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Aspects of pre-diagnostic physical activity in colorectal cancer

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Abstract

Background

Pre-diagnostic physical activity may lower the risk for developing colon cancer and is potentially associated with improved prognosis when diagnosed with colorectal cancer.

Body composition, *i.e.* greater muscle mass and quality, is also associated with improved prognosis in colorectal cancer. Physical activity in middle age prevents or at least delays the development of progressive loss of skeletal muscle mass, strength and function in later years, a condition called sarcopenia.

Immune cell infiltration of the tumour microenvironment is another factor that affects prognosis in colorectal cancer. Physical activity seems to mobilise immune cells into the circulation and peripheral tissues including tumours. Different immune cells react differently to physical activity.

The physical activity guidelines are the same in colorectal cancer patients as in the healthy population and the national health care programme for colorectal cancer in Sweden recommends individualised support for physical activity to all patients. Maintenance of physical activity levels in already physically active individuals might be easier to support than induction of new behaviours in sedentary individuals.

Methods

Studies I and II are retrospective population-based cohort studies matching colorectal cancer cases with the patient's self-reported data on physical exercise. In Study I, physical exercise was matched with immune cell infiltration of cytotoxic T cells and the total T cell population. In Study II, physical exercise was matched with body composition. The combined effect of pre-diagnostic physical exercise and body composition on the prognosis of colorectal cancer was also studied.

Study III is a prospective cohort study using accelerometer data, dual-energy X-ray absorptiometry (DXA) scanning, and physical tests to assess the physical activity, fitness, and body composition of patients diagnosed with colon cancer (Stages I-III) and awaiting curative surgery.

Immune cell infiltration of the tumour microenvironment was assessed using multiplex immunohistochemistry for immune cell types T-helper 1 cells, cytotoxic T cells, regulatory T cells, B cells, and macrophages.

The thesis also included one qualitative interview study examining the attitudes and experiences of physically active individuals diagnosed with colon cancer regarding physical activity while awaiting surgery.

Results

Study I: Pre-diagnostic physical exercise more than three times a week was associated with increased numbers of cytotoxic T cells in the tumour front and centre. The total T cell population was not affected.

Study II: Low pre-diagnostic physical exercise was not associated with sarcopenia (low muscle mass) nor myosteatosis (low muscle quality) at the time of colorectal cancer diagnosis. The combined presence increased the cancer-specific mortality risk.

Study III: After adjusting for multiple testing no significant results were seen either in the descriptive statistics or in the logistic regression model adjusted for age and sex.

Study IV: When analysing experiences and attitudes among physically active individuals diagnosed with colon cancer towards physical activity while awaiting surgery, three main categories were abstracted: “I’ll fight the cancer and come out stronger”; “the diagnosis makes no difference”; and “the cancer is an obstacle for physical activity”.

Conclusions

Self-reported physical exercise may be associated with increased numbers of cytotoxic T cells in the microenvironment of colorectal cancer. However, this was neither confirmed nor rejected when assessing physical activity and fitness more objectively in a smaller cohort of colon cancer patients.

Little physical exercise in middle age was not associated with sarcopenia or myosteatosis at the time of colorectal cancer diagnosis but when present together, the cancer-specific mortality risk was increased.

Physically active individuals have a wide spectrum of attitudes and experiences toward physical activity when diagnosed with colon cancer. Physically active individuals with a diagnosis of colon cancer may use physical activity as a coping strategy while awaiting surgery.

Summary in Swedish

Introduktion

Fysisk aktivitet är troligen associerat med lägre risk att drabbas av tjocktarmscancer samt möjligen förbättrad prognos om man drabbats av tjock- eller ändtarmscancer. Hur och varför fysisk aktivitet påverkar uppkomsten av, samt prognosen vid tjock- eller ändtarmscancer är inte klarlagt vare sig i djurstudier eller kliniska studier på människor.

Sarkopeni, innebär förlust av muskelmassa och muskelstyrka som framförallt drabbar äldre personer. Myosteatos innebär fettinfiltration i muskel, och generellt sett gäller att ju mer fett som finns i en muskel desto sämre är kvaliteten på den muskeln. Både sarkopeni och myosteatos hos en patient med tjock- eller ändtarmscancer försämrar prognosen.

Immuncellsinfiltration i tumören samt dess mikromiljö är ytterligare en faktor som påverkar prognosen vid tjock- och ändtarmscancer. Vid fysisk ansträngning mobiliseras kroppens immunförsvar till blodcirkulationen och i djurstudier finns det resultat som pekar på att immunceller kan mobiliseras till tumörvävnad vid upprepad fysisk aktivitet.

I det nationella vårdprogrammet för tjock- och ändtarmscancer rekommenderas att alla patienter ska erhålla individanpassad stöttning för fysisk aktivitet. Redan fysiskt aktiva patienter som drabbas av en tjocktarmscancerdiagnos är i teorin enklare att motivera till fortsatt fysisk aktivitet än stillasittande individer. Hur dessa, redan fysiskt aktiva individer, upplever att den fysiska aktiviteten påverkas vid tjocktarmscancerdiagnos är inte tidigare kartlagt.

Metod

Den här avhandlingen består av fyra delarbeten där studie I och II är retrospektiva studier där personer med tjock- eller ändtarmscancer från svenska cancerregistret samkördes med Västerbottenkohorten. I studie I undersöktes sambandet mellan självrapporterad fysisk träning och immuncellsinfiltrationen av två cellmarkörer (CD8 markör för cytotoxisk T cell och CD3 generell T cellsmarkör). I studie II undersöktes sambandet mellan självrapporterad fysisk träning och förekomst av

sarkopeni och/eller myosteatos vid diagnos av tjock- eller ändtarmscancer.

Studie III är en studie där individer diagnostiserade med icke spridd tjocktarmscancer tillfrågades att delta. Inkluderade patienters fysiska aktivitet, form/fitness och kroppssammansättning analyserades med accelerometer, fysiska tester och bentäthetsmätning innan planerad operation. Immuncellsinfiltrationen av fem olika celltyper undersöktes med en metod som möjliggör analys av flera immuncelltyper samtidigt.

Studie IV är en kvalitativ intervjustudie där analysmetoden innehållsanalys användes. Erfarenheter och attityder gentemot fysisk aktivitet hos sedan tidigare fysiskt aktiva individer nyligt diagnostiserade med icke spridd tjocktarmscancer undersöktes.

Resultat

Studie I: Fysisk träning innan diagnos mer än tre gånger i veckan var associerat med ökad förekomst av cytotoxiska T celler i tumörernas omgivning. Ingen association sågs för den totala T cellpopulationen.

Studie II: Fysisk träning innan diagnos var inte associerat med förekomst av vare sig sarkopeni eller myosteatos vid diagnos av tjock- eller ändtarmscancer. Risken för cancerspecifik dödlighet var högre hos individer som både hade låg fysisk träning innan diagnos samt förekomst av antingen sarkopeni eller myosteatos jämfört med ensam förekomst av antingen låg fysisk träning eller sarkopeni/myosteatos.

Studie III: Efter statistisk korrigerings för många tester kvarstod inga statistiskt signifikanta associationer mellan immuncellsinfiltration och fysisk aktivitet, form/fitness eller muskelmassa.

Studie IV: Tre huvudkategorier som representerade skillnader i erfarenheter och attityder gentemot träning hos fysiskt aktiva individer diagnostiserade med tjocktarmscancer kom fram vid analysen av telefonintervjuerna i studie IV. De tre huvudkategorierna var

- 1) Jag ska bekämpa cancer och komma ut starkare
- 2) Diagnosen gör ingen skillnad
- 3) Diagnosen är ett hinder för fysisk aktivitet.

Sammanfattning

Självrapporterad fysisk träning kan vara associerat med ökad densitet av cytotoxiska T celler i tjock- och ändtarmstumörers omgivning. Vid mer objektiv analys av fysisk aktivitet på en mindre patientpopulation med tjocktarmscancer såg vi inga övertygande resultat vare sig för ökad eller minskad infiltration av de fem analyserade immuncellerna.

Låg självrapporterad fysisk träning innan diagnos var inte associerat med sarkopeni eller myosteatos vid diagnos av tjock- eller ändtarmscancer men vid förekomst tillsammans var den cancerspecifika mortalitetsrisken ökad.

Fysiskt aktiva individer har ett brett spektrum av attityder och erfarenheter gentemot fysisk aktivitet vid diagnos av tjocktarmscancer. Fysisk aktivitet kan möjligen användas för att mentalt hantera tjocktarmscancerdiagnosen och den stundande kirurgin hos fysiskt aktiva individer nyligen diagnostiserade med tjocktarmscancer.

List of Papers

This thesis is based on the following publications and manuscript. They are referred to in the text by their roman numerals (I-IV).

- I. Renman D, Gylling B, Vidman L, Bodén S, Strigård K, Palmqvist R, Harlid S, Gunnarsson U, Van Guelpen B.
Density of CD3+ and CD8+ cells in the microenvironment of colorectal cancer according to pre-diagnostic physical activity.
Cancer Epidemiol Biomarkers Prev. 2021 Dec;30(12):2317-2326.
- II. Renman D, van Guelpen B, Anderson F, Axelsson J, Riklund K, Strigård K, Palmqvist R, Gunnarsson U, Gylling B.
Association of pre-diagnostic physical exercise and peri-diagnostic body composition with mortality in non-metastatic colorectal cancer.
Int J Colorectal Dis. 2023 Sep 27;38(1):239
- III. Renman D, Edin S, Gunnarsson U, Blind N, Strigård K, Palmqvist R.
Association of objectively assessed physical activity with immune cell infiltration of the tumor microenvironment in non-metastatic colon cancer.
Submitted.
- IV. Renman D, Strigård K, Palmqvist R, Näsval P, Gunnarsson U, Edin-Liljegren A. Attitudes to and Experiences of Physical Activity After Colon Cancer Diagnosis Amongst Physically Active Individuals - A Qualitative Study.
Cancer Control 2022 Jan-Dec:29:10732748221119352

Abbreviations

2MST – 2-minute step test
6MWT – 6-minute walk test
ANOVA – One-way analysis of variance
ASMI – Appendicular skeletal muscle index
BMI – Body mass index
CCI – Comprehensive complication index
CD – Cluster of differentiation
CIMP – CpG island methylator phenotype
CIN – Chromosomal instability
CMS – Consensus Molecular Subtypes
CPET – Cardiopulmonary exercise testing
CRP – C-reactive protein
CT – Computed tomography
CTLA-4 – Cytotoxic T lymphocyte-associated antigen 4
CUP – Continuous Update Project
DAB - 3,3'-Diaminobenzidine
DAMP – Danger-associated molecular patterns
DNA – Deoxyribonucleic acid
DXA – Dual-energy X-ray absorptiometry
EGFR – Epidermal growth factor receptor
ERAS – Enhanced recovery after surgery
EWGSOP – European Working Group on Sarcopenia in Older People
FoxP3 – Forkhead box P3
HDI – Human development index
HR – Hazard ratio
HRP – Horseradish peroxidase
HU – Houndsfield unit
IBD – Inflammatory bowel disease
ICD – International statistical classification of disease and related health problems
ICFSR – International Practice Guidelines for Sarcopenia
ICC – Intra class correlation
IFN – Interferon
IL – Interleukin
IL6R – Interleukin 6-receptor
IMAT – Intermuscular adipose tissue
IMCL – Intramyocellular lipids
IWGS – International Working Group on Sarcopenia
MAR – Missing at random

MCAR – Missing completely at random
MET – Metabolic equivalents
MMR – Mismatch repair
MNAR – Missing not at random
MONICA – Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease
MSP – Mammography Screening Project
MVPA – Moderate to vigorous physical activity
MRI – Magnetic resonance imaging
Nm – Newton meter
NK – Natural killer
NSHDS – Northern Sweden Health and Disease Study
OR – Odds ratio
PACOS - Physical Activity and its impact on Colon cancer Surgery
PD-1 – Programmed cell death protein 1
PD-L1 – Programmed cell death ligand 1
PMI – Psoas muscle index
RA – Rheumatoid arthritis
RCT – Randomized controlled trial
RR – Relative risk
SBRN – Sedentary Behaviour Research Network
SMD – Skeletal muscle density
SMI – Skeletal muscle index
TAM – Tumor associated macrophages
TFN – Tumor necrosis factor
TGF – Transforming growth factor
Th1 – T-helper cell 1
Th2 – T-helper cell 2
Th17 – T-helper cell 17
TMA – Tumor microarray
TNM – Tumor Node Metastasis
TUG – Timed up and go test
VIF – Variance inflation factor
VISTA – V-domain Ig suppressor of T cell activation
VO₂-max – Maximum oxygen consumption
WHO – World Health Organization

Introduction

Physical activity, exercise and fitness

Physical activity, exercise, and physical fitness are terms with similar but separate definitions. Physical activity is defined as any physical movement conducted during a defined period of time. Physical exercise is a subcategory of physical activity that is planned, structured, with the aim of improving fitness and/or well-being. Physical fitness is rather the result of physical activity and may be expressed as cardiorespiratory fitness, muscle strength, or flexibility (1). The terms sedentary behaviour and physical inactivity can also cause confusion. The Sedentary Behaviour Research Network (SBRN) has defined the two terms with distinct differences in meaning. Sedentary behaviour is defined as “*any waking behaviour characterized by an energy expenditure of ≤ 1.5 metabolic equivalents (MET) while in a sitting or reclining posture*”. Physical inactivity on the other hand describes those who do not meet the physical activity guidelines specified (2).

Since ancient times, physical activity and fitness have been linked to health and well-being. There are descriptions of organised health promotion as far back as ancient China, around 2500 BC. The father of modern medicine, Hippocrates (Fig 1), saw the health and pathogenic process as an equilibrium between the internal bodily fluids and external relationship between the person and the environment (3). The preservation of health was seen as a balance between activity and rest, and between nutrition and excretion. Hippocrates was one of the first who stated that physical activity and fitness may be connected with good health: “*all parts of the body which have a function, if used in moderation and exercised in labours in which is accustomed, become therefore healthy, well-developed and age more slowly, but if unused and left idle they become liable to disease, defective in growth and age quickly*”. Hippocrates, and many with him, may have linked physical activity with health but it took almost 2500 years until 1953 when Morris *et al* reported that conductors on London double-decker buses had less than half the incidence of heart attack as sedentary drivers of the same buses (4). A few years later Taylor *et al* examined two-year mortality amongst US railroad employees. They noticed a lower mortality rate among the most active group (section men) compared to the least active group (clerks) (5). These early papers were not free of bias, for example, one could argue that leaner and healthier men are more likely to be employed for

physically demanding work than obese men. Furthermore, possible confounders such as smoking, obesity, and diabetes were not accounted for. Since then, much research has been conducted regarding physical activity and its relationship to health and disease.

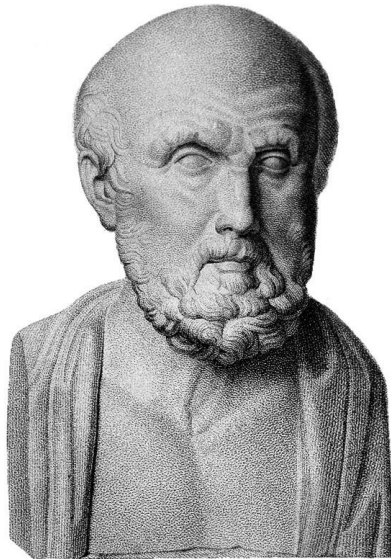


Figure 1. Bust of Hippocrates. Work ID: nndm46ad, from the Wellcome Collection, licensed under Creative Commons BY 4.0.

Low levels of physical activity have been linked with several diseases such as diabetes type 2, cancer, cardiovascular disease, and depression (6). Both questionnaire- and accelerometer-assessed physical activity have been associated with lower overall mortality risk (7, 8). There is a dose-response association between more physical activity (regardless of intensity) and less sedentary time with lower overall mortality risk (8). The dose-response curve is not linear and the greatest effect on overall mortality seems to lie between the first and second quartiles of both overall physical activity, moderate-to-vigorous physical activity (MVPA), and light physical activity (8). Regarding sedentary time and mortality, a previous meta-analysis investigated sedentary time in a population of one million and concluded that those who were not physically active and sat more than eight hours a day had a 27% higher overall mortality risk than those who sat less than four hours. Interestingly, these differences were attenuated by physical activity (9). Daily and lifetime physical

activity varies and can be divided into categories such as occupational, leisure time, and commuting.

Physical activity is associated with several health benefits and the World Health Organization (WHO) recommends that all adults should conduct at least 150-300 minutes of moderate intensity or 75-150 minutes vigorous intensity aerobic physical activity each week, and muscle-strengthening activities twice a week (6). Physical activity may reduce the risk for several cancers including breast, bladder, colon, endometrial, oesophageal, and gastric (10, 11). Physical activity before and after cancer diagnosis, may also be associated with improved survival in breast and colorectal cancer (12). At the time of colorectal cancer, pre-diagnostic physical activity may reduce cancer-specific mortality risk by approximately 15%-20%, while post-diagnostic physical activity conveys a somewhat larger reduction (12, 13, 14). The reason for the beneficial effects of physical activity on the outcome of colorectal cancer is not known and several mechanisms have been proposed such as affecting the insulin-growth factor axis (15), improving immune system function and the tumour microenvironment (16), and an increase in adiponectin (17).

Physical activity might be associated with improved outcome in colorectal cancer and can be recommended generally and to all patients both before and after diagnosis as well as while awaiting surgery. The national healthcare programme for colorectal cancer in Sweden recommends that all patients should receive individualised support for physical activity (18).

Epidemiology and risk factors of colorectal cancer

Colorectal cancer is the third most common cancer worldwide but the second most common in terms of mortality. Colorectal cancer accounts for 1.9 million of 20.0 million cancer cases and 0.9 million of 9.7 million cancer deaths worldwide (19). There are regional differences in the incidence corresponding to the human development index (HDI) explained by different environmental risk factors with higher risks in high HDI countries compared to low (19). The variation in mortality is not as prominent between regions due to higher mortality in low HDI countries (19). In Sweden, around 5,300 new cases of colon cancer, and 2,400 new cases of rectal cancer were diagnosed in 2023 (20, 21). This corresponds to roughly one in nine patients with a newly diagnosed malignancy. Colorectal cancer is also the second most common cause of

cancer-related death in Sweden. Median age when diagnosed in Sweden 2023 was 75 years for colon and 72 for rectal cancer (20, 21) and only 6% of colorectal cancer cases are younger than 50 years when diagnosed (18). However, the incidence of early onset (younger than 50 years) colorectal cancer is rising in high HDI countries (19).

There is no single environmental factor that solely or greatly increases the risk for colorectal cancer but there are several factors that have been observed to affect the risk at the populational level (Fig 2).

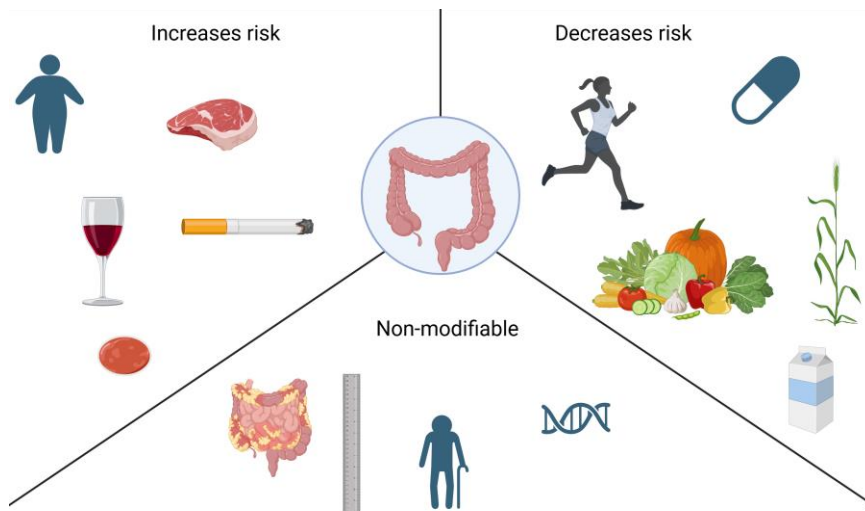


Figure 2. Factors involved in colorectal cancer risk. Increases risk: Obesity, red meat, processed meat, alcohol, and tobacco use. Decreases risk: physical activity, wholegrain, dietary fibre, dairy products, calcium supplements, and hormonal replacement therapy in women. Non-modifiable: age, family history of colorectal cancer, height in adulthood, and history of inflammatory bowel disease. Created with BioRender.com

The Continuous Update Project (CUP) reviews evidence from randomized and prospective cohort studies on different site-specific cancers. CUP published its first report in 1997. Since then, two reports have been published in 2007 and 2018, the latest providing data on diet, nutrition and physical activity in colorectal cancer (22). The report concludes that there is strong and convincing evidence that processed meat (>50g/day), alcohol consumption (>30 grams/day), body fatness, and height in adulthood increase the risk for colorectal cancer. There is further strong and probable evidence that consumption of red meat (>100g/day) increases the risk for colorectal cancer. The report also included factors that decrease the risk where physical activity was the

only factor with strong and convincing evidence for lowering the risk of colon cancer. Consumption of wholegrains (>90g/day), dietary fibre (>10g/day), dairy products, and calcium supplements may also decrease the risk for colorectal cancer however the evidence are strong but probable (22). Apart from factors in the CUP report, further risk factors for colorectal cancer include age, tobacco use, family history of colorectal cancer, history of inflammatory bowel disease, and history of diabetes (23, 24). The use of hormone replacement therapy in postmenopausal women (25) may reduce the risk.

The CUP published a report on cancer survivors in 2024 where they reviewed the evidence on diet, nutrition and physical activity after colorectal cancer diagnosis (14, 26). As stated above, physical activity before and after diagnosis may improve prognosis. In the CUP report, recreational physical activity was the only factor that was associated with lower overall mortality risk, cancer-specific mortality risk, and cancer recurrence risk (14). Other factors that were associated with lower overall mortality risk were high intake of wholegrain and coffee and low intake of sugary drinks (26). However, the level of evidence was low, based on observational studies only, so a causal relationship between these factors and prognosis after colorectal cancer diagnosis is not supported (14, 26).

Colorectal cancer and its subtypes

Colorectal cancer is a heterogenous disease with different molecular patterns and expressions. Almost all colorectal cancers develop from benign adenomas, a process that takes around 10-20 years (Fig 3). There are different molecular pathways leading to this transformation. In 1990, a model for the development of colorectal cancer from an adenoma was described by Fearon and Vogelstein (27). The model described accumulation of mutational changes in adenomas driven by chromosomal instability (CIN). In the CIN pathway, several genomic alterations lead to activation of oncogenes and silencing of tumour suppressor genes. The CIN pathway, or microsatellite stable pathway, is the most common molecular pathway in which an adenoma transforms into colorectal cancer (27). Around 15% of sporadic colorectal cancers have deficient mismatch repair (MMR) genes and are referred to as microsatellite instable. This is a different molecular pathway from CIN where inactivation of an MMR gene/-s results in errors in short repetitive DNA sequences, called microsatellites (28). In microsatellite instable tumours, these mutations drive the pathogenesis and

development of colorectal cancer in an adenoma (28). Microsatellite instability tumours are associated with a higher intratumour immune response, right-sided location, and treatment effect of checkpoint inhibitors (29).

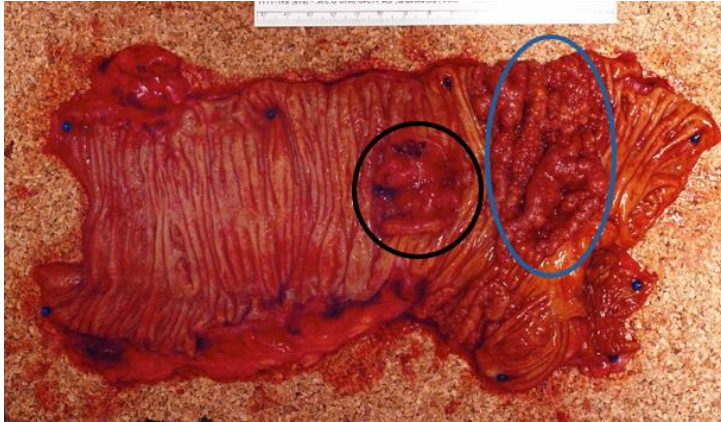


Figure 3. Specimen of colon showing an adenoma, indicated in blue, and adenocarcinoma, indicated in black. Courtesy of Karin Strigård.

DNA has four bases, adenine (A), thymine (T), cytosine (C), and guanine (G). Around 50% of all human genes have a promoter region rich in CpG dinucleotide called CpG islands. The CpG islands can be methylated leading to altered expression of the gene encoded after the promoter region (30). The CpG island methylator phenotype (CIMP) is another molecular pathway in colorectal cancer. CIMP leads to inactivation of tumour suppressor genes and can thereby lead to the development of colorectal cancer (31). There is an association between microsatellite instability, CIMP, and *BRAF*-mutation, and many sporadic microsatellite instable tumours are *BRAF*-mutated and highly methylated (CIMP-high) (32).

KRAS and *NRAS* are members of the *RAS* gene family. *KRAS* is an oncogene that, when mutated, increases the cancer cell potential to metastasise. In metastatic colorectal cancer, the *KRAS*-gene is mutated in approximately 40% of cases. *KRAS* mutations are more frequent in right-sided cancers, and its clinical implication is the predictive efficacy of treatment with epidermal growth factor receptor (EGFR) inhibitors. Anti-EGFR treatment is only recommended for colorectal cancers with wild-type *RAS* (non-mutated *KRAS* and *NRAS*) (33). *BRAF* mutations are rare (14% in patients with Stages II-III and 8% in metastatic disease) and more frequent in right-sided cancer and female patients. *BRAF*

mutation is associated with a poor prognosis, especially in metastatic disease (33). Assessment of *BRAF* mutation is also important in cases with microsatellite instability since the presence of mutated *BRAF* and microsatellite instability favours the plausibility of sporadic pathogenesis and not the Lynch Syndrome (33).

In routine clinical practice, molecular pathology testing should be available for testing of at least microsatellite stability and *NRAS*, *KRAS*, and *BRAF* mutations (33). Testing of microsatellite stability involves assessing the four MMR proteins (MLH1, MSH2, PMS2, and MSH6) immunohistochemically or DNA testing (33).

Since our understanding of the molecular characteristics of colorectal cancer is constantly improving, the Colorectal Cancer Subtyping Consortium in 2015 proposed a new molecular classification of colorectal cancer with four biologically different subtypes called Consensus Molecular Subtypes (CMS)(34). CMS1 tumours generally have enriched immune/inflammatory genes with higher immune cell infiltration, are to a higher extent right-sided, associated with CIMP-high, *BRAF* mutations, and microsatellite instability. CMS2 tumours, the most common sub-type, are usually left-sided and have better survival after relapse. CMS3 tumours are associated with *KRAS* mutations, are CIMP-low, and have upregulation of genes associated with metabolism. Finally, CMS4 tumours have more mesenchymal gene expression and have worse overall and disease-free survival (34).

Exercise prehabilitation in colorectal surgery

Prehabilitation is a term referring to interventions prior to surgery that aim to improve the ability of the patient to recover postoperatively. Prehabilitation can be unimodal but is often multimodal with various processes or interventions initiated during the limited time between diagnosis and surgery. Interventions vary and the term prehabilitation is poorly defined. Some common prehabilitation interventions include physical exercise, psychosocial, dietary, and drug optimisation (35). Exercise prehabilitation indicates that exercise is a part of the prehabilitation programme, either alone or combined with other interventions. Prehabilitation research suffers from great heterogeneity with a wide range of interventions, varying duration of interventions, outcome measures, diagnoses, and patient demographics making it challenging to interpret and draw conclusions from (35). Many studies on prehabilitation have either been feasibility studies or have used

functional fitness as their primary outcome measure, often measured in meters walked in the 6-minute walk test (6MWT). Therefore, many studies are underpowered to detect differences in clinical parameters such as complications or length of hospital stay.

In colorectal surgery, the first randomised controlled trial (RCT) on a preoperative exercise programme was published in 2010 by a research group in Montreal, Canada (36). The study included 112 participants randomised either to a four-week exercise group with cycling exercise and resistance training or to a sham intervention control group doing walking and breathing exercises only. The study showed no difference in the primary outcome measure, distance walked in the 6MWT, between the two groups either preoperatively or four weeks postoperatively. The authors described this as an unexpected finding and one explanation offered was low adherence to the exercise programme in the intervention group (36). In 2018, Barberian-Garcia *et al* reported results from an RCT on participants defined as frail and undergoing prehabilitation before major abdominal surgery. Of the 125 patients in the intention-to-treat analysis, 71 (57%) underwent colorectal surgery. The participants were randomised to either a multimodal prehabilitation scheme for at least four weeks (mean six weeks) including supervised high-intensity exercise and a personalised exercise programme, or to a control group receiving standard care. The primary outcome was postoperative complication defined as any deviation from the normal postoperative course. The intervention group suffered 50% fewer complications. However, no effect was seen on complication severity according to Clavien-Dindo (37). In 2023, a Cochrane review on multimodal prehabilitation before colorectal surgery was published (38) including three RCTs including 250 participants, all published by the same Montreal research team mentioned above (39, 40, 41). Even though the same research group conducted all three studies there were heterogeneity between the three studies. All studies reported 6MWT outcome and the pooled analysis found no difference in postoperative 6MWT between the intervention and control groups, but a minor difference could be seen with a greater distance walked in the prehabilitation group prior to surgery. Regarding postoperative complications no differences were seen (38). In 2023, Molenaar *et al* (42) published an RCT on multimodal prehabilitation in colorectal cancer surgery including 250 participants with an intervention length of four weeks. The prehabilitation group showed lower postoperative complication rates, mainly medical complications, as well as better functional capacity. Another RCT from 2022 by Berkel *et al* (43) investigated the effects of a three-week home-based exercise programme

in 57 frail colorectal cancer patients and found an almost 50% reduction in the rate of complications according to Clavien-Dindo. However, no differences were seen when using the comprehensive complication index (CCI) or on other clinical parameters such as length of stay, readmission, or reintervention (43). In 2022, Onerup *et al* published an RCT on 668 participants scheduled for colorectal cancer surgery randomised to a home-based exercise prehabilitation programme over two weeks or standard care (44). They found no difference in the primary outcome measure, self-reported physical recovery, nor complication at 30 or 90 days between groups. They have also recently published a 12-month follow-up on 616 participants where no differences in self-reported physical recovery, reoperation, or readmission were seen (45).

In conclusion, the effect of exercise prehabilitation in colorectal cancer remains uncertain and the anecdotal or observational beneficial effect of physical activity has not been reproduced in intervention studies. Exercise prehabilitation was until recently recommended in both the national guidelines for colorectal cancer in Sweden and in the Enhanced Recovery After Surgery (ERAS®) concept prior to elective colorectal surgery. In the previous ERAS® guidelines from 2018, exercise prehabilitation was recommended as a “weak recommendation” (46) but was changed to “no recommendation” in the updated guidelines published in June 2025 (47). It is important to distinguish between preoperative physical activity and exercise prehabilitation, and conclusions drawn from one do not always concur with those from the other. In the light of lacking solid evidence of positive effects from exercise prehabilitation in colorectal cancer it may be unnecessary, or even wrong, to recommend an exercise program to these individuals whilst higher physical activity may be associated with better prognosis (13). For an already physically active individual the possibilities to maintain physical activity might be greater than for a sedentary individual to start exercising in the time between colorectal cancer diagnosis and surgery (48).

Immune cell infiltration

Overview of the immune system

The immune system may be divided into three lines of defense. The first line of defense constitutes physical barriers such as the skin or mucosae. The second line of defense, the innate immune system, is activated as the presumed pathogen breaks through the first barrier and attacks it instantly or at least within minutes. Among cell types responsible for the

innate immune system are monocytes (which differentiate into macrophages or dendritic cells), natural killer cells (NK cells), neutrophils, and eosinophils. The third line of defense, the adaptive immune system, evolves over time and has the ability to develop immunological memory (49). The adaptive immune response is broadly divided into two classes, the antibody response and the cell-mediated response. These involve two classes of lymphocytes: the antibody response is mediated through B cells and the cell-mediated response through T cells (49). The surface of every immune cell is covered by numerous molecules having different functions. These molecules are called cluster of differentiation (CD), and the different molecules have unique CD numbers. Different CDs are expressed depending on the cell type and stage of cell maturity. These characteristics are used in both clinical practice and research to detect different cell types in blood and tissue. For example, CD3 is expressed on T cells and can be used as a general T cell marker, CD8 on cytotoxic T cells, CD20 on B cells, and CD68 on macrophages (50).

The immune system and cancer progression

The immune system is closely linked to tumour development and progression, expressed as tumour immunity. In the widely accepted hallmarks of cancer proposed by Hanahan and Weinberg in 2000 (51) and updated in 2022 (52), two hallmarks are linked to tumour immunity: “avoiding immune destruction” and “tumour-promoting inflammation”. The processes of tumour immunity are complex, and tumour-immune reactions depend on several interlinked factors such as cytokines, immune checkpoint molecules, antigens, and the innate and adaptive immune systems. For the immune system to kill cancer cells, the cancer-immune cycle needs to be activated and repeated. A tumour cell expresses characteristic molecules on its surface that cells from the innate immune system recognise and activate processes to eliminate the diseased cell. A damaged or injured cell releases danger-associated molecular patterns (DAMP) which are molecules that antigen-presenting cells bind and react to which is one way of initiating an immune response. Another mechanism involves neoantigens from dead cancer cells being presented to dendritic cells that transport them to the draining lymph node. Dendritic cells are a link between the innate and adaptive immune systems and can, via cytokine production, promote both cytotoxic activity and recruit other cells from the innate immune system. When the antigen is presented to T cells a cascade of cell-activating reactions is initiated. Effector T cells that can migrate and invade the tumour microenvironment are activated when presented with

the antigen. Once in the tumour microenvironment, the cytotoxic cells kill cancer cells which releases additional neoantigens and the cycle is repeated and amplified (53). Tumours that escape the cancer-immune cycle show sparse infiltration of immune cells in the tumour microenvironment and are sometimes referred to as immunologically “cold” tumours. These cold tumours have developed an escape mechanism from one or more of the cycle-steps such as elimination of immunogenic antigens, inhibition of dendritic cell maturation by cytokines, suppression of T cell activity, or inhibition of T cell migration and/or infiltration (53).

Cytokines are soluble proteins secreted from different immune cells that can be pro- or anti-inflammatory (or both) and stimulate both tumour progression and suppression (54). One single cytokine can be secreted by different cell types and have either pro- or anti-inflammatory action depending on the context or milieu (54). Examples of proinflammatory cytokines are IL-1 β , IL-6, IL-12, IFN- γ , and TNF- α , and anti-inflammatory cytokines IL-4, IL-10, IL-11, IL-13, and TGF- β (54). Immune checkpoint molecules are membrane receptors that regulate the immune response and prevent host damage by excessive immune activation (55). These receptors are upregulated on activated immune cells and each receptor has its specific ligand. The ligands are presented by antigen-presenting cells and interaction can result in prevented or reduced activity of the immune cell or immune response exhaustion (anergy) (55). Immune cells that are exposed to a persistent antigen or where negative co-stimulation fails become exhausted or anergic (56). This state of inactivity leads to loss of cytokine and effector protein production and less checkpoint molecule expression (56). Examples of immune checkpoint molecules are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), protein cell death protein 1 (PD-1), B and T cell lymphocyte attenuator (BTLA), and V-domain Ig suppressor of T cell activation (VISTA) (55). Researchers behind drugs targeting these immune checkpoint molecules were awarded the Nobel Prize in 2018. At present, approved checkpoint inhibitor drugs target CTLA-4, PD-1, and programmed cell death ligand 1 (PD-L1) (57). Treatment of colorectal cancer with checkpoint inhibitors is currently approved for microsatellite instable tumours (58).

Different immune cell types associated with cancer

The tumour microenvironment consists of cells surrounding the tumour and the cell types, cytokines, soluble proteins, and biochemical features within. The most important immune cell type in the tumour

microenvironment is the cytotoxic T cell designed to kill tumour cells after recognition. However, the tumour microenvironment contains several immune cells such as NK cells, macrophages, regulatory T cells, helper T cells, dendritic cells, B cells, and neutrophils (59).

T lymphocytes

T lymphocytes of the adaptive immune system comprise several subgroups. Traditional T cells have the $\alpha\beta$ T cell receptor and express either CD4 or CD8 on their surface. Ninety-five per cent of circulating T cells are $\alpha\beta$ -cells which also are referred to as traditional T cells. The other group includes unconventional or non-traditional T cells. Traditional T cells are divided into T-helper cells, regulatory T cells, and cytotoxic T cells. Non-traditional T cells have the $\gamma\delta$ T cell receptor and differ from traditional T cells in many ways (49). $\gamma\delta$ -cells are found in the epithelium of the intestine, the uterus, and the tongue, and many of their functions remain unclear. It is believed that $\gamma\delta$ -cells react to un-presented antigens similar to cells of the innate immune system (49).

Cytotoxic T cells express the CD-protein CD8 and can, upon activation by an antigen-presenting cell, directly kill tumor cells using one of two pathways. Either by using perforin to create a pore in the cell through which it injects granzyme B or by upregulating Fas-Ligand on the target cell. These both mechanisms initiate apoptosis (60). T-helper cells are identified by CD4 expression and are a heterogeneous population of cells. T-helper cells are usually divided into T-helper cell 1 (Th1), T-helper cell 2 (Th2), and T-helper cell 17 (Th17). Th1-cells are activated by IL-12 and secrete primarily IL-2, IFN- γ , and TFN (49). Th1-cells play an important role in protecting against intracellular pathogens and are a major part of the recruitment of cytotoxic cells and thus in the anti-tumour response. Th2-cells are activated by IL-4 and secrete IL-4, IL-5, and IL-13, and primarily protect against extracellular pathogens (49). Th17-cells secrete IL-17 and primarily protect against fungi. Dysregulation of Th17-cells can result in excessive inflammation and autoinflammatory disease. In tumour progression, the Th17-response might be tumour-promoting, however its effect on prognosis is not entirely understood (61, 62). In addition to CD4, regulatory T cells also express Forkhead box P3 (FoxP3) and CD25. In clinical studies, regulatory T-cells are often identified by FoxP3, however other cell types can upregulate both CD25 and FoxP3. Regulatory T cells act immunosuppressive by downregulating the T cell response and are regarded as a central factor in maintaining immunological homeostasis. There are conflicting results on whether they are tumour promoting or

suppressing, probably because there are different subsets within regulatory T cells that have different roles (62, 63).

Macrophages

Macrophages in the tumour microenvironment usually indicate an unfavourable prognosis. However, there are two types of tumour-associated macrophages (TAM). Macrophages express CD68 on their surface and, depending on the tumour microenvironment, can either assume an antitumour function (M1 macrophages) or a protumour function (M2 macrophages) (64). Recently this classification of TAMs into M1 and M2 types has been questioned and some argue that there is rather a spectrum of M-types where M1 and M2 are the extremes of a wide continuum of functional states (64).

Neutrophils

Neutrophils are a part of the innate immune system and the most common immune cell in circulation, accounting for 60% of all leukocytes. Their role in the tumour microenvironment is not completely understood. Tumour-associated neutrophils are generally found in the periphery in the early stages of tumour development but are later found between tumour cells. Some evidence indicates that N1 and N2 neutrophils act in a similar way to macrophages where N1 cells promote tumour suppression and N2 cells tumour progression (64).

Natural Killer cells (NK cells)

NK cells are cytotoxic effector cells that are a part of the innate immune system and are generally associated with favourable prognosis. As cytotoxic T cells they also mediate their effector function via either perforin or by cell-to-cell contact with Fas-Ligand. The NK cells can be identified by the expression of CD56 (65).

B lymphocytes

Much of the research on tumour immunology is focused on T lymphocytes. However, up to 40% of tumour-infiltrating lymphocytes have been shown to be B cells. Most research concludes that plasma B cells promote tumour growth either by antibody-mediated immune suppression by creating circulating immune complexes or by the production of immunosuppressive cytokines such as IL-10, TGF- β , and IL-35 (66). B cells that secrete cytokines have been termed regulatory B

cells, a heterogeneous population of cells that suppress the inflammatory response (66). At the same time, some studies have shown that tumour-infiltrating B cells are associated with better prognosis in some human cancer types including colorectal cancer (67, 68, 69).

Immune cell infiltration and prognosis in colorectal cancer

In 2006, a research group in Paris, France, published their first study on immune cell infiltration in colorectal cancer. They quantified the densities of the total T cell population (CD3⁺), cytotoxic T cells (CD8⁺), and effector memory T cells (CD45RO⁺) in the invasive margin (front) and centre of tumours using immunohistochemistry and concluded that tumours with high density of any of the three cell types in both regions had a better prognosis compared to tumours with low densities. If densities of two of the cell types were combined the results were even more apparent and their prognosis predictive ability was stronger and independent of TNM-classification (70). To make it applicable across labs and clinical settings, the research group developed the Immunoscore[®], a standardised way of quantifying and determining densities using digital pathology analysis. In the Immunoscore[®] the densities of the total T cell population (CD3⁺), cytotoxic T cells (CD8⁺), but not CD45RO⁺ are determined in the invasive front and centre of the tumour. The combined densities are then scored as high or low. The Immunoscore[®] has been validated in a large international multicentre study and a high Immunoscore[®] indicates a better prognosis (71). Several other immune cells have been associated with a better prognosis in colorectal cancer, and a meta-analysis showed that increased tumour infiltration by cytotoxic T cells (CD8⁺), total T cell population (CD3⁺), B cells (CD20⁺), effector memory T cells (CD45RO⁺), and NK cells (CD56/CD57⁺) was associated with improved prognosis (72). Published results from the above-mentioned meta-analysis regarding the infiltration of regulatory T cells (FoxP3⁺) and macrophages (CD68⁺, CD163/206⁺), however, show greater heterogeneity and while indicating a better prognosis the results in the pooled analysis were not significant (72). A review on infiltrating immune cells in solid cancers described a generally positive prognosis associated with an abundance of all cell types tested apart from Th17 and Th2 cells (73). This was not a meta-analysis, and no pooled analysis was performed.

Physical activity and immune cell infiltration

Following an acute bout of physical activity, levels of lymphocytes in the bloodstream increase dramatically and upon cessation, levels decline to preactivity levels or even lower. Recent evidence has suggested that this

decline is due to redistribution of the lymphocytes to peripheral tissues (74) possibly reflecting an increase in immune surveillance. Cardiorespiratory fitness is associated with an increased proportion of circulating naïve T cells and a lower proportion of senescent T cells, indicating a more effective immune surveillance in physically fit individuals (75). Furthermore, the expected increase in circulating senescent T cells associated with aging was no longer present after adjusting for maximum oxygen uptake (75). Animal studies have shown higher numbers of NK cells (76) and cytotoxic T cells (77) in the tumour microenvironment and increased macrophagic cytolytic activity in mice after physical activity (78). That physical activity can mobilise immune cells to both the bloodstream and peripheral tissues including tumour tissue suggests a possible correlation between physical activity and immune cell infiltration in the tumour microenvironment.

Sarcopenia and myosteatorsis

Sarcopenia is a progressive and generalised skeletal muscle condition defined as the presence of low muscle strength and low muscle quantity or quality (79). Since 2016, the WHO includes sarcopenia as a disease in the International Statistical Classification of Diseases and Related Health Problems (ICD) (80). Sarcopenia is associated with lower quality of life (81) and overall mortality risk (82) and can be prevented or delayed by physical activity (83, 84). From the gastrointestinal surgery point of view, sarcopenia has been shown to increase the risk of postoperative complications and mortality (85). A meta-analysis of 15,000 colorectal cancer patients found an increase in both overall and cancer-specific mortality risk in patients with sarcopenia assessed by computed tomography (CT) (86).

There is no consensus on how sarcopenia should be determined. Methods include questionnaires, skeletal muscle strength tests, physical performance tests, radiologic measurement of skeletal muscle mass or muscle quality alone or together (79). In the clinical setting, a questionnaire and an easily conducted physical test is probably the easiest means of assessment. In research, radiologic assessment of body composition is often more accessible, especially in a cancer population. The European Working Group on Sarcopenia in Older People (EWGSOP) published their consensus guidelines in 2018 where they stated that sarcopenia should be suspected when muscle strength is low and can be confirmed if low muscle quantity or quality is present according to physical testing and/or radiologic assessment. Sarcopenia

may be considered severe if the person not only has low muscle strength and quantity or quality but also has low physical performance (79). In the clinical setting, guidelines from the International Practice Guidelines for Sarcopenia (ICFSR) recommend that objective measurement tools should be used when diagnosing sarcopenia and emphasise the use of dual-energy X-ray absorptiometry (DXA) to determine body composition (87). Body composition can also be measured using CT or magnetic resonance imaging (MRI), which is more expensive and resource-consuming than DXA. Most patients in a cancer population undergo several CT scans in routine clinical management, so CT-assessed body composition is usual in cancer research. Another way of non-invasively determining body composition is the use of electrical bioimpedance. This method uses electric resistance in the body to assess the volume of fat and lean body mass. Electrical bioimpedance is not as accurate as DXA, CT, or MRI but is cheaper and does not require interpretation of images. It is endorsed by EWGSOP, but should not be used if DXA, CT, or MRI are available (79).

Assessment of muscle mass is essential when identifying sarcopenia. Muscle mass may be reported as total body skeletal muscle mass, appendicular muscle mass, or the muscle area of a cross-sectional image. Muscle mass correlates with body size and when comparing values between individuals this must be accounted for. Body size can be adjusted for in various ways, either by dividing the muscle mass by the height squared, weight, or the body mass index (88). There is no consensus in the research community on which of these should be used. The use of height squared is more restrictive than body mass index, meaning that the prevalence of sarcopenia increases if the muscle mass is adjusted for body mass index rather than height squared (88). When adjusting for body size the value is often referred to as the muscle index and depending on how muscle mass is measured the index is named skeletal muscle index (SMI), appendicular muscle index (ASMI), or psoas muscle index (PMI). In research, when CT is used to define sarcopenia, it is common to use a cross-sectional image at the level of the 3rd lumbar vertebra. From this slide, the area of all included muscles or specific muscles (often psoas major) can be calculated. Assessment of skeletal muscle mass at this level correlates well with whole body muscle, in both healthy individuals (89) and cancer patients (90).

Another aspect of body composition is myosteatorsis *i.e.* fat infiltration in muscle tissue. Myosteatorsis is associated with aging and correlates negatively with muscle strength but it is not synonymous with sarcopenia since it appears to be independent of muscle mass (91).

Myosteatorsis has been associated with increased mortality risk in colorectal cancer patients (92, 93) and can, as with sarcopenia, be prevented by physical activity (94). Fat can be distributed in different compartments in and around the muscle: intermuscular adipose tissue (IMAT); extracellular adipose tissue found between muscle groups; intramuscular adipose tissue found within the muscle; and intramyocellular lipids (IMCL). The gold standard of assessing myosteatorsis is by biopsy which gives exact measurement of the adipose tissue in all the compartments mentioned above. Due to its invasiveness, muscle biopsy is impractical and rarely used. Myosteatorsis can also be assessed with imaging modalities such as CT, MRI, or ultrasound, but not DXA. CT is the most widely used in research and myosteatorsis assessed with CT is measured in Hounsfield units (HU). The Hounsfield unit is a measurement of radiation waves that pass through water. Higher density has higher measurement and *vice versa*. The lower the density, the lower the Hounsfield unit, and the greater the myosteatorsis (91). MRI can also quantify myosteatorsis by estimating the adipose tissue in and around the muscles, however, muscle density cannot be calculated using MRI data (91). Ultrasound can be used to measure the thickness and echogenicity of muscles and can be used to assess myosteatorsis. In ultrasound, inter-instrument validity is unclear and issues with inter-examiner variability make it difficult to use in clinical research (91). As with muscle mass, when myosteatorsis is assessed with CT or MRI a single scan at the 3rd lumbar vertebra is often used. The mean Hounsfield unit score of either the total muscle area or the area of a specific muscle is calculated and used. This measurement is often referred to as skeletal muscle density (SMD). Simplified, the skeletal muscle index can be thought of as the quantity of muscle and skeletal muscle density as the quality.

Testing for physical performance and fitness

Due to the multifactorial aspects of physical activity, quantification in the clinical and research setting is a challenge. First it must be decided whether it is physical activity, cardiorespiratory fitness, or functional fitness that is to be measured and thereafter which tool is best given the resources available. Physical activity improves cardiorespiratory fitness and cardiorespiratory fitness is a consequence of physical activity. Nevertheless, some physically active individuals may have relatively low cardiorespiratory fitness and *vice versa*. The gold standard for measuring physical activity is by measuring energy expenditure using the doubly labelled water method (95). This is an expensive method that

requires advanced laboratory equipment, several samples of body fluids, and complicated analyses which is why its use in clinical practice and larger studies is limited. Cardiorespiratory fitness can also be measured, and the gold standard for determining this is cardiopulmonary activity testing (CPET). CPET measures the maximum oxygen consumption ($VO_2\text{-max}$) which is expressed as $\text{ml O}_2 \times \text{kg}^{-1} \times \text{min}^{-1}$ (96). CPET is non-invasive but requires a laboratory setting with specialised equipment and a maximal effort from the participant being tested making it difficult to conduct in some research settings. When resources are limited or the participant too frail, one alternative for estimating cardiorespiratory fitness and $VO_2\text{-max}$ is by using submaximal tests that are verified against CPET and thereafter extrapolate the results to an estimated $VO_2\text{-max}$. High physical activity (7, 8) and cardiorespiratory fitness are associated with lower overall mortality risk (97, 98) but the effects on mortality seem to correlate more strongly with cardiorespiratory fitness (99). The relationship between physical activity and cardiorespiratory fitness has been shown to be modest at the most ($r=0.37\text{-}0.49$) when assessing physical activity objectively or with doubly labelled water (100) and cardiorespiratory fitness with CPET (99), indicating its complexity.

MET can be used to make quantification of physical activity comparable between individuals in research. One MET corresponds to oxygen consumption when at rest and is equal to $3.5 \text{ ml O}_2 \times \text{kg}^{-1} \times \text{min}^{-1}$. MET can also be converted to energy and one MET approximately equals $1 \text{ kcal} \times \text{kg}^{-1} \times \text{h}^{-1}$. The compendium of physical activities, first published in 1993 and updated in 2000, 2011, and 2024 (101) is an extensive list of energy expenditures of different activities based on a rigorous literature search (101). In this compendium sedentary behaviour is defined as 1.0-1.5 MET, light intensity physical activity as 1.6-2.9 MET, moderate intensity physical activity as 3.0-5.9 MET, and vigorous physical activity as ≥ 6 MET (101).

More accessible ways of estimating physical activity than doubly labelled water, and the two most commonly used methods in research, are self-reported and sensor-based assessment of physical activity. Sensor-based assessment usually involves an accelerometer. Accelerometers are validated against both step-counting (102) and energy expenditure (103). The benefits of accelerometers are the objective outcome data which produces a more exact output of the actual physical activity a person conducts over a period of time. Accelerometers are more time-consuming and generate a large amount of data that must be processed. Its benefit is therefore limited in a setting where time or resources are scarce. The measuring time required for reliable estimates of habitual

physical activity is often defined as ten or more hours wearing an accelerometer over three to five days in adults and somewhat longer in adolescents (104). Data from accelerometers are respected due to their objective nature. However, when interpreting population-based studies on participants invited to participate, it is important to bear in mind the risk of sample-bias since more motivated and active participants are likely to be included (105). Self-reported assessment is easier to conduct, less time-consuming, and is therefore often used in large studies and population-based cohorts. However, this lacks objectivity and very few physical activity questionnaires have both good reliability and validity (106). In view of this, physical activity data from questionnaires must be interpreted with caution. In some settings, however, it is the only alternative available for quantification of physical activity. A previous study that evaluated physical activity questionnaires showed that even though the concurrent validity was low to moderate the predictive validity showed a clear dose-response curve in predicting presence of the metabolic syndrome from a single-item questionnaire on physical activity (107). Perhaps physical activity questionnaires fail to quantify the exact physical activity conducted but could be a surrogate measure for other forms of behaviour.

There are several physical tests measuring strength, physical fitness, or function in more easily conducted and less strenuous settings. More easily conducted tests that assess a more limited aspect of an individual's function are usually called physical function tests. Physical function tests are often designed to test either cardiopulmonary function, muscle strength, or balance. These easily conducted tests have the advantage of being possible to perform when time and resources are limited, but they cannot provide exact measurements of physical activity or fitness. To attain a comprehensive view of an individual's functional fitness, particularly in geriatric individuals, several tests may be combined. One example of such a functional fitness assessment tool using several tests is the Senior Fitness Test developed by Rikli and Jones (108). Tests for lower body strength, upper body strength, aerobic endurance, lower body flexibility, upper body flexibility, and dynamic balance are combined to achieve a more comprehensive evaluation of functional fitness. A problem with physical function tests is the vast supply of different tests with only small differences in test set-up making comparability between studies difficult. For example, there are several variants of step tests with different platform heights, stepping cadence, and duration. Some examples of different step tests are the 2-minute step test (2MST), 1-minute step test, 5-minute step test, Maritz step test, the Canadian home fitness test, Queens college step test, and Harvard

step test, just to mention a few (109). This makes generalisation and interpretation of results from studies using different step tests difficult.

Some often-used physical function tests are 6MWT, 2MST, sit-to-stand test, timed up-and-go test (TUG), standing balance test, and test of handgrip strength. In Study III, participants performed the 2MST, sit-to-stand test, and handgrip strength using a hand-held dynamometer. 2MST is a self-paced test where the participant steps on the spot raising the knees to a predefined height halfway between the patella and iliac crest. The number of times the right leg reaches the minimum height during the two predefined minutes is recorded and calculated. The 2MST has been validated in the elderly (108) and middle-aged population (110) and the number of steps correlate with VO_2 -max ($r=0.62$ in elderly and $r=0.54$ in middle-aged). From the study on the middle-aged population, a linear regression model was developed that could estimate 64% ($r^2=0.64$) in the variation of VO_2 -max and could possibly be used to predict VO_2 -max (VO_2 -max = $0.15 \times 2MST + 0.26 \times \text{height in cm} - 0.27 \times \text{waist circumference in cm} - 3.85$) (110). In the sit-to-stand test, participants rise and sit from a chair as many times as possible in 30 seconds. The sit-to-stand test is primarily a measurement of functional fitness and lower-limb strength and has been validated against muscle strength-measurement of the lower limbs (111). The use of a hydraulic dynamometer to test handgrip strength is a good way to assess muscle strength since it correlates well with general muscle strength (79).

Qualitative research and content analysis

Depending on the aim of research and that being studied, a research worker can use either quantitative or qualitative methods. Qualitative and quantitative research differ in terms of truth value and how data are evaluated and interpreted. In qualitative research, there are different concepts used when describing the trustworthiness of the research. Trustworthiness can be expressed as how well the results of the study reflect reality. It is up to the researcher to achieve trustworthiness by being transparent during the preparation, data collection, analysis, interpretation and reporting of the data. The three concepts credibility, dependability, and transferability (112, 113) are often used and the qualitative researcher should try to explain the process of data collection, analysis and the interpretation of the results. It is then up to the reader to define its trustworthiness. Credibility, sometimes referred to as the “internal validity” in qualitative research, is ensuring that the correct qualitative research method was used, appropriate participants have

been included, that the data cover the subject of the research, and that the researcher has enough background information about the topic. Having diversity of researchers and participants help ensure rich variation of data. Dependability, sometimes referred to as the “reliability” in qualitative research, is the consistency of data collection and interpretation over time. Transferability reflects how well the results can be transferred to another context or group. To achieve transferability the researcher should describe the context, culture, and characteristics of the participants, research workers, and the process of analysis. Even here it is up to the reader to judge whether the data is transferable to the context of interest (112). Transferability can be referred to as the “external validity” in qualitative research (113). Of course, both qualitative and quantitative research strive for rigorous scientific results that are based on scientifically solid evidence. However, quantitative and qualitative research originate from different ontological and epistemological backgrounds and their different ways of achieving rigorous results are therefore methodologically different. Where quantitative research has a positivistic epistemology and tries to explain the “objective” truth by studying a part of a sample, the qualitative researcher has an epistemology originating from interpretivism and tries to understand the “subjective” truth by studying and interpreting the sample from as many different perspectives as possible.

In qualitative research, data may be collected from text, audio files, video, or images. These data can be gathered from webpages, social media, TV-shows, observations, interviews, focus groups, etc. Data can be analysed deductively or inductively. When using the deductive approach, the framework or codes are created before analysis begins and the data are later applied to these patterns. In the inductive approach, the codes are generated during analysis of the data (114). There are numerous methodologies in qualitative research with different strengths and weaknesses. Some common methods are phenomenology, grounded theory, thematic analysis, and qualitative content analysis (115).

Phenomenology studies the individual and focuses on what was experienced and how it was experienced. With phenomenology, the researcher examines a phenomenon from the perspective of the individual. Therefore, in-depth interviews are a common approach to data collection in phenomenology (115).

Grounded theory is an inductive research method where the theory and codes are built from data. It was developed by Barney Glaser and Anselm Strauss in the 1960s. The idea of grounded theory is that the researcher

should start the analysis as a “blank paper” without any preconceived theories and as little prior knowledge as possible. The research evolves over time as data is collected and towards the end the codes of the data are related to each other (115).

Thematic analysis aims at defining and analysing patterns and themes in the data. Searching for repeated patterns that could be “hidden” in the deeper understanding of the data. When conducting thematic analysis, the data is coded, and these codes are sorted into different themes. A theme should capture something important from the data related to the research (116, 117). In this thesis, a method similar to thematic analysis was used in Study IV, qualitative content analysis (112). In qualitative content analysis, the research process aims to discover and interpret the manifest and latent content. The manifest content is the information closest to the text that can be understood without exaggerated interpretation and is captured in categories. The latent content can be explained as a red line that runs through the data material and is captured in main-categories or themes (118). The process is non-linear and starts with raw data. The data are first decontextualised, where content-bearing units (meaning units) of the data are separated from the context. The next step is recontextualisation where the researcher abstracts categories from codes and in this way enters a new context which gives a deeper understanding of the data. Ideally the analysis is performed by more than one researcher with different backgrounds to enable abstraction with wider and broader experiences.

Aims

The overall aim of this thesis was to evaluate pre-diagnostic physical activity, assessed using self-reported questionnaire, from physical tests, by using an accelerometer, using interviews and its relationship with colorectal cancer with regard to immune cell infiltration of the tumour, body composition, and the patient's own experiences.

Specific aims:

- To investigate the association between prospectively collected self-reported data on pre-diagnostic physical exercise, and immune cell infiltration by all T cells (CD3⁺) and cytotoxic T cells (CD8⁺) in the tumour microenvironment of colorectal cancer using immunohistochemistry (Study I).
- To investigate the association between prospectively collected self-reported data on pre-diagnostic physical exercise and sarcopenia and myosteatosis at diagnosis of non-metastatic colorectal cancer. And furthermore, to investigate the combined effect of the combination of pre-diagnostic physical exercise and sarcopenia or myosteatosis in relation to overall and cancer-specific mortality (Study II).
- To assess the association between objectively assessed preoperative physical activity, fitness, and body composition and immune cell infiltration in the tumour microenvironment of non-metastatic colon cancer using multiplex immunohistochemistry (Study III).
- To explore attitudes and experiences of physical activity in physically active individuals diagnosed with colon cancer awaiting curative surgery (Study IV).

Patients and Methods

In this thesis, three distinct methodologies were used in the four studies included. Studies I and II were retrospective studies where individuals diagnosed with colorectal cancer were extracted from the Swedish Cancer Register and linked with the prospective population-based cohort of the Västerbotten Intervention Programme. Study III was a prospective cohort study where colon cancer patients were asked to participate at the time of diagnosis. Study IV was a qualitative interview study where physically active individuals diagnosed with colon cancer prior to planned curative surgery were interviewed by telephone.

Studies I and II

Study population and study design

Studies I and II were based on the population of Västerbotten County, Sweden. Participants were all part of the Västerbotten Intervention Programme, a population-based cohort included in the Northern Sweden Health and Disease Study (NSHDS). NSHDS contains three subcohorts where the Västerbotten Intervention Programme is one, and the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease cohort (MONICA) and the Mammography Screening Project cohort (MSP) are the other two.

The Västerbotten Intervention Programme was initiated in 1985 and from the beginning was called the Norsjö Project. Analyses had shown that Västerbotten County and in particular a little community called Norsjö (2,000 inhabitants) had an exceptionally high cardiovascular mortality rate (119). To address this question and try to reverse the trend, a healthcare initiative was started. From its inception, the Västerbotten Intervention Programme contained a combination of a population-based strategy and health promotion in the community. The goal was to contact all middle-aged inhabitants and invite them to participate in screening and health counselling (119).

The Västerbotten Intervention Programme was later expanded to cover the whole of Västerbotten County. Between the years 1985 and 1992, more and more cities and villages in the county were gradually included. Today residents in Västerbotten County are invited to take part in a health examination when they are 40, 50, and 60-years-of-age. This

examination consists of a medical examination, collection of blood samples including a fasting blood sample, and a questionnaire regarding their diet and lifestyle habits (119). Participation rates have varied over the years from around 55% in the beginning to 66-67% today. A previous study examined the observed rate of cancer in the Västerbotten Intervention Programme from the beginning until 2003 and found the expected number of cases was as expected except for lung cancer where the observed number of cases were lower than expected (120). This consistency of cancer incidence supports the population-based nature of the cohort.

Participants in the Västerbotten Intervention Programme between 1986-2016 diagnosed with colorectal cancer became the eligible population, in all 927 patients, and the final study populations comprised 592 patients in Study I and 519 patients in Study II (Fig 4).

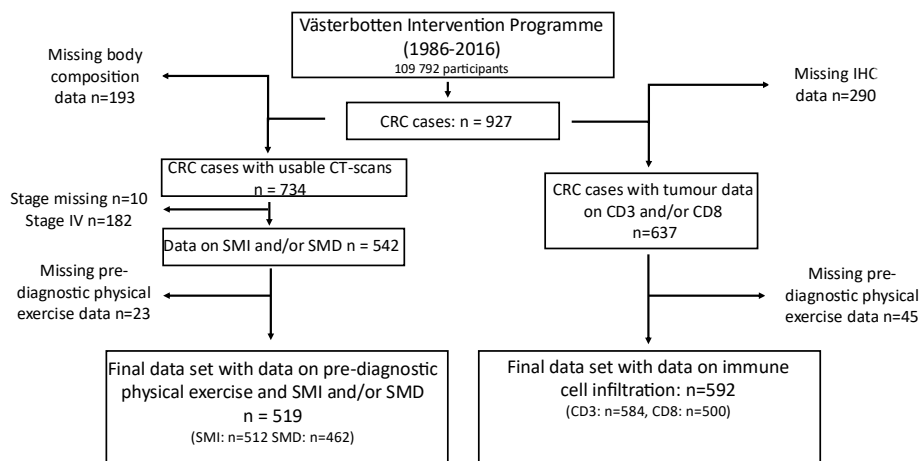


Figure 4 Flow-chart of the study populations and exclusions in Studies I and II. CRC – colorectal cancer; CT – computed tomography; SMI – skeletal muscle index; SMD – skeletal muscle density.

The exposure variable in the two studies was self-reported physical exercise collected from a single-item questionnaire in the Västerbotten Intervention Programme (119). The question was phrased as “How often have you exercised in exercise clothing over the past three months, with the intent to improve your fitness and/or well-being?”. The answers were reported on a five-level scale (“never”, “now and then”, “1-2 times a week”, “2-3 times a week” and “>3 times a week”). In Study I, the

original five-level scale was used as the exposure variable. In Study II we defined the two lowest categories together as “low level physical exercise” and the other three combined as “high level physical exercise”.

Other variables collected from the Västerbotten Intervention Programme were age at baseline, sex, body mass index, diabetes, smoking habit, alcohol use, education level, blood pressure, and C-reactive protein (CRP). Clinical variables, collected after or at diagnosis, were age at diagnosis, tumour location, and tumour stage.

Because of the possible and plausible deterioration of the body composition in Stage IV disease, these patients were excluded from the analysis in Study II.

Data collection

Tumour tissue samples used in Study I were collected from routine clinical samples retrieved from the local biobank (Biobanken Norr). The samples were taken between 1992 and 2016. The immune cell density was determined by immunohistochemistry using an anti-CD8 polyclonal antibody and a primary polyclonal CD3 antibody used at a dilution of 1:50 (Fig 5).

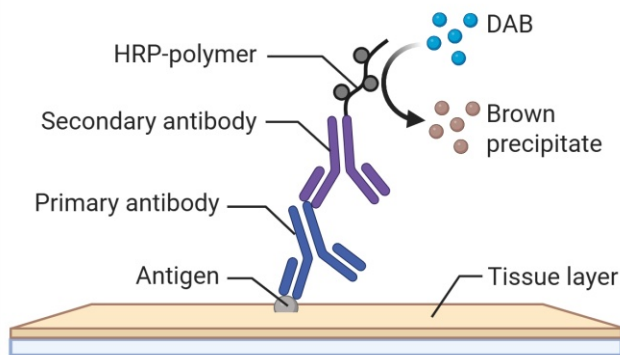


Figure 5 Schematic picture of the immunohistochemistry process. Created with BioRender.com

DAB – 3,3'-Diaminobenzidine; HRP – HorseRadish Peroxidase

The immune cell density was scored for both the cytotoxic T cell marker (CD8) and the general T cell marker (CD3) representing all T-cells (Fig 6). Scoring in the tumour front, centre, and in the intra-epithelial compartment was performed by a single observer. Inter-rater reliability was assessed by a senior consultant in gastrointestinal pathology who

analysed 35 samples with a weighted Cohen Kappa score of 0.61-0.87 indicating moderate to strong agreement. Immune cell density scores were given from one to four representing low to high immune cell density. A total score was calculated for each cell type generated from the sum of the immune densities in each location (front, centre, and intra-epithelial).

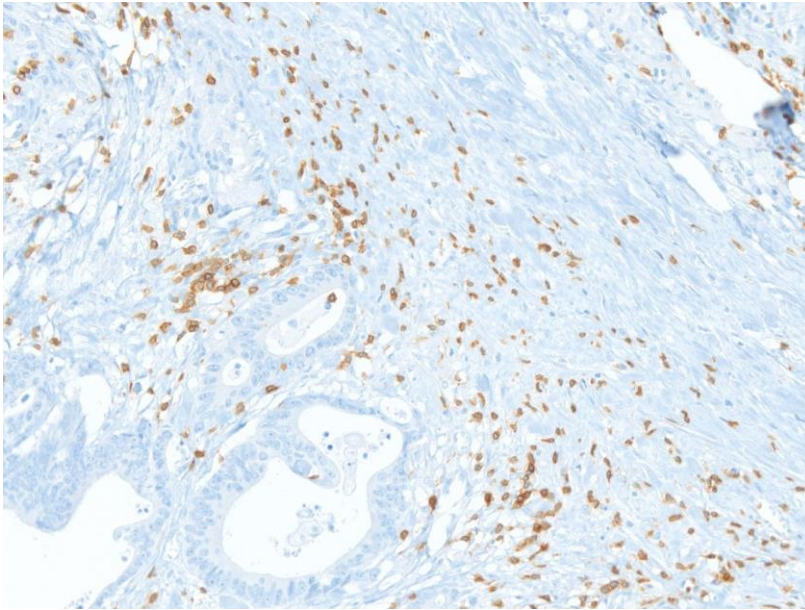


Figure 6. Immunohistochemistry analysis of CD3-positive cells (brown). Courtesy of Björn Gylling.

In Study II, SMI and SMD were calculated using peri-diagnostic CT-images from the most caudal slice with the vertebra L3 visible (Fig 7). SMI was calculated using the total muscle area in square centimeters and dividing this by the patient's height in meters squared ($\text{cm}^2 \times \text{m}^{-2}$). SMD was calculated using the mean Hounsfield units of the delineated muscle area using the same CT-slide as for SMI. We defined sarcopenia and myosteatorsis from SMI and SMD as in a previous study on the same patient material (93) and defined sarcopenia as the lowest sex-specific tertile of SMI and myosteatorsis as the lowest sex-specific tertile of SMD.



Figure 7. Computed tomography scan at the L3-level. Different tissues marked with different colours. Green – skeletal muscle; Red – intra-abdominal fat; Yellow – subcutaneous fat, Purple – intra-muscular fat; Grey/white – other (intra- and retroperitoneal organs and lumbar vertebrae). Courtesy of Björn Gylling.

Outcome measure

In Study I the primary outcome measure was immune cell infiltration by cytotoxic T cells (CD8⁺) in the invasive front of the tumour microenvironment. Secondary outcome measures were immune cell infiltration by CD8⁺ cells in the tumour centre, the intra-epithelial compartment, and total score as well as infiltration of all T cells (CD3⁺) in the tumour front, centre, intra-epithelial compartment, and total score.

The primary outcome measure in Study II was sarcopenia at the time of diagnosis. A secondary outcome measure was myosteatosis at the time of diagnosis.

In Study II overall and cancer-specific mortality were additional secondary outcome measures. Data from the Northern Sweden Cancer Register and individual patient records were used to determine mortality status. Death with known disseminated or recurrent disease was defined as cancer-specific mortality while death from all causes was defined as overall mortality. Patients were followed from the date of diagnosis until May 2nd, 2022. Two patients were excluded from the survival analysis due to death within 30 days of diagnosis.

Study III

Study population and study design

This was a prospective cohort study where participants diagnosed with colon cancer and planned for elective curative surgery without previous treatment were included. The study was a pilot study and part of the Physical Activity and its impact on Colon cancer Surgery (PACOS) study. PACOS was a two-part study, one focusing on the association between physical activity and postoperative complications, and the second, Study III in this thesis, investigating the association between physical activity and immune cell infiltration in the tumour microenvironment of colon cancer.

Inclusion and exclusion criteria in the PACOS study are presented in Table 1. In the original PACOS study, 84 patients were included between January 2019 and February 2024, exclusions for Study III are presented in Fig 8. The final study population comprised 70 colon cancer cases available for immune cell infiltration analysis.

Inclusion criteria
Age \geq 18 years
Colon cancer diagnosis
Planned curative surgery (Stages I-III)
No neoadjuvant therapy
Able to speak and understand Swedish

Table 1. Inclusion criteria of Study III.

Due to the COVID19 pandemic the clinical research centre in Umeå was closed during two long periods and inclusions between March 2020 and April 2021 fell significantly. During this time only one (n=1) patient was asked to participate and was not included in the final study population of PACOS. During the inclusion period a total of 328 patients visited the outpatient clinic at Umeå University Hospital with a newly diagnosed colon cancer. Seventy-five patients visited the clinic between March 2020 and April 2021 when the clinical research centre was closed due to COVID19 restrictions and could therefore not be asked to participate in the study. In all, 253 patients visited the outpatient clinic when there were no COVID19 restrictions and of these, 159 were asked to participate. Seventy-five of the 159 were excluded for several reasons (Fig 8).

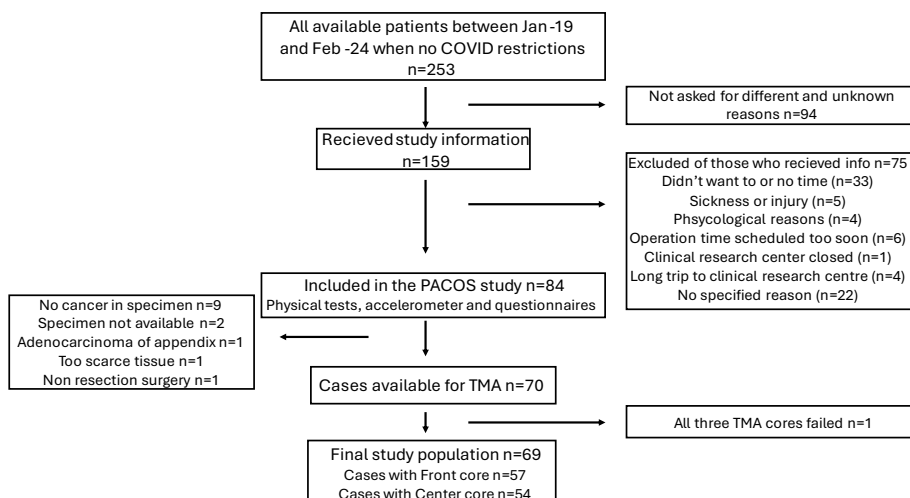


Figure 8 Flow-chart of Study III including exclusions.

Data collection

Before their scheduled surgery, PACOS participants were given an appointment at the clinical research centre in Umeå. This visit was planned as early as possible after the diagnosis had been made. The visit to the clinical research centre took about one hour and consisted of physical function tests (2MST, hand-grip strength, and 30 second sit-to-stand test), and a whole-body DXA-scan. The participants also received an accelerometer (Actigraph™ model GT3Xplus) to carry while awake during one week. If time until surgery was less, the device was carried for as long as possible.

Accelerometer data were reported as minutes MVPA per day. Six hundred minutes of wear time a day for four days was considered acceptable. For participants who were not able to wear the accelerometer for four days prior to surgery, three days of consecutive wear time over 600 minutes was accepted. The 2MST and 30-second sit-to-stand test were reported as the number of repetitions of the right leg and rising from the chair respectively. The hand-grip strength test was reported as force developed in Newton-meters (Nm).

To achieve a variable with a wider assessment of the participant's fitness, a combined variable of the three physical tests was constructed. Participants who had results above age-stratified median for both the 2MST and 30-second sit-to-stand test as well as above sex-specific median for hand-grip strength were included in the "fit" group and the

others in the “unfit” group. Participants with missing values in any of the three tests were registered as “missing” for the combined variable.

ASMI was calculated from DXA scans by adding the lean mass of arms and legs in kilograms and dividing it with the height in meters squared. ASMI was consequently reported in $\text{kg} \times \text{m}^{-2}$.

Tumour specimens were retrieved from the final study population (n=70) and a tumour microarray (TMA) was prepared in November 2024. From every participant, three cores were prepared from tumour areas chosen by a consultant gastrointestinal pathologist. Two cores from the invasive tumour front and one core from the tumour centre.

Immune cell infiltration was evaluated using multispectral quantitative automated pathology imaging using the Vectra 3[®] system. The TMA was prepared and the TMA blocks cut in 4 μm slices and mounted onto slides. They were sequentially stained immunohistochemically using primary antibodies against T-bet (Th1 cells), CD8 (cytotoxic T cells), CD20 (B cells), FoxP3 (regulatory T cells), CD68 (macrophages), and pan-Cytokeratin. For each step in the staining process, the primary antibody incubation was followed by incubation with a secondary antibody with a horseradish peroxidase (HRP) enzyme attached, and finally with a tyramide fluorophore with a distinct emission spectrum (Opal[™], Akoya Biosciences). The HRP enzyme activates the fluorophore resulting in its covalent deposition onto the tissue in close proximity to the reaction. Thereafter the antibodies were stripped using microwave heating followed by incubation with the next primary antibody, and the process repeated (Fig 9). After the staining procedure, the slides were analysed in the Vectra 3[®] system. The Vectra 3[®] system with its software inForm[®] allows for distinction of the different wavelengths from the different fluorophores which indicate different antigens. In the next step inForm[®] was used for spectral unmixing to quantify the signals from each fluorophore.

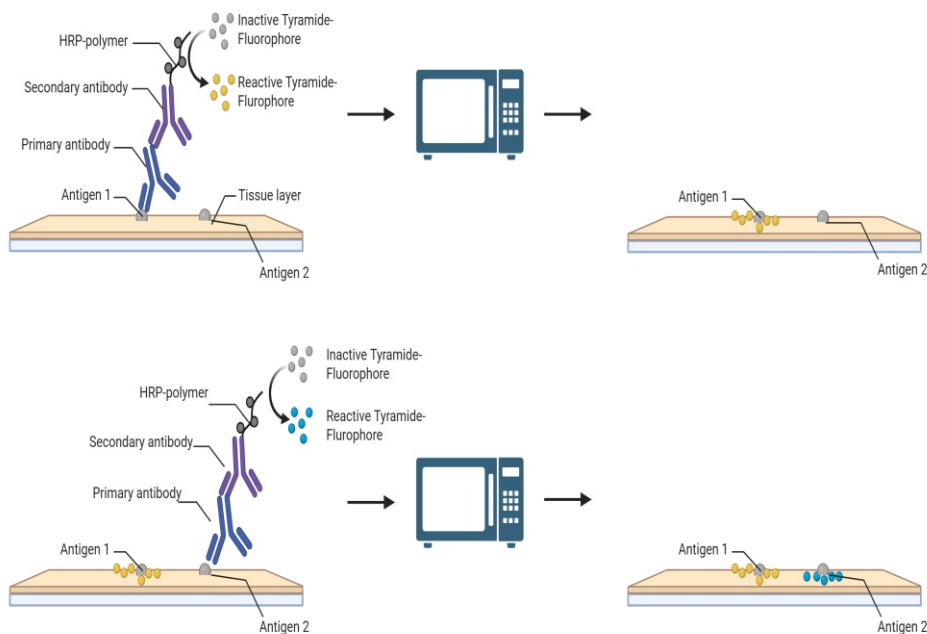


Figure 9 Schematic picture of the staining procedure for multispectral quantitative automated pathology imaging. Created with BioRender.com. HRP – HorseRadish peroxidase

After the staining and imaging procedure, 187 cores (89%) from the final study population were available for immune cell infiltration analyses. The images of scanned slides were then imported and uploaded in the inForm[®] software that was used to quantify the immune cell infiltration. The inForm[®] algorithm was trained by the researchers using 15 representative TMA cores to distinguish tumour tissue from stromal tissue (tissue segmentation), cells from non-cells (cell segmentation), and between different cell types (cell phenotyping).

Of the 187 cores available for immune cell infiltration analysis, 137 cores from 69 patients were available for analyses: 57 of the cases with immune cell infiltration data from the invasive tumour front and 54 with immune cell infiltration data from the tumour centre (Fig 10).

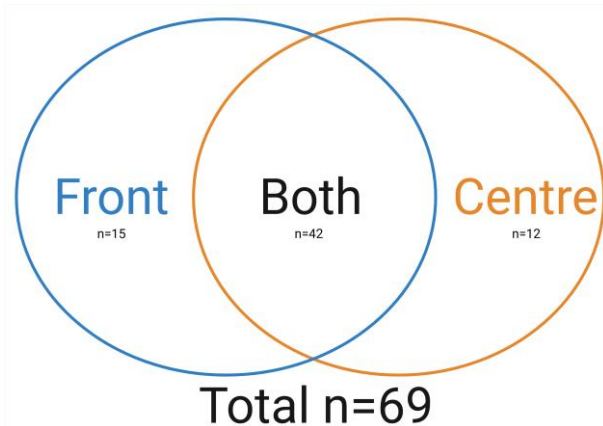


Figure 10 Venn diagram of core availability from front, centre or both.
Created with BioRender.com

Outcome measure

Immune cell density was reported as cells per mm² for each immune cell type in the invasive tumour front and centre. When two cores were available for analysis in the invasive tumour front the total amount of cells and tissue area were added and cells per mm² calculated from the added sum. All immune cell types were quantified in the stromal part of the tumour and cytotoxic T cells, Th1 cells, and macrophages were also quantified in the intra-epithelial part of the tumour.

Immune cell infiltration was divided into “high” and “low” where the two lowest tertiles were defined as “low” and the highest tertile as “high”. This division into two groups was made for all immune cell types in each location.

The primary outcome measure was immune cell infiltration by cytotoxic CD8⁺ T cells in the stromal part of the invasive front. Secondary outcome measures were immune cell infiltration by each additional immune cell type in both the invasive front and centre of the tumour.

Statistical analyses

Descriptive statistics

For comparison of baseline characteristics different descriptive statistical methods for hypothesis testing may be used based on the variables tested. When comparing independent categorical variables between groups, the chi-squared test (χ^2) may be used if the expected

frequency in each cell is over five. If the expected frequency is under five, the Fischer exact test gives a more accurate estimate. When comparing two independent groups with a continuous variable one must determine if the variable is normally distributed or not. This can be done graphically with a histogram or mathematically with, for example, the Shapiro Wilks test. If the two groups contain normally distributed continuous variables, the independent t-test comparing the means should be used. When comparing more than two groups with normally distributed continuous variables, one-way analysis of variance (ANOVA) can be used for comparing means. If the assumption of normal distribution cannot be met, a non-parametric test, comparing medians instead of means should be used. When comparing two groups the non-parametric version of the t-test, Mann-Whitney-U test may be used. If there are more than two groups, the non-parametric version of one-way ANOVA, the Kruskal Wallis test, may be used.

In Study I, baseline characteristics were compared using the original five-level scale for pre-diagnostic physical exercise. All continuous variables were tested for normal distribution using the Shaprio-Wilks test and all variables were defined as non-normally distributed. χ^2 was used for categorical variables and Kruskal-Wallis for continuous variables. In Study II, no statistical method was used for the descriptive variables. In study III, the density of each immune cell was compared to clinical variables. Immune cell density was defined as not normally distributed and so Mann Whitney U-test was used when comparing two groups and Kruskal Wallis test when comparing more than two groups. In study III, the physical test results, physical activity and body composition were also compared to immune cell density for each cell type grouped as high or low. The MVPA per day was defined as normally distributed so the t-test was used in calculations including MVPA per day. The other physical measurements were defined as not normally distributed and Mann Whitney U-test was used.

When conducting modern research using effective computers and powerful statistical software, it is easy to fall into the temptation to conduct just one more test. When conducting several tests some considerations are important. The α -level used in most research is 0.05 and a p-value below this is often considered significant. p reflects the probability of the results occurring at random and $p=0.05$ indicates a 5% risk that the observed difference between the groups is not a true difference. If multiple tests are conducted the chance of rejecting the null-hypothesis when it is actually true (type I-error) increases. The probability of making these type I-errors is also called family wise error

rate and can be calculated using the formula $(1-(1-\alpha)^n)$ where α indicates the significance level and n the number of tests performed. If 15 tests are conducted, the chance of finding at least one $p < 0.05$ is approximately 54% ($1-(1-0.05)^{15}=0.537$). A simple, and intuitive, method of handling multiple comparisons is the Bonferroni method. In the Bonferroni method, the α -level is divided by the number of tests. In the example above the Bonferroni corrected significance level when conducting 15 tests would be $0.05/15=0.0033$. The Bonferroni method is intuitive and easy but with many test the risk of not rejecting the null-hypothesis when it is actually false (type II-error) increases. One other way of adjusting for multiple testing that comes with lower risk of type II-errors is the Benjamini-Hochberg method (121). With the Benjamini-Hochberg method the p-values are ranked, and a critical value is calculated for each p-value depending on the numbers of tests and the false discovery rate decided. The highest p-value that is smaller than the critical value is detected and all lower p-values are considered significant (121). In Study III, a large number of statistical tests were used due to the inclusion of five immune cell types from both tumour front and centre being analysed against three physical tests, accelerometer data, and data on body composition. We used both the Bonferroni corrected p-values and the Benjamini-Hochberg method to lower the risk of type-I error.

Regression analysis

In observational studies there is no randomisation of study participants, and the groups cannot be considered similar. Due to this, descriptive statistics without adjusting for covariables are usually not considered sufficient. The choice of statistical method usually falls on one kind of regression analysis with the possibility of adjusting for covariates.

In Study I, the outcome variable immune cell infiltration in the tumour microenvironment, was reported on an ordinal scale which is why we used ordinal logistic regression. The proportional odds assumption was tested using the Brant test. When the assumption of proportional odds was violated, generalised ordinal regression was used (122, 123). In studies II and III, sarcopenia, myosteatosis, and immune cell density were defined on a binary scale (yes/no and high/low), and logistic regression was used as the regression model.

When analysing the relationship between an exposure and an outcome the risk of confounding needs to be considered, otherwise the possible association seen could wrongly be interpreted as a causal effect when the association is explained by a third variable, a confounder. For a variable

to be considered a confounder the variable needs to be associated with both exposure and outcome as well as not be part of the causal chain between exposure and the outcome. In observational studies, the risk of confounding can never be eliminated partly because of the risk of unknown confounding variables. Adding confounding variables in a multivariable model also decreases the power of the analysis and is why the sample size often limits the numbers of confounding variables that can be included.

Different approaches to choosing variables in the multivariable models were used in the three quantitative studies in this thesis. In Study I, two multivariable models were created, one minimally and one fully adjusted model. The minimally adjusted model included sex, age at diagnosis, and tumour stage. In the fully adjusted model, additional variables were included. In the CD8⁺ analysis tumour location, age at baseline, CRP, and year when diagnosed were added and in the CD3⁺ analysis tumour location, BMI, smoking, systolic blood pressure, and alcohol intake were added. In Study II, age at diagnosis, tumour location, tumour stage, sex, and educational level were included in the multivariable model. In Study III, only age and sex were included.

In multivariable regression models, the independent variables are assumed to be independent of each other. Multicollinearity occurs when one independent variable is highly correlated to another independent variable. A variance inflation factor (VIF) above ten is generally considered a threshold for the risk of multicollinearity (124). To further lower the risk of multicollinearity we used the value five as the threshold in the studies included of this thesis. No multivariable regression analysis had a VIF above five and multicollinearity was not considered a problem in the studies included in the thesis.

Missing data

When conducting retrospective research and using population-based cohorts, the problem of missing data is unavoidable. Missing data are usually divided into three distinctly different types according to the cause of the absence as first described by Donald Rubin in 1976 (125). The categories described were: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR) (126). MCAR means that the missing data are independent of both the observed and the missing data, an example being a survey where one page was missing for 5% of the participants. When missing data are MCAR there is no systematic explanation for the missingness, and the

risk of bias is therefore small. When missing data are MAR, they are related to the observed values but not the missing values. For example, a self-reported health survey where the relationship between physical activity and length is studied. If people who conduct more physical activity are more likely to report their length, then the missing length data may be considered MAR, since they are related to the physical activity data. MNAR is considered the missing category with the highest risk of bias. This is because the missingness is systematically related to the missing values. For example, if blood pressure is measured and the cuff only measures up to 140 mmHg. All observations above 140 mmHg will be missing, which means that participants with high blood pressure will systematically have missing blood pressure values. Both MAR and MNAR imply a risk of systematic errors in the missing data, bias (126).

There are several possible methods for handling missing data ranging from removal of observations to imputing the missing values. For outcome and exposure variables in all three quantitative studies, we decided to use listwise deletion, which is the removal of all observations missing either of the variables. For covariables, with less than 20 observations missing, we imputed the missing values using sex-specific medians for continuous variables and sex-specific modes for categorical variables. One covariate, alcohol intake in Study I, had more than 20 data values missing. For this variable, the missing data were included as a separate category in the regression models.

Survival analysis

Survival analysis assesses the relationship between an exposure and the time to the occurrence of an event. In survival analysis, there is a possibility to censor an observation. An observation where the event has not yet happened is censored, either at the end of a study or if lost to follow-up. One common approach in survival analysis is to use the Kaplan-Meier curve to graphically visualise survival curves between two groups. To statistically test if two survival curves are different from each other, the log-rank test can be used (127). The log-rank test cannot adjust for additional covariables which is why the semiparametric test Cox proportional hazard model is often used for multivariable survival analysis. The Cox proportional hazard model allows the hazard to change over time but assumes that the hazard of an event between the groups is proportional. The proportionality assumption can be checked in different ways. One way is to plot the log-log survival curves of the variables of interest. Parallel lines indicate proportionality. Another way is to check the proportionality assumption by using the Schoenfeldt's

residual-based test where a significant result indicates non-proportionality.

In Study II, survival analysis with Cox regression was conducted for both sarcopenia and myosteatorsis for both overall and cancer-specific mortality respectively. To investigate the possible combined effect on mortality if both sarcopenia/myosteatorsis and low exercise level were present we created cross-combined variables with four groups for both sarcopenia and myosteatorsis. The combination with the best expected survival, no sarcopenia/myosteatorsis + high exercise, was used as the reference category.

All the models included in Study II were tested for proportionality assumption and when the assumption was not met the model was stratified on the variable or variables that violated the assumption. No model included violated the proportional hazard assumption and the results were presented as final. A log-likelihood test comparing the multivariable model including an interaction term between physical exercise and sarcopenia/myosteatorsis with the multivariable model without the interaction term was conducted for sarcopenia and myosteatorsis respectively for both overall and cancer-specific mortality. All log-likelihood tests were non-significant and we concluded that the models without the interaction term fitted the models most appropriately and were used.

Study IV

Study IV was a qualitative study using content analysis according to Graneheim and Lundman (112). Participants diagnosed with colon cancer in northern Sweden (Region Västernorrland, Region Västerbotten, and Region Norrbotten) were recruited for semi-structured interviews between September 2020 and November 2021. Inclusion criteria were that the patients should be above 18-years-of-age, able to speak and understand Swedish, diagnosed with colon cancer, awaiting primary elective surgery with curative intent (Stages I-III), no neoadjuvant chemotherapeutic treatment, and self-reported physical activity level following the national recommendations of the Public Health Agency in Sweden (128). Eligible participants were contacted by telephone by the author of this thesis after they had received written information on the study by mail. For the assessment of physical activity, a two-item questionnaire (Table 2) regarding their recreational physical

exercise and everyday physical activity was completed over the telephone (129).

Question 1
During a normal week, how much time do you spend exercising on a level that makes you short of breath, for example running, fitness class, or ball games?
1 – 0 minutes/no time 2 – Less than 30 minutes 3 – 30-60 minutes 4 – 60-90 minutes 5 – 90-120 minutes 6 – More than 120 minutes
Question 2
During a normal week, how much time are you physically active in ways that are not exercise, for example walking, bicycling, or gardening? Add together all activities lasting at least 10 minutes.
1 – 0 minutes/no time 2 – Less than 30 minutes 3 – 30-60 minutes 4 – 60-90 minutes 5 – 90-150 minutes 6 – 150-300 minutes 7 – More than 300 minutes

Table 2 Questions in the two-question questionnaire to assess the physical activity level of the participants. Scores were given according to the number indicated in bold.

The answers to the two questions were scored and a score of eleven or more indicated adherence to the physical activity recommendations of the Public Health Agency in Sweden (128), which are the same as those of the WHO (6). If written and verbal informed consent was given and the participant fulfilled all inclusion criteria, an appointment for a telephone interview was scheduled.

Eighty-four participants were eligible for inclusion. Of these, 21 did not meet the physical activity recommendations, 16 were not reached in time, 15 declined participation, nine did not answer the telephone, and three were not able to speak or understand Swedish. Only the 20 participants included and the 21 who did not meet the physical activity recommendations were asked regarding their physical activity, all other participants were excluded before being questioned on physical activity. The final sample size was 20 informants. A question guide for semistructured interviews consisting of nine main questions (Fig 11) was constructed and discussed among three of the authors. Telephone

interviews using the question guide were then carried out with the participants. The interviews were recorded and later transcribed word-by-word. The transcribed interviews were read and re-read. The text was divided into shorter meaning units which were further condensed. From the condensed meaning units, the data were labelled and divided into codes. The analysis process continued, and the codes were sorted and abstracted into subcategories, categories, and main categories. Only codes related to the aim were included in the final abstraction after discussion among the authors. Data were continuously analysed when a new interview was transcribed, and the codes and categories were regularly and repeatedly discussed among the authors.

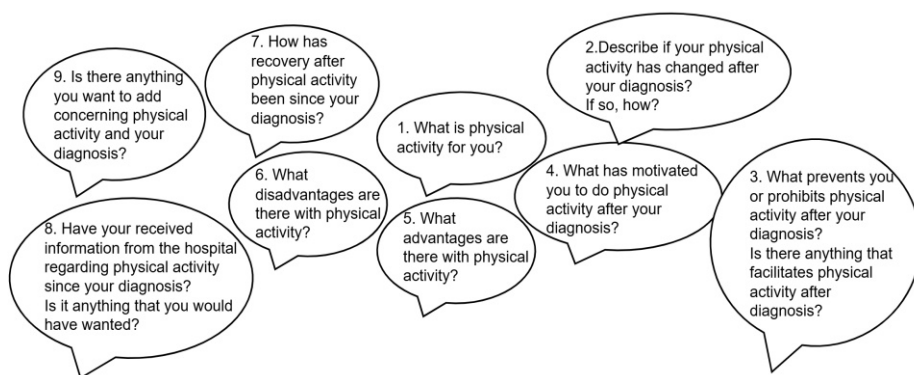


Figure 11 Semistructured interview guide. Follow-up questions such as “Can you give an example”, “Can you elaborate”, “What did you mean by that”, were asked when appropriate.

Ethics

All studies included in this thesis are approved by our ethics review board. Application for Study IV was filed after the Swedish Ethics Review Authority reorganised from being several regional boards to being a national authority. Studies I, II, and III were approved by the Regional Ethics Review Board in Umeå, Sweden (Study I Dnr: 2015-243-31 and amendment 2020-00081; Study II Dnr: 2016/221-31, 2015-243-31M and 2017-172-32M; Study III Dnr: 2018-52-31M, 2018-299-332, 2018-332-32M and 2019-03091). Study IV was approved by the Swedish Ethics Review Authority (Dnr: 2020-01661 and 2021-00553). An amendment to Study III has also been approved by the Swedish Ethics Review Authority (Dnr: 2023-00146-02).

Results

Study I

In the final study population (n=519), participants with higher pre-diagnostic recreational physical exercise had shorter times between baseline and diagnosis (p=0.009), higher education level (p=0.002), and lower systolic blood pressure (p=0.043). When observations with missing data for physical exercise and stage were excluded, both CD3⁺ and CD8⁺ Total Score were associated with female sex, right-sided colon cancer, and higher tumour stage (Table 3 and 4). This was also evident when all observations were included in the analysis, however for CD3⁺ Total Score in right-sided colon cancer was not significant (p=0.051).

	CD8 ⁺ Total Score				p-value
	N = 474	1 low n=194	2 medium n=126	3 high n=154	
Age at diagnosis, n (%)					
<55	54	24 (12.4)	18 (14.3)	12 (7.8)	0.204
55-65	120	53 (27.3)	30 (23.8)	37 (24.0)	
65-75	22	95 (49.0)	57 (45.2)	73 (47.4)	
>75	75	22 (11.3)	21 (16.7)	32 (20.8)	
Sex, n (%)					
Male	236	112(57.7)	62 (49.2)	62 (40.3)	0.005
Female	238	82 (42.3)	64 (50.8)	92 (59.7)	
Tumour site, n (%)					
Right colon	174	61 (31.4)	37 (29.4)	76 (49.4)	0.001
Left colon	154	63 (32.5)	44 (34.9)	47 (30.5)	
Rectum	146	70 (36.1)	45 (35.7)	31 (20.1)	
Stage, n (%)					
I and II	266	92 (47.4)	68 (54.0)	106 (68.8)	<0.001
III and IV	208	102 (52.6)	58 (46.0)	48 (31.2)	

Table 3. Clinical characteristics for CD8⁺ Total Score respectively. Statistical calculations using Chi-squared.

	CD3+ Total Score				p-value
	N = 559	1 low n=176	2 medium n=156	3 high n=227	
Age at diagnosis, n (%)					
<55	78	26 (14.8)	26 (16.7)	26 (11.5)	0.124
55-65	160	55 (31.3)	48 (30.8)	57 (25.1)	
65-75	247	79 (44.9)	64 (41.0)	104 (45.8)	
>75	74	16 (9.1)	18 (11.5)	40 (17.6)	
Sex, n (%)					
Male	288	93 (52.8)	97 (62.2)	98 (43.2)	0.001
Female	271	83 (47.2)	59 (37.8)	129 (56.8)	
Tumour site, n (%)					
Right colon	200	56 (31.8)	51 (32.7)	91 (41.0)	0.026
Left colon	180	53 (30.1)	48 (30.8)	79 (34.8)	
Rectum	179	67 (38.1)	57 (36.5)	55 (24.2)	
Stage, n (%)					
I and II	309	69 (39.2)	87 (55.8)	153 (67.4)	<0.001
III and IV	250	107 (60.8)	69 (44.2)	74 (32.6)	

Table 4. Clinical characteristics for CD3+ Total Score respectively. Statistical calculations using Chi-squared.

In the ordinal logistic regression model, pre-diagnostic recreational physical exercise more than 3 times a week was associated with higher infiltration of cytotoxic CD8⁺ T cells in the tumour front (OR 2.77, 95% CI 1.21-6.35 minimally adjusted, and OR 2.91, 95% CI 1.25-6.75 fully adjusted) and tumour centre (OR 2.85, 95% CI 1.28-6.33 minimally adjusted, and OR 2.92, 95% CI 1.31-6.50 fully adjusted). In the model for CD8⁺ Total Score, results for pre-diagnostic recreational physical exercise had ORs around two, however these were not significant due to wide confidence intervals. For infiltration by CD8⁺ in the intra-epithelial

compartment and CD3⁺ in all three locations, and Total Score, no statistically significant results were seen.

In the analysis of subgroups, results were consistent with the total population indicating that there were no differences between men and women, follow-up time between participation in the Västerbotten Intervention Programme and diagnosis longer or shorter than the mean, and normal or overweight according to body mass index.

To provide the reader of this thesis with a clinical evaluation of the results with increased immune cell infiltration by cytotoxic CD8⁺ T cells we conducted a survival analysis not published in the original paper. Participants without data on physical exercise and stage were excluded. Higher abundance of both cytotoxic CD8⁺ T cells and CD3⁺ T cells in the tumour front, centre, and intra-epithelial compartment were all associated with decreased hazard for both cancer-specific and overall mortality (data not shown). This was also evident when adjusting for sex, age at diagnosis, and stage (data not shown).

Study II

Pre-diagnostic physical exercise was associated with sarcopenia (OR 1.62, 95% CI 1.04-2.52) but not myosteatorosis (OR 1.20, 95% CI 0.77-1.86) in univariable logistic regression. When adjusting for tumour stage, tumour location, age at diagnosis, sex, and education level no significant results were seen for sarcopenia (OR 1.37, 95% CI 0.86-2.19) or myosteatorosis (OR 0.95, 95% CI 0.59-1.51).

In the survival analyses for overall mortality, both sarcopenia and myosteatorosis were associated with increased hazard of mortality in the univariable analyses. When adjusting for tumour stage, tumour location, age at diagnosis, sex, and education level, the HR for sarcopenia was 1.49 (95% CI 1.09-2.04) and for myosteatorosis 1.38 (95% CI 0.99-1.92). In the cancer-specific models, no significant association with mortality risk was seen for sarcopenia and myosteatorosis in both uni- and multivariable analyses. In cases with low pre-diagnostic exercise, no significant mortality risk was seen in overall and cancer-specific mortality models.

When combining sarcopenia and myosteatorosis respectively with pre-diagnostic physical exercise, the presence of sarcopenia and low physical exercise in the multivariable cancer-specific mortality model showed increased mortality risk (HR 1.94, 95% CI 1.00-3.76). For the other

combinations in the multivariable cancer-specific mortality model, no significant associations were seen. The same pattern was seen for myosteatorsis and cancer-specific mortality where myosteatorsis and low physical exercise were associated with increased mortality risk in both the uni- and multivariable analyses (HR 2.24, 95% CI 1.13-4.43) and (HR 2.39, 95% CI 1.16-4.94) respectively.

In the overall mortality models the combination of myosteatorsis and high exercise was associated with increased mortality risk (HR 2.08, 95% CI 1.12-3.88) while the combination of myosteatorsis and low exercise were non-significant and with a smaller effect size (HR 1.58, 95% CI 0.93-2.66). For the other combinations in the multivariable overall mortality model, no significant association was seen.

Two sensitivity analyses were performed. The first where cases with physical exercise reporting within one year of diagnosis were excluded (n=19) and the second where cases with CT-scans after surgery (n=19) were excluded. Neither of the sensitivity analyses affected the main results.

Study III

In the tumour front, higher ASMI and higher number of repetitions in the 2MST were associated with decreased densities of cytotoxic CD8⁺ T cells and CD20⁺ B cells. In the tumour centre, more minutes MVPA per day and higher number of repetitions in the 30-second sit-to-stand test were associated with lower densities of Tbet⁺ Th1-cells. No other significant associations (p<0.05) were seen for the other physical measurements and immune cell types in the tumour front or centre. When correcting for multiple testing with the Bonferroni method (0.05 / 40 = 0.00125) and the Benjamini-Hochberg method all associations were considered non-significant in both methods.

Uni- and multivariable logistic regression analyses were performed for all immune cell types in the stromal compartment of both tumour front and centre cores. The 30-second sit-to-stand test was associated with lower density of Tbet⁺ Th1 cells even when adjusted for age and sex (OR 0.82, 95% CI 0.70-0.96, p=0.016). Although after correcting for multiple testing with both the Bonferroni method (0.05 / 30 = 0.0016) and the Benjamini-Hochberg method the result was interpreted as not significant in both methods. No other significant findings were seen in the tumour front or centre, however a trend towards significance was

seen for ASMI and cytotoxic CD8⁺ T cells in the tumour front (OR 0.47, 95% CI 0.22-1.01) in the multivariable model.

When analysing the three physical tests, MVPA per day and ASMI with sex, tumour location, and stage, almost all measurements had results indicating better physical performance in male sex, left-sided cancer, and higher stage (Table 5).

	n	MVPA per day¹	n	2-minute step test²
Sex				
Male	37	30.0 (8.6-60.9)	38	101 (22.5)
Female	24	27.7 (7.3-49.2)	24	92.8 (22.3)
Tumour location				
Right	30	18.0 (2.7-39.7)	30	94 (25.8)
Left	31	38.3 (16.0-76.0)	32	102 (18.8)
Stage				
I	13	16.8 (4.2-50.3)	14	88 (27.5)
II	29	34.8 (8.6-62.8)	28	99 (22.3)
III	19	31.0 (16.0-48.8)	19	102 (16.1)
		30-second sit-to-stand test²	n	Hand grip strength²
Sex				
Male	42	17 (5.4)	43	43.2 (9.3)
Female	25	15 (5.5)	25	26.9 (5.9)
Tumour location				
Right	33	15 (6.0)	33	35.6 (11.6)
Left	34	17 (4.7)	35	38.7 (11.1)
Stage				
I	14	16 (6.5)	14	33.0 (10.4)
II	30	17 (5.7)	31	39.1 (12.1)
III	22	15 (4.0)	22	37.0 (10.8)
		ASMI²		
Sex				
Male	44	7.6 (0.9)		
Female	25	6.4 (1.0)		
Tumour location				
Right	34	6.8 (1.2)		
Left	35	7.5 (0.9)		
Stage				
I	14	7.0 (1.3)		
II	31	7.1 (1.1)		
III	22	7.4 (0.9)		

Table 5. Distribution of results from accelerometer, DXA-scan, and physical tests according to sex, tumour location and stage.

¹ – results displayed as median (25-75th percentile)

² – results displayed as mean (SD)

When analysing the combined fit variable, no results were significant but ORs were generally above 1 except for cytotoxic CD8⁺ T cells and regulatory FoxP3⁺ T cells in the tumour centre. In all analyses the confidence intervals were wide indicating low power.

Study IV

Characteristics of the study participants in Study IV are displayed in Table 6.

Study Participants	
Sex, n (%)	
Men	10 (50)
Women	10 (50)
Age, mean (range)	70.4 (50-88)
Body mass index, mean (range)	25.1 (20.8-32.4)
Highest level of education, n (%)	
Elementary school	6 (30)
Secondary school	6 (30)
Post-secondary education	5 (25)
Missing	3 (15)
Social status, n (%)	
Married	16 (80)
Partnership (not married)	1 (5)
Single	1 (5)
Widow/widower	1 (5)
Missing	1 (5)
Distance to treating hospital, km mean (range)	76.8 (0.7-250)
County of abode, n (%)	
Region Västernorrland	6 (30)
Region Västerbotten	10 (50)
Region Norrbotten	4 (20)

Table 6. Characteristics of the study participants in Study IV

Physical activity varied between participants including walking, cross-country skiing, heavy weightlifting, running, and step training. For many of the participants, physical activity was a large part of their lifestyle, given priority, and was referred to as being sociable and fun.

Three different main categories were abstracted from eleven categories.

Main category 1: "I'll fight this cancer and come out stronger"

This main category was abstracted from four categories (Fig 12) and the experiences of increased motivation and greater determination for physical activity after having their colon cancer diagnosed were

described. A belief in the future and a will to be able to maintain physical activity after receiving their diagnosis was experienced. Increased motivation for physical activity was described as founded by the desire to manage the operation planned. Participants also experienced greater motivation for physical activity to improve their postoperative recovery.

“I am more determined now to do it (=physical activity) every day [...] To perform the activity. Not skipping it, making sure to do it”, Female 79 years.

“I try to walk more than 10,000 steps per day and I’m also dancing 30 to 60 minutes each day. This is new, I didn’t do this before (=the diagnosis). Maybe three times per week before but now it’s every day”, Female 70 years.

“It is even more motivating because you read about the diagnosis. And you read that it can be good and positive for both rehabilitation and the disease. I feel even more motivated (=to exercise)”, Male 72 years.

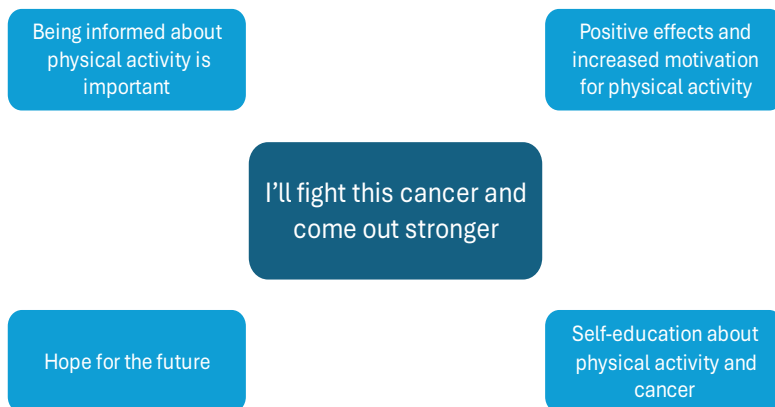


Figure 12 Main category 2 “I’ll fight this cancer and come out stronger” and its subcategories.

Main category 2: “The diagnosis makes no difference”

The diagnosis makes no difference main category (Fig 13) contains experiences of unchanged lifestyle and physical activity after the diagnosis. Experiences of that the life was lived as before without alterations including physical activity and exercise habits. Most of the experiences of not altering physical activity stemmed from being symptomless between diagnosis and surgery which presumably made it easier carry on living as before the diagnosis.

“The physical activity is not changed compared to before. It is roughly as before”, Female 79 years.

“No I haven’t changed anything and I will not change anything. Why should I do that?”, Female 77 years.

“There is no difference compared to before. It’s the same. I don’t conduct hard physical activity so I don’t see any change in recovery”, Male 73 years.

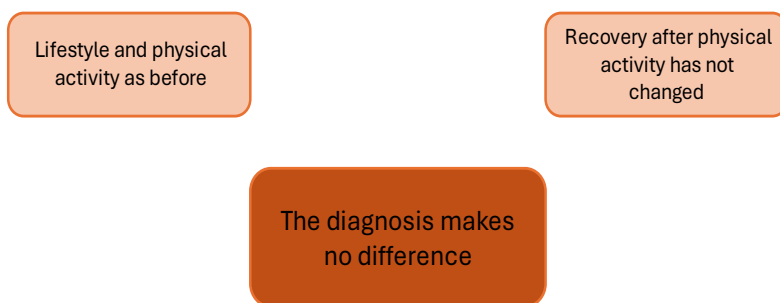


Figure 13 Main category 2 “The diagnosis makes no difference” and its subcategories.

Main Category 3: “The diagnosis is an obstacle to physical activity”

For some, the motivation to do physical activity was lower after than before diagnosis. Experiences of difficulties in being physically active after the diagnosis were also reported. Participants who expressed these motivational and physical difficulties expressed physical or mental symptoms that affected their ability to conduct physical activity. For some participants receiving the diagnosis, hospital visits and examinations required were time-consuming, affecting their ability to perform physical activity. Several experiences in this main category were worries about the time after surgery and/or disturbing thoughts about the diagnosis. Thoughts regarding how much physical activity was safe and possible after surgery were also expressed. Disturbing thoughts that altered the ability to conduct physical activity in a negative way were also evident (Fig 14).

“It is the mental thoughts that are an obstacle to it (=physical activity). You wonder and think, that’s it”, Male 76 years.

“I’ve had problems with my bowels and not been able to do physical activity when I’d like. I’ve had to wait a while before it gets better during the morning [...] so I’ve had to skip physical activity in the morning”, Female 64 years.

“Before I spent time on physical activity; but now that time is spent dealing with the disease; visiting the hospital and healthcare centre, making phone calls to doctors and nurses et cetera”, Male 75 years.

“It’s all the thoughts going on that prevent physical activity – constant thoughts like: That’s it! What’s going to happen? Then again; That’s it! Will I manage? Will it end up well? Will I survive the operation?”, Male 76 years.

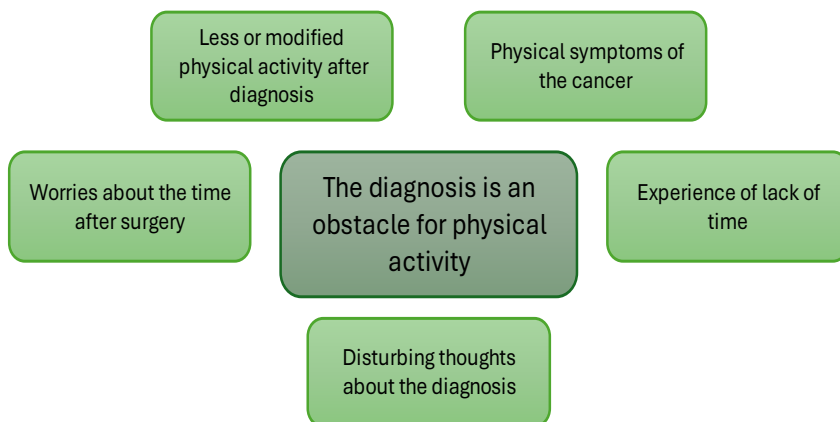


Figure 14 Main category 3 “The diagnosis is an obstacle to physical activity” and its subcategories.

Discussion

That physical activity is beneficial to general health is well-known and physical activity provides better mental and physical health and may lower the risk for several forms of cancer (6). When delving into research on physical activity you are quickly faced with the wide definition and interpretation of “physical activity”. For example, physical activity and exercise are not the same. Physical activity conducted at work *i.e.*, occupational, may have other effects on health than physical activity in general. How vigorous the physical activity is must also be taken into consideration. Fitness, a natural consequence of physical activity, can be divided into cardiorespiratory, functional, flexibility/mobility, muscular strength, and muscular endurance. When struggling to define physical activity one must also consider sedentary behaviour because given the same amount of physical activity during the week, what is done at other times may also affect the individual’s health.

Further problems when defining physical activity arise when trying to quantify it both in a social context, a clinical context, and especially in the research setting. The gold standard for quantifying physical activity, the doubly labelled water method, is very resource demanding and its use in research is limited. How and when to quantify physical activity is a difficult problem. Questionnaires are easily conducted, cheap, and can reach many individuals but they lack in objective assessment and suffer from bias (106). Moreover, the number of questionnaires available is high and choosing which questionnaire to use is another problem facing a researcher conducting research into physical activity as the exposure. Accelerometers are another alternative that are more expensive but provide objective data, however, which week in an individual’s life is the most representative? When the individual has also recently been diagnosed with cancer, the question of representativeness is even more relevant. If the researcher instead wants to quantify fitness there are also several questionnaires and more or less easily conducted tests assessing various aspects of fitness.

When a researcher decides on how and when to quantify physical activity, further difficulties emerge, particularly in research on cancer patients. If you are interested in hard outcome data, such as risk for getting the disease or mortality after diagnosis, the time aspect is important. An RCT is really not an option using physical activity as exposure and mortality or risk for cancer as endpoint. Consequently, the

degree of physical activity needs to be collected beforehand as part of another study or cohort, collected around the time of diagnosis (risking results being affected by the disease), or retrospectively assessed using self-reported questionnaires. All these methods suffer from limitations in not knowing the actual physical activity level of the individual. Prospectively collected cohort data, such as data from the Västerbotten Intervention Programme, has an obvious advantage compared to retrospectively collected physical activity data due to minimal recall bias. In Sweden, data from the military conscription examination, is another valid source of prospective data where actual physical testing for muscular strength and cardiorespiratory fitness are conducted. The unique identification number that every citizen possesses in Sweden enables linking data to population-based registers providing interesting and objective data on fitness in young adults at the population level. Historically, however, data on women are lacking. Onerup *et al* published a study describing associations between cardiorespiratory fitness in young adults (at conscription) and hazard ratio of developing site-specific cancer, including colon and rectum (11). Over 1 million men were included and just over 84,000 developed a cancer during the mean follow up of 33 years, of which 3,222 cases were colon and 2,337 were rectal cancer. When cardiorespiratory fitness was graded into a nine-level scale during conscription, being in the two highest categories was associated with a reduction in risk for colon cancer by 18%, while the association was weaker and non-significant for rectal cancer with only a 5% reduction (11). When cardiorespiratory fitness was reported on its original continuous scale, using maximal Watt generated per kg, a more objective measurement, the risk was significantly reduced by 21% for colon cancer and 15% for rectal cancer (11). Another Swedish study included almost 140,000 males with cardiorespiratory fitness data from both the conscription cohort and from a database with data on cardiorespiratory fitness in adulthood. The better the cardiorespiratory fitness at conscription the lower the risk for colorectal cancer also when cardiovascular comorbidity was adjusted for (130). The latter study reported colorectal cancer as one entity and not colon and rectal cancer separately. In another study from Ekblom-Bak *et al*, a cohort based on the same database was studied but only with data on cardiorespiratory fitness in adulthood. They investigated the association between cardiorespiratory fitness and both colon cancer risk and cancer-specific mortality and concluded that the risk for colon cancer was lower with moderate to high cardiorespiratory fitness. The cancer-specific mortality risk was lower in the crude model, but no association was seen after adjusting for covariables such as education level, diet habits, comorbidity, and smoking (131).

In epidemiological research there is always some degree of uncertainty whether associations seen are causal. Given all obstacles and limitations there is strong evidence that moderate to vigorous physical activity decreases the risk for colon cancer (22). For rectal cancer evidence for physical activity lowering the risk is not as convincing as with colon cancer, and the previous mentioned CUP report does not conclude a risk-lowering effect in rectal cancer (22). The evidence is difficult to interpret since almost all data available come from self-reported information in observational studies. In a meta-analysis from 2013, 16 observational studies had data on physical activity and colorectal cancer risk at different sites and reported an RR of 0.76 (95% CI 0.70-0.83) for right-sided colon cancer, an RR of 0.77 (95% CI 0.71-0.83) for left-sided colon cancer, and an RR of 0.98 (95% CI 0.88-1.08) for rectal cancer (132). Two later published meta-analyses included 31 (133) and 23 (134) observational studies respectively (only one observational study was included in both meta-analyses) on colorectal cancer with a pooled RR of 0.84 (95% CI 0.77-0.93) (133) and RR 0.77 (95% CI 0.71-0.83) (134) respectively. When analysing the pooled risk estimates from the studies reporting risk by site, the results differed between colon and rectal cancer with pooled an RR of 0.81 (95% CI 0.75-0.88) for colon cancer and an RR of 1.07 (95% CI 1.07-1.24) for rectal cancer in the first meta-analysis (133) and an RR of 0.79 (95% CI 0.67-0.91) for colon cancer and an RR of 0.80 (95% CI 0.54-1.07) for rectal cancer in the latter (134). There is also some evidence for a risk-lowering effect in rectal cancer. In 2016, Moore *et al* published pooled data from 12 prospective cohorts with self-reported physical activity covering 1.44 million individuals. They reported decreased risk for colon cancer (HR 0.84, 95% CI 0.77-0.91) and for rectal cancer (HR 0.87, 95% CI 0.80-0.95) in physically active individuals (135).

Regarding prognosis, two recent meta-analyses of observational studies reported an association between pre-diagnostic physical activity and overall and cancer-specific mortality in colorectal cancer (13, 136). In a meta-analysis by Wu *et al*, eight observational studies were included. The pooled RR was 0.81 (95% CI 0.71-0.91) for colorectal cancer-specific mortality and 0.79 (95% CI 0.71-0.89) for overall mortality (136). Qiu *et al*, included 12 observational studies with data on pre-diagnostic physical activity and of those, six were not included in Wu's analysis. Qiu reported a pooled RR of 0.85 (95% CI 0.77-0.93) for colorectal cancer-specific mortality and 0.81 (95% CI 0.76-0.87) for overall mortality (13). Of the 14 observational studies included, only seven reported separate data for colon and rectal cancer. Those seven studies included 15,978 cases with colorectal cancer of which 4,801 were rectal cancer cases.

Three studies reported a significantly lower risk of overall mortality in patients with a high level of physical activity for colon cancer (26-49% lower) but non-significant findings for rectal cancer (137, 138, 139). Only one of the studies reported colorectal cancer-specific mortality risk with HR 0.72 (95% CI 0.51-1.02) for colon cancer and HR 0.76 (95% CI 0.49-1.18) for rectal cancer (137). Contrary to the findings of these studies, two studies reported a significantly lower risk for overall mortality in rectal cancer cases and non-significant findings for colon cancer. The first of these studies reported a HR of 0.86 (95% CI 0.77-0.96) for overall mortality and a HR of 0.85 (95% CI 0.75-0.97) for colorectal cancer-specific mortality in rectal cancer cases scoring just one point higher on a combined score constructed from information on adherence to specific recommendations from the American Institute for Cancer Research (AICR) where physical activity is included (140). The second study used self-reported pre-diagnostic physical activity assessed after diagnosis. Rectal cancer cases reporting more than 30 MET hours per week had a HR of 0.49 (95% CI 0.25-0.95) compared to cases reporting zero MET hours per week. For colorectal cancer-specific mortality the corresponding HR was 0.60 (95% CI 0.29-1.40) whereas for colon cancer HRs for overall and colorectal cancer-specific mortality were non-significant (141). Another study from Scotland reported non-significant findings in both colon and rectal cancer (142) In an Australian study, cases were divided into right-sided colon, left-sided colon, and rectal cancer. When these cancer locations were analysed separately they observed a 50% lower risk of colorectal cancer-specific mortality in cases with self-reported activity greater than “none at all” in right-sided cancer whereas no significant findings were seen for left-sided colon or rectal cancer (143). In a study from Korea, data from 275 colorectal cancer deaths were linked to a prospective cohort consisting of more than 200,000 individuals providing self-reported data on pre-diagnostic physical activity. In the overall mortality model, individuals with self-reported “exercise” and colon cancer had a significantly lower risk of mortality (HR 0.68, 95% CI 0.49-0.94), which was not seen in rectal cancer (144). In the colorectal cancer-specific mortality model, there were only nine cases in the high activity group with rectal cancer. Even so the risk estimate for rectal cancer was HR 0.57 (95% CI 0.28-1.17) and for colon cancer HR 0.84 (95% CI 0.52-1.37) (144).

In summary, there is not sufficient evidence to support the notion that pre-diagnostic physical activity lowers the risk for rectal cancer. Regarding prognosis, data on colon and rectal cancer diverge somewhat. When combining the two diagnoses as colorectal cancer, risk estimates often lean towards significance. However, when assessed individually it

has not been shown without doubt that this is true for either colon or rectal cancer. It is important to remember that risk for developing a disease and prognosis of the foresaid disease are two different entities. Prognosis, especially mortality outcome is affected by treatment, while the risk is not. The treatment of non-metastatic rectal cancer is generally tougher than for non-metastatic colon cancer, more often including neoadjuvant treatment and more extensive surgery. Rectal cancer also shows poorer 3- and 5-year mortality and recurrence rates compared to colon cancer (20, 21).

No study in this thesis was designed to confirm or deny a potential causal relationship between physical activity and immune cell infiltration or body composition in colorectal cancer, but rather to broaden our knowledge.

Physical activity and immune cell infiltration in colorectal cancer

The immune landscape in colorectal cancer is multifaceted and difficult to interpret. Different cell types have different functions in different surroundings. There are some immune cell types where evidence for a better prognosis is solid, for example cytotoxic CD8⁺ T cells (70, 71), effector memory CD45RO⁺ T cells (70, 72) and Th1 cells (62). Cytotoxic T cells are activated by Th1-cells and their activity and presence is highly correlated. CD56⁺ NK cells, which are a part of the innate immune system, also exert cytotoxic action and their presence seems to be associated with improved prognosis (72). The general T cell marker, CD3, is a part of the Immunoscore[®] and when levels are high there is an association with improved prognosis, probably due to abundance of cytotoxic T cells and Th1 cells. The prognostic power of regulatory FoxP3⁺ T cells, Th2, and Th17 cells is not so clear (62). Tumour-associated macrophages can be divided into M1 and M2 cells, where M1 cells are generally seen as antitumour and M2 as protumour. CD68, used as one of the five markers in Study III in this thesis, is a general macrophage marker and does not distinguish between the two types. CD20 is a B cell marker, however not all B cells express CD20. In colorectal cancer, CD20⁺ cells may indicate a positive prognosis (62, 68). The immune cell types analysed in this thesis were cytotoxic T cells (CD8⁺, Studies I and III), all T cells (CD3⁺, Study I), regulatory T cells (FoxP3⁺, Study III), Th1 (Tbet⁺, Study III), B cells (CD20⁺, Study III), and macrophages (CD68⁺, Study III).

In Studies I and III, physical activity, exercise, fitness, and body composition were investigated in relation to immune cell infiltration in different ways. Despite having similar hypotheses, they differed substantially in terms of study design, measurement of physical activity, and immune cell infiltration analysis. In study I, pre-diagnostic recreational physical exercise more than three times a week was associated with higher immune cell infiltration by cytotoxic CD8⁺ T cells assessed by immunohistochemistry in the tumour front and centre, but not in the intra-epithelial compartment, nor infiltration by CD3⁺ T cells. Cytotoxic CD8⁺ T cells can be mobilised to the bloodstream and tumour microenvironment by physical activity (77, 145, 146). The mobilisation of cytotoxic CD8⁺ T cells and NK cells could be the result of adrenergic mechanisms (76, 147). Different immune cells and T cell subsets have varying densities of adrenergic receptors on their surface, with CD8⁺ and NK cells having the highest densities of these receptors (148, 149). This could explain why previous animal studies have shown a greater effect of physical activity on cytotoxic CD8⁺ T cell mobilisation and why we in Study I only saw increased densities of cytotoxic CD8⁺ T cells in the front and centre, and not CD3⁺ T cells. The results of Study I indicate that pre-diagnostic physical exercise may affect the mobilisation and infiltration of cytotoxic CD8⁺ T cells in a different manner than the total T cell population in colorectal cancer.

In Study I, we found no association between pre-diagnostic recreational physical exercise and cytotoxic CD8⁺ T cell infiltration in the intra-epithelial compartment which did not support our hypothesis. There are different subtypes of cytotoxic CD8⁺ T cells and upon activation, depending on how and when they are activated, they can evolve into central memory or effector memory cells where the effector cells are the cell type with cytotoxic qualities (150). The effector memory subtype is mobilised to a greater extent after activity (146). These subsets of cytotoxic CD8⁺ T cells express different adhesion molecules and may therefore infiltrate different tumour areas and tissues in different ways (77). This could explain why we only observed increased cytotoxic CD8⁺ T cell infiltration in the front and center only.

Intervention studies with physical activity as the exposure and immune cell infiltration in the tumour microenvironment as the outcome, in general and in colorectal cancer in particular, is rather uncommon. Individuals with Lynch Syndrome have a hereditary defect in the mismatch repair gene and therefore a high life-time risk for microsatellite-unstable colorectal cancer. A non-randomised intervention study on 21 participants with Lynch Syndrome, comparing a group doing

45 minutes cycling sessions three times a week for 52 weeks with a group following usual healthcare, showed increased cytotoxic CD8⁺ T cell and NK cell density in the colon mucosa in the exercise group (151). Only eight participants were analysed immunohistochemically, and the results should be interpreted with caution. To our knowledge, other human intervention studies or studies assessing physical activity objectively when investigating the association between physical activity and immune cell infiltration in colon, rectum or colorectal cancer, have not been published. Kurz *et al* used a cohort of nine patients with pancreatic ductal adenocarcinoma who underwent a preoperative exercise programme and compared this with historical controls. They showed increased infiltration by cytotoxic CD8⁺ T cells in the tumour microenvironment in the exercise group (152). A Danish research group studied the effect of exercise intervention on tumour infiltration by NK cells (153), CD3⁺ T cells (154), and cytotoxic CD8⁺ T cells (154) in 30 men with prostate cancer awaiting radical prostatectomy. Twenty patients were randomised to 30 minutes high-intensity training four times a week over two to eight weeks depending on the time remaining before surgery and the other ten receiving standard care. Immune cell infiltration was analysed both in core biopsies and in the surgical specimen after radical prostatectomy. No differences were seen for any of the cell types in the intention-to-treat analysis. In the per protocol analysis of 11 participants in the intervention group, there was a significant increase in NK cell infiltration (153, 154). To our knowledge, these are the only intervention studies examining the effect of physical activity on immune cell infiltration in human solid cancer to be published.

There are observational studies that have investigated the effects of self-reported physical activity in human cancer. Huang *et al*, evaluated self-reported physical activity and its relationship to immune cell infiltration in prostate cancer. They showed less infiltration of CD4⁺ T cells and macrophages in men reporting higher levels of physical activity. No differences were seen for cytotoxic CD8⁺ T cells, NK cells, or B cells (155). Sasamoto *et al*, analysed the association between prospectively collected self-reported data on physical activity and immune cell infiltration by CD68⁺ macrophages and M2 CD163⁺ macrophages in 306 ovarian cancer cases. No association between self-reported physical activity and tumour infiltration by macrophages was seen (156). Another study on breast cancer patients, also using self-reported physical activity data, showed no association between physical activity and immune cell infiltration by cytotoxic CD8⁺ T cells, CD4⁺ T cells, CD20⁺ B cells, or CD163⁺ M2 macrophages (157). When compiling the results from human

intervention and observational studies it is difficult to draw far-reaching conclusions regarding the effect of physical activity on immune cell infiltration in cancer. The above-mentioned studies show heterogeneity in their study design and results. From these studies and the two studies in this thesis, we cannot confirm or deny any effect of physical activity or exercise on immune cell infiltration in solid cancer.

There are more studies published using animal models, but again research on colorectal cancer is limited. A previous study from Gouez *et al* investigated the effect of exercise in mice with subcutaneously injected colorectal tumour while receiving both cytostatic treatment (oxaliplatin and capecitabine) and immune checkpoint inhibitors (anti-PD-1)(158). The exercise intervention consisted of forced treadmill running five days a week starting around five days after tumour inoculation. In the group receiving both treatment and exercise, the densities of cytotoxic CD8⁺ T cells and CD4⁺ T cells in the tumour microenvironment were increased compared to exercise alone and treatment alone. In the group only exercising and not receiving treatment, increased immune cell infiltration was not seen. This indicates that the positive effects seen from exercise were dependent on the effects of treatment and also that the treatment effects were augmented by exercise (158). McClellan *et al* studied the effects of treadmill running six days a week over 12 weeks on intestinal polyp formation in an Apc^{Min/+} mouse model, which is a breed with a hereditary defect in the *Apc* gene making the mice predisposed to intestinal adenoma formation. Exercising mice had a similar polyp count to those not, but fewer large polyps (145). Gene expression analysis of mucosal scrapings showed decreased gene expression of CD206 and CCL22 both related to M2 macrophages. Further gene expression analysis showed increased numbers of cytotoxic CD8⁺ T cells and fewer regulatory FoxP3⁺ T cells (145). Other animal studies on physical activity and colorectal cancer models have not been published to our knowledge.

Most of the preclinical research published on physical activity and immune cell infiltration has been on either breast cancer or melanoma models. In a previous study on mice by Rundqvist *et al* (77), exercise was shown to reduce tumour growth and increase cytotoxic CD8⁺ T cell infiltration in mammary tumours. They also injected sedentary mice with cytotoxic CD8⁺ T cells from exercising donors and showed enhanced survival and reduced tumour growth in the mice with cytotoxic CD8⁺ T cells injected (77). Turbitt *et al* investigated immune cell infiltration by cytotoxic CD8⁺ T cells and CD4⁺ T cells from gene expression analysis of mammary tumours in mice (159). The mice were divided into an exercise or a sedentary group, and the exercise

intervention began eight weeks before inoculation of the tumour. The two groups were further divided into either energy restriction or not. The study showed increased infiltration by cytotoxic CD8⁺ T cells in the exercising mice that had energy restriction, while no increased infiltration was seen in the exercise group without energy restriction. No difference was seen for infiltration by CD4⁺ T cells (159). These results were in accordance with another study that studied energy intake without physical activity in mice inoculated with the MC38 colon adenocarcinoma cell line (160). Infiltration by cytotoxic CD8⁺ T cells was significantly lower in mice fed with high fat diet. Furthermore, when mice were treated with antibodies against cytotoxic CD8⁺ T cells, the excess in tumour growth seen in the high fat diet group was no longer evident indicating that tumour growth was to some extent controlled by infiltration by cytotoxic CD8⁺ T cells (160). In another mouse study on mammary cancer, treadmill running five days a week showed an increased number of activated cytotoxic CD8⁺ T cells, but no effect on the total number of cytotoxic CD8⁺ T cells in the tumour microenvironment (161). Gomes-Santos *et al* also studied immune cell infiltration in breast cancer models in mice and found higher immune cell infiltration by cytotoxic CD8⁺ T cells in mice exercising 45 minutes a day. No effect on immune cell infiltration was seen for CD4⁺ T cells, regulatory FoxP3⁺ T cells, NK cells, or macrophages (162). Different results were reported by Hagar *et al* who studied endurance exercise in mice injected with mammary cancer. Immunohistochemistry analysis of cytotoxic CD8⁺ T cells and regulatory FoxP3⁺ T cells showed no difference between sedentary or exercising mice. However, when analysing the CD8⁺/FoxP3⁺ ratio it was significantly increased in exercising mice indicating a more beneficial immune profile of the tumour microenvironment (163). Buss *et al* investigated voluntary wheel running in two mice model (melanoma and mammary cancer) and observed reduced number of cytotoxic CD8⁺ T cells and CD3⁺ T cells in the breast model while no effects were seen in the melanoma model with exercise (164). Interestingly, when combining exercise with PDL1 treatment the numbers of cytotoxic CD8⁺ cells were increased in the breast cancer model, which was similar finding to those of Gouez *et al* (158). Pedersen *et al* investigated immune cell infiltration in mice subcutaneously injected with melanoma tumour tissue (76). The exercise group underwent six weeks wheel running. Tumours in the running mice had higher immune cell infiltration as shown by flow cytometry of NK cells, CD3⁺ T cells, and dendritic cells. No difference was seen for cytotoxic CD8⁺ T cells, CD4⁺ T cells, B cells, or Myeloid-derived suppressor cells (76). In another model using intravenously injected melanoma cells, exercise intervention of four weeks of wheel running

was associated with greater immune cell infiltration for only NK cells when using flow cytometry. However, when analysing the degree of infiltration by immunohistochemistry, greater infiltration was seen for NK cells, cytotoxic CD8⁺ T cells, and CD4⁺ T cells (76). The authors also investigated the effects of exercise on athymic mice *i.e.* mice without T cells. They showed that 66% of the tumour volume persisted, indicating that the tumour suppressing effect of exercise was not only exerted through T cells. When treating athymic mice with antibodies against NK cells, the tumour suppressing effect of exercise was completely abolished (76) indicating that the tumour suppressing effect was dependent on both cytotoxic CD8⁺ T cells and NK cells. In a model where mice conducted swim exercise daily for four weeks after inoculation of breast cancer, the exercise intervention had no effect on infiltration by CD4⁺ T cells, cytotoxic CD8⁺ T cells, Th1-cells, and regulatory FoxP3⁺ cells (165). Results from animal studies are difficult to extrapolate to humans, and the studies mentioned above highlight the difficulties in interpreting how physical activity affects immune cell infiltration in cancer. Most results tend to indicate that infiltration by cytotoxic CD8⁺ T cells and NK cells increases in exercising animals (76, 77, 145, 162), while others indicate no effect (161, 163), or only if combined with either treatment (158, 164) or energy restriction (159).

In Study III, body composition was assessed using ASMI from DXA-scans. A higher ASMI, indicating greater muscle mass, was associated with lower densities of cytotoxic CD8⁺ T cells in the descriptive statistics, but results were not significant when adjusted for multiple testing or analysed by multivariable logistic regression. No previous study on the association between body composition and immune cell infiltration in colorectal cancer has been published. In a recent study on 52 patients with non-metastatic breast cancer where body composition was related to immune cell infiltration, increased infiltration by cytotoxic CD8⁺ T cells, CD45RO⁺ T cells, and CD20⁺ B cells was seen, but not CD3⁺ T cells in patients with both lowest tertile for total adipose tissue and highest two tertiles for skeletal muscle mass (166). However, there were two important limitations in that study: half of the patients were treated with neoadjuvant chemotherapy which was not adjusted for in the multivariable regression models; and non-metastatic breast cancer patients were not routinely CT-scanned (the CT-scans used in the study were opportunistic increasing the risk of sample bias) (166). In another study on 62 patients with renal cell carcinoma, immune cell infiltration was assessed using gene expression analysis and its association with SMI was assessed using CT-scans (167). The study showed increased macrophage and Th1-cell infiltration and decreased Th17 infiltration in

patients with low SMI. No differences were seen for other immune cell types analysed such as cytotoxic T cells, Th2-cells, B cells, NK cells, and regulatory T cells (167). These studies also differed from each other in design and study population and the results diverged. ASMI could reflect physical activity prior to diagnosis but it can also be affected by the disease itself so conclusions regarding an individual's level of physical activity are difficult to draw based on ASMI alone. Given the results from Study III together with existing evidence from observational studies on body composition and immune cell infiltration in cancer in general and colon cancer in particular conclusions regarding a potential association cannot be made.

Immune cell infiltration in colorectal cancer is generally higher in females, right-sided cancer, and lower stage (34). In Study I, the distribution of immune cell infiltration was as expected indicating a representative data material. The representativeness was also supported by the survival analyses conducted in this thesis showing decreasing mortality risk with increasing immune cell infiltration. In Study III, there were no consistent results supporting the hypothesis that increased immune cell infiltration is seen in individuals with higher physical activity, better physical fitness, or increased muscle mass index. One possible explanation for this is skewed distribution of participants. When comparing accelerometer data, ASMI, and results from the physical tests, better results were seen in males, higher stage, and left-sided cancer. These results were surprisingly consistent in almost all measurements. Even if differences seen were small for some cell types, the consistent pattern seen for all cell types and tests should have affected the results. The trend towards a negative correlation between physical activity and fitness with immune cell infiltration could be explained more by sex, tumour location, and stage rather than physical activity and fitness.

Studies I and III did not adjust for conditions and comorbidities affecting the immune system. In a recent mendelian randomisation study, Rheumatoid Arthritis (RA), an autoimmune disease characterised by chronic inflammation, was associated with lower risk for colorectal cancer (168). In another recent mendelian randomisation study, however, the risk for colorectal cancer was slightly increased in RA patients (169). IL6 plays a major role in the pathogenesis of RA and treatment aimed at the IL6-receptor (IL6R) blocking IL6 binding is an important part of RA treatment. The first-named study also analysed the association between IL6R and immunity in colorectal cancer and showed a higher correlation between IL6R and various immune cells in

colorectal cancer compared to other cancers (168). Other autoimmune diseases have been studied. One recent study used mendelian randomization to investigate colorectal cancer risk in several autoimmune diseases excluding inflammatory bowel disease (IBD). They concluded that there is an increased risk for cancer in patients with primary sclerosing cholangitis and psoriasis but not in the other five autoimmune diseases investigated (170). Research into immune cell infiltration and its association with diseases affecting the immune system is limited. Little is known about how the tumour microenvironment in colorectal cancer is affected by autoimmune disease. IBD is an exception and patients with IBD have an increased risk for developing colorectal cancer (170). If and how immune cell infiltration is affected by IBD is not well studied, and IBD patients are often excluded from colorectal cancer research focusing on the tumour microenvironment. A study from 2019 used the Vectra 3[®] quantitative multispectral pathology imaging system to quantify infiltration by CD3⁺ T cells, cytotoxic CD8⁺ T cells, regulatory FoxP3⁺ T cells, and PD-L1 in the invasive front in 24 specimens from patients with colitis-associated colorectal cancer and 48 with sporadic colorectal cancer (171). The colitis-associated cancers had significantly lower densities of all immune cell types tested. This could partly be explained by higher stages in the colitis-associated group (33.3% stage IV disease in colitis-associated versus 8.4% stage IV disease in sporadic) (171). Summarising, there is not sufficient evidence to say that autoimmune disease is a confounding factor when assessing the association between physical activity and immune cell infiltration in colorectal cancer. There is an increased risk in IBD but it is uncertain whether immune cell infiltration is altered in IBD-associated cancer. We therefore did not adjust for autoimmune disease or IBD in Studies I and III of this thesis.

Physical activity and body composition in colorectal cancer

The way in which physical activity prevents sarcopenia and myosteatorsis is not completely understood. Potential mechanisms are the prevention of age-related effects seen in sarcopenia such as; increased insulin resistance, impaired mitochondrial function, increased oxidative stress, and increased inflammation (172). Physical activity prevents or delays the onset of sarcopenia (83, 84, 173) and myosteatorsis (94). Intuitively one would expect that physical activity and body composition are tightly linked. Both sarcopenia and myosteatorsis seem to be affected by resistance and endurance exercises (174) where resistance activity might

have more effect on muscle mass (174). At the same time, physically active individuals develop sarcopenia, and not all non-exercising individuals develop sarcopenia. The questionnaire in Study II on self-reported physical exercise did not distinguish between resistance and endurance exercise. The physical exercise data were derived well before the diagnosis was made (median 9.6 years) and the participants had a mean age of 53.2 years (participation was at 40, 50, or 60-years-of-age as described in the Methods section) when included in the Västerbotten Intervention Programme. Thus, physical exercise levels were assessed at middle-age. A Japanese study on healthy individuals over 65 years old showed that self-reported physical exercise levels higher than “never” between the ages of 25 and 50 years can prevent sarcopenia (175) defined according to the EWGSOP criteria (79). Another Japanese study on 1,607 participants defined sarcopenia as a combination of low muscle mass, low muscle strength according to hand grip strength, and low physical performance assessed by gait speed. They found lower odds for sarcopenia in men with high self-reported exercise levels in adolescence and older age, but not for women (176). However, their results are difficult to compare with ours because of the restrictive definition of sarcopenia in their study with a prevalence of 6.6% in men and 1.7% in women (176). Both these Japanese studies used retrospectively collected data on physical activity which increases the risk of recall bias. A study from Reykjavik using prospectively collected self-reported physical activity showed decreased odds for sarcopenia defined according to EWGSOP criteria (79) in participants engaging in MVPA at baseline (84). However, the amount of MVPA at baseline was not associated with the rate of loss of muscle mass, hand grip strength, or result in the 6MWT (84). Even though activity data were prospective, the participants were already 74.9 years old at baseline and follow-up was a mean of around five years *i.e.*, physical activity was not assessed in middle age. Another study on 1,918 UK inhabitants aged around 55 years at baseline with an average of 6.1 years follow-up used accelerometer data to define physical activity. Higher MVPA at baseline was associated with decreased odds of sarcopenia as defined by the EWGSOP criteria (79). There was however no association with probable sarcopenia (defined as low hand grip strength but normal SMI) (173). The lack of association between physical exercise in middle age and sarcopenia or myosteatosis when diagnosed with colorectal cancer in Study II could be that we had too wide a definition of sarcopenia/myosteatosis (lowest sex-specific tertile of skeletal muscle index/density) or that the self-reported questionnaire failed to distinguish between exercising individuals.

The combined body composition and pre-diagnostic exercise level results in Study II showed increased risk estimates in the cancer-specific mortality model but not in the overall mortality model. In a recent meta-analysis of sarcopenia and prognosis in colorectal cancer (86), pooled-analysis hazard ratios showed similar values for overall mortality (HR 1.83, 95% CI 1.57-2.14) and cancer-specific mortality (HR 1.77, 95% CI 1.40-2.23). Another meta-analysis on myosteatosis and prognosis in stages I-III colorectal cancer also had similar pooled hazard ratios for overall mortality (HR 1.52, 95% CI 1.38-1.67) and cancer-specific mortality (HR 1.67, 95% CI 1.40-1.99) (177). The same pattern was evident when analysing physical activity and prognosis in colorectal cancer with similar risk estimates for both overall and cancer-specific mortality (13). The cancer-specific mortality model included fewer events, and those events were more likely to be related to the cancer than to other comorbidities. The fact that the results were only seen in the cancer-specific model when physical exercise was added, provides some evidence that the combined effect on mortality could be linked to tumour progression rather than deterioration in general health, and at least partly due to the effect of physical activity.

When investigating associations between physical activity, body composition, and mortality, comorbidities are of importance. In our investigation we only had comorbidity data on diabetes at the time of inclusion in the Västerbotten Intervention Programme. Other comorbidities affect mortality, so we tried to at least partly adjust for the lack of comorbidity data by analysing both overall and cancer-specific mortality. A previous study using retrospective data showed that individuals with cardiovascular disease may suffer higher cancer mortality than individuals without (178) so our adjustment method has limitations. We had access to surrogate markers of comorbidity such as smoking and education status. Smoking is related to several cancers, cardiovascular disease, diabetes, and respiratory disease (179), and a higher education level is associated with better health and increased lifespan (180). Smoking status and diabetes when included in the Västerbotten Intervention Programme did not differ between the physical activity groups (p-value for diabetes 0.714 and smoking status 0.334). There was a difference in education status between the groups, which is why we included education status but not smoking and diabetes in the multivariable analyses. Adjusting for this surrogate marker partly adjusted for presumed comorbidity.

Physical activity reporting

In the studies of this thesis, data on physical activity and exercise were collected and reported in four different ways. In Studies I and II, answers to the same five-scale self-reported question were used but were dichotomised between high and low in Study II. In study III, a triaxial hip-worn accelerometer was used, while physical activity was assessed by telephone interview using a self-reported form in Study IV.

The use of prospectively collected data from the Västerbotten Intervention Programme allowed for assessment of physical exercise prior to the diagnosis of colorectal cancer, reducing the risk of recall bias. However, when interpreting studies based on self-reported physical activity and exercise, the reader must be aware of the problem of poor correlation (106) as discussed briefly in the Introduction to this thesis. In Studies I and II, we used a single-item question from the composite Cambridge index used in the Västerbotten Intervention Programme (181). In the validation study, the Spearman coefficient for this single-item question was low to moderate with coefficients 0.27 for MVPA and 0.29 for daily energy expenditure. However, the recreational index showed a dose-response correlation for both MVPA and daily energy expenditure with the most evident difference for the highest category. This indicates that participants reporting the highest level of physical exercise were individuals that had the highest physical activity (181). The problem of low correlation is evident in many questionnaires on self-reported physical activity. A systematic review compared and calculated the reliability and validity of 65 studies reporting the results of physical activity questionnaires. They showed a median reliability coefficient of 0.62-0.71 and validity coefficient 0.30-0.39 for existing questionnaires and similar numbers for new questionnaires (106). In other words, physical activity questionnaires usually give the same results when one individual is tested two or more times (reliability) but estimates of physical activity correlate worse with objective measurement (validity). This weak, however positive, association has also been reported in other reviews when comparing physical activity questionnaire results with accelerometer data (182). Assessment of physical activity in the Västerbotten Intervention Programme must be interpreted with this in mind while understanding that no other prospective physical activity data were available for most of the individuals included in Studies I and II.

The self-reported activity form used in the interview in Study IV has been validated against both accelerometer data and cardiorespiratory

fitness assessed with a submaximal cycle test (129). The Spearman correlation coefficients between the questionnaire results and accelerometer data (minutes MVPA per day) and $\text{VO}_2\text{-max}$ were both 0.27 (129). The determined cut-off used had a sensitivity of 0.63 and a specificity of 0.67 compared to individuals not adhering to the recommendations (129). Even in Study IV, the correlation coefficient comparing self-reported physical activity and actual physical activity was low but in line with other questionnaires (106). The questionnaire in Study IV was chosen because it had only two questions which made it easy to conduct over the phone and had been correlated against the physical activity recommendations both from WHO (6) and the Public Health Agency in Sweden (128). However, all individuals included in the study considered themselves as adhering to the recommendations. Since Study IV explored the experiences and attitudes towards physical activity in physically active individuals, their view of themselves as physically active individuals might be more important than actual physical activity level.

Activity devices such as the accelerometer must be validated so that the researcher knows that the data collected can be trusted. There are numerous accelerometer devices and methods for measuring and calculating the data output which hinder interpretation of which tool is valid and why. Validation studies can be done in the laboratory setting or in the free-living environment. There are several studies on accelerometer data validated against manual step count, doubly labelled water, or indirect calorimetry, as well as against various devices, observations, or diaries (183). Triaxial accelerometers, such as the Actigraph GT3X used in Study III, are the ones most used (183). The Actigraph accelerometer has been validated in healthy adults against step counts (102) and energy expenditure (103). A recent meta-analysis including studies validating Actigraph GT3X against energy expenditure with indirect calorimetry included eight studies and 436 participants. The authors concluded that there is no difference between the two methods when estimating energy expenditure (103). The minimum wear-time commonly used is four days with at least 600 minutes a day. Some participants in Study III had only a short time until surgery, so to minimise the number of missing values, we accepted a wear-time of three days with a minimum of 600 minutes a day if surgery was scheduled within four days of receiving the accelerometer. A previous study compared accelerometer-derived physical activity data in 52 participants with a mean age of 69 years keeping a physical activity log for 21 days (184). To achieve an intraclass correlation (ICC) of 0.85 for MVPA using Actigraph, three days with 600 minutes wear-time a day

was sufficient. However, when analysing sedentary time, seven days were needed for an ICC of 0.85 (184). In the population of Study III with approved wear-time the mean MVPA per day were 38 minutes. Of the 61 individuals, 37 (61%) had more than 21.4 minutes MVPA per day (*i.e.*, 150 divided by seven). A mean MVPA of 38 minutes corresponds to 266 minutes per week and is within the recommendations given by WHO (6) and the Public Health Agency in Sweden (128). The WHO guidelines on physical activity and sedentary behaviour (6) were updated in 2020. Before that, MVPA bouts were required to last longer than ten minutes to count. In the updated guidelines from 2020, all MVPA time should be included since all conducted physical activity is associated with improved health outcome (6). MVPA lasting at least ten minutes could represent more planned structured activities, while all MVPA also includes short sporadic activities. A recent study from Tromsø on 5,918 participants with a mean age of 63 years showed a mean MVPA time of only 13.4 minutes when including ten minutes sessions only but 41.0 minutes using all MVPA time (185). MVPA time in Study III was similar to this which indicates that our cohort had representative physical activity levels even though only recently diagnosed with colon cancer.

Assessment of body composition

In the two most recent meta-analyses on sarcopenia and prognosis after surgery (85, 86) a total of 68 studies were included. The definition of sarcopenia varied depending on which assessment modality was used (CT, bioimpedance, or combined modalities from both imaging and physical status). The usual definition of sarcopenia was SMI from cross-sectional CT scans at the 3rd lumbar vertebral level. Definitions also varied according to cut-off points: optimal stratification cut-off, lowest quartile, lowest tertile, or predefined cut-offs based on references. Sarcopenia was defined in 44 ways in the 68 studies included and the prevalence of sarcopenia varied from 11.9% to 78% (85, 86). In a meta-analysis of 40 studies on myosteatosi including 21,000 patients, all studies used Hounsfield units from cross-sectional CT-scans to define myosteatosi. Despite this, myosteatosi was defined in 20 different ways and the prevalence varied between 11% and 80% across the studies (92). This implies considerable heterogeneity in the definition of sarcopenia and myosteatosi and must be considered when interpreting research on sarcopenia and myosteatosi.

Optimal stratification is a way to determine which cut-off value to use to define sarcopenia and myosteatosi. With optimal stratification, the cut-off value for the exposure variable (SMI or SMD) is statistically

determined to see the greatest effect on the outcome variable (for example overall or cancer-specific mortality). The most common statistical method used for optimal stratification is by using the log-rank test and determining the cut-off value with the lowest p-value. Optimal stratification is not without problems; the most prominent being that a cut-off value is created by defining the value that has the largest effect on the outcome variable and therefore the highest chance of obtaining a significant result (186). Other common ways of defining sarcopenia or myosteatorsis are using lowest quartile, lowest tertile, mean, or median. There are of course problems with using a predefined proportion of the population since an individual can be defined as having sarcopenia and/or myosteatorsis in one study cohort but not in another. For sarcopenia, 13 of the 68 studies included, used predefined cut-off values from a retrospective study on 250 patients with respiratory tract, colorectal, or "other" cancer stages I-IV (187). This study used optimal stratification to define sex-specific cut-off values for SMI. Regarding myosteatorsis, 15 of the 40 studies included used predefined cut-off values from a retrospective study on lung and gastrointestinal cancer by Martin *et al* (188). The cut-off values defined by Martin were also derived from optimal stratification calculation on their data. The advantage of using a predefined cut-off value is that an individual will be defined as having myosteatorsis regardless of cohort. However, when the cut-off is defined by optimal stratification the problem with this method remains. In study II, the lowest tertile was used to define both sarcopenia and myosteatorsis since it had been used on the same patient cohort (93) showing consistent and expected results on prognosis.

The problem with heterogeneity is also evident in public health research on sarcopenia where a combination of physical tests and body composition to define sarcopenia is more frequently used than CT-scans. CT-scanning is not as accessible to healthy individuals as it is to those with cancer, and body composition is more frequently assessed with either bioimpedance or DXA-scan. The most frequently used combination method for defining sarcopenia is that stated by EWGSOP. Individuals are screened with a muscle strength test and only those with low strength, <27kg handgrip strength for men and <16kg for women or >15s for five chair climbs are then assessed for muscle mass to define sarcopenia (79). Public health research also suffers from discrepancy in the definition of sarcopenia. In a meta-analysis on physical activity and sarcopenia onset (83), 18 studies were included using ten different definitions of sarcopenia, the usual being the EWGSOP criteria (n=8). Two studies used a combined criteria definition by the International Working Group on Sarcopenia (IWGS) where individuals are screened

using gait speed, and those with low gait speed are assessed for muscle mass to define sarcopenia (189). The guidelines proposed by ICFSR recommend using a combination of objective testing of muscle strength and muscle mass when defining sarcopenia (87). In a research setting, especially retrospective research, this is seldom possible. When results from both tests are not possible, it is a question of whether not to conduct the research or to conduct the research with muscle mass assessment only. In Study III, we were able to define sarcopenia according to the EWGSOP guidelines. When measuring handgrip strength, only two (n=2) individuals came under the level suggestive of sarcopenia and none of them was under the ASMI cut-off for sarcopenia. This definition could therefore not be used in our study, so we decided to use ASMI as a continuous variable. When interpreting research on sarcopenia the reader must be aware of how sarcopenia is defined and account for this when interpreting results.

Experiences of physical activity

The experiences of physical activity among physically active participants after a colon cancer diagnosis varied from the diagnosis being an obstacle to maintaining physical activity to a will to increase physical activity, fight the cancer, and come out stronger. In a meta-synthesis of qualitative literature, the diagnosis of colorectal cancer was linked with varied experiences that required multiple adaptations and/or coping strategies (190). Our results covering a similar subject concurred with the results of this meta-synthesis, implying that physical activity is closely linked to the individual's lifestyle and affected by the general nature of the individual.

Previous qualitative research has not investigated attitudes towards and experiences of physical activity of individuals receiving a colon cancer diagnosis who are already physically active and awaiting surgery. In a recent systematic review of qualitative and mixed-method studies aimed to explore barriers to and facilitators of physical activity in patients living with and beyond cancer (191), only two studies, ours being one, studied patients prior to treatment. In accordance with our results, cancer symptoms, lack of time and lack of motivation were examples of barriers to physical activity (191). In the review, physical activity prior to diagnosis (pre-diagnostic) was considered a facilitator of physical activity throughout cancer disease and treatment (191). This was partly the case in our study, but attitudes and experiences varied even though all participants were physically active prior to their diagnosis. A previous

Swedish qualitative study investigated attitudes toward preoperative physical activity in patients awaiting colorectal cancer surgery (192) concluding that there is a gap between awareness and action, and participants felt that physical activity was possible with the right motivation. The multifactorial base of attitudes towards preoperative physical exercise was also highlighted. Previous experiences of both healthcare and physical activity were important (192). Our results confirmed the multifactorial base and differing attitudes towards preoperative physical activity. Previous physical activity is not the only factor affecting attitudes to preoperative physical training, they also depend on many aspects of life such as the cancer disease itself and the patient's ability to cope with the new reality. In a qualitative study by Burke *et al* on ten participants with locally advanced rectal cancer undergoing a prehabilitation programme, the structured exercise programme induced feelings of increased vitality, a positive attitude, enhanced social connections, and a sense of purpose (193). In contrast to our results, there were no negative experiences or attitudes towards physical activity expressed in that study. An important difference between our study and theirs is that the participants in the study were involved in a specific study on prehabilitation. Individuals that could have experienced a negative attitude toward activity after their cancer diagnosis might already have refused participation in the study and thus not included in the sample (193). Many cancer patients feel the need for a coping strategy, and many patients diagnosed with colorectal cancer experience distress during treatment (194). The process of receiving a colon cancer diagnosis and maintaining the everyday life may be understood in the light of Lazarus and Folkman's theory of stress, appraisal, and coping (195). When a person encounters a stressor, they choose a primary appraisal pattern. A secondary appraisal is made if the encounter is considered "harmful". One of the two coping strategies can be chosen, problem-focused or emotion-focused and one individual may cope in several different ways using coping strategies from both groups (195). The first main-category "*fight the cancer and come out stronger*" could be seen as an example of problem-focused coping where physical activity can be perceived as an active way to manage the cancer challenge. Whereas in the main-category "*the diagnosis is an obstacle to physical activity*" the emotions are directed toward the obstacles rather than using physical activity as a coping strategy. In the last main-category "*the diagnosis makes no difference*" could possibly be explained as that the individual does not use physical activity as a coping strategy and copes in other ways. Our study implies that physical activity may be used as a coping strategy in physically active individuals diagnosed with colon cancer.

In quantitative research, the sample size required to detect expected differences can be estimated using a power calculation. In qualitative research there is no such mathematical or statistical way to determine sample size. Instead, you strive for a sample that contains all possible experiences on the aim with as wide a range of participants as possible. A concept, information power, indicates that the more information a sample holds the lower is number of participants required. It is not possible to decide on a sample size in advance, it must be reviewed as the study progresses. When deciding on sample size, the study aim, sample specificity, methodology used, interview quality and context, and analysis strategy must all be considered (196). In our study physically active colon cancer participants were interviewed over the phone. This was quite a specific sample, the use of telephone interviews enabled more participants to accept participation, and content analysis is an established methodology. All these traits of the study enable a fairly low sample size. At the same time some of the interviews were short (the shortest 3 minutes) and the interviewer (the author of this thesis) was not an experienced qualitative researcher, which would generally require more participants in the sample. During the study period, interviews were continuously analysed and data from previous interviews processed at the same time as other interviews were being conducted. After 15 interviews, no more information related to the aim was obtained, a phenomenon sometimes referred to as saturation. There is no clear objective definition of saturation, and there is a risk that this infers “heard it all” rather than adequate information power of the data already collected (196). Since the first author was inexperienced in conducting qualitative research, we decided to include five more interviews to achieve higher information power.

There was a wide distribution of participant demographics in the study, and patients were included and treated at a university hospital and a county hospital. The three authors interpreting the data also had different sex, backgrounds, and ages. This wide distribution of participants and researchers increases the credibility of this study. The participants included lived in northern Sweden and all but one were born and raised in Sweden. Furthermore, they were all diagnosed with colon cancer where the intent of treatment was curative and who fulfilled the physical activity recommendations of WHO. The transferability of our results is therefore limited to similar settings *i.e.*, not strictly to colon cancer patients but also to others awaiting surgery for disease with curative intent. Since our aim was to investigate this specific group of patients, transferability of the study is somewhat affected from the beginning. The interviews were conducted by the first author over a

period of almost a year. During this time, the analysis process was continuous, and the findings, interpretations and codes repeatedly discussed among the authors to increase consistency of data interpretation and analysis and hence increase dependability of the study (112, 113).

Future Perspectives

Physical activity provides many health benefits but studying it is difficult. At first glance pre-diagnostic physical activity appears to improve colorectal cancer risk and prognosis. However, when distinguishing the specific risk and prognosis patterns of colon and rectal cancer, the evidence is not as clear. It seems that pre-diagnostic physical activity has a beneficial effect on prognosis in both rectal and colon cancer, but this has not been firmly established. In many studies colorectal cancer is examined as a single entity, and separate data for colon and rectal cancer are often not available. Although stratifying cancer location may reduce statistical power, future research on pre-diagnostic physical activity should study colon and rectal cancer separately to better understand any differences between the two.

Physical activity is a behavioural trait and being physically active may come with other health-promoting behaviour and possibly comorbidity. In the quantitative studies of this thesis, we lacked data on potential comorbidities that we tried to account for in various ways as described previously. Future research should include comprehensive data on comorbidities to better control for potential confounding in the association between pre-diagnostic physical activity and prognosis in cancer.

In this thesis positive effects were seen with self-reported data for pre-diagnostic physical exercise and immune infiltration of cytotoxic CD8⁺ T cells and on cancer-specific mortality risk when combined with data for body composition. However, when more objective physical activity measurements were made and more advanced tools for assessment of immune cell infiltration were used, no consistent associations were seen. The two retrospective studies lack in its self-reported data on physical exercise while the prospective cohort study had a small sample size and thus low power. In future studies, a combination of larger sample size and objective assessment of physical activity, body composition, and immune cell infiltration in colorectal cancer will provide more information regarding any potential association. It is highly unlikely, for

instance, that a prospective study including 600 individuals who undergo cardiopulmonary testing and have their immune cell infiltration quantified by multiplex immunohistochemistry will ever be conducted due to the resources required. However, the use of accelerometers to quantify physical activity is an easy and inexpensive method that may be used on large populations yet still provides objective data on physical activity. Another example of achieving objective data in a more reasonable way is the linking of large prospective cohorts with objective data, for example the Swedish military conscription cohort, with biobanks or databases with radiologic imagery. This would require extensive collaboration between regions in Sweden using several biobanks which would be difficult, expensive and resource demanding.

Few intervention studies on physical activity and immune cell infiltration in colorectal cancer have been published. Intervention studies with physical activity or exercise as the intervention and immune cell infiltration as the outcome are reasonably realistic for researchers familiar with immunohistochemistry. The already existing data from exercise prehabilitation studies could be used as the intervention since tissue specimens are often available for a long time after surgery. This could shed more light on the potential effects of physical activity on immune cell infiltration in colorectal cancer.

Conclusions

- Self-reported pre-diagnostic physical exercise more than three times a week was associated with increased immune cell infiltration by cytotoxic CD8⁺ T cells both in the invasive front and centre of colorectal tumours. No association was seen for CD3⁺ T cells.
- Self-reported pre-diagnostic physical exercise less than once a week in middle age was not associated with CT-assessed sarcopenia or myosteatorsis at the time of non-metastatic colorectal cancer diagnosis. The combined presence of self-reported pre-diagnostic physical exercise less than once a week and sarcopenia or myosteatorsis was associated with increased hazard for cancer-specific mortality but not overall mortality.
- After correction for using multiple tests, no consistent association between objectively assessed data on physical activity, fitness or muscle mass index with immune cell infiltration in colon cancer tumour samples was seen.
- Physically active individuals diagnosed with colon cancer awaiting curative surgery expressed different attitudes and experiences towards physical activity varying from the diagnosis being an obstacle to continue physical activity to a will to increase physical activity and thereby fight the cancer.

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