. Introduction

Testing for fecal hemoglobin (f-Hb) in average-risk individuals is an integral part of colorectal cancer (CRC) screening programs [1,2]. Fecal samples are taken to measure f-Hb, often using the nonquantifiable guaiac fecal occult blood test (gFOBT) or the quantifiable fecal immunochemical test (FIT) [3–5]. The test result is positive if a sufficient level of hemoglobin is detected, which, for the gFOBT, is approximately 80 µg Hb/g feces [6]. A positive gFOBT result is usually followed by invitation for a diagnostic colonoscopy. F-Hb has been found to be a strong predictor of CRC and a viable initial investigation target in screening programs seeking to reduce CRC mortality [7–10]. For example, a recent study found that screening participants with a positive gFOBT had higher all-cause mortality rates and were more likely to die from causes other than CRC compared to those with a negative test. This association persisted after adjusting for possible confounding factors, such as age, sex and social deprivation [11]. A Taiwanese research group conducted a similar study among screening participants investigated with the FIT and their risk of cardiovascular disease and found an increase in incidence and mortality rates as FIT levels increased [12].

These results might indicate the possible predictive potential of f-Hb for post-gFOBT/FIT non-CRC survival. However, existing studies lack individual-level adjustment for socioeconomic status, comorbidity and long-term follow-up. Therefore, the aim of this study was to investigate the potential impact of f-Hb on mortality and cause of death by comparing gFOBT-positive and -negative individuals after 33 years of follow-up.

2. Materials and Methods

2.1. Study Population

We followed participants from the randomized controlled Hemoccult-II (HM-II) trial cohort for more than three decades using the original trial data enriched with data from national registers on health and population [13–15]. The HM-II trial enrolled participants in 1985 and 1986 from the Danish region of Funen and then conducted nine rounds of biennial gFOBT-based screening using the Hemoccult-II test, terminating in 2002. Inclusion criteria were age 45–75 and no prior history of CRC or other abdominal surgery. A total of 30,967 people were invited to submit a gFOBT stool sample in the first round of screening and were subsequently invited for colonoscopy if the test was positive. Only participants agreeing to participate in the first screening round were reinvited for subsequent rounds. Written informed consent was obtained from participants before they entered the study in 1985, and the Danish Data Protection Agency approved the study protocol. All individuals with a positive gFOBT were invited for an interview, physical examination and colonoscopy. The study is described in detail elsewhere [14]. In our study, we included all participants from the trial with one or more completed gFOBTs during the trial.

2.2. Data Sources

The trial cohort was followed for 33 years. Individual follow-up lasted from the date of inclusion in 1985–1986 and until death, emigration or the 31st of December 2018 (median: 23 years, IQR: 13.8–32.6). Follow-up was conducted using Danish national registers on health and population (Figure 1). Data from the HM-II trial were collected from the Danish National Archives. In addition, we used the Danish Register of Causes of Death (DRCD) to establish cause and time of death [16]. We extracted data from the National Patients Register to identify relevant diseases diagnosed at any Danish public hospital [17]. We used the Danish Education Register [18] and the Income Statistics Register [19] to ascertain socioeconomic status. In the HM-II trial, CRC-related deaths were reviewed using a panel of experts that reviewed all death certificates from 1985 to 2002 where doubt about the actual cause of death was present. These death records were used in the underlying period for CRC deaths. From 2002 onwards, we used the registered cause of death in the DRCD. We used the period from 1980 to 1985 to establish baseline income and conditions. We achieved complete follow-up on all participants for all outcomes but excluded some participants

due to missing data on education and income. The raw data are available in the Danish National Archives and the national registers and can be accessed by researchers.

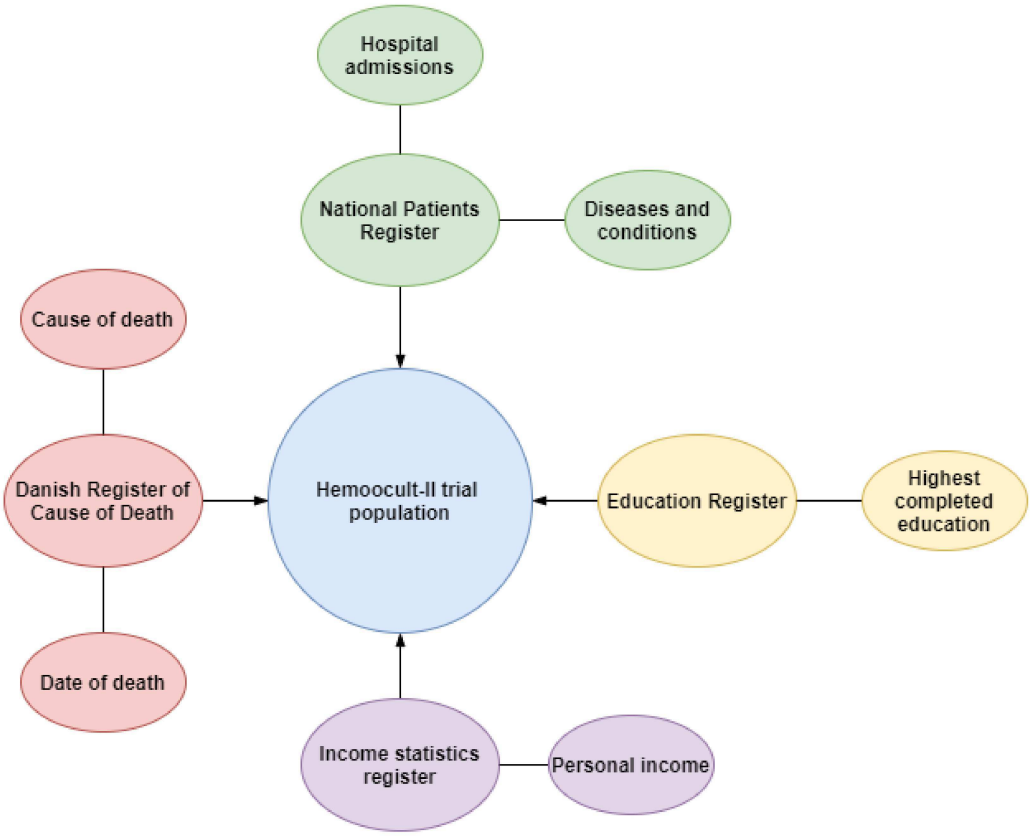

Figure 1. Data sources.

2.3. Outcome Measures

All-cause mortality and the separate causes of death are included as outcomes in this study. All causes of death were recorded using ICD-8 (1985–2001) or ICD-10 (2001–2018). We used the categories of cause of death used by Libby et al. to increase comparability [11]. This included death due to: CRC, non-colorectal cancer, cardiovascular disease, respiratory disease, digestive disease, neuropsychological disease, hematological and endocrine disorders and external causes (Table S1). External causes included accidents and other non-disease-related causes of death. We used individual-level data to follow all participants from their date of inclusion until death using the DRCD in combination with trial records. We used both the underlying and the primary contributing causes of death registered in the DRCD as our primary outcome measurements. The choice to use both was made to reduce the significance of registration errors in the DRCD [16].

2.4. Exposure and Covariate Measurements

We extracted gFOBT results from all nine rounds of screening and identified all participants with a least one positive test result. Since participants could participate in up to nine rounds of screening and have more than one positive gFOBT during the trial, we found it necessary to account for differences in the accumulated levels of exposure of each participant by introducing gFOBT results as a time-varying exposure. This allowed participants up to nine entries in the dataset with either a negative or a positive test result for each entry. Participants were included over time as a control in the screening rounds in which they tested gFOBT negative and as a case in those where they tested gFOBT positive. By doing so, we handled the individual trajectories of each participant appropriately.

Each participant underwent a baseline interview and examination from which we extracted age and sex. Age at baseline was divided into three groups "<55", "55–65" and ">65". Income and education were both included as socioeconomic status indicators. We divided income into tertiles from lowest to highest based on the five-year average annual income before the inclusion date. The highest completed education was estimated at baseline and divided into "Primary", "Secondary" and "Higher". The first category covers elementary school, the second covers high school and vocational educations and the last covers short, medium and long periods of higher education. We included known conditions and diseases suspected of affecting the result of the gFOBT by causing gastrointestinal bleeding up to five years before inclusion (Table S2). In addition, we adjusted for the effects of comorbidity from the date of inclusion and five years backwards in time using the Charlson Comorbidity Index [20].

2.5. Statistics

X^2-tests were used to compare the gFOBT-positive and -negative participants. We investigated the presence of effect modification on all outcomes for all covariates that were significant in the X^2-test. Kaplan–Meier curves were used to depict survival. We used Cox proportional hazards regression models considering positive gFOBT as time-varying exposure to estimate the crude and adjusted hazards ratios (HRs and aHRs) and their 95% confidence intervals. We conducted both univariate and multivariate analyses on all outcomes. Log–log plots were used to assess the proportional hazards assumptions, and we excluded outcomes if the assumptions were not met. For our primary analyses, we chose to only use participants with no missing data on any covariates. To investigate the impact of this decision, we also conducted a sensitivity analysis investigating whether the exclusion of all participants with missing values for education impacted our results. All analyses were performed in Stata 16.0 [21].

3. Results

3.1. Demographics

During the first round of the HM-II trial, 30,967 people were invited to submit a stool sample. We included all 20,694 (66.8%) participants in the trial who submitted a stool sample in the first round. At the end of our study period, 15,542 (75.1%) had died. They had a mean age at death of 80 (IQR, 74–87) years. Therefore, 1766 (8.5%) gFOBT-positive participants were included in the analysis, representing a total of 1866 positive gFOBTs. One hundred participants had two or more positive gFOBTs during the nine rounds of screening, with a maximum of four. A total of 40.2% of participants were 55–65 years old at inclusion. More females (52.9%) participated than males (46.1%), but more males tested gFOBT positive (54.5%) than females (45.5%). Most of our population had primary education as their highest completed education (42.1%), but many participants had missing registrations on education (27.3%). The Charlson Comorbidity Index showed that 428 participants (2.1%) scored 2 points or higher (signifying extensive comorbidity) at baseline (Table 1). We observed 8566 participants (41.4%) who participated in all nine rounds of screening. A total of 10,070 (48.0%) participants had no missing values and were eligible for analysis. Causes of death are presented in Table 2.

Table 1. Demographics stratified by gFOBT result.

	(+)ve gFOBT (*n* = 1766) N (%)	(−)ve gFOBT (*n* = 18,928) N (%)	Group Comparison
Gender			
Female	804 (45.53)	10,150 (53.62)	
Male	962 (54.47)	8778 (46.38)	<0.001
Age group at baseline			
<55	573 (32.45)	6471 (34.19)	
55–65	779 (44.11)	7543 (39.85)	
>65	414 (23.44)	4914 (25.96)	0.002
Education			
Primary	749 (42.41)	7972 (42.12)	
Secondary	402 (22.03)	4156 (21.96)	
Higher	157 (8.89)	1618 (8.55)	0.58
Missing data	458 (25.93)	5182 (27.38)	
Income			
1st tertile	554 (31.37)	6340 (33.50)	
2nd tertile	552 (31.26)	6342 (33.51)	
3rd tertile	658 (37.26)	6235 (32.94)	0.002
Missing	<10	<10	
Charlson Comorbidity Index			
0	1708 (96.72)	18,322 (96.80)	
1	24 (1.36)	212 (1.12)	
>2	34 (1.93)	394 (2.08)	0.606
Status			
Alive December 31st 2018	377 (21.35)	4775 (25.23)	
Dead December 31st 2018	1389 (78.65)	14,153 (74.77)	
Age at death (Median, IQR)	81 (75–87)	80 (74–87)	
Conditions suspected of causing bleeding at baseline			
Yes	52 (2.94)	404 (2.13)	
No	1714 (97.06)	18,525 (97.87)	0.027

Table 2. Cause of death by gFOBT result for participants with non-missing data.

	(+)ve gFOBT (*n* = 946) (%)	(−)ve gFOBT (*n* = 9122) (%)
All-cause mortality	946 (100.00)	9122 (100.00)
All-cause excl. CRC	882 (93.23)	8813 (96.59)
CRC	64 (6.45)	311 (3.41)
Non-CRC	277 (29.28)	2751 (30.17)
Cardiovascular disease	340 (35.94)	3328 (36.48)
Respiratory disease	236 (24.95)	2160 (23.67)
Digestive disease	57 (6.03)	444 (4.87)
Endocrine and hematological disease	73 (8.35)	595 (6.52)
External conditions	31 (3.28)	335 (3.67)

Abbreviations: gFOBT, guaiac fecal occult blood test; CRC, colorectal cancer.

3.2. Mortality and Cause of Death

Kaplan–Meier curves comparing gFOBT-positive to -negative participants showed a difference in mortality rate between the two groups (Figure 2). Participants with a positive gFOBT appear more likely to have died during the course of the study compared to those with a negative gFOBT.

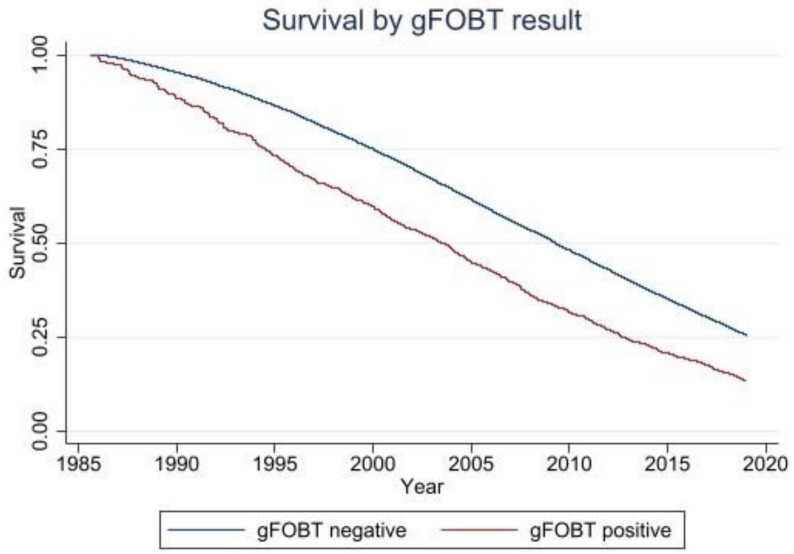

Figure 2. Survival by gFOBT result. Abbreviations: gFOBT, guaiac fecal occult blood test.

We performed Cox proportional hazards regressions on all participants with no missing values for any covariates. We investigated all nine outcomes and found significant differences between screening participants with a positive gFOBT and those with a negative gFOBT (Figure 3).

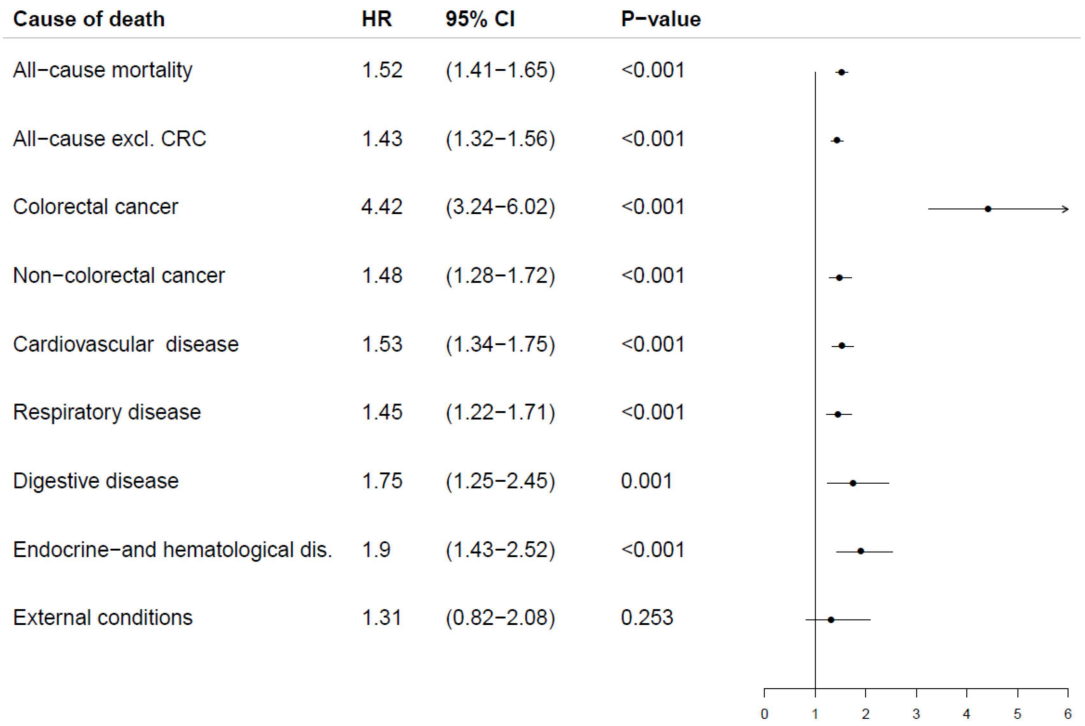

Figure 3. Cause of death and gFOBT result by univariate Cox regressions.

Multivariate analyses considering all potential covariates revealed that those testing gFOBT positive were more likely to die in the study period from all causes (aHR: 1.28, 95% CI: 1.18–1.38). We observed an association between CRC and gFOBT results in which those with f-Hb had a higher risk of dying from CRC (aHR: 4.07, 95% CI: 3.00–5.56). Furthermore, the same participants were also more likely to die from all causes, excluding those who died from CRC (aHR: 1.20, 95% CI: 1.10–1.30), and from non-colorectal cancers (aHR: 1.30, 95% CI: 1.12–1.51). Cardiovascular disease as a cause of death was associated with

f-Hb (aHR: 1.22, 95% CI: 1.07–1.39). The same was true for endocrine and hematological diseases as the underlying cause of death (aHR: 1.58, 95% CI: 1.19–2.10). We also found an association between respiratory disease as the cause of death and f-Hb (aHR: 1.19, 95% CI: 1.01–1.40). Digestive diseases also appeared to be more common as a cause of death among participants with detectable f-Hb (aHR: 1.50, 95% CI: 1.07–2.10). We did not observe any association between external conditions as a cause of death and the gFOBT result (aHR: 1.09, 95% CI: 0.69–1.74) (Figure 4). The interpretation of the log–log plots on proportional hazards assumptions led to the exclusion of neuropsychological diseases as a cause of death from our analyses.

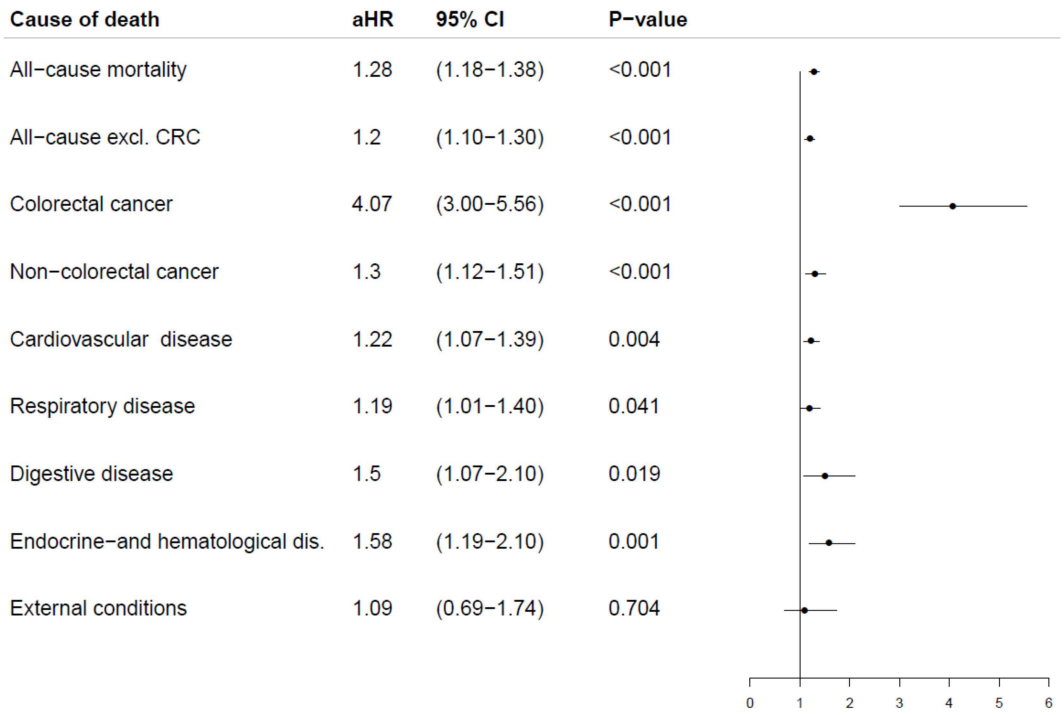

Figure 4. Cause of death and gFOBT result by multivariate Cox regressions. Adjusted for: age, gender, income, education, bleeding at baseline and comorbidity at baseline. Abbreviations: aHR, adjusted hazard ratio; CRC, colorectal cancer.

3.3. Sensitivity

We conducted a sensitivity analysis in which we included those with missing values for the educational level to see if this would affect our results. Although most of the aHRs changed slightly, only the aHR of respiratory disease became statistically insignificant (aHR: 1.13, 95% CI: 0.98–1.30) (Table S3).

4. Discussion

We investigated the association between f-Hb and mortality in a large, randomized population that, to our knowledge, represents the most extended follow-up in the current literature. Our analyses showed a modest association between detectable f-Hb and mortality that persisted after adjusting for all available confounding factors. We observed an association between f-Hb and death caused by CRC. We also found modest but significant associations between f-Hb and death from other cancers, endocrine and hematological disease, cardiovascular disease, respiratory disease and digestive diseases. Life expectancy did not appear to be significantly shorter in the gFOBT positive.

Recently, the notion that f-Hb may be an indicator for diseases other than CRC was proposed in a study by Chen et al., who found an increased risk of mortality in the population with f-HB [7]. This led to the suggestion that f-Hb may reflect serious non-CRC conditions that affect life expectancy. Findings by Libby et al. supported the results by

showing an increased non-CRC-related mortality rate among participants with a positive gFOBT. The authors also reported an association between f-Hb and some causes of death other than CRC. Correcting for medication that could cause gastrointestinal bleeding did not change the conclusions [11]. A recent study from Taiwan by Chien et al. supported the Scottish findings by concluding that f-Hb was associated with cardiovascular mortality [12]. A Korean study supported this by presenting an association between f-Hb and ischemic stroke, myocardial infarction and all-cause mortality [22]. Another Taiwanese study suggests that f-Hb is associated with oral cancer and its precursor lesions [23]. Moreover, f-Hb measured by the FIT has also been associated with cancer in the stomach, small intestine and esophagus [24].

A study by Libby et al. investigated medicine consumption as a proxy measure for disease. They found a strong association between f-Hb and prescription medication for heart disease, hypertension, diabetes and depression [25]. A potential explanation could be that f-Hb may be a surrogate marker for a number of lifestyle-related risk factors, such as a Western lifestyle, and their derived effects on changes to the microbiome. The observed association between f-Hb and increased rate of death may therefore reflect an association between lifestyle and increased mortality. The association between f-Hb and cause of death remained after adjusting for known lifestyle surrogates, income and education, and it is therefore unlikely that lifestyle factors can explain our findings alone. Another potential explanation is that the association between mortality and f-Hb is only partly understood. Libby et al. suggested that the presence of subclinical colonic inflammation could be reflected by f-Hb levels. This state may be a surrogate marker for systemic inflammation and, therefore, also a marker of pathogenesis with an inflammatory component [11]. Supporting this argument is a Taiwanese study that found an association between high f-Hb levels and inflammatory-driven metabolic syndrome [26].

Similar findings were presented by a Japanese study, further strengthening this hypothesis [27]. If later studies confirm that f-Hb may be caused by the subclinical inflammatory state, several personalized treatment measures and initiatives could become available. An example could be a newly suggested approach utilizing the anti-inflammatory potential of the cytotoxic polyacetylenic oxylipins falcarinol and falcarindiol derived from carrots. These substances inhibit the enzyme cyclooxygenase 2 (COX-2), as well as tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6), which are members of the proinflammatory cytokine cascade and have been implicated in carcinogenesis [28]. The reported associations in both our and other studies suggest that f-Hb might have clinical potential. Although additional studies are needed, it appears that f-Hb levels can be used as a biomarker for several diseases amenable to preventive measures. In a population-based screening population, participants testing positive for traces of f-Hb with no suspected cause of bleeding detected at a subsequent endoscopy could be a viable group for general follow-up diagnostic initiatives. Future studies exploring the potential of f-Hb in different clinical settings and in combination with disease-specific diagnostic or monitoring modalities are needed. A Chinese study suggested the prognostic use of f-Hb to predict complications and survival after R0 gastrectomy [29]. Another one from Scotland suggested using f-Hb measured by the FIT as a prioritization tool for endoscopic investigations in patients with iron deficiency [30]. These preoperative approaches represent targets for future studies and initiatives that could elaborate on the clinical potential of f-Hb.

The strengths of this study include a long period of follow-up in a large, randomized population, in addition to the extensive individual-level data retrieved through the Danish registers.

Limitations of our study include the potential misclassification of cases by the DRCD due to the quality of the input data. However, this was addressed by taking contributing causes of death into account for each cause of death. We also have no reason to suspect that potential misclassification is differential. The unquantifiable nature of the gFOBT also represents a weakness, as it does not allow for further stratification into f-Hb levels, which might add valuable insight into the nature of the observed association. Another limitation

is the lack of data on prescription medication, obesity and other lifestyle risk factors such as physical activity, smoking and diet.

Although we cannot directly adjust for all of these variables, we believe to have addressed a significant part of the expected effect of between-group differences in lifestyle-related factors by including income and education as proxy variables in our regression analyses. Using prescription medications, such as diabetic and antihypertensive drugs, as proxies for diseases at baseline would have been a reasonable addition to our project. However, since we already adjusted for the effects of comorbidity at baseline using hospital-registered diagnoses, we do not expect that this addition would have a substantial effect on our HRs or the interpretation of our results. Medication causing gastrointestinal bleeding, such as anticoagulants, could influence the risk of a positive gFOBT and would be relevant to adjust for in a multivariate regression model. The only available source for prescription medication when addressing our topic retrospectively is the Danish Medical Statistics Register, which began registering prescriptions in 1994, almost 10 years after our baseline. We were therefore not able to obtain this information. We did, however, adjust for the presence of diseases or conditions that may cause gastrointestinal bleeding at baseline, which should address some of the expected effects on the risk of positive gFOBT. Future prospective studies exploring the associations presented here should address the consumption of relevant drugs as a potential confounding factor. Clinical trials investigating the association between lifestyle factors and f-Hb are needed.

We have a proportion of missing data on educational registrations. We believe that this may result from the lack of registrations in the early years of the register and, in part, the age of the participants at register creation. This was addressed by completing our analysis without these participants, and it was found that it only significantly affected mortality from respiratory disease. The FIT has gradually replaced the gFOBT, which sees little clinical application today. This somewhat limits the direct translation of our results regarding modern CRC screening programs. However, due to the FIT being a recent addition to screening, obtaining a long-term follow-up using a FIT-positive population was not possible, and the gFOBT remains our best alternative.

5. Conclusions

We conducted what is, to our knowledge, the most extensive follow-up in a randomized CRC screening population, with more than three decades of follow-up time. We found a modest association between f-Hb measured by the gFOBT and death from several causes other than CRC. Our results indicate that f-Hb may reflect a (patho-) physiological state more prone to morbidity and mortality, reflected by a higher risk of death from other cancers than CRC, as well as endocrine, hematological, cardiovascular and digestive diseases. Limitations include a lack of information on diet, lifestyle, body mass index and prescription medication. Studies to clarify the role of f-Hb and its association with specific diseases are needed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cancers14010246/s1, Table S1: Causes of death and the corresponding ICD-8 and ICD-10 classification codes; Table S2: Diseases and indications potentially contributing to digestive bleeding; Table S3: Cause of death, allowing for missing data on education and income

Author Contributions: L.K., I.A.-N. and G.B. conceptualized the study. Data curation was performed by L.K. and U.D. L.K., U.D. and G.B.-B. completed the formal analysis. L.K. and G.B. obtained funding for the project. L.K., I.A.-N., U.D., G.B.-B., R.J.C.S., M.K.-L., A.S., M.R. and G.B. developed the methodology. L.K. handled project administration. Supervision was conducted by G.B. Writing of the original draft was performed by L.K. and supported by I.A.-N. All authors contributed to the review and editing process. U.D. had access to and verified the underlying data. All authors have read and agreed to the published version of the manuscript.

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Supplementary

Supplementary tables

Table S1. Causes of death and the corresponding icd-8 and icd-10 classification codes.

Cause of death	ICD-8 classification	ICD-10 classification
Colorectal cancer	153-154	C18-C20
Non-colorectal cancer	140-152, 155-209	C00-C17, C21-C99
Cardiovascular disease	390-458	I00-I99
Respiratory disease	460-519	J00-J99
Digestive disease	520-577	K00-K99
Endocrine-and hematological disease	280-289, 240-258	D00-D99, E00-E99
Neuropsychological disease	290-358	G00-G99
External conditions	800-999	S00-S99, T00-T99, U00-U99, V00-V99, W00-W99, X00-X99, Y00-Y99, Z00-Z99

Abbreviations: ICD, International Classification of Disease.

Table S2. Diseases and indications potentially contributing to digestive bleeding .

Diseases And Indications	ICD-8 classification	ICD-10 classification
Hemorrhage of anus and rectum	-	K625-K626
Inflammatory Bowel Disease	561, 563	K50-K52
Diverticular disease	562	K57
Hemorrhoids	455	I84
Colorectal fissures	565	K60
Gastrointestinal ulcers	531–534	K25–K28
Gastritis	535	K29

Abbreviations: ICD, International Classification of Disease.

Table S3. Cause of death, allowing for missing on education and income.

	Positive gFOBT (*n* = 1,389)	Negative gFOBT (*n* = 14,153)	HR (95% CI)	*P*-value	aHR (95% CI)*	*P*-value
All-cause mortality	1389 (100.00)	14,153 (100.00)	1.46 (1.37–1.56)	<0.001	1.25 (1.17–1.34)	0.000
All-cause excl. CRC	1286 (92.58)	13,722 (96.95)	1.35 (1.26–1.45)	<0.001	1.16 (1.09–1.25)	0.000
Colorectal cancer	103 (7.42)	431 (3.05)	5.21 (4.10–6.63)	<0.001	4.48 (3.76–6.08)	<0.001
Non-colorectal cancer	353 (25.41)	3729 (26.35)	1.41 (1.24–1.61)	<0.001	1.24 (1.09–1.42)	0.001
Cardiovascular disease	567 (40.82)	5948 (42.03)	1.48 (1.34–1.64)	<0.001	1.24 (1.12–1.37)	0.000
Respiratory disease	323 (23.25)	3264 (23.06)	1.34 (1.16–1.54)	<0.001	1.13 (0.98–1.30)	0.091
Digestive disease	78 (5.62)	695 (4.91)	1.15 (1.56–2.04)	0.003	1.33 (1.00–1.78)	0.048
Endocrine-and hemato-logical disease	110 (7.20)	981 (6.93)	1.64 (1.30–2.07)	0.000	1.42 (1.12–1.79)	0.003
External conditions	46 (3.31)	548 (3.87)	1.16 (0.80–1.70)	0.435	1.01 (0.69–1.48)	0.964

*__Adjusted for__: Age, gender, income, education, bleeding at baseline, comorbidity at baseline. **Abbreviations**: gFOBT, guaiac fecal occult blood test; HR, Hazard Ration; aHR, adjusted Hazard Ratio NB. Each participant may occur with more than one cause of death in this table.