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Effect of preoperative radiotherapy for rectal cancer on spermatogenesis

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Introduction

Dose-dependent endocrine testicular failure with acute decline in serum testosterone, followed by a regulatory increase in luteinizing hormone, has been reported in men treated with preoperative radiotherapy (RT) for rectal cancer[1]. In light of the increasing incidence of rectal cancer in young adults and trends towards expanding use of RT to enable organ preservation, the effect of RT on spermatogenesis has gained interest[2–6].

Spermatogenesis refers to the development of mature spermatozoa from male germ cells[7]. This process takes approximately 70 days and is regulated by the hypothalamic–pituitary–gonadal axis[7]. Follicle-stimulating hormone (FSH) stimulates testicular Sertoli cells to induce spermatogenesis and release inhibin B, with a negative feedback signal on FSH secretion[7]. Analysis of semen, containing spermatozoa and secretions from accessory sex glands, enables assessment of spermatogenesis and ejaculatory tract function[8].

Raised FSH levels have been reported after RT for rectal cancer, indicating Sertoli cell damage, but effects on inhibin B and spermatogenesis have not been described[9–15]. The aim of this study was to explore the effects of RT for rectal cancer on spermatogenesis.

Methods

The association between testicular radiation dose and change in total number of spermatozoa (TNS), semen volume, FSH, and inhibin B was investigated based on data from a longitudinal prospective cohort study of 115 men who had surgery for stage I–III rectal cancer. Results for testicular dose and endocrine testicular function have been published previously[1,16]. Semen and blood samples were collected before oncological treatment (baseline), and 1 and 2 years after surgery. Men who underwent RT had an additional blood sample taken the day before surgery. Men aged less than 55 years had the option to preserve semen according to national healthcare regulations.

Testicular doses (TDs) were calculated from planning CT images and reported as mean cumulative TD[1,16]. Relative TD was calculated based on the assumption that RT regimens for rectal cancer are bioequivalent, and referred to as proportion of the prescribed dose absorbed by the testes[1]. Semen samples were collected at the Department of Reproductive Medicine of Karolinska University Hospital after 72 h of sexual abstinence and analysed according to the WHO 2010 standard[8]. The primary outcome, TNS (millions of spermatozoa per ejaculate), was calculated by multiplying spermatozoa concentration (millions of spermatozoa per millilitre semen) by semen volume (millilitres of semen per ejaculate)[8]. Serum levels of FSH and inhibin B were analysed using commercial assays and enzyme-linked immunosorbent assay respectively, with a coefficient of variation of 7 per cent between 19 and 260 ng/l for inhibin B[1].

Owing to the limited number of semen samples, the hormonal response was compared between participants with (group A) and those without (group B) a semen sample to explore the dose–response relationship between TD and changes in FSH and inhibin B in all participants.

Statistical analysis

Groups were compared by Wilcoxon rank-sum and signed-rank tests with a two-sided 0.05 level of statistical significance. Longitudinal regression models based on generalized estimating equations were used to estimate mean population effects of TD on changes in TNS, semen volume, FSH, and inhibin B. Longitudinal profiles of inhibin B and FSH were compared between groups A and B by testing time–group interactions. TD was categorized by quantiles to explore the dose–response relationship.

Results

Of 104 participants included at baseline, 21 had at least one semen sample (group A; 20 of 21 had RT) and 83 had no semen samples (group B; 72 of 83 had RT). Follow-up was not completed for three participants in group A and 42 in group B (*Table 1*). Median age was lower in group A (52 *versus* 63 years; $P < 0.001$). Groups were comparable regarding BMI, ASA grade, tumour distance from anal verge, clinical TNM stage, type of surgery, and

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relative TD (2.6 *versus* 1.8 per cent; $P = 0.305$). Median time between RT and surgery was shorter in group A (4 *versus* 43 days; $P = 0.003$) as more participants received short-course RT (25 Gy) (16 of 21 *versus* 41 of 83).

Semen samples and hormonal response

TNS decreased from 164.0 million per ejaculate at baseline to 1.8 million ($P = 0.012$) 1 year after surgery, and increased to 32.6 million by 2 years ($P = 0.021$) (*Table 1*). A significant decline in semen volume was observed after 1 year, which persisted at 2 years after surgery. The frequency of oligospermia (TNS below 39 million per ejaculate) among those with semen samples at all three visits increased from 2 of 8 at baseline to 7 of 8 and 5 of 8 respectively 1 and 2 years after surgery. The hormonal response was similar in both groups, with a significant increase in FSH 1 year after surgery and a decrease by 2 years, without reaching baseline levels. In compensation, the inhibin B level had declined significantly by 1 year, with signs of recovery after 2 years, without reaching baseline levels.

Longitudinal regression analysis

Longitudinal regression analysis confirmed the mean change in TNS and semen volume described above. The change in TNS was associated with TD (–14.6 (95 per cent c.i. –26.1 to –3.0) million/Gy; $P = 0.013$) and relative TD (–4.0 (–6.9 to 1.0) million/per cent; $P = 0.008$). This was not the case for semen volume (*Table 2*). In univariable models, age, BMI, ASA grade, type of surgery/RT regimen, and use of chemotherapy were not related to change in TNS or semen volume.

Models for FSH and inhibin B showed comparable baseline levels and changes over time between groups A and B. Age, BMI, ASA grade, type of surgery/RT regimen, and use of chemotherapy were not statistically associated with the hormonal response after adjustment for time between RT and surgery, and did not change point estimates by more than 10 per cent. The dose–response relationship between TD and FSH or inhibin B was therefore assessed using models including all participants and categorized TD/relative TD values (*Table 2*). The second to fourth quartiles of TD, and the fourth quartile of relative TD were associated with the change in FSH. The third and fourth quartiles of TD and relative TD were associated with the inhibin B profile.

Discussion

These findings indicate that multimodal treatment for rectal cancer results in impaired spermatogenesis, and ejaculatory tract and Sertoli cell function. The study design does not allow separate direct analysis of the effects of RT and surgery on spermatogenesis and the ejaculatory tract. However, the data show an association between TD and changes in TNS, FSH, and inhibin B suggestive of Sertoli cell dysfunction. The hormonal changes, verified in the entire cohort, were present after RT and before surgery, and showed a dose–response relationship with TD, which reinforces the hypothesis that RT has a negative impact on spermatogenesis. Within 24 months after treatment, signs of partial recovery of TNS and hormone levels were observed, but on average they did not reach baseline levels and the proportion of men with oligospermia was higher than at baseline. The negative impact of rectal cancer treatment on ejaculatory tract function, measured by semen volume, did not recover within 24 months or relate to TD.

Parts of the ejaculatory tract are exposed to the entire prescribed dose owing to its location in the target volume, which in combination with injuries to the vas deferens, seminal vesicles, prostate, and superior hypogastric nerves during surgery may explain the absence of recovery within 24 months and relation to TD. Age, BMI, ASA grade, and type of surgery/RT regimen had no important effect on semen measurements, and did not alter the hormonal response. Data on the gonadotoxicity of chemotherapeutic agents mainly used in this study (capecitabine, 5-fluorouracil, oxaliplatin, leucovorin, irinotecan) are limited, but have not shown a very pronounced effect[17,18]. Chemotherapy was not a significant predictor of post-treatment changes in TNS, semen volume, FSH, and inhibin B.

RT threatens fertility in men treated for rectal cancer, and cryopreservation of semen before the start of RT is recommended

Table 1 Profile of semen samples and hormonal response

	At least 1 semen sample (group A)			No semen sample (group B)			
	n	Median (range)	P†	*n*	Median (range)	P†	P‡
TNS (millions per ejaculate)							
Baseline	19 of 21	164.0 (5.9–1140.0)					
1 year after surgery	9 of 18	1.8 (0–216.0)	0.012				
2 years after surgery	12 of 18	32.6 (0–187.0)	0.021				
Semen volume (ml)							
Baseline	19 of 21	3.3 (1.5–5.7)					
1 year after surgery	9 of 18	1.2 (0.1–4.5)	0.017				
2 years after surgery	12 of 18	1.1 (0.6–3.1)	0.003				
FSH (units/l)							
Baseline	20 of 21	4.1 (1.8–12.0)		81 of 83	5.2 (1.2–33.0)		0.060
After RT, before surgery*	18 of 21	5.9 (2.6–15.0)	0.227	58 of 77	10.0 (2.0–30.0)	< 0.001	0.004
1 year after surgery	18 of 18	11.0 (0.1–24.0)	0.001	50 of 50	12.0 (2.7–40.0)	< 0.001	0.448
2 years after surgery	18 of 18	5.4 (0.5–14.0)	0.218	41 of 41	6.6 (0.1–41.0)	0.014	0.526
Inhibin B (ng/l)							
Baseline	16 of 21	185.0 (66.0–280.0)		70 of 83	149.0 (5.0–340.0)		0.262
After RT, before surgery*	15 of 21	114.0 (6.3–214.0)	0.002	45 of 77	43.0 (4.0–198.0)	< 0.001	0.021
1 year after surgery	17 of 18	58.0 (10.0–159.0)	0.002	48 of 50	42.0 (4.9–262.0)	< 0.001	0.497
2 years after surgery	18 of 18	132.5 (14.0–294.0)	0.013	41 of 41	100.0 (10.0–285.0)	< 0.001	0.229

* One participant in group A and 11 in group B did not have preoperative radiotherapy (RT) and did not provide blood samples at this visit. *n*, Number of samples analysed as a proportion of number of participants included at each study visit; TNS, total number of spermatozoa; FSH, follicle-stimulating hormone.
† Longitudinal comparison *versus* baseline values (Wilcoxon signed-rank test); ‡ Between-group comparison (Wilcoxon rank-sum test).

Table 2 Longitudinal regression analysis of total number of sperm, semen volume, follicle-stimulating hormone, and inhibin B in relation to testicular dose

	Coefficient	P
Estimated mean change in TNS (millions per ejaculate) (group A, *n* = 21)		
Baseline	Reference	
After RT, before surgery	n.a.	
1 year after surgery	−198.8 (−303.3, −94.4)	< 0.001
2 years after surgery	−169.1 (−277.6, −60.6)	0.002
Relative TD (%)	−4.0 (−6.9, −1.0)	0.008
Estimated mean change in semen volume (ml) (group A, *n* = 21)		
Baseline	Reference	
After RT, before surgery	n.a.	
1 year after surgery	−1.79 (−2.7, −0.9)	< 0.001
2 years after surgery	−2.00 (−2.7, −1.3)	< 0.001
Relative TD (%)	0.02 (−0.0, 0.1)	0.412
Estimated mean change in FSH (units/l) (groups A + B, *n* = 104)*		
Baseline	Reference	
After RT, before surgery	2.80 (0.8, 4.8)	0.006
1 year after surgery	7.22 (5.4, 9.1)	< 0.001
2 years after surgery	1.29 (−0.4, 3.0)	0.133
Relative TD (%)		
Q1: 0.00–1.18	Reference	
Q2: 1.21–2.07	0.54 (−1.6, 2.7)	0.621
Q3: 2.12–3.89	1.73 (−0.9, 4.4)	0.201
Q4: 4.00–57.48	5.88 (2.3, 9.4)	0.001
Estimated mean change in inhibin B (ng/l) (groups A + B, *n* = 104)*		
Baseline	Reference	
After RT, before surgery	−65.5 (−89.9, −41.0)	< 0.001
1 year after surgery	−88.7 (−109.7, −67.7)	< 0.001
2 years after surgery	−27.3 (−50.4, −4.2)	0.020
Relative TD (%)		
Q1: 0.00–1.18	Reference	
Q2: 1.21–2.07	−19.2 (−43.2, 4.7)	0.116
Q3: 2.12–3.89	−37.1 (−65.0, −9.1)	0.009
Q4: 4.00–57.48	−53.5 (−77.1, −29.9)	< 0.001

Values in parentheses are 95 per cent confidence intervals. Relative mean testicular dose (TD) was calculated as TD/prescribed dose × 100. TNS, total number of spermatozoa; RT, preoperative radiotherapy; n.a., not available; FSH, follicle-stimulating hormone; Q, quantile. * Model adjusted for time between preoperative radiotherapy (RT) and surgery.

in patients who wish to have children. The observed recovery of spermatogenesis may become an issue regarding anticonception for cancer survivors.

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