Karolinska Institutet

Institutionen för molekylär medicin och kirurgi

Rectal Cancer

Aspects on Radiotherapy, Androgens and Body Composition

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Aspects on Radiotherapy, Androgens and Body Composition

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“You don´t need a weatherman to know which way the wind blows.”
Bob Dylan

ABSTRACT

Rectal cancer is diagnosed among 1200 men in Sweden every year. Current treatment for local and regional disease implies surgical resection of the rectum often in combination with preoperative radiotherapy (RT). This treatment results in a cancer-specific survival of approximately 90% after three years and a cumulative incidence of local recurrence of 5% after five years. The oncological benefits frequently come at the price of impaired bowel and sexual function with consequences for quality of life. To design a research project with the aim to investigate the effects of preoperative RT on testicular function and sexual health in men treated for rectal cancer a review of the available literature was performed.

The findings of this review (Paper I) showed that the testicular dose (TD) was on average 3% to 17% of the prescribed dose for RT. No reports on semen analysis in men treated for rectal cancer were identified. The androgen levels decreased in men treated with RT and the relative risk to have low serum testosterone (T < 8 nmol/l) was 2.7 (95% CI 1.6 to 4.7; p<0.001) after four years. Low serum T was also related to post-treatment erectile dysfunction.

Based on the results of Paper I, a cohort study with preoperative RT as exposure was initiated. One hundred and five men with rectal cancer stage I to III were included between April 2010 and May 2014. To increase the sample size of the unexposed group 63 men with prostate cancer planned to robot-assisted prostatectomy were included additionaly. All participants had a baseline and two follow-up visits 12 and 24 months after surgery to collect blood samples, patient-reported outcome measures and semen samples. Men receiving preoperative RT had an additional blood sample the week prior to surgery.

The planned TD was calculated with the treatment planning system based on the planning computed tomography (CT) in 101 men (Paper II). The median planned TD for short course RT was 0.57 Gy (range 0.06 to 14.37 Gy) and 0.81 Gy (range 0.36 to 10.80 Gy) for long course RT. In 32 men the delivered TD was assessed for each RT fraction with repeated cone beam CT. The comparison between planned and delivered TD show that the planned TD is an accurate estimate of the delivered dose. The within-person variability of the delivered TD is related to the position of the testes in men with moderate to high TD.

The androgen levels at baseline of the entire cohort were similar and independent of the type of preoperative RT or the type of cancer (Paper III). Preoperative RT resulted in a significant decrease of T and increase of luteinising hormone (LH) and LH-T ratio. The risk of low serum T (T < 8 nmol/l) increased from 14.6% at baseline to 35.5% at the time of surgery in men treated with RT corresponding to a relative risk of 2.41 (95% CI 1.57 to 3.71, p<0.001). These findings confirm that preoperative RT leads to primary testicular failure. The preliminary analysis indicates a dose-response relationship between the TD and the negative impact on testicular function.

The biochemical signs of testicular failure persisted in 40 men analysed 12 months after surgery (Paper IV). The cross-sectional area of the psoas and erector spinae muscle, assessed on routinely acquired planning and follow-up CT, was related to the level of bioavailable T. The area of subcutaneous tissue was not related to androgen levels. The decrease in these muscle groups is an androgen-dependent sign and identified men with testicular failure that had T levels in the grey zone of hypogonadism one year after surgery.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals as indicated below:

I. Testicular function after radiotherapy for rectal cancer – A review

C. Buchli, A. Martling, S. Arver, T. Holm
J Sex Med 2011;8:3220-3226. DOI: 10.1111/j. 1743-6109.2011.02455.x

II. Assessment of testicular dose during preoperative radiotherapy for rectal cancer

C. Buchli, M. Al Abani, M. Ahlberg, T. Holm, T. Fokstuen, M. Bottai, J-E Frödin, I. Lax, A. Martling
Manuscript

III. Prospective cohort study assessing acute effects of preoperative radiotherapy on sexual hormones in men with rectal cancer

C. Buchli, A. Martling, M. Al Abani, J-E. Frödin, M. Bottai, I. Lax, S. Arver, T. Holm
Manuscript

IV. Testosterone and body composition in men after treatment for rectal cancer

C. Buchli, J. Tapper, M. Bottai, T. Holm, S. Arver, L. Blomqvist, A. Martling
J Sex Med 2014 Nov 12 Epub ahead of print, DOI: 10.1111/jsm. 12751

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List of Abbreviations

APE	Abdominoperineal excision
BED	Biologically effective dose
CI	Confidence interval
COX-2	Cyclooxygenase-2
CRC	Colorectal cancer
CT	Computed tomography
CTV	Clinical tumour volume
CV	Coefficient of variance
DEXA	Dual energy X-ray absorptiometry
FSH	Follicle-stimulating hormone
GTV	Gross tumour volume
HCG	Human chorionic gonadotropin
HLA	Human leukocyte antigen
HR	Hazard ratio
IIEF	International index for erectile dysfunction
LARS	Low anterior resection syndrome
LET	Linear Energy Transfer
LH	Luteinising hormone
LOH	Late-onset hypogonadism
MDT	Multidisciplinary team conference
MR	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
ns	Statistically not significant
OR	Odds ratio
p	p value
PDE5-inhibitor	Phosphodiesterase type 5 inhibitor
15-PGDH	Hydroxyprostaglandin dehydrogenase 15
PROM	Patient reported outcome measures
PTGS2	Prostaglandin-endoperoxide synthase 2
PTV	Planning tumour volume
RR	Relative risk
RT	Radiotherapy
SAT	Subcutaneous adipose tissue
SHBG	Sex hormone-binding globulin
SM	Skeletal muscle
SMR	Standardized mortality ratio
T	Testosterone
TD	Testicular dose
TME	Total mesorectal excision
VAT	Visceral adipose tissue

BACKGROUND

Rectal cancer

The rectum, the final segment of the gastrointestinal tract, extends 15 cm from the anal verge in oral direction and is a part of the large intestine with specific functions. The faeces accumulate in the rectum to enable controlled discharge in cooperation with the anal canal. Malignant transformation of the mucosa in the large intestine results in an adenocarcinoma named colorectal cancer (CRC). Colorectal cancer accounts for 10% of the total cancer burden [1]. It is the second most common cancer in women and the third most common cancer in men worldwide. The age-standardized incidence rates of CRC vary ten-fold across the world with the highest estimated rates in Australia/ New Zealand (32.2 and 44.8 per 100 000 in women and men respectively) and the lowest rates in Western Africa (3.8 and 4.5 per 100 000). The geographical variability in mortality rates is six-fold in men and four-fold in women, indicating poorer survival in less developed regions of the world. Sweden is one of the countries with very high incidence rate of CRC [2]. One third of CRC occur in the rectum resulting in 1200 men and 800 women with diagnosis of rectal cancer in Sweden every year [3]. The age-standardized incidence has been stable between 1980 and 2012 and mortality is slightly decreasing.

Determinants of colorectal cancer

Age and family history

The lifetime risk of CRC is 5.9% in men and 5.5% in women [4]. Age is the most prominent risk factor as 90% of new cases occur after age 50 [5]. About 25% of affected patients have family history of CRC. The relative risk (RR) of CRC is 2.24 (95% CI 2.06 to 2.43) for one first-degree relative with CRC and 3.97 (95% CI 2.60 to 6.06) for at least two first-degree relatives [6]. Specific inherited syndromes account for 5% of CRC. The lifetime risk of CRC in Lynch syndrome families is 43% in women and 66% in men with a median age at diagnosis of 47 and 42 years respectively [7]. Familial adenomatous polyposis is the second most common cause of hereditary CRC. Affected individuals develop hundreds to thousands of adenomas in the colon and rectum early in life. The lifetime risk of CRC approaches 100% by age 40 [8].

Personal history

Personal history of CRC increases the risk of a second, metachronous CRC, which is the reason for postoperative colonoscopic surveillance. The postoperative adenoma-free survival is related to sex, age and number of synchronous lesions at the time of surgery [9]. Removal of high-risk adenomas in the personal history results in a standardized mortality ratio (SMR) of 1.16 (95% CI 1.02 to 1.31) for CRC after a median follow-up of 7.7 years [10]. In contrast the CRC mortality is lower after removal of low risk adenomas, SMR 0.75 (95% CI 0.63 to 0.88).

The risk of CRC in inflammatory bowel disease is debated but severe long-standing colitis and primary sclerosing cholangitis seem to be of importance. The meta-analysis of eight population-based cohort studies, which includes five studies from Scandinavian countries, reports a relative risk of CRC for ulcerative colitis of 2.4 (95% CI 2.1 to 2.7) [11]. Young age at diagnosis, male gender and extensive disease are risk factors. The absolute risk of CRC in this pooled study population with ulcerative colitis is 1.6% including the sporadic cases of CRC during a median follow up of 7 to 24 years. The cumulative probability of CRC is considerably lower than reported earlier with a cancer risk of 2% after 10 years, 8% after 20 years and 18% after 30 years [12]. The standardized incidence ratio for CRC risk in Crohn´s disease seems even smaller, 1.7 (95% CI 1.01 to 2.5) [13]. Diabetes mellitus increases the risk of CRC and mortality, HR 1.26 (95% CI 1.12 to 1.34) and HR 1.30 (95% CI 1.15 to 1.47) respectively, in study populations with predominantly type 2 diabetes [14].

Overweight and obesity are suspected to account for 20% of all cancers worldwide [15]. The relative risk of CRC in obese women is inconsistent and two of three meta-analysis report an increase, RR 1.15 (95% CI 1.06 to 1.24) [16]. The association is stronger in men with RR ranging from 1.37 (95% CI 1.21 to 1.56) to 1.95 (95% CI 1.59 to 2.39).

Behavioural factors (diet, tobacco, alcohol and physical activity)

Mediterranean diet, composed of vegetables, fruits, cereals, olive oil and moderate amount of red wine, has a protective effect regarding CRC, RR 0.86 (95% CI 0.80 to 0.93) [17]. The use of diet supplements containing multi-vitamins, RR 0.92 (95% CI 0.87 to 0.97), or calcium, RR 0.86 (95% CI 0.94 to 0.99), seem to be protective whereas the associations are inconsistent for supplements with vitamin A, C, D or E, garlic and folic acid [18]. Daily intake of 100g red meat or 50g processed meat results in RR 1.27 (95% CI 1.16 to 1.40) and 1.29 (95% CI 1.10 to 1.53) respectively [19]. The effect of tobacco use on CRC risk is inconsistent and smokeless tobacco, Scandinavian moist snuff (snus), is not associated with an elevated risk for CRC in men [20]. Smoking increases the risk for cancer in the colon, HR 1.08 (95% CI 0.99 to 1.19), and in the rectum, HR 1.16 (95% CI 1.04 to 1.30). A meta-analysis of prospective studies yields similar findings with RR 1.15 (95% CI 1.00 to 1.32) in current smokers and 1.20 (95% CI 1.04 to 1.38) in former smokers [21]. This publication also reports a stronger association for smoking with rectal than colonic cancer and there is evidence for a dose-response relationship between smoking and CRC incidence. The relation of alcohol consumption and CRC mortality seems to be dose-dependent as only the intake of more than 50 g of ethanol per day is associated with an elevated risk for death due to CRC, RR 1.21 (95% CI 1.01 to 1.46) [22]. The results regarding the protective effect of physical activity on CRC risk are inconsistent. The latest systematic reviews report a reduced risk to develop colon cancer but no effect on the risk for rectal cancer [23] [24]. The findings of a case-control study from Western Australia suggest that vigorous physical activity is required to reduce the risk for CRC and that the effect is pronounced for the distal colon [25]. Vigorous physical activity reduces the risk for rectal cancer in men but not in women. Physical activity has also an impact on survival [26]. Any amount of physical activity before the diagnosis of CRC reduces cancer-specific mortality, RR 0.75 (95% CI 0.65 to 0.87). Physical activity after the diagnosis of CRC has a similar effect on cancer-specific survival, RR 0.74 (95% 0.58 to 0.95). The insulin-like growth factor axis is supposed to play a central role in the inverse relationship between physical activity and CRC survival [27].
The joint effect of these behavioural factors is of interest as these factors are recognized as healthy lifestyle and can be present simultaneously. The HR for CRC is 0.87 (95% CI 0.76 to 0.98) for the presence of two healthy lifestyle factors and decreases to HR 0.63 (95% CI 0.54 to 0.75) if five healthy lifestyle factors (BMI<25kg/m2, high physical activity, non-smoker, adequate alcohol consumption and healthy diet) are present [28]. The findings of this publication suggest that 16% of new CRC cases are attributable to not fulfilling the five healthy lifestyle factors.

Aspirin (acetylsalicylic acid)

Aspirin and other non-steroidal anti-inflammatory drugs are substances that have repeatedly been studied regarding their preventive effect on CRC development. Recent findings suggest a heterogeneous effect of aspirin related to the hydroxyprostaglandin dehydrogenase 15 (15-PGDH) expression in the colonic mucosa [29]. The 15-PGDH enzyme is a metabolic antagonist of the prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2), and is down regulated in CRC. Regular aspirin use is not associated with the risk of CRC in individuals with low expression of 15-PGDH mRNA, HR 0.90 (95% CI 0.63 to 1.27). High colonic expression of 15-PGDH and aspirin use is related to marked reduction of CRC risk, HR 0.49 (95% CI 0.34 to 0.71). Aspirin has also promising effects after treatment of CRC. The adjuvant use of aspirin does not enhance cancer-specific or overall survival in individuals with wild-type CRC for the gene encoding phosphatidylinositol-4,5-bisphosphonate 3-kinase catalytic subunit alpha polypeptide (PIK3CA), HR 0.96 (95% CI 0.69 to 1.32) and HR 0.94 (95% CI 0.75 to 1.17) respectively [30]. Up-regulation of phosphatidylinositol 3-kinase (PI3K) increases PTGS2 activity and synthesis of prostaglandin E2, which inhibits apoptosis of CRC cells. The regular use of aspirin results in improved cancer-specific and overall survival among patients with mutated-PIK3CA cancers, HR 0.18 (95% CI 0.06 to 0.61) and HR 0.54 (95% CI 0.31 to 0.94). This astonishing effect of aspirin on CRC survival is confirmed in participants of a randomized trial investigating the effect of rofecoxib, a selective COX-2 inhibitor, on CRC survival [31]. The

recurrence-free survival, HR 0.11 (95% CI 0.001 to 0.832), and the overall survival, HR 0.29 (95% CI 0.04 to 2.33), improve in patients with mutated-PIK3CA cancers that had a long-term low dose aspirin therapy (< 100 mg per day) at the time of randomization or commenced with low dose aspirin during follow-up. Rofecoxib has no benefit in mutated-PIK3CA cancers. In contrast to the two previous studies the analysis of PTGS2 expression and PIK3CA mutation seems not to identify individuals with benefit of aspirin in a Dutch cohort study assessing the effect of aspirin use on CRC survival [32]. In this study patients with colon cancer expressing the HLA class I antigen have a significantly longer survival, RR 0.53 (95% 0.38 to 0.74), if they use aspirin after CRC diagnosis. The loss of HLA class I antigen expression in the tumours is associated with no survival benefit from aspirin use, RR 1.03 (95% CI 0.66 to 1.61). The molecular mechanisms resulting in an anticancer effect of aspirin remain incompletely understood. The discussed studies explain the high interest that this old and commonly used substance actually has regarding chemoprevention and adjuvant therapy of CRC.

Milestones in treatment of rectal cancer

The ancient Egyptians collected knowledge of anorectal diseases. Several rectal procedures and medications are documented on the Ebers and the Chester Beatty Medical Papyrus [33]. Herodotus, a fifth century Greek historian, concluded after his studies at the Library of Alexandria that rectal cancer was a disease without cure [34]. It took more than a millennium until Giovanni Morgagni proposed to resect the rectum for treatment of rectal cancer during the 18th century. Several French surgeons contributed to advances in rectal surgery [35]. The first diverting procedure is assigned to Henry Pillore that performed an elective cecostomy to release an obstructing cancer of the rectum in 1776. Jean Zulema Amussat, experienced from battlefield operations during the Napoleonic wars, established the formation of a colostomy as treatment for rectal cancer. The excision of a distal rectal tumour by Jacques Lis-Franc 1826, using a perineal approach, is the first documented resection for rectal cancer. Success at the time was the ability of the patient to leave the hospital after survival of postoperative haemorrhage. These early reports of rectal cancer treatment sent a shiver down the reader´s spine, as general anaesthesia and bleeding control other than compression were unavailable. The introduction of anaesthesia, asepsis and bowel anastomosis techniques facilitated the progress in surgery for rectal cancer. The Swiss surgeon Theodor Kocher evolved 1874 a technique with closure of the anus to avoid spillage, partial sacrectomy to enhance exposure for the resection of the rectum and the formation of an artificial sacral anus [36].

Abdominoperineal excision

William Ernest Miles performed 57 perineal resections between 1899 to 1906 and 95 per cent of the patients had local recurrences within three years [37]. From the findings of the post-mortem examinations Miles concluded that the zone of upward spread of cancer from the rectum has to be removed in analogy to clearance of the axilla in cases of breast cancer. He proposed an en bloc resection of the pelvic colon, the pelvic mesocolon and iliac lymph nodes in combination with a wide perineal excision to prevent the cylindrical spread of cancer cells. The abdominoperineal excision (APE) was performed in 75 to 90 min with the patient in right lateral semi-prone position during the perineal excision. The postoperative mortality was 41.6% after 12 operations. The frequency of local recurrence decreased from 95% to 29.5% in APEs performed by Miles [38].

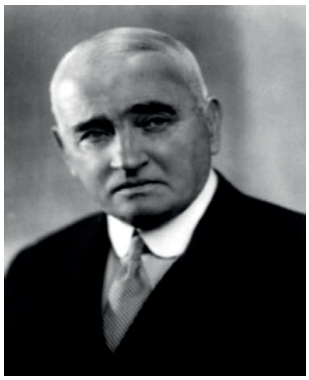


Figure 1. Sir William Ernest Miles

The need of a permanent stoma and the consequences of radical APE regarding functional outcomes encouraged sphincter-saving procedures. Claude Dixon, a surgeon at the Mayo Clinic, reported a hospital mortality of 5.9% and a five-year survival of 67.7% in 426 anterior resections performed during 1930 to 1947 for cancer in the upper or mid rectum. The development of stapling devices, introduced to clinical practice of Humer Hultl in Hungary 1908, facilitated the consecutive advancement in surgical treatment of rectal cancer [39]. A scientific research program, initiated in the Soviet Union during World War II, resulted in stapling guns used for intestinal and vascular anastomosis. This technique fascinated Mark Ravitch, a paediatric surgeon from the United States, during his visit to the Soviet Union in 1958. The following American modifications of the devices promoted the widespread use of mechanical suturing.

Total mesorectal excision

The principles of surgical oncology, defined by an en bloc resection of the affected bowel segment with the surrounding lymphatic tissue as described for example by W. E. Miles, were not applied consistently. The blunt dissection in the pelvis, accompanied by a characteristic sucking noise, frequently left "blocks of fatty lymphovascular tissue" along the pelvic sidewall. Serious intraoperative bleeding and cumbersome local recurrence were common to occur [40]. In 1982, Professor Bill Heald from the Basingstoke District Hospital, UK, described total mesorectal excision (TME), the state-of-the-art technique for surgical treatment of rectal cancer today [41]. Sharp scissor dissection under direct vision along embryological planes resulted in adequate cancer clearance and reduced adverse events due to autonomic nerve injury. None of the 50 patients with complete cancer clearance developed local recurrence after a minimum of two years of follow-up. In 1986 the five-year results of 115 patients with curative anterior resection in TME technique showed an overall survival of 87.5% and a cancer-free survival of 81.7%. The estimated probability of local recurrence was 3.7% without the use of radiation or chemotherapy. Professor Phil Quirke, pathologist in Leeds, reported the same year that 14 of 52 rectal cancer specimens had tumour spread to the lateral resection margin and 12 of these patients developped local pelvic recurrence [42]. Tumour involvement of the circumferential margin, defined as cancer cells within 1 mm from the lateral surface of the specimen, turned out to be an important predictor of local recurrence and survival, HR 3.68 (95% CI 2.32 to 5.83) and 2.16 (95% CI 1.53 to 3.05) respectively [43]. The contributions of Heald and Quirke enabled to set standards for anterior resection and the pathologic assessment of the specimen with a positive impact on patient outcome. The work-shops in Stockholm, initiated 1994, resulted in significant improvements regarding frequency of permanent stoma formation, local recurrence and cancer-specific mortality in a study population with a frequency of preoperative radiotherapy around 50% [44].

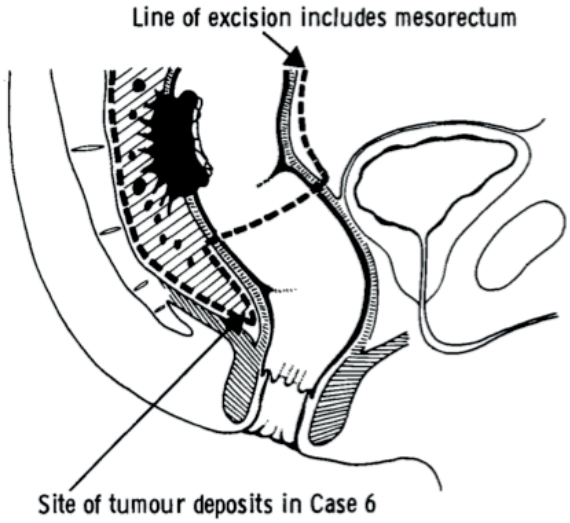


Figure 2. Plane of excision in TME [41] (reprint with permission)

Radiotherapy

While Heald described the TME technique, several randomized trials explored the effect of radiotherapy (RT) on local recurrence to define the optimal biologically effective dose (BED), fractionation and time-point (pre- or postoperative) for RT in rectal cancer. Many of these studies were conducted in Sweden.

The Uppsala Trial compared preoperative short course RT (5 x 5.1 Gy) versus postoperative long course RT (30 x 2 Gy). The local recurrence rate was lower for preoperative short course RT (13% vs. 22%, p=0.02) after a minimum follow-up of five years with similar overall survival [45].

In the Stockholm I Trial participants were assigned to surgery alone or preoperative short course RT (5 x 5 Gy) followed by surgery. The risk of pelvic recurrence was lower in patients treated with RT (14% vs. 28%, p<0.01) but no effect on overall survival was registered [46]. The increased postoperative mortality, observed in patients treated with RT (8% vs. 2%, p<0.01), was mainly due to cardiovascular events in patients over age 75.

The Stockholm II Trial had a similar study design but with a four-field box RT technique to decrease the target volume and was restricted to patients under the age of 80. The incidence of local recurrence was similar (12% vs. 25%, p<0.001) in favour of RT and the 30-day postoperative mortality decreased (2% vs. 1%, ns) [47]. The intention-to-treat analysis showed no effect on overall survival. Patients with curative surgery, defined as R0 resection in stage I to III disease and a specimen with tumour free margins, had a benefit in overall and cancer-specific survival, HR 0.77 (95% CI 0.61 to 0.98, p=0.03) and HR 0.60 (95% CI 0.45 to 0.80, p<0.001) respectively.

The Swedish Rectal Cancer Trial had the same protocol as the Stockholm II Trial and included participants from 70 Swedish hospitals. In addition to a comparable effect on local recurrence (11% vs. 27%, p<0.001) a survival benefit in the intention-to-treat analysis was reported [48]. The difference in overall survival, HR 0.79 (95% CI 0.66 to 0.92), and cancer-specific survival, HR 0.69 (95% CI 0.55 to 0.83), was regarded reliable after comparison with the population-based Swedish Cancer Registry [49].

These benefits of RT regarding local recurrence and survival were observed in randomized trials without standardized surgical technique for anterior resection and APE. The Dutch TME Trial closed this gap by randomizing patients with stage I to III disease between TME surgery alone and preoperative short course RT (5 x 5 Gy) followed by TME surgery. A total of 1748 participants were evaluated after exclusion of 57 patients with macroscopically incomplete (R2) resection. The proportion of specimens with positive circumferential margin was 16% in both groups. After 10 years of follow up the cumulative incidence of local recurrence was 5% vs. 11% (p<0.0001) in favour of preoperative RT [50]. No difference in overall or cancer-specific survival was observed and 25% vs. 28% (p=0.21) developed distant metastasis. The effect of preoperative RT was most convincing in stage III disease and increased cancer-specific survival in patients with negative circumferential margin.

The MRC CR07/ NCIC-CTG C016 Trial recommended TME technique and randomly assigned patients with resectable tumours to preoperative short course RT (5 x 5 Gy) or selective postoperative chemoradiotherapy (25 x 1.8 Gy) in case of circumferential margin involvement [51]. Preoperative short course RT resulted in enhanced local control, HR 0.39 (95% CI 0.27 to 0.58, p<0.0001), and cancer-specific survival, HR 0.76 (95% CI 0.62 to 0.94, p=0.013). The proportion of specimens classified with good (mesorectal) plane of surgery was 52% and 11% had an involved circumferential margin [52]. The plane of surgery was an important prognostic factor for local recurrence and the combination of preoperative RT and correct plane of surgery almost abolished the risk of local recurrence.

Other European studies assessed the effect of long course RT (prescribed dose between 45 to 50.4 Gy) and the addition of chemotherapy. The European Organisation for Research and Treatment of Cancer (EORTC) initiated the EORTC 22921 Trial in 1993, which included 1011 patients (T3/ T4 M0 tumours) in four arms to evaluate the effects of long course RT (25 x 1.8 Gy) in combination with fluorouracil-based chemotherapy [53]. Surgical resection was performed in 966 participants with 787 R0 resections. The addition of neoadjuvant chemotherapy enhanced preoperative downsizing and downstaging [54]. The surgical technique was not standardized as TME was recommended just after 1999 and the type of surgery was unknown in about 50% of resections. After 10 years of follow-up the proportion of local recurrence was significantly increased for patients treated only with RT (22%, p=0.0017). Adjuvant chemotherapy had no effect on disease-free or overall survival but less than half of the patients assigned to postoperative chemotherapy could be treated with the intended dose [55]. The German trial CAO/ARO/AIO-94, comparing pre- and postoperative chemotherapy in combination with long course RT, reported similar findings and difficulties to deliver postoperative chemotherapy [56]. Preoperative chemotherapy had a benefit on local control but no differences regarding survival or cumulative incidence of distant metastasis. The French trial FFCD 9203 also found an advantage in local control if preoperative chemotherapy was added to long course RT [57]. In T4 or recurrent rectal cancer preoperative chemoradiotherapy improved local control and cancer-specific survival compared to long course RT (25 x 2 Gy) [58]. Two similar trials compared preoperative short course RT (5 x 5 Gy) to preoperative chemoradiotherapy (28 x 1.8 Gy) and found no difference in survival, local control or toxicity [59 60].

In summary neoadjuvant RT in rectal cancer reduces the RR of local recurrence by 50% to 70% and may enhance survival [61]. Radiotherapy for rectal cancer is best given before surgery. The effect on local recurrence and survival is probably not different in short or long course RT but at the price of increased costs and resource requirements for long course RT due to the number of RT fractions and the need of concomitant chemotherapy. Experiences from the Stockholm III Trial, that completed inclusion in 2013, indicate a similar effect on downsizing for short course RT and delayed surgery [62]. The addition of chemotherapy to preoperative RT enhances local control and a significant survival gain was only observed in locally advanced cancers. The results of randomized trials do not support the idea that preoperative (chemo)radiotherapy increases the proportion of sphincter-saving anterior resections [63].

Revised abdominoperineal excision

The Dutch TME Trial with surgeons trained in TME detected an alarming difference in survival between APE and anterior resection [64]. Increased proportion of positive circumferential margins, intraoperative perforations and inadequate planes of resection were identified as potential factors for the inferior outcome of APE. The pooled analysis of five randomised clinical trials on rectal cancer resulted in an increased risk of margin involvement, OR 2.52 (95% CI 1.69 to 3.76, p<0.001), local recurrence, HR 1.36 (95% CI 1.07 to 1.72, p=0.011), and decreased cancer-specific survival, HR 1.31 (95% CI 1.11 to 1.56, p=0.002), for APE [65]. The TME technique defines the plane of surgery in anterior resection and is the standard to be reproduced and teached. This is not the case for conventional APE, often the only choice in low rectal cancer growing into the anal sphincter or levator ani muscle. Professor Torbjörn Holm from the Karolinska University Hospital, Stockholm, refined the technique of APE by combining the TME principles, precise dissection along defined anatomical planes free of tumour growth, with the technique for the perineal part of the operation described by Miles. The extended APE begins as TME in the abdomen yet avoids dissecting the mesorectum off the levator muscles. The patient is then turned into prone position to complete the perineal excision by resecting anus, lower rectum and levator muscles en bloc [66].
The resulting specimen has in contrast to the conventional APE no waist at the proximal end of the anal canal. The technique described by Holm is also called extralevator APE (ELAPE or eAPE) due to the plane of surgery during the perineal excision or cylindrical APE due to the shape of the resulting specimen. The amount of tissue removed outside the smooth muscle layer increases with extralevator APE and the proportion of specimens with involved circumferential margin or in-traoperative perforation decreases [67]. In 193 patients treated at Karolinska University Hospital for low T3 or T4 rectal cancer on preoperative magnetic resonance imaging (MR) since 2000 the proportion of circumferential margin involvement and intraoperative perforation was 10% each [68]. The rate of local recurrence was 6% and 24% died from rectal cancer after a median follow-up of three years not considering 3% 30-day mortality. The core issue of extralevator APE, elimination of waisted APE specimens, has been applied earlier at the Mayo Clinic with excellent oncologic outcome under the label standard APE [69]. In analogy to the extralevator APE two additional variants of the perineal part can be defined [70]. The intersphincteric APE combines TME with resection of the anal canal preserving the external anal sphincter and the pelvic floor in patients not suitable for bowel reconstruction without tumour growth into the anal sphincter or levator ani muscle. The ischioanal APE includes a wide excision of the perianal skin and the ischioanal adipose tissue by lateral dissection toward the ischial tuberosity. The plane of surgery follows the fascia of the internal obturator muscle up to the lateral insertion of the levator ani muscle and is applied in patients with tumour perforation through the pelvic floor or anal abscesses/ fistulae with tumour growth. In the light of modern rectal cancer treatment a randomized trial to compare extralevator versus conventional APE, a surgical technique without definition of anatomical planes, seems not feasible and ethically controversial. The meta-analysis of comparative studies until May 2013 showed marked advantages of extralevator APE regarding the frequency of circumferential margin involvement, intraoperative perforation and local recurrence [71]. The results of population-based cohort studies failed to show an advantage of extralevator APE, however, the validity of these results is questionable [72] [73]. The Spanish study used a propensity score to perform a one-to-one match of 457 individuals in each group from originally 1490 conventional APEs and 480 extralevator APEs. This study design raises concerns, as several matching factors (i.e. R0, circumferential margin and quality of mesorectal excision) are intermediate factors between exposure (type of APE) and outcome (local recurrence). This implies high risk of selection bias due to overmatching. In the Swedish study the exposure status, conventional or extralevator APE, could not be defined in 44.5% of the study population. After exclusion of

the “not stated” group the two groups available for analysis, conventional versus extralevator APE, differ regarding tumour height and frequency of preoperative chemoradiotherapy usually applied in more advanced tumours. This selection results in comparison between low and advanced tumours in the extralevator APE group versus higher and less advanced tumours in the conventional APE group.

Extensive surgery for locally advanced or recurrent rectal cancer

Despite the introduction of screening programs and multimodal treatment of rectal cancer some patients still present with primary rectal cancer growing into other pelvic structures or pelvic recurrence of rectal cancer. Uncontrolled pelvic tumour growth has a disastrous impact on patient´s life and median survival for locally recurrent rectal cancer treated with supportive care is limited to few months [74]. Extensive surgery including multivisceral resection (pelvic exenteration), sacral resection and hemipelvectomy are procedures to enable tumour clearance as R0 resection is a strong predictor for the oncologic outcome in these patients [75 76]. The status of the resection margin is more important for long-term survival than the fact that surgery is performed for primary advanced or recurrent rectal cancer [77]. The five-year overall survival after treatment with curative intent of recurrent rectal cancer has improved over time and can reach 50% in selected series [78 79]. The Beyond TME Collaborative has published a consensus statement for staging and treatment of these patients with complex presentation of rectal cancer diseases [80]. This type of demanding pelvic surgery seems to require a minimum of 14 cases for a surgical team headed by an expert colorectal surgeon to reduce perioperative complications [81].

Current multimodal therapy of rectal cancer stage I to III

Curative treatment for primary rectal cancer should aim for less than 5% risk of residual disease in the pelvis and the expected gains of additional treatments such as RT, chemotherapy and more extensive surgery should motivate the increased morbidity [82]. “Name it, stage it, treat it” is an established sequence for cancer care. Medical history, physical examination and venous blood sample (blood count, liver and renal function, carcinoembryonic antigen) are completed with digital rectal examination and rectoscopy with biopsy to confirm diagnosis of primary rectal cancer. To enable individualized risk assessment dedicated MR of the pelvis and computed tomography (CT) of the chest and abdomen are required to stage the disease. Synchronic lesions of the colon are excluded by complete colonoscopy and endoscopic rectal ultrasound may be useful to assess the depth of invasion in the earliest tumours. This workup should provide morphological verification and TNM classification of the rectal lesion. The distance between the lower end of the tumour and the anal verge, extramural vascular invasion, tumour growth in relation to the mesorectal fascia and invasion of other pelvic structures/ organs are additional criteria to discuss the treatment strategy at the multidisciplinary team conference (MDT). Risk stratification on four levels is recommended to choose the treatment modalities in rectal cancer stage I to III and patient´s preferences/ conditions are considered for individualized therapy. Complete surgical resection is the most important treatment modality and should be performed according to standardized techniques. Radiotherapy and chemotherapy are preferably given before surgery, as the preoperative treatment is more effective and less toxic than the postoperative treatment.

Table 1. Tailored treatment for primary rectal cancer stage I to III (adapted [82])

Risk group	Height	Clinical stage	Treatment
Very early	any	T1 sm1(-2?) N-	Local excision Complete with resection (or CRT) if sm ≥ 2, high grade or vascular invasion
Early ("good")	upper	T3a/b N±, mrf-, EMVI-	Standard resection Complete with Cx or CRT if CRM+ or pN2
	middle	T3a/b N-, mrf-, EMVI-	
	low	T1-2 N-, mrf-, EMVI-	
Intermediate ("bad")	upper	T3mrf-, N+, EMVI+ limited T4a N-	Preop RT/ CRT and standard resection
	middle	T3mrf-, N+, EMVI+ limited T4a N-	
	low	T2 mrf-	
Advanced ("ugly")	any	T3 mrf+, T4, lateral nodes+	Preop CRT and extended resection alt. preop RT and delayed extended resection if Cx not tolerated

T=tumour stage, N=nodal stage, sm=submucosal invasion level, mrf=mesorectal fascia, EMVI=extramural vascular invasion, CRM=circumferential resection margin, RT=radiotherapy, CRT=chemoradiotherapy, Cx=chemotherapy

The postoperative MDT conference is the opportunity to perform a product analysis based on a standardized specimen evaluation by the pathologist. The pathologist personally should examine the specimen before and after fixation to ensure high interrater reliability [83]. Chemotherapy is the weakest modality for treatment of rectal cancer. In analogy to treatment of colon cancer postoperative chemotherapy can be given for stage III or "high-risk" stage II diseases, however, the level of evidence is lower for rectal cancer [84].

National results of rectal cancer treatment in Sweden

The report on rectal cancer from the Swedish Colorectal Cancer Registry with 98% national coverage displays the following results in 2013. Preoperative RT was given to 64.5% of patients treated with surgery and the five-year cumulative incidence of local recurrence was 5%. Three-year cancer-specific survival was 89.7% for stage I to III disease treated with curative intent, though 18.8% of these patients developed distant metastasis after three years. Current rectal cancer treatment offers good local control and room for improvement in systemic control. Chemotherapy is effective in treating microscopic disease and is currently tested in combination with short course RT as neoadjuvant treatment in the "Rectal Cancer And Pre-operative Induction Therapy Followed by Dedicated Operation" (RAPIDO) trial [85].

Organ preservation

Preoperative treatment can result in complete disappearance of detectable cancer growth in the specimen. The fact of complete pathological response rose questions about the need of resection in these selected patients. In 2004 Professor Angelita Habr-Gama, from the University of São Paulo School of Medicine, published the results of a wait-and-see policy in low rectal cancer [86]. After preoperative chemoradiotherapy 71 patients not resected after complete clinical response were compared to 22 patients resected with complete pathological response. No pelvic recurrence was observed and two patients not resected developed an endorectal recurrence treated by local excision or brachytherapy. Rectal resection showed no overall or cancer-specific survival benefit. The patients not resected are meticulously followed with repeated digital, endoscopic and radiologic examinations challenged to discriminate between post-treatment fibrosis and residual/ recurrent cancer growth. A Dutch pilot study also found promising outcomes but large observational and randomized studies are to come [87 88].

Adverse effects of rectal cancer treatment

The perioperative mortality is 2% after rectal cancer surgery and 11% to 12% develop an anastomotic leak or pelvic sepsis [89]. The oncologic outcomes improved during the past decades and functional outcomes with an impact on life of cancer survivors have gained more attention. Resection of the rectum, localized in the posterior compartment of the narrow pelvis, has an impact on bowel function due to the loss of storage capacity. Even urinary and sexual function may be affected. The widespread use of RT, associated with specific adverse effects, makes it difficult to differentiate between the negative impacts of surgery and RT on pelvic function. In the Stockholm I and II Trials the majority of postoperative deaths were due to cardiovascular disease and more common in patients treated with preoperative RT. After reduction of the target volume by changing from two-field to four-field RT technique the difference in mortality disappeared [90]. The acute adverse events of short course preoperative RT in the TME Trial were limited to a slight increase of total number of postoperative complications, 100ml higher intraoperative blood loss and more perineal wound infections after APE [91]. No effect on postoperative mortality was observed.

Perineal wound healing after abdominoperineal excision

Wound healing after APE is complicated by several factors that may be more pronounced after extralevator APE due to the increased tissue loss of the pelvic floor. The remaining pelvic cavity can lead to small bowel displacement with consecutive small bowel obstruction or perineal herniation. In women the vagina and the uterus are displaced towards the sacrum resulting in decreased vaginal calibre and "cupping" of the upper vagina associated with vaginal discharge and dyspareunia [92]. Primary closure of the defect of the pelvic floor is seldom possible and the perineal wound cavity is surrounded by irradiated tissue in an area keen to bacterial contamination. The frequency of wound problems after primary perineal wound closure increases from 15% to 30.2% in conventional APE and 37.6% in extralevator APE if RT is added [93]. Biological mesh closure of the pelvic floor may decrease perineal wound problems after extralevator APE and randomized trials comparing mesh closure to primary or gluteus maximus myocutaneous flap closure are going on. Additionally, patients treated with APE have to cope with a permanent stoma associated with certain morbidity beside potential stigmata and taboos. Apart from inappropriate stomal site and early postoperative complications late adverse events such as stomal prolapse, stomal stenosis or parastomal herniation may occur [94]. However, quality of life with a permanent stoma is not necessarily inferior compared to patients treated with sphincter-saving procedures [95].

Low anterior resection syndrome

Reconstruction of bowel continuity with anastomosis of the left colon and the remaining part of the rectum or anal canal after anterior resection results in problems regarding bowel function characterized by urgency, frequent bowel movements and occasional faecal incontinence [96]. The low anterior resection syndrome score is a reliable and validated questionnaire to assess bowel function after anterior resection [97]. Preoperative RT, short length of remaining rectum, anastomotic leakage, female gender and age over 64 years have a negative impact on severances of the anterior resection syndrome [98 99]. Impaired bowel function is also related to restricted quality of life [100]. The reason for urgency and frequent bowel movements might be physiological changes based on neural damages resulting in a hyperactive postprandial response of the neorectum [101]. The colonic J-pouch showed improved functional outcomes compared to a straight anastomosis but was not better than the side-to-end anastomosis [102 103 104]. The small bowel is also affected by treatment for rectal cancer with increased risk for postoperative obstruction due to adverse effects of surgery and/ or RT. Diarrhoea, bleeding and rarely malabsorption, necrosis or perforation are attributed to small bowel damage following RT [99].

Urinary function

The negative impact on urinary function seems to be less pronounced and gradual improvement within six months might be expected [105]. Injuries of autonomic nerves during pelvic dissection contribute to a greater extent than RT to negative effects on bladder function [106 99]. Evaluation of urinary function with dedicated questionnaires in Danish women resulted in a frequency of 77% and 63% respectively for urgency and incontinence [107]. The type of surgery was not related to the negative impact on urinary function and RT increased only voiding difficulties in these long-term survivors.

Sexual function

The autonomic nerves of the pelvis gained a lot of attention during introduction of TME, as they are located close to the mesorectal fascia, the recommended plane of surgery. The hypogastric nerves unite the superior hypogastric plexus, containing sympathetic fibres anterior to the abdominal aorta, with the bilateral inferior hypogastric plexus or pelvic plexus at the pelvic sidewall. The inferior hypogastric plexus has connections with the sacral roots S2 to S4 containing parasympathetic fibres and proceeds in form of the neurovascular bundles to the pelvic organs. Resection of the superior hypogastric plexus/ nerves results in retrograde ejaculation in men [108]. Unilateral resection of the inferior hypogastric plexus impairs penile erection. In women parasympathetic fibres from the inferior hypogastric plexus mediate the increase of vaginal blood flow during sexual arousal leading to an increase of vaginal transudate with lubricating effect [109]. Anatomy and physiology of structures important for orgasm in women are still a matter of debate [110]. While male sexual dysfunction after rectal cancer treatment has been well described, considerably less data have been published about the impact on women [111]. Data from the Dutch TME Trial indicate that a majority of patients with resectable rectal cancer are sexually active at the time of diagnosis (79% of men, 52% of women) [112]. Among women, 60% experienced an increase in general sexual dysfunction, dyspareunia and vaginal dryness. In men, problems with increased sexual dysfunction, erection and ejaculation were reported by almost 75%. Radiotherapy and presence of a stoma was associated with increased sexual dysfunction in both sexes [113]. The convenience in assessment of sexual response and the discovery of phosphodiesterase type 5 inhibitors (PDE5-inhibitor) might have contributed that data on erectile function and ejaculation dominate the available literature of sexual dysfunction after rectal cancer treatment. However, sexual health is more than erection and ejaculation in men. Physiologic, psychological and context-related factors (i.e. couple dynamics, sociocultural issues, abuse) should be considered in evaluation of sexual function according to the Standards Committee of the International Society for Sexual Medicine. A physiologic sexual response requires integrity of hormonal, vascular, nervous, muscular, connective and immune systems and classification of sexual disorders involves desire, arousal, orgasm and pain. Studies assessing sexual function in patients with rectal cancer should also account for the high prevalence of sexual complaints in the general population of comparable age [114 115]. Several questionnaires assessing sexual disorders exist but it is not clear if the existing instruments are able to reliably assess the impact of rectal cancer treatment on sexual function [116].

The positive effect of PDE5-inhibitors in men with vasculogenic erectile dysfunction is well documented. The benefit of PDE5-inhibitors in treatment of erectile dysfunction caused by rectal cancer treatment is not well established and might be impaired as surgery puts penile innervation at risk and RT may induce penile fibrosis. Further it has not been studied at which time-point PDE5-inhibitor treatment should be initiated. Currently three PDE5-inhibitors (sildenafil, vardenafil and tadalafil) with different pharmacokinetics are available. Sildenafil completely reversed or satisfactorily improved erectile dysfunction in a randomized placebo-controlled trial among men 5 years after rectal resection due to rectal cancer or inflammatory bowel disease [117]. Rehabilitative and protective effects of PDE5-inhibitors on penile function are discussed for men undergoing nerve-sparing radical prostatectomy for prostate cancer, but there is still little clinical evidence [118]. The lack of spontaneous erections and consecutive decreased oxygenation of the cavernous tissue during the state of erectile nerve dysfunction may be a potential mechanism leading to long-term erectile dysfunction. In men after radical prostatectomy the medication with 5mg tadalfil once daily was more effective than 20mg tadalafil on demand to restore erectile function during the first 9 months post-operatively [119]. This randomized controlled trial failed to show a clear advantage of early continuous PDE5-

inhibitor intake on postoperative erectile function but the postoperative decrease in flaccid penile length, a consequence of penile fibrosis, was significantly lower.
The effects of PDE5-inhibitors have also been tested in treatment of female sexual dysfunction with conflicting results. According to a review from 2009, some trials showed a beneficial effect especially for women with underlying conditions such as multiple sclerosis, diabetes, spinal cord injury, and use of antidepressant medications [120]. The results of self-reported effects on female sexual function are mixed despite reproducible significant physiologic effects on genital vascular congestion. The combination of testosterone and PDE5-inhibitors on demand might be promising for treatment of hypoactive sexual desire disorders in women [121].

As PDE5 inhibition results in smooth muscle relaxation and increased pelvic blood perfusion, several other effects may be of interest in patients treated for rectal cancer [122]. Sildenafil improved the bursting pressure of colonic anastomosis in the presence of intra-abdominal infection and reduced peritoneal adhesions in rats [123]. PDE5-inhibitors enhanced and prolonged the induction of DNA damage in chemotherapy for gastrointestinal cancer [124]. Even positive effects of PDE5-inhibitors on skeletal muscle function are reported [125].

Specific late adverse effects of radiotherapy

Specific late adverse effects attributed to RT are increased risk of secondary cancers, pelvic or femoral insufficiency fractures and thromboembolic disorders [99]. Many of these results were observed in studies with old RT technology and may decrease or even disappear with the use of more refined RT technology [61]. The acute and late effect of RT on ovarian or testicular function may have an impact on fertility and hormone homeostasis [106]. The sensitivity of the ovaries to irradiation and chemotherapy results in high risk of impaired fertility and premature menopause [126]. Radiation doses over 24 Gy result in permanent ovarian ablation. This could be of importance even in postmenopausal women as the ovaries produce half of the testosterone in women [127]. The effects on testicular function are discussed later. In analogy to the findings in RT for prostate cancer exposure of the neurovascular bundles and the penile base to radiation may result in impaired erection due to vascular and nervous injuries [128]. Characteristic vaginal findings after RT are atrophic pale mucosa and fibrosis with consecutive loss of lubrication and elasticity or even narrowing and shortening of the vagina leading to dyspareunia or impossibility of penetrating intercourse [129]. The genital organs are not routinely included in normal tissue at risk of radiation damage during treatment planning of RT [130]. The positive effects of RT on oncologic outcomes and the potential of complete response enabling organ preservation are in contrast to the negative effects on postoperative and functional outcomes. So the future use of RT in rectal cancer treatment is debated [131].

Basics of radiation biology

The basics of radiation biology are retrieved from the handbook for teachers and students published by the International Atomic Energy Agency [132].

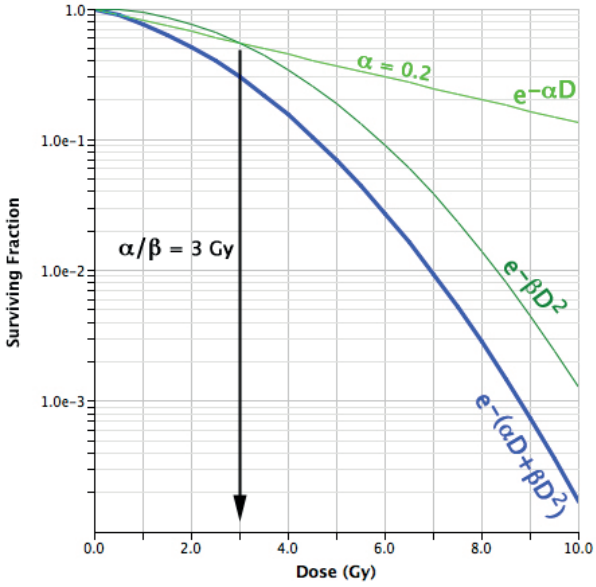
Electromagnetic radiation (e.g. X-rays or γ-rays) is indirectly ionizing as energy of photons, left behind during passage through biological tissue, produces secondary electrons (charged particles), which results in biological effects. Particulate radiation (e.g. α and β particles, protons, neutrons) also causes ionization and specially the charged elementary particles cause intense damage as they lose their energy within short distance of tissue. The density of energy deposition in the tissue is called Linear Energy Transfer (LET) and is important as the biological effect depends on LET. Electromagnetic radiation has generally lower LET than particulate radiation.

The radiation dose is the amount of energy absorbed per mass of tissue measured in the units of Gray (1 Gy = 1 Joule/kg). The equivalent dose is calculated by multiplying the absorbed dose with a weighting factor (quality factor) and has the units of Sievert (Sv). The absorbed and equivalent doses for low LET radiation are equal as the quality factor is one (1 Sv = 1 Gy).
The loss of energy of radiation after physical interaction leads to ionization and excitation of atoms and molecules,

which may convert into free radicals. When ionizing radiation energy is deposited in DNA or lipids of the cell membrane the effect is direct. Radiation energy can also be deposited in cellular water leading to indirect effects by water radiolysis. Certain compounds may scavenge radicals of water radiolysis and act against the indirect effects of radiation. A dose of 1 to 2 Gy induces about 1000 base damages, 1000 single strand breaks and 40 double strand breaks in the DNA of a single cell. The double strand breaks play a critical role for cell killing but different enzymatic DNA repair mechanism can limit the effects of these radiation induced damages. The loss of reproductive integrity (i.e. apoptosis, necrosis, mitotic catastrophe, induced senescence) is seen as equivalent for cell kill by radiation. In contrast to apoptosis cell necrosis is accompanied by an inflammatory response.

Survival of cells during radiotherapy

Radiosensitivity of cell populations is measured by the proportion of cells surviving irradiation with intact ability to undergo more than five to six cell divisions. Survival curves plot surviving fraction against radiation dose and the most common model to describe the decline of survival by increasing dose is the linear-quadratic model.

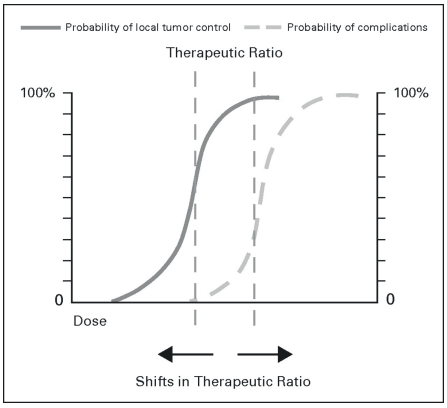


Radiosensitivity differs during the cell cycle and cell survival is increased if the same dose is given in two fractions with more than two hours in between due to cellular repair. The time for full cell repair takes six to eight hours or even longer. The repair during delivery of radiotherapy is negligible in external beam RT with a dose rate of 1 to 5 Gy/min but significant in brachytherapy with 1.6 to 150 cGy/min. Oxygen and pyrimidine analogues (e.g. 5-fluorouracil administered during long course RT) are examples of molecules that increase radiosensitivity.

Figure 3. A survival curve using the standard LQ formula $e^{-(\alpha D+\beta D2)}$ where α = 0.2 and α/β = 3. The components of cell killing are equal where the curves $e^{-\alpha D}$ and $e^{-\beta D2}$ intersect. This occurs at dose D = α/β = 3 Gy in this example. (www.eyephysics.com/tdf/models.htm)

Tumour control

The growth of a solid tumour is the result of proliferation of tumour cells and development of supporting stroma and vasculature by angiogenesis. The effect of RT on these cells is the tumour response to irradiation. Complete tumour control by RT means that all cancer stem cells, defined by unlimited proliferative capacity, must be killed.



Hypoxia and apoptosis of endothelial cells are factors influencing the microenvironment of tumour cells and may reduce radiosensitivity of tumour cells. Theoretically the required dose for tumour control depends on the number of cancer stem cells, estimated from the tumour size, and their radiosensitivity. The expected level of cell survival for a given dose can be predicted by a Poisson distribution and shows a sigmoid relation to dose with the slope reflecting radiosensitivity.

Figure 4. Sigmoid dose-response curves showing the relationship between increasing dose and tumour control probability and normal tissue complication probability. The therapeutic ratio is determined by the horizontal separation between the curves [133].

Fractionated radiotherapy

Fractionation of the radiation dose enables RT with higher doses as repair of radiation damages and proliferation of surviving cells can occur between fractions. The repair capacity of normal tissue is greater compared to tumour tissue, which favours fractionated RT. The effect of RT on biological tissue depends on the proliferation rate of the tissue and becomes evident during cell divisions after irradiation. The loss of function in renewal tissues like the bone marrow or the mucosa of the gastrointestinal tract correlates with the loss of proliferation activity of stem cells. The acute adverse effects appear within few weeks. Late responses are the result of damages to parenchymal cells with low proliferation or damages to supporting stroma/ vasculature. Late adverse effects become evident months or years after RT and often confine the total dose of RT regimens.

Biological factors affecting the response of tumours and normal tissues in fractionated RT are also called the five "R": Repair, repopulation, redistribution, reoxygenation and radiosensitivity.
The capacity to repair radiation damages is indicated by the shoulder of a cell survival curve and depends on the type of tissue. Repopulation occurs between fractions due to proliferation of surviving cells and may increase specially in early-responding normal tissue and tumours. Radiosensitivity differs during the phases of the cell cycle and surviving cells redistribute to more sensitive phases enhancing the effect of fractionated RT. Hypoxia increases radioresistance and surviving cells become reoxygenated between fractions hence also favouring the fractionated RT.

The biological effect of different fractionation schedules can be compared with models based on the linear-quadratic equation. It is assumed that every fraction has equal effect and that isoeffective schedules result in the same survival fraction. The constant [α/β] for a particular tissue can be determined by comparison of two isoeffective regimens with different fractionation to predict other isoeffective schedules. The parameter α defines the initial slope of the survival curve and parameter β defines the curvature of the curve. The values of the constant [α/β] are in the range of 2 to 4 Gy for late responding tissues and 8 to 12 Gy for early responding tissues. Many tumours have [α/β] values similar or higher to those of early responding tissues. The duration of a fractionated schedule is not considered in the LQ model, which is an important limitation in early responding tissues and tumours characterized by high proliferation. The equation to calculate the biologically effective dose (BED) of early responding tissues includes therefore a repopulation term that can be omitted for calculation of BED in late responding tissues [134].

$$\text{BED} = \text{log cell kill} - \text{repopulation term}$$

$$\text{BED} = n*d(1 + d/[\alpha/\beta]) - \log_e 2*(T-T_k)/\alpha*T_p$$

n = number of fractions
d = dose per fraction
T = treatment time
T_k = time to start of delayed repopulation
T_p = cell doubling time

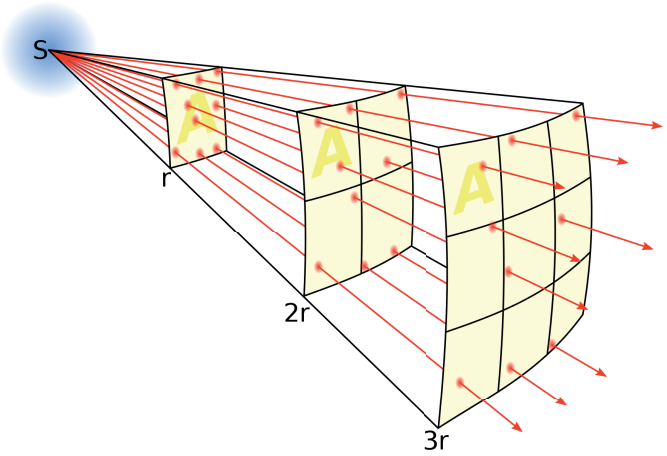
This formula is useful to describe BED of different fractionation schedules for tissues exposed to radiation doses resulting in cell kill.

Planning and delivering of external beam radiotherapy

Current external beam RT uses high-energy electromagnetic radiation (X-rays) from a linear accelerator. The electron gun produces free electrons that are sent to the accelerator tube. The high-frequency electromagnetic waves in the tube accelerate the electrons that ride on top of electromagnetic waves and boost their energy to the desired level. The beam exiting the accelerator is then diverted and sent to the treatment head. Blocks, filters and multileaf collimators shape the beam before leaving the gantry that can rotate 360° around the patient. The precise positioning of the radiation source and the patient are important to deliver accurate RT.
During treatment simulation the patient is immobilized on the treatment couch to acquire a planning CT and to

mark relevant reference points of the patient´s body by skin tattoos. The target volume and organs at risk are contoured on each slice of the planning CT. The gross tumour volume (GTV) includes visible and palpable tumour based on clinical and radiologic investigations. The clinical target volume (CTV) contains regions with high risk of microscopic disease. The CTV is extended to the planning target volume (PTV), which accounts for physiological organ movements and set-up errors. The planning organ at risk volume defines organs at risk in the treatment field that can cause changes to treatment plans and doses.

In conformal planning the treatment volume is determined virtually with assistance of treatment planning software based on the three-dimensional planning CT. The beams with blocks and multileaf collimators can be seen and selected digitally. Multiple treatment fields, used for PTVs located deep in the body, provide homogeneous dose distribution in the PTV and reduce doses to normal tissues outside the treatment volume. The final treatment plan describes the dose distribution and contains dose-volume histograms for the target and organs at risk. The authorized final treatment plan is then sent online to the treatment machine where RT can be initiated.



For each fraction the patient is positioned with help of the tattooed reference marks on the treatment couch and adjusted according to the electronic portal imaging device. Delivery of the photon beam takes not more than a few minutes.

Figure 5. The inverse square law states that physical intensity is inversely proportional to the square of the distance from the source of the intensity (adapted from www.wikipedia.org)

Distance reduces dose is a simple but important role in radiation. The inverse square law states that a physical intensity is inversely proportional to the square of the distance from the source of intensity. Dose-volume histograms are a more sophisticated way to summarize the three-dimensional dose distribution in a two-dimensional graph. First the dose bins, dose intervals of equal size, are determined arbitrarily. The differential dose-volume histogram looks like a usual histogram and the column height indicates the proportion of the volume of interest receiving the dose given by the bin. Cumulative dose-volume histograms are preferred over differential dose-volume histograms and are the result of a bin-by-bin integration. The cumulative dose-volume histograms look like a line graph for small bin sizes displaying the proportion of the volume of interest receiving more or equal to a determined dose.

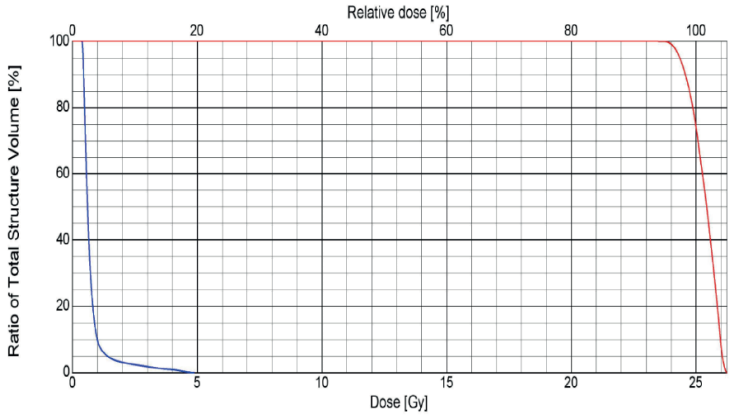


Figure 6. Cumulative dose-volume histogram for RT of rectal cancer (prescribed dose of 25 Gy); red line = PTV, blue line = Testes (mean testicular dose 0.73 Gy)

Hypogonadism

Testosterone in men

The pulsatile release of gonadotropins from the pituitary is the result of gonadotropin-releasing hormone (GnRH) pulses occurring every 60 to 90 min in the hypothalamus [135]. Follicle-stimulating hormone (FSH) and intratesticular testosterone (T) stimulate spermatogenesis by acting on Sertoli cells. Luteinising hormone (LH) increases the T synthesis in the Leydig cells of the testes. Adult men after normal puberty produce 3 to 10 mg of T per day corresponding serum T levels of 10.4 to 34.7 nmol/l [136]. The diurnal variation with a decrease during daytime in serum T is most prominent in men younger than 45 years [137]. Serum T can be assessed at any time before 2 p.m. in men older than 45 years without misleading results. In men from the north of Norway seasonal variation in serum T with a small peak in February and a prominent peak in autumn have been registered [138]. Glucose load and physical activity may also have an impact on serum T levels [139].

In plasma approximately half of the serum T is tightly bound to sex hormone-binding globulin (SHBG) and not available for cells. The remaining T is loosely bound to proteins such as albumin and 1% to 2% of T is free in plasma. The non-SHBG-bound T or bioavailable T is freely dissociated and participates in tissue interaction [140]. Thus, conditions that influence SHBG may affect bioavailable T. SHBG increases with age, hyperthyroidism, liver disease and decreases with obesity, diabetes mellitus and glucocorticoid use. The presence of other steroids in high concen-tration and potentially very low levels of T in children, women and hypogonadal men complicate the measurement of T plasma levels [141]. The reference methods for direct measurement of free T and bioavailable T, equilibrium dialysis and ammonium-sulphate precipitation respectively, are technically challenging and not widely available. The radioimmunoassay is expensive, time intensive and creates radioactive waste. So enzyme immunoassays are widely used to measure T. They often overestimate low concentrations and have a considerable inter-laboratory variability [142]. Mass spectrometry is actually promoted as the most accurate method for clinical steroid measurement. However, inter-laboratory variability is still a problem and validated immunoassays seem sufficient to detect subnormal T concentrations for diagnosis of hypogonadism in men [143].

To evaluate men with signs of hypogonadism the measurement of serum T in a morning sample with a validated immunoassay or mass spectrometry is recommended. Low serum T levels should be confirmed with a second blood sample due to considerable variation even in young healthy men [144]. In addition to serum T the concentrations of SHBG and albumin should be measured in the second sample to calculate bioavailable T and free T based on equations [145]. LH and prolactin measurement in the second sample allow for differentiation between primary and secondary testicular failure as explained below.

Testosterone acts directly via androgen receptors in the cytoplasm and nucleus of target cells. Polymorphism of the androgen receptor, for example different length of CAG repeats in exon 1 of the androgen receptor gen, may influence the androgen action of T in several tissues [146] [147]. The 5α-reductase of the prostate, external genitalia and skin, converts 5% to 8% of total T to the locally active metabolite dihydrotestosterone. A small amount of T (0.5%) is converted to oestradiol by aromatase in adipose tissue and liver. The joint action of T and oestrogens in adult men suppresses accretion of adipose tissue and bone loss and is important for maintaining normal libido and erectile function [148].

The adrenal cortex also produces androgens such as dehydroepiandrosterone (DHEA), its sulphate and androstenedione. These androgens can be converted into T, however, serum T levels drop by 95% after surgical androgen deprivation by orchiectomy to levels below 0.69 to 1.73 nmol/l [149]. So the adrenal gland cannot compensate a significant decline in serum T in men due to pathologies of the hypothalamic-pituitary-gonadal axis. Adrenal androgens may become important during androgen deprivation in advanced prostate cancer. Observations under this condition indicate that the adrenocorticotropic hormone (ACTH) and LH play a role in regulation of adrenal androgen synthesis [149].

The hypothalamic-pituitary-gonadal axis

In men disruption of the hypothalamic-pituitary-gonadal axis leads to hypogonadism, sometimes referred to as testosterone deficiency. Primary testicular failure results in primary hypogonadism characterized by low T levels, impaired spermatogenesis and elevated gonadotropins. Common causes of testicular failure are Klinefelter´s syndrome (47, XYY), testicular tumours, Mumps-related orchitis, radiation, chemotherapy, testicular trauma and torsion. Low T levels, reversible reduced spermatogenesis and low or inappropriately normal gonadotropins characterize secondary hypogonadism. Apart from isolated congenital gonadotropin deficiency acquired conditions such as hyperprolactinaemia, pituitary damages, systemic disease, obesity, diabetes, eating disorders, excessive exercise or medications can lead to hypogonadotropic hypogonadism. Serum T levels below the lower reference limit of young adults are not uncommon in men over age 60 and due to a yearly decline in serum T of about 1% after the age of 20 to 40 years. Names like andropause or male climacterium generate confusion with the physiologic condition of menopause in women and should be avoided. Late-onset hypogonadism (LOH) describes a mixed form of hypogonadism observed in older men with combined primary and secondary testicular failure [142]. The serum T levels are low and gonadotropin levels indicate whether primary testicular or hypothalamic-pituitary failure dominates. Hypogonadism due to androgen receptor defects or 5α-reductase deficiency result in androgen insensitivity syndromes and are rare.

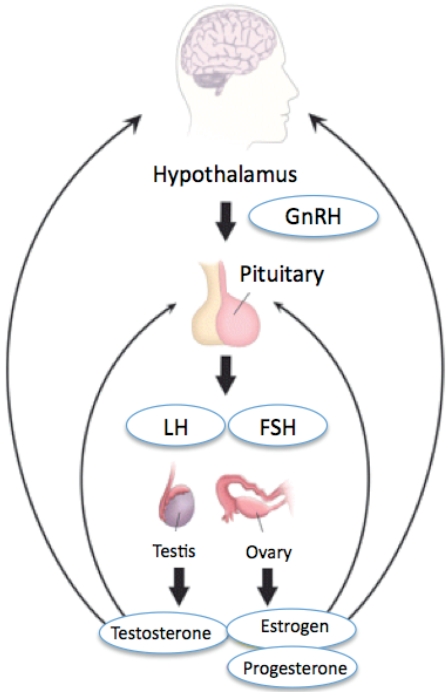


Figure 7. Hypothalamic-pituitary-gonadal axis (www.dsdgenetics.org; reprinted with permission)

Signs of hypogonadism

Prepubertal onset of hypogonadism results in incomplete sexual development (micropenis, small testes and prostate), infertility, eunochoidal proportions (delayed epiphyseal fusion), gynaecomastia, high-pitched voice and decreased body hair, bone and muscle mass. Adult men that develop hypogonadism after normal puberty have normal skeletal proportions, penile length, voice and prostate size. Postpubertal onset of hypogonadism affects sexual function, body composition and mood. The clinical symptoms occur at different levels of serum T [150].

Clinical manifestations suggestive for postpubertal onset of hypogonadism:

Sexual function	Decreased sexual interest/ libido
	Decreased frequency of spontaneous (morning) erections
	Erectile dysfunction
Body composition	Decreased body hair/ shaving
	Visceral obesity and gynaecomastia
	Decrease in lean body mass and muscle strength (sarcopenia)
	Decrease in bone mineral density
	Insulin resistance, metabolic syndrome, hot flushes, sweat
Mood	Decreased energy, motivation, concentration and memory
	Fatigue, dysthymia, depressive thoughts, anger
	Sleep disturbances

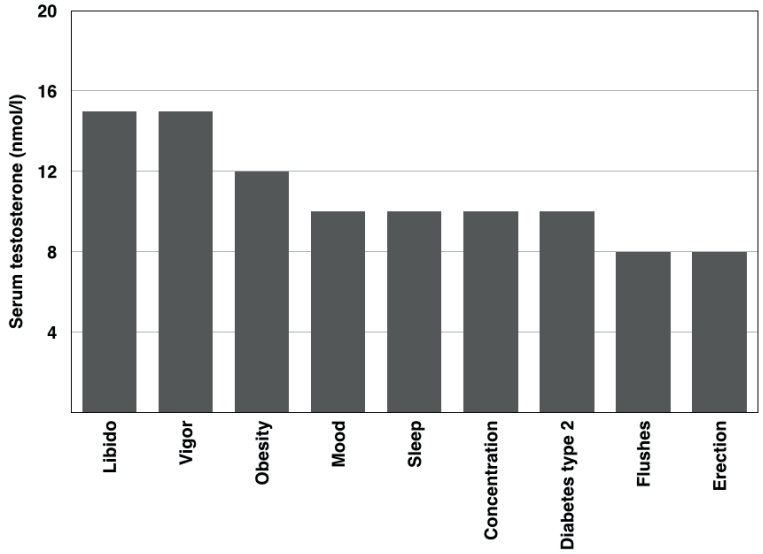


Figure 8. Levels of serum T below prevalence of respective symptom increases (adapted [150])

Diagnosis of hypogonadism

The diagnosis of hypogonadism is challenging as clinical manifestations of hypogonadism and physiological signs of ageing overlap. The diagnosis based on T levels alone is not appropriate as biochemical methods are restricted to measure plasma levels of T and gonadotropins, a surrogate of androgen action submitted to considerable variation. By definition 2.5% of normal men lie outside the mean ± two standard deviation reference range between 10 to 30 nmol/l and men in the upper half of the reference range (serum T 20 to 30 nmol/l) only can become hypogonad if the T decline exceeds 50% [151] [142]. Neither does the reference range respect the age-related decline of T, which overestimates the prevalence of hypogonadism in older men [152]. A practical approach is to regard serum T levels below 8 nmol/l as suggestive for hypogonadism and serum T between 8 to 12 nmol/l as grey zone of hypogonadism [150]. Recent results confirmed that persistent serum T below 8 nmol/l is sufficient for the diagnosis of hypogonadism and free T below 220 pmol/l strengths the diagnosis in the grey zone of 8 to 11 nmol/l in serum T [153]. This study also reported a syndromic association for three sexual symptoms (decreased frequency of sexual thoughts, decreased frequency of morning erections and erectile dysfunction) and low T values. Other physical or psychological symptoms suggestive for hypogonadism were unrelated or weakly related to low serum T. Apart from low serum T values the isolated elevation of LH above 9.4 IU/l, specially in non-obese men, may indicate a precursor of primary age-dependent hypogonadism, also called compensated hypogonadism, with relevant risk of subsequent primary testicular failure [154] [142]. In summary it can be concluded that the diagnosis of hypogonadism should be restricted to men with persistent low serum T in combination with clinical manifestations of hypogonadism. Screening of low serum T should be avoided [151]. These requirements for diagnosis of hypogonadism are not completely applicable in men treated for pelvic malignancies as treatment-related damages of autonomic nerves, vascular structures or cavernous bodies restrict the ability of having an erection.

Late-onset hypogonadism

The European Male Aging Study (EMAS) analysed 3369 men aged 40 to 79 years and invited by random to participate in a baseline assessment followed by a visit after 4 years in eight countries [155]. The proportion with late-onset hypogonadism, defined by the combination of low serum T and three sexual symptoms, was 2.1% with an age-dependent increase from 0.1% in men aged 40 to 49 years to 5.1% in men aged 70 to 79 years [155]. The association of LOH with obesity or co-morbidity (poor general health) was also confirmed. Increasing age was related to primary testicular failure whereas obesity-related hypogonadism was not associated with increased gonadotropins. Men with severe LOH (serum T < 8 nmol/l) had substantially higher all-cause and cardiovascular mortality [156]. Low serum T and oestrone were also related with decreased self-rated health in men over age 70 and low serum oestrone levels were predictive for longitudinal deterioration in self-rated health [157]. In a small non-comparative study low serum T was associated with a increased risk for postoperative complications after gastrointestinal surgery [158].

Testosterone-replacement therapy

Testosterone-replacement therapy (TRT) is an option in hypogonadal men without signs of hormone-responsive tumours (prostate, breast), severe lower-urinary-tract-symptoms, haematocrit > 50%, severe sleep apnoea or uncontrolled congestive heart failure [136]. Sexual motivation, erectile function and number of successful intercourses increases with TRT in men with serum T levels < 12 nmol/l but the impact on overall sexual satisfaction are conflicting and the effect of TRT declines over time [159]. The combined treatment with PDE5-inhibitors and testosterone restores erectile function in hypogonadal men more effectively than monotherapy with PDE5-inhibitors or testosterone [160]. Improvement of sexual function under TRT is most marked in less obese men and those without simultaneous depression [161]. Lumbar spine bone-mineral density increases with intramuscular but not with transdermal TRT and the effect on the femoral neck is not conclusive [162]. A meta-analysis summarized the effect on body composition in men between 50 to 78 years of age [163]. The body weight did not change but on average the fat-free mass increased by 1.6 kg and the total body fat decreased by the same amount. Muscle strength tended to improve and total cholesterol decreased. Low T and sexual dysfunction is a common finding in obese men with metabolic syndrome and diabetes [164]. TRT may improve the risk profile of cardiovascular diseases in this group but the results of TRT in men with diabetes or metabolic syndrome are conflicting [165]. In depressed patients with hypogonadism TRT was antidepressive and the route of administration could be important [166]. In men over age 60 improvements of memory have been observed under TRT and the positive effect on verbal memory seems to be related to aromatisation of T to oestradiol [167]. The treatment monitoring includes assessment of erythrocytosis by haematocrit and prostate by digital rectal examination and PSA to detect adverse effects of TRT. The evidence regarding benefits and risks of TRT is mainly based on small studies with suboptimal design and duration, so the results of on-going randomized studies are awaited with interest [142].

Body composition

Models of body composition assessment

Direct measurement of body fat is despite a variety of methods for assessment of body composition not possible [168]. The four-compartment (4C) model measures the chemical composition of the body and is often considered the reference method for assessment of body composition. The model uses minimal assumptions and quantifies the amount of water, mineral, protein and fat. Total body water and bone mineral are measured directly using isotope dilution of deuterated (heavy) water and dual energy X-ray absorptiometry (DEXA). Total body density is calculated after assessment of total body volume by subtraction of underwater weight from weight in air or alternatively by whole body plethysmography. The amount of fat and protein, the unmeasured fraction, can be calculated applying assumed densities of fat, water, bone mineral and protein [169].
The two-compartment (2C) model separates the body into fat and fat-free/ lean mass (i.e. total body water, bone mineral and proteins). The accuracy of such a model has been questioned as it is based on the assumption of con-stant and fixed hydration/ bone mineralisation ignoring age- and sex-related differences. Skinfold thickness assessment and bioelectric impedance analysis are cost effective and widely available methods to assess the two-compartment model but accuracy is limited [170].
The three-compartments (3C), fat, bone mineral and soft-lean mass, can be assessed by DEXA, the method of choice to quantify bone mineralisation. This X-ray based method assumes constancy of water and lipid content for skin, muscle and bone, which in vivo may vary.
Anthropometric methods based on weight such as the BMI cannot distinguish between fat and muscle and problems arise in individuals with shape different from the norm. Considering sitting height or leg length can improve the value of these indices [170].

Cross-sectional imaging techniques, CT and MR, can also be used to assess body composition on the tissue level. Different attenuation of adipose tissue, skeletal muscle and bone enable tissue delineation on two-dimensional slic-

es to compute volumes of interest. These imaging methods can assess quantity and distribution of body fat by separate measurement of subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and even intra-organ adipose tissue of skeletal muscle (SM) or liver. The skeletal muscle mass but also muscle groups or individual muscles are assessable. The accuracy of CT and MR is comparable with an expected error of 2% for skeletal muscle and 4% for SAT [171]. The measurement error for VAT is around 10% for CT and MR. The difference between CT and MR in measurement of SAT and SM is 5% approximately. Automated image analysis is available and can be combined with manual correction to improve accuracy and spare time. Different values of attenuation in Hounsfield units allow separating adipose tissue (-50 to -100 HU) from skeletal muscle (40 HU) in a standardized way on CT. This is more demanding for MR analysis as different approaches for segmentation are used. The analysis of a single cross-sectional abdominal image results in accurate measures of muscularity or adiposity of the whole body [171]. The level of vertebra L3 is often used to assess the area of SM and the L4-5 level appears appropriate to assess VAT.

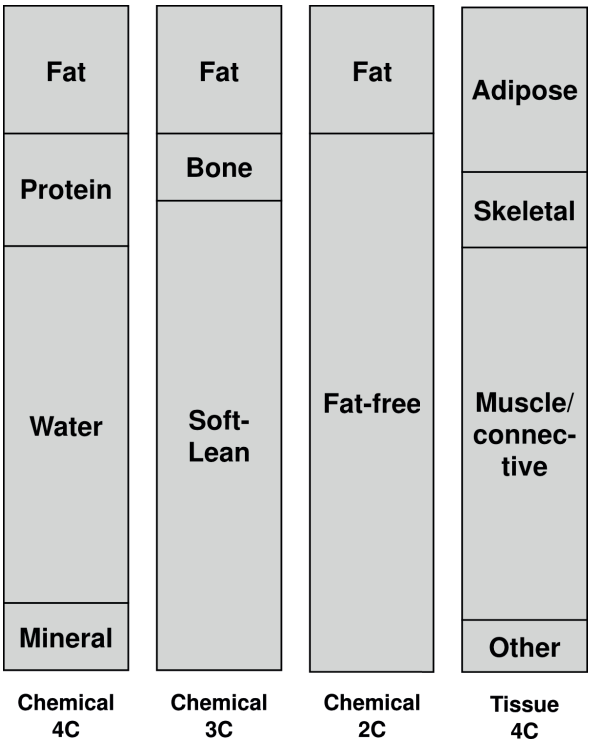


Figure 9. Models for chemical and anatomical assessment of body composition

Sarcopenia

Patients with rectal cancer have cross-sectional imaging for staging, planning of RT and follow-up. Routine use of the information on body composition, available without additional costs or exposure to radiation, has not yet reached clinical practice. The diagnosis of sarcopenia, defined as age-related loss of skeletal muscle mass and strength, is based on low muscle mass in combination with low muscle strength or low physical performance [172]. The proposed cut-off values for the three signs are two standard deviations below the mean reference value derived from healthy young adults. Normative values of standardized assessment of skeletal muscle mass on abdominal CT are available and validated [173 174]. Handgrip strength can be used to assess muscle strength in clinical practice and physical performance is evaluated with basic tests (i.e. usual gait speed, timed get-up-and-go test, stair climbing or short physical performance battery). Sarcopenia may be present in cachexia and frailty, conditions diagnosed based on medical history and clinical examination. Cachexia is a complex metabolic syndrome with severe underlying disease characterized by loss of muscle with or without loss of fat mass [175]. Frailty is a geriatric syndrome describing an age-related decline across multiple physiologic systems leading to increased vulnerability for adverse health outcomes and includes psychological and social dimensions [176].

In patients treated with surgery for CRC stage II-IV sarcopenia increases the risk of postoperative infections and the need of inpatient rehabilitation [177]. According to a recent review, sarcopenia is also associated with increased 30-day mortality, 5-flurouracil toxicity and diminished survival for stage IV CRC [178].

The size of skeletal muscle matters, however, the quality of muscle also is of concern. Fat can be found in myocytes (intramyocellular lipid droplets) and between muscle bundles in adipocytes [179]. The extracellular fat of the skeletal muscle is known as intermuscular adipose tissue (IMAT) and related to cardiovascular risk, age and little physical activity [180]. The intracellular lipid droplets could serve as fuel for mitochondrial oxidation and are the result of en-

durance exercise. In obese men accumulation of intracellular lipid droplets was related to insulin resistance. The increment of intracellular fat in skeletal muscle is also called myosteatosis and leads to changes in muscle attenuation detectable on CT or MR [181]. In a large cohort with patients treated for respiratory or gastrointestinal tract malignancies sarcopenia and low attenuation of psoas muscle were independent predictors for lower survival [182].

Testosterone and skeletal muscle

The anabolic effect of androgens on skeletal muscle is the reason for the popularity among abusers [183]. Multiple mechanism are involved in the androgen action on muscle, which results in promotion of muscle protein anabolism and differentiation of pluripotent stem cells toward myogenic lineage [184]. Apart of this myotrophic effect of endogenous and exogenous T, training enhances the T effect on skeletal muscle. Physical exercise increases the expression of androgen-synthesizing enzymes (e.g. 5α-reductase) in the skeletal muscle that promote synthesis of T and dihydro-T from circulating DHEA resulting in intracrine androgen action [185]. Several studies report an association of endogenous T levels and muscle mass, muscle strength and physical function but these results are not consistent and often restricted to free or bioavailable T [186]. The supplementation of T in young and older men results in a dose-dependent increase in muscle mass and a reciprocal decrease in fat mass [187] [188] [189]. Improvements in muscle strength are modest especially for near physiologic TRT [186] [190]. An interesting alternative to supraphysiolog-ic T replacement aiming to increase muscle mass and strength are selective androgen receptor modulators with myotrophic effect in absence of androgenic effects on prostate, hair and skin [191]. Findings in androgen receptor knockout mouse models suggest that androgens are involved in growth and maintenance of skeletal muscle mass as well as muscle development and function [192]. In animal studies the androgen sensitivity of skeletal muscle was often investigated in muscles supporting masculine reproductive functions [193]. Examples are copulatory perineal muscles of rodents, vocal muscles used in mate-attraction or limb muscles in birds that display spectacular athleticism dur-ing courtship. In pigs the androgen effect on skeletal muscle growth differs among muscle groups [194]. Androgen deprivation therapy in men with prostate cancer may result in more pronounced loss of muscle mass and strength of the upper compared to the lower extremity [195]. Differences in androgen receptor expression observed in trapezius and vastus lateralis muscle of humans may explain this variation in androgen sensitivity of different skeletal muscle groups [196] [147].

AIMS OF THE THESIS

The general aim of the research project was to investigate the effects of contemporary rectal cancer treatment on testicular function and the consecutive impact on sexual function and quality of life. The available literature on radiation exposure of the testes during radiotherapy and testicular function in men treated for rectal cancer was systematically reviewed in Paper I to summarize current knowledge and generate relevant hypotheses. The reviewed data served as basis to design and implement a cohort study assessing identified research questions. In the present thesis the following specific aims are addressed:

Paper II

To calculate the testicular dose during contemporary radiotherapy for rectal cancer based on planning CT and to evaluate the difference between planned and delivered testicular dose.

Paper III

To assess the acute effects of preoperative radiotherapy for rectal cancer on androgen levels.

Paper IV

To investigate the association between longitudinal changes in testosterone levels and body composition in men treated with preoperative radiotherapy for rectal cancer and to evaluate the clinical value of such an association to support decision-making in men with testosterone levels in the grey zone of hypogonadism.

REVIEW OF LITERATURE

Paper I

Methods

In cooperation with a librarian, we assessed PubMed, Embase, and Cochrane applying a search design using the MeSH-term "Rectal Neoplasms" in combination with: "Sexual Behaviour", "Sexual Dysfunctions, Psychological", "Sexual Dysfunctions, Physiological", "Gonadal Hormones", "Radiotherapy", "Radiation", "Quality of Life", "Spermatozoa" and "Testis". Original articles providing data on testicular radiation exposure, testicular function or the impact of hypogonadism on sexual function and quality of life in men with primary rectal cancer were included. No restrictions regarding study design were applied.

Results

The search strategy resulted in inclusion of three cohort studies and seven case series out of 188 identified abstracts. No randomized control trials or systematic review were identified. According to the "New levels of evidence" published by the Centre for Evidence-Based Medicine (University of Oxford; http://www.cebm.net) regarding treatment harms the evidence is at level 4 (step 4). The lack of high-quality studies and the heterogeneity of the identified publications precluded a systematic data synthesis.

Testicular radiation exposure during radiotherapy for rectal cancer

Six studies reported the testicular radiation exposure during RT for rectal cancer [197 198 199 200 201 202]. The testicular dose (TD) was estimated either by scrotal dosimeters, by treatment planning software based on CT or by equations derived from phantom measurements. The mean cumulative TD varied between 1.24 Gy and 8.4 Gy (range 0.06 to 13.7 Gy) for external beam RT. This corresponds to 3% to 17% of the prescribed dose.

Table 2. Testicular radiation exposure during radiotherapy for rectal cancer

	Radiotherapy schedule			Cumulative testicular dose	
First author	Type	Dose per fraction	Prescribed dose	Mean	Range
Dueland [197]	EB	2	46-50	8.4	3.7 – 13.7
Piroth [198]	EB	1.8	50.4	1.6	0.98 – 3.19
Hermann [199]	EB	2	50 + 6	3.56	0.7 – 8.4
Mazonakis [200]	EB	1.8	45	1.9	1.1 – 2.8
Yau [201]	EB	1.8	45-50.4	1.24	0.06 – 7.8
	BT	6.5	26	0.27	0.14 – 0.65
Yoon [202]	EB	1.8	50.4 + 3.6	4*	1.5 – 8.9

Dose values in Gy, EB=External beam, BT=Brachytherapy, * Median cumulative testicular dose

The distance between the testes and the lower radiation field edge, the patient thickness along the beam axis and the photon energy were factors influencing TD. Treatment field elongation and wedged lateral beams were of minor importance [203 200].

In most studies the patients were treated in the prone position to reduce the irradiated small bowel volume. In order to enhance this effect, a single-hole belly board (SHBB) was used [198]. The double-hole belly board (DHBB) with an additional hole for the testes was developed to decrease the testicular dose. The number of radiation fields, the positioning devices (SHBB vs. DHBB) and the height of the tumour influenced the testicular dose significantly [204]. A conventional gonad shield based on the placement of a lead block on a shadow tray reduced the TD by 27–41%. With the round gonad shield made of 1.27 cm thick lead, a reduction of 66–74% was seen [200]. The lowest proportion of the prescribed dose (0.5–2.5%) was delivered to the testes using a high-dose-rate brachytherapy for tumours located in the lower and middle third of the rectum. The patient was in lithotomy position and using a jockstrap to move the testes away from the target volume [201].

Spermatogenesis in men with rectal cancer

No reports on semen analyses in men treated for rectal cancer were identified.

Testosterone levels in men treated with radiotherapy for rectal cancer

The use of RT decreased serum T levels in all studies when comparing pre- to posttreatment levels or patients with and without radiotherapy. The reduction in serum T was statistically significant in three of four studies and observed within two months after RT and persisted after follow-up of at least 3.9 years [197 205].

Table 3. Serum testosterone in men treated with external beam radiotherapy for rectal cancer

	Mean (± SD) serum testosterone (nmol/l)			Frequency of serum testosterone < 8 nmol/L (%)	
First author	Before/without RT	After/with RT	*P* value	Before/without RT	After/with RT
Dueland [197]	14.4 (4.9)	10.6 (4.3)	0.001	8	35
Hermann [199]			0.07	40	70
Bruheim [205]	13.4 (5)	11.1 (4.7)	<0.001	10	27
Yau † [201]				0	17.6
				0*	2.7*
Yoon ‡ [202]	15.4 (6.1)	12.1 (4.6)	<0.001		

SD=standard deviation, RT=preoperative radiotherapy

* Brachytherapy, † Only patients with normal baseline hormonal values, ‡ Only patients reporting sufficient erectile function for intercourse included

The frequency of low serum T (< 8 nmol/l) before RT or among men treated with surgery alone was 0% to 40%, increasing to 17% to 70% after RT. From these data, an absolute risk increase of 17% to 30% to have low serum T after RT can be deducted. In the study with the largest number of patients (N = 290), the relative risk for low serum T was 2.7 (95% CI 1.6 to 4.7; p<0.001) four years after RT [205].
In multiple regression analyses, a low radiation field edge and the use of a two-field technique were risk factors for low T levels [205]. No significant effect was found for time since treatment, treatment position (prone vs. supine), chemotherapy (5-fluorouracil, leucovorine), or the timing of radiotherapy (pre- vs. postoperative). External beam RT with higher testicular doses resulted in a trend to lower T levels compared to brachytherapy [201].

Testosterone levels and sexual function in men with rectal cancer

Based on the Norwegian Rectal Cancer Registry, one publication assessed serum T levels and sexual function with the International Index for Erectile Dysfunction (IIEF). The prevalence of moderate to severe erectile dysfunction was 86% in patients treated with RT compared to 55% treated with surgery alone ($P < 0.001$) [206]. Age, RT and low serum T (< 8 nmol/l) were related to moderate-to-severe erectile dysfunction in the multivariate analysis. Men treated with RT also had significantly lower scores for orgasmic function and overall satisfaction with sex life.

Discussion

The studies investigating the TD during RT for rectal cancer and the effects on testicular function were sparse with low level of evidence. Systematic reviews or randomized controlled trials were lacking and the two population-based cohort studies could analyse less than 60% of the eligible study population [205 207]. The sample size of the other stud-ies was limited and power calculations were not published. The TDs were measured with different methods (i.e. scrotal dosimeters, treatment planning system or phantom-derived equations) resulting in dose values that represent the dose of a certain point of the scrotal skin (i.e. scrotal dosimeters) or the mean dose of the testicular volume (i.e. treatment planning system). The prescribed dose of RT, the RT technique (e.g. external beam vs. brachytherapy, number of fields, beam energy) and the time of RT (pre- vs. postoperative) varied between the studies. The methods of testosterone measurement could not be identified in three publications [199 201 202] and was different in the remain-ing two studies [197 205]. In addition the baseline measurement of T was after surgery in the longitudinal study with postoperative RT [202]. Yau et al. excluded men with abnormal androgen profiles [201]. These methodical aspects lead to heterogeneity of the reported outcomes and imply a risk for bias that resulted in the decision not to perform a quantitative synthesis of the results.

Testicular dose during radiotherapy for rectal cancer

The accuracy of the methods to assess the TD was not reported. According to Piroth et al., the variation of TDs measured by dosimetry was substantial [198]. Different positions of the testes or dislocation of the scrotal dosimeters during the treatment session were mentioned as possible reasons. Gonadal shields could reduce TDs substantially [200]. Although they should be used with caution as they can induce cremaster reflex that decreases the distance between the lower radiation field edge and the testes. Gonadal shields located in the field of radiation produce secondary electrons that increase TD [208].

Effects of testicular dose on germinal epithelium

A single dose of 4–6 Gy reduced the number of spermatozoa significantly in healthy prisoners and time to complete recovery of spermatogenesis was dose dependent [209]. A single-dose exceeding 6 Gy likely caused permanent azoospermia [210]. Testicular doses between 0.2 to 0.7 Gy during fractionated RT for Hodgkin's disease caused a transient dose-dependent decrease in sperm concentration and a return to normospermia within 1 to 2 years [211]. No recovery of spermatogenesis was found after TDs of 1.4 to 2.6 Gy in eight men treated with fractionated RT for testicular seminoma, while two men with TD of 1.2 Gy returned to normospermia [212]. Similar findings were reported in 11 men that received fractionated pelvic radiation with TDs between 1.18 to 2.28 Gy [213]. During the follow-up period of 35–107 weeks, six men remained azoospermic, three recovered to oligospermia, and two returned to normospermia. The threshold of fractionated RT inducing permanent azoospermia may be a cumulative TD around 1.2 to 1.4 Gy.

Effects of testicular dose on Leydig cells

The significant increase of LH with maintained T levels after single doses above 0.75 Gy and fractionated doses above 2 Gy indicate a negative yet compensated impact on Leydig cell function [209 214]. A mean TD of 2.07 Gy in men treated with external beam RT for prostate cancer patients resulted in a significant decrease of serum T levels after three months, which, however, was not considered to be of clinical relevance [215]. In contrast a mean TD of 1.24 Gy during RT for rectal cancer resulted in biochemical hypogonadism (serum T < 8 nmol/L) in 15.5% of men with normal pretreatment T levels [201]. So the results of studies that assessed the effect of pelvic RT for other malignancies cannot be compared directly with results in men treated for rectal cancer.
The majority of the studies included in this review do not report symptoms or risk factors related to hypogonadism. Only the Norwegian study found an association between low serum T (< 8 nmol/l) and moderate-to-severe erectile dysfunction [206].

Conclusion

Patient care

This review suggests an increased risk of infertility and hypogonadism in men treated with RT for rectal cancer. Patients should get this information before start of RT to enable semen cryopreservation if requested and physicians should be alert to screen clinically for signs of hypogonadism.

Research

This review generates several hypotheses for further research. The testes are exposed to direct and/ or scattered radiation during RT for rectal cancer but the accuracy of TD calculation based on planning CT to estimate the delivered TD during all treatment fractions is not known. The acute and late effects of RT on testicular function may be related to a posttreatment decline in androgen-dependent physiological/ psychological functions and infertility. A dose-response relationship between TD and the effects of RT has not been established. The effects of hypogonadism on quality of life have not been assessed in men with rectal cancer and semen analyses are lacking.

SUBJECTS AND METHODS

Cohort study

Hypothesis

The testicular radiation exposure has acute and late adverse effects on testicular function resulting in an increased risk of transient or permanent infertility and hypogonadism with a relevant impact on patient reported outcome measures (PROM) in men treated with RT for rectal cancer.

Study design

Based on the findings of the review article (Paper I) we designed a cohort study with prospective data collection and preoperative RT as exposure. The exposure status was defined at the pre-therapeutic MDT conference prior and independent to any assessment of outcome measures. Serum T was chosen as the primary endpoint and spermatogenesis, sexual function and quality of life were secondary endpoints.

Power calculation

The sample size of the exposed group was estimated under the assumption of a longitudinal decline in mean serum T of 3 nmol/l (standard deviation 6.5nmol/l) during the first two years after rectal cancer treatment with two-sided confidence intervals of 0.95 (1-alpha) and a minimal power of 0.80 (1-beta). The power of this setting exceeded 0.80 with 40 pairs of observations. The proposed sample size was 100 considering that two thirds are given preoperative RT and one third is lost to follow up.

Study participants and setting

Men with rectal cancer stage I to III and scheduled for rectal resection were eligible if they were over age 18, fluent in Swedish and had given informed consent.

Exclusion criteria:	Planned local excision of rectal cancer
	History/ evidence of urogenital cancer
	History of pelvic radiation for other diseases
	Androgen deprivation therapy, T replacement, androgen abuse

Men treated with preoperative RT were assigned to the exposed group and men treated with surgery alone to the unexposed group.

Eighteen months after start of inclusion, less than 10% of the participants were treated with surgery alone . To increase the sample size of the unexposed group, men with prostate cancer stage I to III and scheduled for robot-assisted prostatectomy were deemed suitable to paricipate, if they met the study criteria regarding age, command of language and informed consent.

Exclusion criteria:
- History/ evidence of other pelvic malignancy
- History of pelvic radiation for other diseases
- Androgen deprivation therapy, T replacement, androgen abuse

The participants with rectal cancer were enrolled at a tertiary (Karolinska University Hospital) and a secondary (Ersta Hospital) referral centre in Stockholm between April 2010 and May 2014. At the Urology depart-ment of the Karolinska University Hospital in Stockholm participants with prostate cancer were included between May 2012 and January 2013.

All participants had a baseline visit before the start of any oncologic treatment to collect fasting morning venous blood samples, semen samples, PROMs and data concerning demographics, medical history and physical examination. Men under 55 years of age were offered semen cryopreservation. Men treated with preoperative RT for rectal cancer had an additional venous blood sample during the week before surgery. Data on surgery, adverse events and histopathology were retrieved from clinical records.
One and two years after surgery the participants had follow-up visits with identical data collection as at baseline and testicular stimulation test with human chorionic gonadotropin (HCG), a LH analogue. The study visits took place at the outpatient clinic of the Centre for Andrology and Sexual Medicine, Karolinska University Hospital. The participants could choose if they wanted to participate in hormone analysis, semen sample and/ or PROMs. Semen sampling and cryopreservation was not performed in men with prostate cancer.

Table 4. Study visits and assessments of the cohort study

Baseline	Prior to surgery*	12 months	24 months
Androgens	Androgens	Androgens	Androgens
		HCG test	HCG test
PROMs		PROMs	PROMs
Semen sample		Semen sample	Semen sample

PROM=patient reported outcome measures, HCG=human chorionic gonadotropin
* men with RT (exposed group),

Participants were offered to meet an andrologist after every visit to evaluate treatment of sexual dysfunction or symptoms of LOH. At any time of the study treatment of erectile dysfunction was possible. The T replacement in severe LOH was restricted to the period after the first follow-up visit 12 months postoperatively.

Approvals and registrations

We registered the cohort study "Sexual Function and Wellbeing in Males Diagnosed With Rectal Cancer" at ClinicalTrials.gov under the identification number NCT01216206. The Regional Ethical Review Board in Stockholm approved the study (2009/1860-31/2) and the amendments for inclusion at Ersta Hospital, Södersjukhuset, Danderyd Sjukhus AB, Department of Urology Karolinska University Hospital (2010/1768-32; 2011/2097-32) and for analysis of radiologic images performed during cancer treatment (2012/2173-32). The collection of five cone beam CT in 30 men treated with short course RT was also approved by amendment (2012/668-32) and by the Radiation Protection Authority (K1180-2012 (2012_27)).

Radiotherapy and chemotherapy

The pre-therapeutic MDT conference recommended the treatment modalities according to the guidelines of the European Society for Medical Oncology (ESMO) [82]. Preoperative RT was given as short course (5 x 5 Gy) or long course RT (25 x 2 Gy or 28 x 1.8 Gy). Long course RT with a prescribed dose of 50.4 Gy was combined with concomitant chemotherapy (capecitabine). Men with short course RT had usually surgery the following week. Participants included at the same time to the experimental arm of the "Rectal Cancer And Pre-operative Induction Therapy Followed by Dedicated Operation" (RAPIDO) trial had short course RT and six cycles preoperative chemotherapy (capecitabine and oxaliplatin) [85].
The oncologists delineated the gross tumour volume (GTV) and the clinical target volume (CTV) on the planning CT using the treatment planning system Eclipse (Varian, Palo Alto, CA, USA). The CTV covered, apart from the primary tumour and the mesorectum, lymph nodes outside the mesorectum at risk of containing cancer cells [82]. The pelvic floor was included to the CTV in men planned for extralevator or ischioanal APE. The planning target volume (PTV) was defined by addition of an isotropic margin around the CTV (6 mm for short course RT and 9 mm for long course RT) to account for set up errors. External beam RT was delivered to the undressed patient in supine position without specific gonadal protection using a four-field conformal technique with 15 MV or 18 MV photons (Varian). Two participants had Volumetric Modulated Arc Therapy (6 MV).
The expected gains of adjuvant chemotherapy were discussed at the postoperative MDT conference and administered on a selective basis.

Outcome measures

Venous blood samples

The blood samples were analysed at the Karolinska University Laboratory. Serum T, LH, FSH, SHBG and Inhibin B were measured in the serum. Bioavailable T was calculated under the assumption of constant albumin concentrations (43g/l) according to the method of Vermeulen et. al. [145]. The LH-T ratio was calculated by dividing LH with serum T. Low serum T was defined as a level of serum T below 8 nmol/l.
Serum T was measured by chemiluminescent immunoassay using the Access Immunoassay System (Beckman Coulter, Inc., Stockholm, Sweden). with a coefficient of variance (CV) < 10% in the range of 6.94 to 34.70 nmol/l. Serum LH and FSH were determined with an AutoDELFIA hLH/hFSH assay (PerkinElmer Life and Analytical Sciences, Turku, Finland), which is an immunoradiometric assay with CV < 2%. SHBG was assessed by immunoassay (Beckman Coulter) with CV 5%. Inhibin B, a marker of spermatogenesis, was measured with the OBI Inhibin B ELISA (DSL, Oxford, UK), which has a CV of 7% at 339 ng/l and 15% at 28 ng/l. Chromatography was used to determine HbA1c in the blood and the values were reported according to the International Federation of Clinical Chemistry in mmol/mol.

Semen analysis

We asked men that consented to semen analysis to avoid ejaculation 48h prior to the study visits. Semen samples were analysed following the World Health Organisation guidelines from 1999 at the Centre for Andrology and Sexual Medicine, Karolinska University Hospital.

Patient reported outcome measures

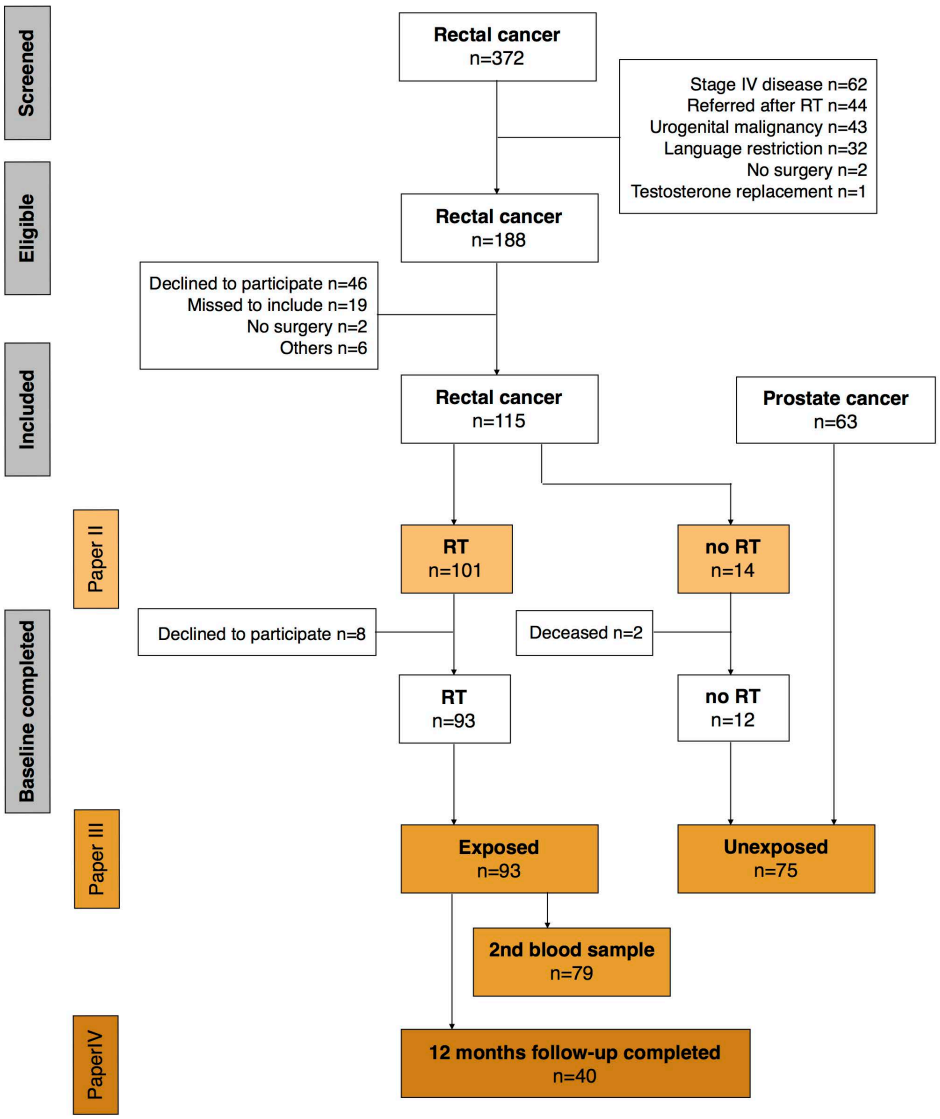
Sexual function, symptoms of androgen deficiency, quality of life and anxiety/ depression were assessed by questionnaires that the participants filled in during the study visits to ensure completeness.

Sexual function	International Index of Erectile Function (IIEF)[216]
	Sexual Complaints Screener for Men (SCS-M)
Hypogonadism	Aging Males' symptoms rating scale (AMS) [217]
Quality of life	European Organization for the Research and Treatment of Cancer (EORTC) Core Quality of Life Questionnaire (EORTC QLQ-C30) [218] and colorectal cancer module (EORTC QLQ-CR29) [219]
	Life Satisfaction checklist (Li-Sat-11) [220]
Anxiety/ Depression	Hospital Anxiety Depression Scale (HADS) [221]

Enrolment

The eligibility for this study was assessed in 372 men with rectal cancer and 115 men were included. The number of eligible men with prostate cancer was 298 and 63 men were included to the unexposed group of this study. The reasons for non-participation were language/ communication restrictions, residence outside the Stockholm County and declined participation.

Figure 10.
Flow chart



SPECIFIC METHODS PAPER II TO IV

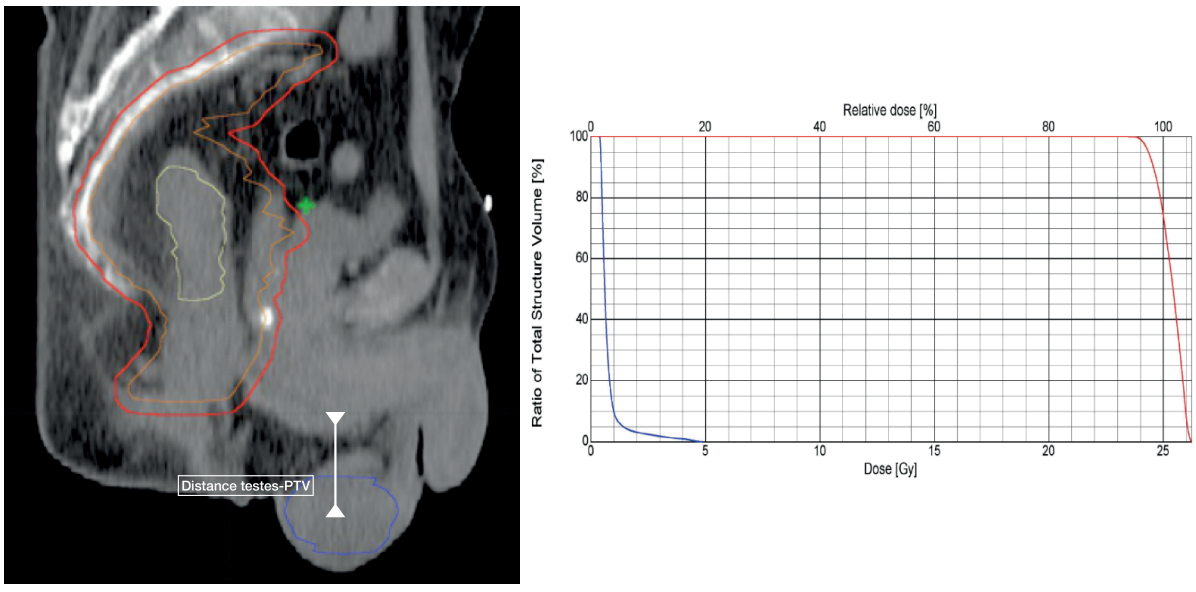
Paper II

The expected testicular radiation exposure during preoperative RT for rectal cancer was calculated for 101 participants with available planning CT. Men treated with short course RT between August 2012 and May 2014 had repeated cone beam CTs (CBCT) to assess longitudinal differences of TD during the course of RT. The CBCTs were acquired in treatment position after each fraction.

Assessment of planned testicular dose (cross-sectional analysis)

The testicular tissue was delineated on planning CT as one volume excluding other scrotal structures such as skin, soft tissue or epididymis to assess the planned TD. The Analytical Anisotropic Algorithm (AAA) of the Eclipse system was used to calculate the dose distribution of the testicular volume. The mean TD (mTD), the minimum TD and the maximum TD for the complete RT were reported based on the dose-volume histogram as physical doses. The shortest distance between the centre of the testicular volume and the lower end of PTV/ beam (distance testes-PTV) in the sagittal view was also registered. The beam model in Eclipse was not primarily optimized outside the geometrical beam. Thus all TDs were adjusted by the factor 1.15 to correct for this systematic underestimation.

Figure 11. Delineation of target volume and testes (left), respective cumulative dose-volume histogram (right)



Red line=Planning target volume (PTV)
Blue line=Testicular volume

Assessment of delivered testicular dose (longitudinal analysis)

The delivered TD for each RT fraction was calculated based on CBCTs with the treatment planning system as described above and multiplied by five (number of fractions) to compare the planned and delivered TDs. The image quality of CBCT was lower compared to planning CT and the volume of the body scanned with CBCT did not cover the complete PTV. To decrease systematic differences of TD calculation between planning CT and CBCT, the size of the testicular volume on CBCT was matched with the size on the respective planning CT. The loss of photon scattering from the "missing PTV" on CBCT was corrected with individual factors ranging from 1.1 to 1.6. These factors were estimated by comparing the effect of an equivalent "missing PTV" on the respective planning CT. The observed within-person variability of the delivered TD is due to differences in the position of the testes under the assumption of negligible systematic differences of TD calculation between planning CT and CBCT and small set up errors.

Statistical analysis

The distribution of mTD was skewed and the relationship with the distance testes-PTV was hyperbolic. The distribution of mTD was compared between groups of patients with non-parametric tests (Wilcoxon rank-sum and Kruskal-Wallis). We estimated quantile regression models for the 25^{th}, 50^{th} and 75^{th} percentile of the inverse mTD as a quadratic function of the distance testes-PTV, exploited the invariance property of the quantiles and estimated the quantiles for mTD by inverting the estimates from the above model [222].

Paper III

Preoperative RT for rectal cancer determined the exposure status in this cohort study. Men with rectal cancer treated with preoperative RT were assigned to the exposed group. The unexposed group consisted of men with rectal cancer planned to surgery alone or men with prostate cancer planned to robot-assisted prostatectomy. The primary endpoint was serum T (continuous variable) and low serum T (categorical variable). Secondary endpoints were bioavailable T, LH and the LH-T ratio. All participants had a visit at baseline and men treated with preoperative RT had a second blood sample the week prior to surgery. The androgen status relevant at the time of surgery was the baseline measurement for the unexposed group and the follow-up measurement for the exposed group. The elapsed time between the start of RT and the second blood sample was also registered.

Quantification of exposure

The planned TD calculated with the treatment planning system based on planning CT in Paper II was used to quantify the exposure. To account for the additional testicular radiation exposure due to CBCTs, 0.04 Gy was added to the individual mean, maximal and minimal TD for every CBCT. The dose-response relationship between TD and change in androgen levels was restricted to men treated with short course RT as a different effect for long course RT cannot be excluded.

Statistical analysis

Groups of patients were compared using t-tests or non-parametric tests as appropriate. Fisher's exact tests were used for count data. A general estimating equation (GEE) approach was chosen to analyse the longitudinal data and

to report population-averaged outcomes while allowing for correlation between repeated observations. We compared sexual hormones of all study participants at baseline with sexual hormones in men after preoperative RT for rectal cancer prior to surgery to assess the effect of RT on the androgen status at the time of surgery.

Paper IV

This was a longitudinal observational study in men exposed to preoperative RT from the described cohort study that had planning and one-year follow-up CT available by May 2014. The changes in serum and bioavailable T between baseline and the visit 12 months after surgery were the exposure and changes in body composition the outcome. Elapsed time denotes the time between the last day of RT and the venous blood sample 12 months after surgery.

Outcome measures

Data on body composition were retrieved from abdominal CT examinations on a dedicated workstation (Advantage Workstation 4.1, GE Medical Systems using AW4.1 Basic Display software). The CT section at the level of the inferior surface of the L3 vertebra was selected to delineate manually the area of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), psoas muscle (PM) and erector spinae muscle (EM). The skeletal muscle of the abdominal wall was not traced due to potential confounding effects of laparotomy and stoma formation such as denervation and incisional/ parastomal hernia on the longitudinal analysis. The psoas and erector spinae muscle were assessed separately and intramuscular adipose tissue (IMAT) was excluded. The average amount of disagreement, considering all assessed tissue areas (PM, EM and SAT), was 0.4% for the primary investigator and 0.3% for the second investigator.

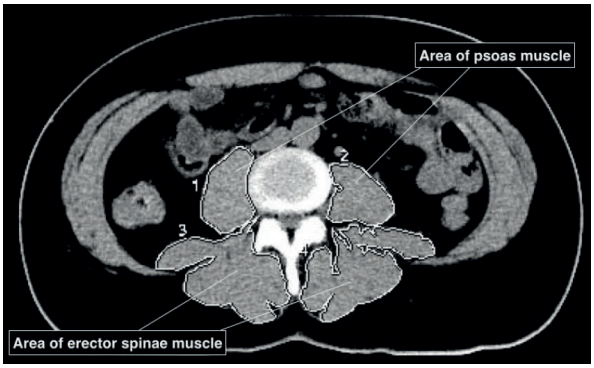


Figure 12. Delineation of skeletal muscle at L3

Statistical analysis

Summary statistics were compared between groups of patients using two-sided paired t-tests, Wilcoxon rank-sum, Kruskal-Wallis and Fisher's exact tests. For dependent count data the McNemar's test was used. The association of T levels and body composition during the first year of follow up was analysed with random-effects linear regression models. We considered weight, age and LH as potential confounders. Age and LH, however, changed the estimated coefficients associated with the exposure variable of interest by less than 10% and were excluded from the final models.

RESULTS PAPER II TO IV

Paper II

The testicular dose was analysed in 101 individuals. Seventy-six men were planned to short course RT and 25 men were planned to long course RT. The median planned mTD for short course RT was 0.57 Gy (range 0.06 to 14.37 Gy) and 0.81 Gy (range 0.36 to 10.80 Gy) for long course RT.

Cross-sectional analysis of planned testicular dose

Increasing distance testes-PTV or distance of the tumour from the anal verge as well as exclusion of the anal canal from PTV or surgery with preservation of the pelvic floor (i.e. low anterior resection, intersphincteric APE or Hartmann´s procedure) were factors resulting in decreased planned mTD in the stratified analysis. The distance between testes-PTV remained a significant predictor in the multivariate quantile regression analysis for the 25th and 75th percentile of planned mTD.

Table 5. Stratified analysis of the planned testicular dose

	Short course RT	p value	Long course RT	p value
Number of men	76		25	
mTD	0.57 (0.06 to 14.37)		0.81 (0.36 to 10.80)	
minTD	0.33 (0.0 to 7.73)		0.48 (0.21 to 1.73)	
maxTD	1.08 (0.18 to 25.91)		1.87 (0.56 to 42.74)	
mTD by distance testes-PTV				
0-1 cm	4.32 (2.21 to 14.37)		6.09 (5.40 to 10.80)	
1-5 cm	0.78 (0.33 to 2.35)		1.31 (0.99 to 2.97)	
>5 cm	0.27 (0.06 to 0.51)	<0.001†	0.58 (0.36 to 0.81)	<0.001†
mTD by tumour distance from anal verge				
0-5 cm	1.35 (0.18 to 14.37)		1.16 (0.43 to 10.80)	
6-10 cm	0.50 (0.15 to 14.19)		0.81 (0.47 to 6.09)	
11-15 cm	0.29 (0.06 to 1.92)	<0.001†	0.70 (0.36 to 1.67)	0.52†
mTD by relation of anal canal and PTV				
Anal canal included in PTV	0.91 (0.15 to 14.37)		1.33 (0.43 to 10.80)	
Anal canal not in PTV	0.34 (0.06 to 0.77)	<0.001††	0.69 (0.36 to 2.59)	0.08††
mTD by type of surgery				
Pelvic floor resected	1.34 (0.18 to 14.37)		1.37 (0.43 to 10.80)	
Pelvic floor preserved	0.37 (0.06 to 4.32)	<0.001††	0.70 (0.36 to 6.09)	0.10††

Doses reported in Gy as median (range), RT=preoperative radiotherapy, mTD=mean testicular dose, minTD=minimal testicular dose, maxTD=maximal testicular dose, PTV=planning target volume
† Kruskal-Wallis test, †† Wilcoxon ranksum test

Longitudinal analysis of planned and delivered testicular dose

The delivered mTD could be assessed on 127 CBCTs acquired from 32 men treated with short course RT. The median planned and averaged delivered mTD was similar (0.52 Gy vs. 0.51 Gy; p=0.84) and the graphical analysis showed no systematic difference between mTD assessed on planning CT and CBCT. The estimated median difference between planned and delivered mTD expected for a given planned mTD of 1 Gy was smaller than 0.5 Gy but this difference increased for higher planned mTDs.

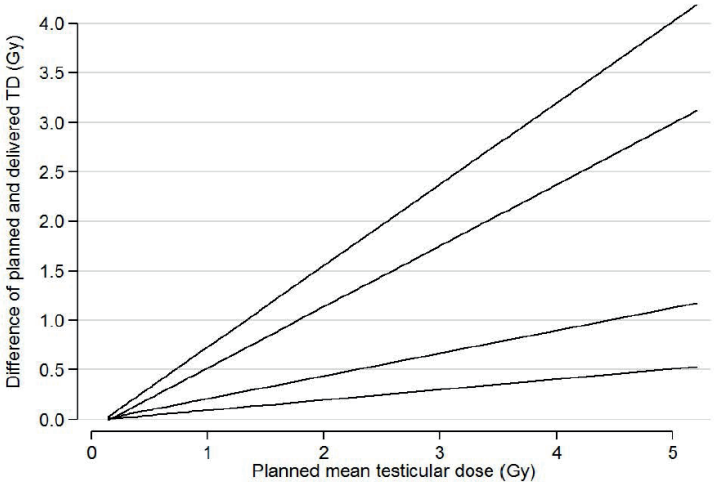


Figure 13. Estimated 25th, 50th, 75th and 90th percentile of absolute difference between planned and delivered mean testicular dose for a given planned mean testicular dose.
TD=testicular dose

The longitudinal assessment of mTD calculated on planning CT and repeated CBCT can be displayed as spaghetti plot. The longitudinal regression analysis confirmed the graphical impression that mTD did not change significantly over the time of planning and delivering of short course RT. The differences in the position of the testes during planning and delivering of RT, estimated by the distance testes-PTV, had a heterogeneous effect on the within-person variability of mTD. The distance testes-PTV had no significant effect on within-person variability of mTD in men with low mTD (25th percentile). In men with moderate and high mTD (50th and 75th percentile) the distance testes-PTV had a significant effect on within-person variability of mTD.

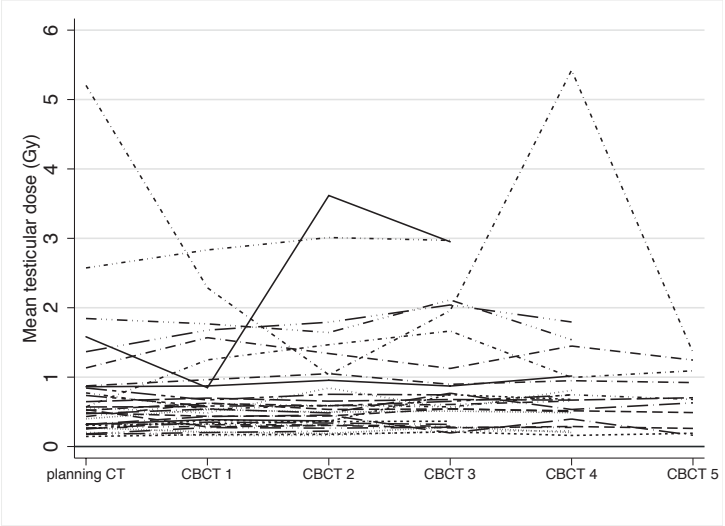


Figure 14. Spaghetti plot of planned and delivered mean testicular dose for 32 men treated with short course RT (25 Gy).

CBCT=cone beam computed tomography

Paper III

This cohort study consisted of 168 participants, 105 men with rectal cancer and 63 men with prostate cancer. The 93 men treated with preoperative RT and surgery for rectal cancer were assigned to the exposed group. The number of participants in the unexposed group was 75. Twelve of these men had rectal cancer and were treated with surgery alone. The remaining 63 men had robot-assisted prostatectomy for prostate cancer.
In the exposed group 68 men had short course RT and preoperative chemotherapy was given to 34 men. Mean age and BMI were higher and the proportion of American Society of Anesthesiologists (ASA) score equal to three was lower in the unexposed group.

Androgen status at baseline

The levels of serum T, bioavailable T and LH were similar at baseline and no significant differences in the LH-T ratio or proportion of low serum T were detected between the exposed and unexposed group. The type of cancer and the type of RT were not associated with androgen status at baseline. The regression analysis yielded the same finding.

Table 6. Sexual hormones at baseline according to exposure status

	No RT (unexposed group)	RT (exposed group)	P value t-test
Number of participants	75	93	
Serum T (nmol/l)	11.9 ± 4.0	11.3 ± 3.4	0.26
Bioavailable T (nmol/l)	5.3 ± 1.5	5.0 ± 1.6	0.15
LH (IU/l)	4.1 ± 2.3	4.5 ± 2.2	0.38
LH-T ratio (IU/nmol)	0.38 ± 0.31	0.43 ± 0.28	0.25
Proportion of serum T < 8 nmol/l (%)	15.1	14.3	0.53*

Hormone measures reported as mean ± standard deviation, RT=preoperative radiotherapy, T=testosterone, LH=luteinising hormone
* Fisher´s exact test

Acute effect of preoperative radiotherapy on androgen status

A second blood sample was collected in 79 of 93 men of the exposed group. Preoperative RT resulted in a significant decrease of serum T and increase of LH and LH-T ratio independent of the type of RT. The decrease in bioavailable T was significant in men treated with short course RT but not in men with long course RT.

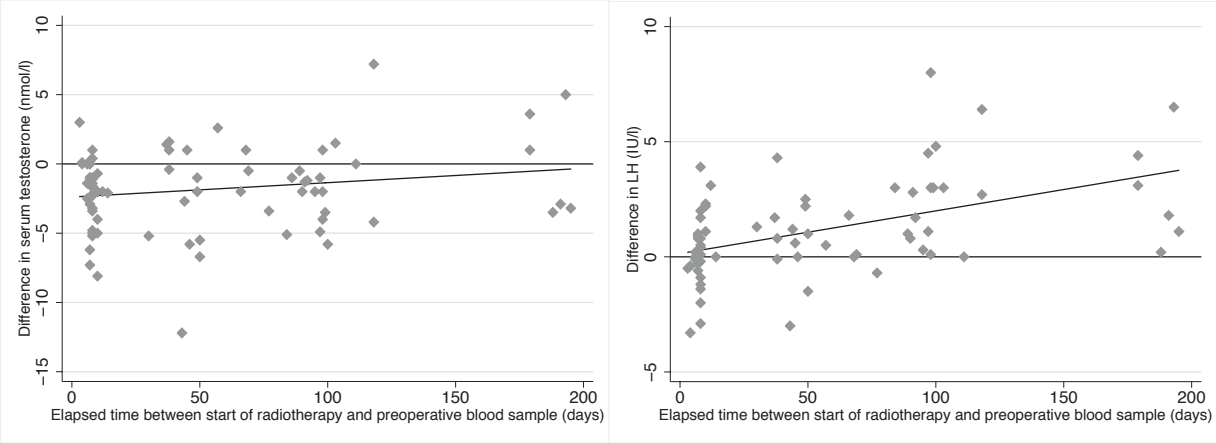
Table 7. Difference of sexual hormones in men treated with preoperative radiotherapy for rectal cancer

	Short course RT	p value	Long course RT	p value
Number of patients	59		20	
Mean testicular dose (Gy)	0.65 0.06 to 14.37		1.02 0.36 to 10.80	
Elapsed time between start of RT and preoperative blood sample (days)	10 3 to 195		96 57 to 118	
Difference in serum T (nmol/l)	- 2.0 ± 3.0	<0.001	-1.3 ± 3.0	0.03
Difference in bioavailable T (nmol/l)	-0.9 ± 1.4	<0.001	-0.5 ± 1.6	0.06
Difference in LH (IU/l)	0.8 ± 2.0	0.003	2.1 ± 2.2	<0.001
Difference in LH-T ratio (IU/nmol)	0.27 ± 0.43	<0.001	0.49 ± 0.89	<0.001

Mean testicular dose and elapsed time reported as median (range), hormone levels as mean ± standard deviation, p values of Wilcoxon signed-rank test, RT=preoperative radiotherapy, T=testosterone, LH=luteinising hormone

The elapsed time between the start of RT and the second blood sample was depended on the type of neoadjuvant treatment. A decline of the difference in serum T but an increase of the difference in LH was observed with increasing elapsed time.

Figure 15. Estimated effect of elapsed time on the difference of serum testosterone and LH in men with rectal cancer exposed to preoperative radiotherapy (reference line: difference=0)



The longitudinal regression analysis confirmed the results above with a significant decrease in serum and bioavailable T combined with a significant increase in LH and LH-T ratio in men treated with preoperative RT. Age, BMI, ASA score, preoperative chemotherapy and elapsed time had no confounding effect on the outcome estimates nor could an effect modification of these covariates be detected.

Risk of low serum testosterone at the time of surgery

The proportion of all study participants with low serum T at baseline was 14.6 per cent (n=164) and increased to 35.4% in men treated with preoperative RT for rectal cancer at the time of surgery (n=79). The absolute risk increase of 20.8% for low serum T resulted in a number needed to harm (NNH) of 4.8 for preoperative RT of rectal cancer. The relative risk of low serum T at the time of surgery was 2.41 (95% CI 1.57 to 3.71, $p<0.001$) for men treated with preoperative RT compared to all study participants at baseline. The regression analysis did not result in relevant confounding or effect modulation of age, BMI, ASA score, preopera-tive chemotherapy or elapsed time.

Dose-response relationship between testicular dose and serum testosterone

The preliminary results of the dose-response analysis restricted to men treated with short course RT resulted in a significant interaction between planned mTD and follow-up visit for the models with bioavailable T ($p=0.011$), LH ($p<0.001$) and LH-T ratio ($p=0.004$) and a borderline significant interaction for the model with serum T ($p=0.055$).

Paper IV

The longitudinal changes in androgen levels and body composition were analysed in 40 men treated with preoperative RT that had completed the study visit 12 months after surgery and the CT for one-year cancer follow-up was available by May 2014. The average age of these non-obese participants was 60 years and 30 of 40 men had short course RT. Almost half of the participants had an extralevator APE and 15 of 40 patients had additional tissue/ organs resected.

Longitudinal change in androgen levels and body composition

Half of the study population experienced a decrease in serum T resulting in a slight decrease of mean serum and bioavailable T and a significant increase in mean LH levels. The frequency of low serum T (< 8 nmol/l) and elevated LH (> 9 IU/l) increased significantly.
The mean SAT increased significantly during the first year after surgery for rectal cancer and the mean skeletal muscle area did not change.

Table 8. Endocrine assessment and body composition data for all study participants (n = 40) at baseline and 12 months after preoperative radiotherapy and surgery for rectal cancer

	Baseline	Follow-up	P value
Serum T (nmol/l)	11.5± 3.0	10.9 ± 3.1	0.16*
Bioavailable T (nmol/l)	5.05 ± 1.7	4.96 ± 1.23	0.09*
LH (IU/l)	4.3 ± 1.9	6.1 ± 3.1	0.0001*
Frequency of serum T <8 nmol/l (%)	7.5	15	<0.0001†
Frequency of LH >9 IU/l (%)	2.5	23	<0.0001†
SHBG (nmol/l)	43.0 ± 16.8	40.4 ± 12.4	0.41*
HbA1c (mmol/mol)	38.4 ± 3.8	37.4 ± 4.8	0.09*
BMI (kg/m²)	24.1 ± 3.2	24.4 ± 3.4	0.43*
SAT (mm²)	14713 ± 5940	16062 ± 5831	0.004*
VAT (mm²)‡	16002 ± 10428	15853 ± 8913	0.86*
Psoas mucle area (mm²)	2221 ± 568	2292 ± 525	0.06*
Erector spinae muscle area (mm²)	5145 ± 1211	5048 ± 1121	0.26*

Values are reported as mean ± standard deviation. T=testosterone, LH=luteinising hormone, SHBG=sex hormone binding globulin, HbA1c=glycated haemoglobin, BMI=body mass index, SAT=subcutaneous adipose tissue area, VAT=visceral adipose tissue area
* t-test, † McNemar's test, ‡ n=27

Association between androgen levels and body composition

The regression analysis adjusted for weight and elapsed time between the last day of RT and the second venous blood sample (Model 2) showed no association between the level of serum T and the area of SAT or skeletal muscle (PM and EM). The level of bioavailable T was related to the area of the assessed skeletal muscle groups (PM and EM) at any time of the study. The area of SAT was not related to bioavailable T.

Table 9. Regression coefficients of serum and bioavailable testosterone from the random-effects linear regression models with area of skeletal muscle and subcutaneous adipose tissue as outcome

	Psoas muscle (mm²)	Erector spinae muscle (mm²)	SAT (mm²)
Regression coefficients for serum testosterone as predictor			
Model 1*	2,0 (-21,6 to 25,7)	8,0 (-46,5 to 62,5)	-343,6 (-608,8 to -78,4)
P value	0,866	0,774	0,011
Model 2†	11,4 (-12,7 to 35,5)	40,2 (-16,6 to 97,0)	-140,2 (-358,1 to 77,8)
P value	0,355	0,166	0,207
Regression coefficients for bioavailable testosterone as predictor			
Model 1*	49,8 (-1,7 to 101,3)	118,5 (-1,4 to 238,5)	-323,5 (-907,9 to 260,9)
P value	0,058	0,053	0,278
Model 2†	64,3 (14,4-114,2)	185,9 (67,9-304,0)	8,7 (-442,4 to 459,8)
P value	0,011	0,002	0,970

Regression coefficients (95% confidence interval), SAT=subcutaneous adipose tissue area
* Adjusted for elapsed time, † Adjusted for elapsed time and weight

The cross-sectional area of psoas muscle as an androgen-related sign

At the visit 12 months after surgery 24 of 40 participants had serum T levels in the grey zone of hypogonadism (serum T between 8 to 12 nmol/l). The area of psoas muscle increased in 17 and decreased in 7 of 24 potentially hypogonad men. The mean LH and mean LH-T ratio were significantly higher in men with loss of psoas muscle. Differences in age, BMI, co-morbidity, SHBG and Hba1c were not significant.

Table 10. Men treated with preoperative radiotherapy for rectal cancer in the grey zone of hypogonadism (serum T 8-12 mmol/l) 12 months after surgery stratified on the change in psoas mucle area

	Increase of psoas muscle area (n=17)	Decrease of psoas muscle area (n=7)	P value
	Mean ± SD		
Age (years)	60,9 ± 11,6	64,1 ± 11,5	0,45*
BMI (kg/m²)	25,2 ± 3,6	23,9 ± 4,3	0,39*
Charlson comorbidity index	0,41 ± 0,51	0,14 ± 0,38	0,21*
Bioavailable T (nmol/l)	4,3 ± 0,8	4,7 ± 0,6	n.a.
LH (IU/l)	4,7 ± 2,1	7,7 ± 3,2	0,02*
LH- T ratio	0,472 ± 0,221	0,743 ± 0,253	0,01*
SHBG (nmol/l)	42,2 ± 9,2	36,9 ± 9,3	0,19*
HbA1c (mmol/mol)	37,3 ± 4,4	39,3 ± 5,6	0,40*
	Median (range)		
Tumour distance from anal verge (cm)	5 (1 to 13)	4 (1 to 12)	0,50*
	Count data		
Pathological tumour stage			
Stage I	4	3	0,13‡
Stage II	6	4	
Stage III	7	0	
Radiotherapy regimen			
5 x 5 Gy	10	7	0,07‡
28 X 1,8 Gy	7	0	
Type of operation			
Low anterior resection	9	3	1,00‡
Abdominoperineal excision	8	4	
Postoperative chemotherapy	7	1	0,35‡
Adverse events			
Clavien ≥ III	3	2	1,00‡
Distant metastasis	2	0	

T=testosterone, SD=standard deviation, BMI=body mass index, LH=luteinising hormone, SHBG=sex hormone binding globulin, HbA1c=glycated haemoglobin

* Wilcoxon rank-sum test, ‡ Fisher's exact test

DISCUSSION AND CONCLUSION PAPER II TO IV

Discussion Paper II to IV

Interpretation Paper II to IV

The average TD calculated with the treatment planning system was 4.7% of the prescribed dose in contemporary RT for rectal cancer, which is low compared to 3% to 17% in the review (Paper I). In a recent publication with similar proportion of low rectal cancer and identical method of dose calculation, the TD was 7.7% of the prescribed dose despite radiation in prone position on double-hole belly board [223]. The spatial proximity of the testes to the PTV was, as expected by the inverse square law, the strongest predictor of the TD and had a hyperbolic relation. This leads to considerable values of TD in men with short distances testes-PTV of concern regarding spermatogenesis and Leydig cell function as discussed in the review article [224]. The effect of the other three investigated predictors of TD (i.e. distance of the tumour from the anal verge, relation of anal canal to PTV and type of surgery regarding pelvic floor resection) was weaker. The distance testes-PTV and the distance of the tumour from the anal verge can be used to identify men with low TD as men with a distance testes-PTV larger than 5 cm or with rectal cancer in the upper third of the rectum (11 to 15 cm from the anal verge) have a planned mTD below 2.0 Gy. The relation of the anal canal to PTV and the type of surgery seem not to be appropriate to identify individuals with planned mTD below 2.0 Gy.

The longitudinal analysis based on TD calculation by planning CT and repeated CBCT showed that the planned TD was similar to the delivered TD. The absolute differences between planned and delivered TD increased with increasing planned TD. The observed differences between planned and delivered TD (within-person variability) are essentially due to variation in position of the testes during RT. This effect is not important in men with low mTD (25th percentile) and the accuracy of the planned TD as an estimate of the delivered TD is within 0.5 Gy for TDs up to 1 Gy. The variation in position of the testes has a significant impact on within-person variability in individuals with median to high TD. This might be a target group with particular profit of gonadal protection [200, 225].

The results of the cohort study (Paper III) indicate that preoperative RT has an acute negative effect on androgen levels with a relative risk of 2.4 to have low serum T at the time of surgery. One out of five men treated with preoperative RT is expected to drop below the threshold of biochemical hypogonadism (8 nmol/l) and the simultaneous increase in LH and LH-T ratio indicate a primary testicular failure. So we can confirm the findings of smaller case series regarding the acute effect of RT on Leydig cell function [197 199 202]. Studies with a follow-up of two to four years report similar values for the risk of biochemical hypogonadism, which indicates that the negative impact of RT could be permanent in selected individuals [201 205]. The decline in serum T was evident in our study already during the first week after RT with a trend of improvement over time. The LH levels increased steadily after RT. So it is possible that a proportion of men with serum T above 8 nmol/l in fact have compensated hypogonadism characterized by elevated gonadotropins and borderline T. The testicular vulnerability seems not to depend on age, BMI, physical status according ASA and preoperative chemotherapy. These risk factors of hypogonadism had no impact on the acute adverse effect of RT. The preliminary results of the dose-response analysis indicate that the extent of the acute adverse effect of RT on Leydig cell function is related to the TD, which sustains the causal relationship. The clinical consequences of the observed preoperative androgen decline on perioperative adverse events or oncological outcomes are unknown. Low perioperative T may increase morbidity after abdominal surgery and androgens are important for maintenance of skeletal muscle mass and function [158 189].

The skeletal muscle mass, assessed by the cross-sectional area of the psoas and erector spinae muscle, is androgen-dependent and related to the non-SHBG-bound, bioavailable T. The androgen analysis in this subgroup of participants exposed to RT confirms the adverse effect of RT on Leydig cell function. The primary testicular failure is evident 12 months after surgery from a significant increase in LH combined with a doubled frequency of low serum T. The increment in LH suggests a compensatory attempt to increase T levels and indicates a compromised testicular function as described for compensated LOH. The negative effect of RT on testicular function persists and implies an elevated long-term risk for development of hypogonadism with important consequences on cardiovascular mortality, all-cause mortality and health-related quality of life [156] [157]. The cross-sectional area of the psoas and erector spinae muscle, available from routinely performed imaging during rectal cancer treatment, can also be used as androgen–dependent sign to assist in clinical diagnosis of hypogonadism. This is important in men after pelvic surgery as typical signs of hypogonadism, decreased frequency of morning erections and erectile dysfunction, are not reliable due to high frequency of impaired erection based on autonomic nerve injury.
Subcutaneous adipose tissue was not related to androgens in our study after adjustment for weight . The amount of adipose tissue seems to be related to the joint action of T and oestrogen [148]. The detected minor change of mean serum T has a small impact on circulating oestrogen, as oestrogen levels mainly depend on the amount of adipose tissue and not on a lack of substrate for conversion to oestrogen. The longitudinal analysis of VAT seems not to be reliable in small groups of patients treated for rectal cancer as position of diaphragm and bowel content at the moment of CT acquisition affect the area of VAT. The amount of resected/ displaced mesocolon and greater omentum during abdominal surgery may also interfere with VAT assessment.
The value of detecting sarcopenia, a risk factor for perioperative adverse events and impaired oncological outcome, is debated and seems to be of further interest [177] [226] [227]. It could be of value to investigate sarcopenia and LOH simultaneously as preoperative RT for rectal cancer can result in primary testicular failure with detectable loss of skeletal muscle.

Validity of the results Paper II to IV

The subgroup analysed with repeated CBCTs was recruited consecutively without further restrictions beside prescribed dose of 25 Gy. We have reduced measurement bias due to systematic differences between dose calculation on planning CT and CBCT. The calculated mean doses may be compared in volumes that have different dose distributions for each fraction under the assumption of identical radiation sensitivity throughout the testicular volume. So the testicular mean dose is a relevant dose measure to relate to clinical effects of testicular irradiation. The risk of cremaster reflex induction and undetected dislocation of the dosimeter is absent [198]. For these reasons the treatment planning system is preferred over scrotal dosimetry for testicular dose calculation. The effect of different fractionation schemes on testicular function is unknown, so we presented the results separated for short and long course RT or adjusted in the regression analysis. The quantile regression respects the skewness of the distribution of TD and weight or BMI had no confounding effect as suggested by Budgell et al. [203]. The results for long course RT and individuals with high TD may be affected by the restricted sample size.

The assignment to preoperative RT occurred during the pre-therapeutic MDT conference without knowledge of androgen or participation status of the patient based mainly on morphologic criteria of rectal cancer disease. The baseline androgen levels were similar between the exposed and unexposed group indicating low probability for selection bias. The clinical importance of statistically significant differences in age, BMI and ASA score was small and these factors were not identified as confounders or effect modifiers in later data analysis. The rationale to include men with prostate cancer planned to robot-assisted prostatectomy in the unexposed group was the following: Both cancer types are adenocarcinoma of the pelvis and can be treated with surgery alone. Robot-assisted prostatec-tomy is suitable in men with local disease and low risk of nodal involvement, thus not planned to RT or androgen deprivation, which are exclusion criteria for the study. The risk of developing prostate cancer is not associated with serum levels of androgens and prostatectomy seems not to have an impact on serum T [228, 229].
The androgen levels between men with prostate and rectal cancer were similar in our study. The reason for missed blood sample after RT was of logistic nature and not related to androgen levels. The increased ASA score in the exposed men missed to follow-up could lead to an underestimation of the association

as hypogonadism is related to co-morbidity [153]. The risk for misclassification of exposure was absent and the quantification of exposure by calculation of the TD with the treatment planning system based on planning CT results in accurate estimates of the delivered TD (Paper II) Misclassification of outcome related to the variation of T level within and between the participants must be assumed. But the misclassification of outcome is non-differential leading to a potential underestimation of the relative risk for low T at the time of surgery. The longitudinal regression analysis with GEE produces valid estimates of population-averaged outcomes unrelated to the correlation structure of the responses and permitted to use all available observations. The results are unadjusted, as relevant confounding or effect modulation by age, BMI, ASA score, SHBG, preoperative chemotherapy or elapsed time was not present.

The association between androgen levels and specific skeletal muscle groups is explored in a subgroup but the risk to find a different physiological interaction between psoas muscle and androgens in an alternative study population is small. The observation of psoas muscle increase under T replacement in hypogonad men would strengthen our findings of androgen-dependency of the investigated muscle groups. The reproducibility of the tissue area measurements with an amount of disagreement below 0.5% was very high. This precision allows for an assessment of the changes in body composition probably superior to common measurements such as lean mass or anthropometric measures. The within- and between-person variation of serum T levels could be a reason for the absent association in this small group of participants. The analysis is adjusted for the confounding effect of weight but physical activity was not assessed specifically. The differences in physical ability, assessed by the physical functioning score of the EORTC QLQ-C30 questionnaire, seem not to explain the observed changes in area of the assessed skeletal muscle groups in this study.

The internal validity of the studies presented in Paper II and IV may be restricted due to the non-comparative design and the small sample size with a certain risk for type II errors. But important conclusions of these publications are verified in the comparative study presented in Paper III with robust internal validity. The external validity might be restricted by the fact that the frequency of rectal cancer treatment with surgery alone (11%) or anterior resection (63%) was rather low. This indicates that the proportion of advanced and low rectal cancer in the study participants is higher compared to the general population with rectal cancer, which is not surprising, as the participants were enrolled at referral centres.

Conclusion Paper II to IV

Paper II

Testicular dose calculation on planning CT results in an accurate estimation of the delivered TD and the distance testes-PTV is the most important predictor of the TD. This permits to identify individuals with low TD if the TD is not calculated from the planning CT. The within-person variability of the delivered TD is related to differences in the position of the testes in men with moderate to high TD and should be considered during positioning of the patient.

Paper III

The exposure of the testes during RT results in an acute decline of androgen levels due to primary testicular failure, which may be dose dependent. One of five men treated with RT is expected to develop low serum T but the impact on Leydig cell function implies even a risk of compensated hypogonadism in a larger proportion of patients.

Paper IV

The negative effect of preoperative RT on testicular function persists 12 months after surgery. The cross-sectional areas of the psoas and erector spinae muscle are androgen dependent and can be used as clinical sign of hypogonadism.

FUTURE PERSPECTIVES

We will verify the results of Paper IV in the whole cohort. The relation between radiation-induced changes of androgen levels and skeletal muscle mass might be of importance for acute and late outcomes of rectal cancer treatment. A simplified method of muscle measurement on cross-sectional images might enhance the clinical application of this potentially underestimated information present already today.

Further follow-up of the cohort study will provide longitudinal data on androgens, sexual function and spermatogenesis one and two years after surgery for rectal cancer. The late adverse effects of RT on testicular function and the consequences on quality of life will be assessed. The dose-response relationship between the testicular dose and the outcomes mentioned above will also be further explored.

These findings will form the basis of a randomized trial testing the hypothesis whether early intervention can reduce the impact of rectal cancer treatment on sexual function. PDE5-inhibitors and testosterone are interesting substances in the context of penile rehabilitation and treatment of testicular failure. Their effects on erectile function in men treated for rectal cancer are not well studied and may even have an impact on penile fibrosis, sexual desire or body composition.

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