Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study

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Summary

Background Treatment of childhood cancer can result in impaired spermatogenesis. Intracytoplasmic sperm injection (ICSI), however, can enable men to achieve fatherhood, and has focused attention on gamete integrity in men with oligozoospermia. Our aim was to assess testicular function in survivors of childhood cancer.

Methods We assessed testicular function in 33 survivors of childhood cancer and 66 age-matched controls. The median age at diagnosis and at the start of the trial was 10·0 years (range 2·2–16·9) and 21·9 years (16·5–35·2), respectively. We assessed pubertal staging, measured plasma sex steroid hormone concentrations, and analysed semen quality, including spermatozoal DNA integrity.

Findings Ten (30%) individuals were azoospermic and six (18%) oligozoospermic (sperm concentration $<20\times10^6$/mL). Sperm concentration was significantly lower in the non-azoospermic group than in controls (median $37{\cdot}1\times10^6$/mL, IQR $19{\cdot}7\times10^6$ to $89{\cdot}9\times10^6$, *vs* $90{\cdot}7\times10^6$/mL, $50{\cdot}5\times10^6$ to $121{\cdot}5\times10^6$; p=0·002). In the non-azoospermic cancer survivor group, inhibin B concentrations were lower than in controls (mean 153·3 ng/L, SEM 17·8, *vs* 223·7 ng/L, 8·8; p<0·001), and FSH concentrations were higher (6·6 U/L, 0·9, *vs* 3·2 U/L, 0·2; p<0·001). Only 11 (33%) survivors of childhood cancer had normal semen quality. There was no significant difference in sperm DNA integrity between the non-azoospermic and control groups (9%, 5–13, *vs* 11%, 7–16; p=0·06).

Interpretation Sperm concentration is reduced after treatment for cancer. However, the sperm produced seems to carry as much healthy DNA as those produced by the healthy population, suggesting that assisted conception can be considered as a treatment option for these men.

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Introduction

70% of individuals survive childhood cancer, and this proportion continues to increase. As such, attention is being focused on the lasting morbidity associated with radiation and chemotherapy treatment.[1] A frequent and psychologically traumatic late complication of cancer treatment is infertility. Cytotoxic chemotherapy drugs, especially alkylating agents, can produce long-lasting or permanent damage to the germinal epithelium, resulting in oligozoospermia or azoospermia.[2–8] The germinal epithelium is also sensitive to radiotherapy, and doses as low as 1·2 Gy can result in permanent sterility.[9] Recovery from surviving germ cells can happen but is unpredictable and often takes a long time.[7,8] Leydig cells, with their slower rate of turnover, are more resistant to gonadotoxic therapy, resulting in preservation of androgen production even when patients are infertile.[5]

Advances in techniques of assisted reproduction, especially intracytoplasmic sperm injection (ICSI), have enabled some men with oligozoospermia to become fathers.[10,11] Concerns have been raised, however, about the safety of ICSI,[12] since whether or not spermatozoa from men with impaired spermatogenesis carry abnormal genetic information is unkown.[13–15] Data on the health of offspring born after ICSI are broadly reassuring,[16] though there are no data on the health of children born to fathers whose deficit in semen quality is a specific consequence of potentially mutagenic treatment.[17] Results of studies in animals have shown that exposure of the male germ line to chemotherapy agents can disrupt spermatozoal DNA and result in deleterious effects on embryo development.[18] Awareness of the importance of the integrity of sperm DNA for accurate transmission of genetic material to the offspring has necessitated the development of new techniques to assess sperm characteristics in more detail.[19]

Our aim was to investigate testicular function and semen quality in survivors of childhood cancer.

Methods

Patients

We searched the oncology database at the Royal Hospital for Sick Children, Edinburgh, for all male survivors of childhood cancer aged older than 16 years, and identified 51 individuals between December, 1999, and June, 2001. We invited 45 of these men to participate in the study, and excluded six because they no longer lived in the area (five) or were on antidepressant medication (one). Six of the 45 men declined and six did not reply to the invitation. The 18 men who did not participate in our study were comparable for age, diagnoses, age at diagnosis, treatment regimens, and disease-free survival. 33 men participated in the study. For each study participant, we recruited two age-matched controls (n=66). The volunteers were recruited by means of advertisement in local media and through hospital out-patient clinics, and selected on the basis of the absence of any clinical evidence, on history or physical examination, of reproductive health problems. The Lothian Paediatric and Reproductive Medicine research ethics subcommittee approved the study, and all patients provided written informed consent.

Study protocol

We assessed pubertal maturation according to the Tanner criteria, and measured testicular volume with the Prader orchidometer.[20] We noted the mean volume of the two testes as the individual's testicular volume. We identified concentrations of luteinising hormone (LH), follicle stimulating hormone (FSH), and testosterone in venous blood samples (20 mL) with an automated immunoassay analyser (Bayer Immuno 1, Bayer, Newbury, Berkshire, UK), and measured inhibin B concentrations in serum from venous blood samples that had been centrifuged at 2000 *g* for 10 min and stored at –20°C.[21] Semen samples were collected in a room adjacent to the laboratory, by masturbation into sterile, wide-mouthed non-toxic containers, after an abstinence period of at least 48 h. The samples were analysed for ejaculate volume, sperm concentration, motility, and normal morphology, according to WHO protocols.[22] Throughout the study, the laboratory was subject to external quality control.

We measured the extent of DNA fragmentation in spermatozoa with the terminal deoxyribonucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labelling (TUNEL) technique, according to the general methodology of Sun and colleagues,[19] modified as follows. We obtained an unselected population of cells by mixing an aliquot of each sperm sample with Biggers-Whitten-Whittingham medium[23] containing 20 nmol N-(2-hydroxyethyl) piperazine-N′-(2-ethan sulfonic acid) (Invitrogen, Paisley, UK) and 0·3% human serum albumin solution, and centrifuged at 500 *g* for 5 min. After decanting the supernatant, we resuspended the pellet of spermatozoa in 2 mL of phosphate buffered saline (Sigma-Aldrich, Gillingham, UK) and centrifuged at 500 *g* for 5 min. This step was repeated and the spermatozoa were subsequently fixed in 1% formaldehyde (Sigma-Aldrich) in phosphate buffered saline for 60 min at room temperature. The fixed sperm concentration was adjusted to 20×10^6 cells/mL with a Neubauer haemocytometer. We centrifuged the sample at 500 *g* for 5 min and washed it in phosphate buffer saline. The fixed sperm were resuspended in 100 μL prewash buffer, containing single strength One-Phor-All buffer (Amersham Pharmacia Biotech, Buckinghamshire, UK) and 0·1% Triton X-100 (Sigma-Aldrich) for 15 min at room temperature. We centrifuged out the sperm from the buffer at 500 *g* for 30 min and resuspended in 50 μL of TdT buffer, containing 3 μmol biotin-16-dUTP (Roche Diagnostics, Lewes, UK), 6 μmol dATP (Amersham Pharmacia Biotech), and 1 IU/μL of TdT enzyme (Amersham Pharmacia Biotech), and incubated at 37°C for 1 h. After washing in phosphate buffer saline, we resuspended the fixed permeabilised sperm in 100 μL of staining buffer, consisting of 0·1% Triton X-100 (in distilled water) and 1% streptavidin/fluorescin conjugate (Calbiochem-Novabiochem, Nottingham, UK), and incubated the sample in the dark at 4°C for 30 min. The stained cells were spun at 500 *g* for 5 min and resuspended in 500 μL phosphate buffer saline to give a concentration of about 1×10^6 cells/mL. For negative controls, we omitted the enzyme terminal transferase from the reaction mixture. For positive controls, we treated the samples with 0·8 IU/μL DNase I (Roche Diagnostics) for 15 min at room temperature before incubation with the TdT buffer.

Patient	Diagnosis	Chlorambucil total dose (g/m²)	Procarbazine total dose (g/m²)	Vinblastine total dose (g/m²)	Ifosfamide total dose (g/m²)	Cyclophosphamide total dose (g/m²)	Cytarabine total dose (g/m²)	Radiotherapy: area applied/ total dose (Gy)	Sperm concentration ($\times10^6$/mL)
1	ALL	..	..	..	..	..	2·76 (2·00)	Cranium/total body irradiation 24/14·4	0
2	ALL and testis relapse	..	..	..	..	..	2·93 (2·00)	Cranium/testis 18/24	0
3	HD	0·90 (0·67)	16·80 (11·20)	134·00 (96·00)	..	..	..	None	0
4	HD	0·50 (0·50)	8·40 (8·40)	79·20 (72·00)	..	..	..	None	0
5	HD	0·50 (0·50)	8·40 (8·40)	72·00 (72·00)	..	..	..	Mediastinum 30	0
6	HD	0·50 (0·50)	10·00 (8·40)	86·40 (72·00)	..	..	..	Upper mantle 30	0
7	HD	0·34 (0·50)	6·30 (8·40)	54·00 (72·00)	..	..	..	Medias/neck 35	0
8	B cell NHL	..	..	..	..	..	2·20 (2·00)	Cranium/total body irradiation 6/14·4	0
9	Ewing's sarcoma	..	..	..	139·20 (87·00)	..	..	Fibula/lung 45/12·5	0
10	Ewing's sarcoma	..	..	..	165·60 (84·00)	..	..	None	0
11	Teratocarcinoma (maxilla)	..	..	..	..	..	..	Maxilla 60	0·55
12	HD	0·80 (0·67)	14·00 (11·20)	112·00 (96·00)	..	..	..	None	4·55
13	Relapsed ALL	..	..	..	..	5·67 (4·20)	2·70 (2·00)	Cranium 24	5·75
14	Ewing's sarcoma	..	..	..	132·60 (102·00)	4·30 (3·30)	..	Radius 55	12·45
15	ALL	..	..	..	..	..	1·20 (1·00)	None	14·75
16	ALL	..	..	..	..	..	4·80 (3·80)	Cranium 18	19·10
17	ALL	..	..	..	..	..	..	Cranium 15	20·29
18	Pineal germinoma	..	..	..	..	..	..	Cranium 35	20·30
19	ALL	..	..	..	..	..	–(>3) NI	Cranium 24	21·00
20	B cell NHL	..	..	..	..	..	2·28 (2·00)	None	24·65
21	Osteosarcoma	..	..	..	..	..	..	None	33·75
22	Wilms' tumour	..	..	..	..	..	..	None	37·10
23	ALL	..	..	..	..	..	NI	Cranium 24	42·00
24	Ewing's sarcoma	..	..	..	138·60 (126·00)	..	..	None	53·00
25	ALL	..	..	..	..	0·78 (1·20) NI	1·30 (2·00) NI	Cranium 24	66·25
26	ALL	..	..	..	..	0·45 (0·60) NI	NI	Cranium 24	77·00
27	ALL	..	..	..	..	..	..	Cranium 18	85·00
28	Medulloblastoma	..	..	..	..	..	..	Cranium/spine 55/35	94·83
29	ALL	..	..	..	..	..	..	Cranium 21	103·50
30	ALL	..	..	..	..	..	1·86 (2·00)	Cranium 18	113·25
31	Ewing's sarcoma	..	..	..	144·00 (106·00)	..	..	None	125·25
32	ALL	..	..	..	..	..	..	Cranium 18	145·00
33	ALL	..	..	..	..	..	..	Cranium 18	230·00

ALL=acute lymphoblastic leukaemia. HD=Hodgkin's disease. NHL=non-Hodgkin lymphoma.

Table 1: **Diagnosis and exposure to potentially gonadotoxic treatment by sperm count of all study patients**

	Long-term survivors of childhood cancer		
	Azoospermic (n=10)	Non-azoospermic (n=23)	Controls (n=66)
Characteristics			
Age (median, range) (years)	19·5 (16·5–25·3)	22·4 (17·6–35·2)	20·8 (18·0–36·3)
Smoking habit			
Yes	4 (40%)	5 (22%)	25 (38%)
No	6 (60%)	18 (78%)	41 (62%)
Alcohol consumption (units/week) (%)			
0	1 (10%)	6 (26%)	5 (8%)
⩽10	5 (50%)	3 (13%)	6 (9%)
11–20	1 (10%)	11 (48%)	29 (44%)
⩾21	3 (30%)	3 (13%)	26 (39%)
Pubertal staging			
Tanner	5	5	5
Testicular volume (median, IQR) (mL)	10 (7·4–12)*	17·5 (15–21·25)	20 (15–23)

Data are number (%) unless otherwise indicated. *p<0·001 for azoospermic group compared with other two groups.

Table 2: **Testicular function in long-term survivors of childhood cancer and in controls**

We analysed the samples with an Epics XL flow cytometer (Beckman Coulter Corporation, Buckinghamshire, UK) with a 15 mW argon ion laser operating at 488 nm. We measured green fluorescence with the FL1 detector at 525 nm. The flow rate during analysis was controlled at 200 events/s, and we analysed 10 000 events in each sample. Light-scatter and fluorescence data were obtained at a fixed gain setting in the logarithmic mode. Debris were gated out on the basis of forward scatter versus side scatter dot plot, by drawing a region enclosing the cell population of interest, and 10 000 events were collected. We processed the data with an IBM compatible computer installed with System II, version 1.0 (Beckman Coulter Corporation). We identified the proportion of labelled sperm in each sample.

Seven of the ten men identified on semen analysis as being azoospermic had a testicular biopsy done under general anaesthetic, to exclude a diagnosis of obstructive azoospermia. We fixed the specimens in Bouins and stained them with haemotoxylin and eosin. The presence of spermatogonial stem cells was assessed.

Statistical analysis

We did statistical analysis with SPSS (version 10.0). Analysis of variance was done on the endocrine data (non-parametric) and ejaculate volume (Kruskal-Wallis test), the data common to all three groups. We did pair-wise tests to study differences between the groups, if such differences existed. We did *t* tests and Mann-Whitney *U* tests to compare the differences between patient groups and controls with respect to endocrine data and semen data, respectively. For comparisons that involved all three patient groups, three pair-wise two-sample tests were done and Bonferroni corrections applied to the p values. A p value less than 0·05 was judged significant.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The 33 men had a median age of 21·9 years (range 16·5–35·2). Their median age at diagnosis of cancer was 10·0 years (2·2–16·9), and they had a median disease-free survival time of 11·6 years (0·3–24·4). The underlying malignancies included acute lymphoblastic leukaemia (15), Hodgkin's disease (six), Ewing's sarcoma (five), non-Hodgkin lymphoma (two), brain tumours (two), Wilms' tumour (one), osteosarcoma (one), and teratocarcinoma (one). Table 1 shows the patients' diagnoses and details of the potentially gonadotoxic chemotherapy and radiotherapy received. Table 2 shows characteristics of patients and controls.

Of the 33 patients, ten were azoospermic. Five of these individuals had received treatment for Hodgkin's disease with the alkylating agents chlorambucil, procarbazine, and vinblastine (table 1), all of which are gonadotoxic. Two of the azoospermic patients had been treated with ifosfamide for Ewing's sarcoma, two had received total body irradiation, and one had received direct testicular irradiation (table 1). Of the ten azoospermic patients, seven were prepubertal at diagnosis, providing cogent evidence that the prepubertal testis is not afforded protection from cytotoxic insult.

Six (18%) patients were oligozoospermic (sperm concentration $<20\times10^6$/mL), with severe oligozoospermia ($<2\times10^6$/mL) in one individual. In controls, however, oligozoospermia was seen in only three (5%) individuals. Only one of the six men treated for Hodgkin's disease with an alkylating agent-based regimen showed preservation of spermatogenesis (sperm concentration $4{\cdot}55\times10^6$/mL). Three of the oligozoospermic patients had been treated with Medical Research Council Protocols, UKALL II, III, and X, which consisted of combination chemotherapy, including, vincristine, prednisolone, 6-mercaptopurine methotrexate, cytarabine, and cyclophosphamide. Oligozoospermia was seen in one of the five patients treated for Ewing's sarcoma, for whom treatment included ifosfamide and cyclophosphamide. The remaining oligozoospermic patient (sperm concentration $0{\cdot}55\times10^6$/mL) did not receive treatment with agents expected to be gonadotoxic, and the reason for impaired spermatogenesis remains unknown.

Sperm concentration in the non-azoospermic group of individuals treated for cancer was significantly lower than the sperm concentration of controls (table 3, figure 1). Nine (29%) of the patients were asthenozoospermic

	Long-term survivors of childhood cancer			95% CI azoospermic *vs* controls	95% CI non-azoospermic *vs* azoospermic	95% CI non-azoospermic *vs* controls
	Azoospermic (n=10)	Non-azoospermic (n=23)	Controls (n=66)*			
Semen characteristics						
Abstinence (h)	62 (40·5–137·8)	60·4 (36–82·5)	73·5 (59·3–91·5)	..	..	..
Ejaculate volume (mL)	1·9 (1·5–2·3)	2·5 (2·1–3·5)	3·4 (2·5–5·1)	–2·7 to –0·7†	–1·5 to 0·2	–1·7 to –0·3†
Sperm concentration ($\times10^6$/mL)	0	37·1 (19·7–89·9)	90·7 (50·5–121·5)	..	..	–67 to –15†
Progressive motility (%)	..	56·3 (44·4–64·7)	61·9 (55·5–69·1)	..	..	–15·1 to –0·6‡
Normal morphology (%)	..	6·5 (3·7–7·6)	9·3 (6·3–11·0)	..	..	–4·6 to –1·3†
TUNEL damage (%)	..	8·8 (5·1–12·6)	11·4 (7·2–16·3)	..	..	–5·3 to 0·2

Data are mean (IQR). *n=64 for TUNEL assay. †= p<0·01. ‡=p<0·05.

Table 3: **Semen quality in long-term survivors of childhood cancer and in controls**

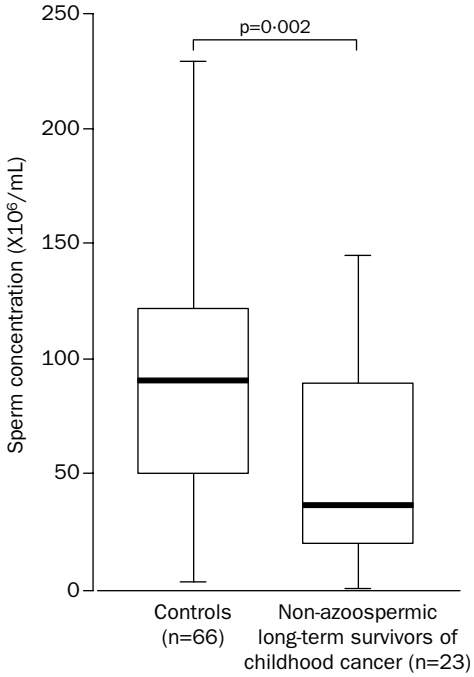

Figure 1: **Sperm concentration in non-azoospermic long-term survivors of childhood cancer and in controls**
Median, IQR, and maximum and minimum values are shown.

(progressive motility <50%) compared with ten (15%) controls. Median progressive motility for the non-azoospermic group was significantly less than for the control group (table 3). The proportion of sperm with normal morphology was also significantly less in the non-azoospermic group than in controls (table 3). From our population of 33 male survivors of childhood cancer only 11 (33%) men had a normal semen analysis as defined by

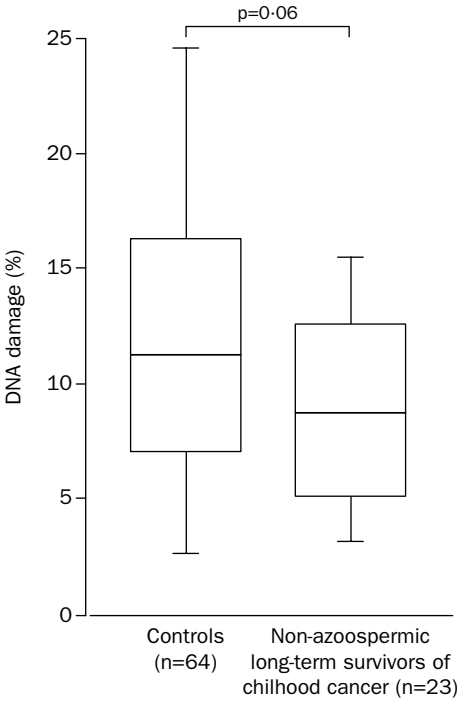

Figure 2: **Sperm DNA integrity in long-term survivors of childhood cancer and in controls**
Median, IQR, and maximum and minimum values are shown.

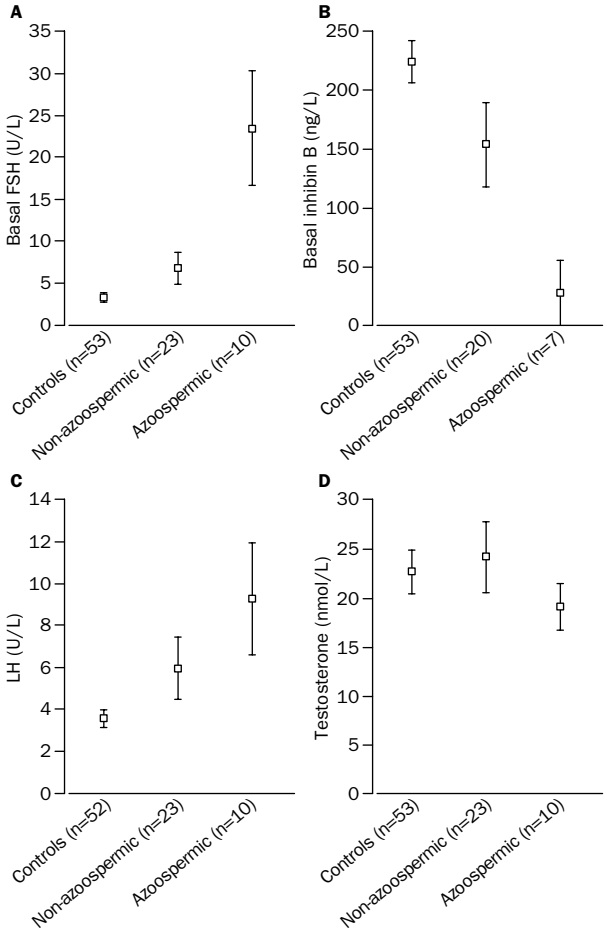

Figure 3: **Comparison of values for follicle stimulating hormone (FSH) (A), inhibin B (B), luteinising hormone (LH) (C), and testosterone (D) in the control group, and in non-azoospermic and azoospermic cancer survivors**
Mean and SEM are shown. Reference ranges for FSH and LH are 1·5–9·0 U/L and for testosterone 10–30 nmol/L. There is no established reference range for inhibin B.

WHO compared with 55 (83%) in the control group. There was no correlation between either age at diagnosis or time out from treatment and sperm concentration in this group of cancer survivors who were non-azoospermic (p=0·109 and p=0·516, respectively). There was a difference in ejaculate volume between the three groups (p<0·001). Ejaculate volume was significantly reduced in the azoospermic and non-azoospermic groups compared with controls (p=0·002 and p=0·006, respectively).

We assessed the integrity of the spermatozoal DNA with the TUNEL assay and the results are shown in table 3. There was no significant difference between DNA fragmentation in the non-azoospermic group and controls (table 3, figure 2).

Testicular biopsies from seven of the ten men with azoospermia on semen analysis showed a Sertoli-cell only picture in all instances, thus excluding obstructive azoospermia.

Analysis of variance for the endocrine measurements, FSH, LH, testosterone, and inhibin B, showed a significant difference for the FSH, LH, and inhibin B values for the three groups (p<0·001), but no significant difference was seen between the groups for testosterone concentrations. Pair-wise tests were subsequently done to assess the differences between the groups, with Bonferroni corrections applied.

Basal serum concentrations of FSH were significantly higher in the azoospermic group than in the non-azoospermic (mean difference –16·6, 95% CI –22·0 to –11·3, p<0·001) and control (–20, –23·1 to –17·1, p<0·001; figure 3A) groups. The concentrations of FSH were also significantly greater in the non-azoospermic group than in the controls (–3·4, –4·8 to –2·1, p<0·001; figure 3A). Inhibin B concentration was barely detectable in the azoospermic group, and was significantly lower in that group than in both the non-azoospermic (129·3, 61·2 to 190·5, p<0·001) and control groups (198·9, 146·6 to 245·9, p<0·001; figure 3B). Inhibin B concentrations were also significantly lower in the non-azoospermic group than in the control group (69·6, 34·6 to 106·3, p<0·001; figure 3B). Serum concentrations of LH were significantly greater in the non-azoospermic and azoospermic groups than in the controls (–2·4, –3·5 to –1·2, and –5·6, –7·1 to –4·2, respectively, p<0·001 in both instances, figure 3C). Testosterone concentrations were within normal limits in all three groups, with no significant differences between the groups (figure 3D).

Discussion

Our results indicate that treatment of childhood cancer is associated with a high risk of impaired spermatogenesis in adulthood. Moreover, in men in whom spermatogenesis continues after treatment, production is generally compromised, with reductions arising in ejaculate volume, sperm concentration, sperm motility, and in the proportion of morphologically normal sperm. Reassuringly, however, the integrity of the genomic DNA carried by the gametes of men treated for cancer seems unaffected. Our findings suggest that the reduction in sperm number is directly attributable to the cytotoxic effects of cancer therapy.

Fertility and sexual function are the principal life-style concerns in more than 80% of men successfully treated for cancer,[24] yet a substantial proportion of survivors invited refused to take part in our study. Cancer survivors are less likely to have children for several reasons other than treatment-induced infertility, including an inability to form relationships, fear of relapse of their disease, and the prospect of leaving a child parentless.[24]

Cytotoxic treatment for childhood cancer should minimise unwanted side-effects without compromising survival. Where there is equal efficacy between regimens, the effect of drugs on reproductive function should be considered when devising the most appropriate therapy.[25] Our data confirm the sterilising effects of treatment for Hodgkin's disease with a standard regimen, ChlVPP (chlorambucil, vinblastine, procarbazine and prednisolone).[7,8] The ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) combination, which does not contain alkylating agents or procarbazine, is much less gonadotoxic, resulting in temporary azoospermia in 33% of patients and oligozoospermia in 21%, with full recovery after 18 months reported in all patients.[26] Consequently, with the introduction of hybrid regimens, three cycles of ABVD with three cycles of ChlVPP or MOPP (mechlorethamine, vincristine, procarbazine, and prednisolone), gonadotoxicity could be greatly reduced.

Three of the sterile men in our study had been treated with either fractionated total body irradiation or testicular irradiation. Although recovery has been reported after several years, the high doses administered in these treatments make recovery unlikely.[9] Furthermore, as a prerequisite of recovery, some stem cells must survive, and in these three men, as indeed in all seven men biopsied, stem cells were absent.

Ifosfamide, an analogue of cyclophosphamide, is potentially gonadotoxic. Five of our patients had received treatment that included ifosfamide for Ewing's sarcoma. Of these, two had a normal semen analysis, one was oligozoospermic, and the remaining two patients were azoospermic. The three patients with sperm present in their ejaculate had been treated more than 11 years previously, compared with the azoospermic patients who had completed treatment 4 and 7 months previously. The two sterile patients were sexually mature at the time of diagnosis and had provided semen samples for cryopreservation before commencing cancer therapy, with sperm concentrations of 124×10^6/mL and 128×10^6/mL. This finding suggests that recovery of testicular function with increasing time since treatment is a possibility. Continued, perhaps yearly, reassessment of semen analysis is warranted in azoospermic patients.

Normal semen quality, as defined by WHO, stipulates that sperm concentration must be greater than 20×10^6/mL and progressive motility greater than 50%.[22] According to these criteria, only a third of the survivors of childhood cancer had a normal semen analysis, compared with most of the control group. Our results are similar to those reported by Lopez Andreu and co-workers.[27]

Oligozoospermia was observed in six (18%) of the long-term survivor patients, compared with three (5%) in the control group. One of the patients, with severe oligozoospermia, had been treated with alkylating agents for Hodgkin's disease. Three of the oligozoospermic patients received treatment with standard protocols for acute lymphoblastic leukaemia, which included cytarabine and in one patient cyclophosphamide. The doses of cytarabine and cyclophosphamide received by these three patients were significantly less than previously reported to be gonadotoxic doses[6] and could indicate individual susceptibility. Lendon and colleagues[28] studied the testicular histology of 44 boys treated for acute lymphoblastic leukaemia and noted a severely depressed tubular fertility index, <40%, in 18 individuals, indicative of germinal epithelial damage. In a follow-up study by Wallace and colleagues,[4] semen analysis in seven of these patients with severe depression of tubular fertility index, who had been off treatment for a median of 10·8 years (range 5·5–15·9), reported azoospermia in four of the patients and full recovery of spermatogenesis in three (sperm concentration $>20\times10^6$/mL)—ie, previous chemotherapy treatment with cyclophosphamide and cytarabine impaired gonadal function, which improved with increasing time after treatment in some patients.

Inhibin B mediates non-steroidal negative feedback from the testes, reflecting the number of spermatozoa produced and regulating FSH secretion.[29,30] Inhibin B secretion in the adult requires the presence of germ cells.[29] Inhibin B concentrations were barely detectable in the azoospermic patients, in whom the germ cells were destroyed, despite preservation of Sertoli cells, as confirmed on testicular biopsy. This finding provides further evidence for the essential role of the germ cell-Sertoli cell interaction for the production of inhibin B, and confirms the value of inhibin B as a non-invasive marker of spermatogenesis after cytotoxic therapy.

The sperm concentration in the non-azoospermic group was significantly less than that of the general population. Although azoospermia after gonadotoxic chemotherapy has been widely investigated there are few data about sperm concentrations in those patients in whom spermatogenesis is preserved. Rautonen and co-workers[31] report median sperm concentrations of 67×10^6 mL (range $0{\cdot}01\times10^6$ to 425×10^6) in 27 patients treated for various

childhood haematological and solid cancers. Sperm concentrations of $20{\cdot}4\times10^6$ mL ($3{\cdot}2\times10^6$ to 43×10^6) were reported in a small study cohort (four) treated for acute lymphoblastic leukaemia (one) and non-Hodgkin lymphoma (three), and 31×10^6 mL ($4{\cdot}5\times10^6$ to 100×10^6) in eight long-term survivors of acute lymphoblastic leukaemia, indicating a general trend towards a lower sperm concentration.[32,33] In view of the fact that intact spermatogenesis requires the presence of stem cells, which are capable of self-renewal in addition to differentiation, the discrepancy in sperm concentrations between the non-azoospermic and control groups is difficult to reconcile. The non-azoospermic group clearly retains a population of intact stem cells capable of undergoing normal spermatogenesis, yet the surviving stem cells do not seem to repopulate the pool to produce sperm concentrations comparable with the general population. This deficit could reflect a more complex picture, emphasising the important role of Sertoli cells in supporting a finite number of stem cells. If there has been subtle damage to the germinal epithelium, involving loss of Sertoli cells, remaining Sertoli cells might already be functioning to full capacity. This possibility is suggested by the subnormal inhibin B concentrations and corresponding rise of serum FSH concentrations in the non-azoospermic group compared with the control population. This finding is analogous to data showing a decline in plasma inhibin B concentrations, directly in proportion to Sertoli cell numbers, after unilateral orchidectomy in rhesus monkeys.[34] In a physiological setting, where the negative feedback control system that regulates the testes is operational, Sertoli cell number is the primary determinant of circulating inhibin B concentrations.[34]

Ejaculate volume was reduced in the non-azoospermic and azoospermic groups compared with the control group, a finding which is unlikely to be attributable to retrograde ejaculation, obstruction, impaired autonomic innervation, or incomplete specimen collection. Testosterone concentrations were normal in both groups of patients, making testosterone deficiency an unlikely cause of decreased ejaculate volume. Damage to the prostate is unlikely, since only three of the patients had radiotherapy treatment involving the pelvis. Whether chemotherapy plays a part is uncertain. In a study[31] of 55 long-term survivors treated with chemotherapy or radiotherapy for various childhood cancers, 18 (33%) were reported to have a low ejaculate volume.

The mutagenic potential of cancer therapy might confer a risk to the fetus conceived with gametes produced after cancer therapy, although current epidemiological data suggest that offspring of cancer survivors do not have an increased incidence of congenital abnormalities or cancer relative to the general population.[35] However, an important concern is that these results are largely based on offspring arising from natural conception, and the consequences of circumventing the natural selection processes of fertilisation involved by means of ICSI, are unknown.[15] There is at least the hypothetical possibility of injection of abnormal spermatozoa carrying abnormal genomic DNA with the potential to increase congenital and other abnormalities among offspring.[19] Techniques to assess spermatozoal DNA integrity have been developed,[14,19] and it has become clear that men from subfertility clinic populations, with abnormalities of the conventional criteria of semen quality, also have increased amounts of damage to the genomic DNA in their gametes. Even among normal populations, sperm chromatin damage has been linked with impaired fecundity.[36] Spermatozoal DNA damage does not preclude pronucleus formation at ICSI,[13] and abnormal DNA within the male gamete is detectable in the early embryo.[37] Concern thus arises in the case of childhood cancer survivors, given that the capacity of ionising radiation and some chemicals to induce transmissible genetic damage in the germ cells of laboratory mammals has been clearly shown.[38] Thus far, evidence on the safety of ICSI has been largely based on its use in populations of men with deficits in spermatogenesis unrelated to potentially mutagenic cancer treatment. This evidence has been broadly reassuring with respect to health risks to the offspring,[16,39] although it is limited by restricted length of follow-up available. We have shown that although the conventional criteria of semen quality are frequently abnormal in long-term survivors of childhood cancer, the sperm produced do not seem to carry a greater burden of damaged DNA. This observation goes some way to providing reassurance about the use of ICSI, which will circumvent the problems associated with severe oligozoospermia and asthenozoospermia, and offer cancer survivors the possibility of paternity in adulthood.

Contributors

A B Thomson participated in study design, patient recruitment, semen and TUNEL analysis, analysis of results, and writing and editing of the report. A J Campbell participated in recruitment of controls, semen and TUNEL analysis in controls, and editing of the report. D S Irvine, C J H Kelnar, and R A Anderson participated in the study design, analysis of results, and editing of the report. W H B Wallace participated in the study design, patient recruitment, analysis of results, and writing and editing of the report. W H B Wallace, C J H Kelnar, and D S Irvine obtained funding.

Conflict of interest statement

None declared.

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