Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study

Daniel M Green, Wei Liu, William H Kutteh, Raymond W Ke, Kyla C Shelton, Charles A Sklar, Wassim Chemaitilly, Ching-Hon Pui, James L Klosky, Sheri L Spunt, Monika L Metzger, DeoKumar Srivastava, Kirsten K Ness, Leslie L Robison, Melissa M Hudson

Summary

Background **Few data define the dose-specific relation between alkylating agent exposure and semen variables in adult survivors of childhood cancer. We undertook this study to test the hypothesis that increased exposure to alkylating agents would be associated with decreased sperm concentration in a cohort of adult male survivors of childhood cancer who were not exposed to radiation therapy for their childhood cancer.**

Methods **We did semen analysis on 214 adult male survivors of childhood cancer (median age 7·7 years [range 0·01–20·3] at diagnosis, 29·0 years [18·4–56·1] at assessment, and a median of 21·0 years [10·5–41·6] since diagnosis) who had received alkylating agent chemotherapy but no radiation therapy. Alkylating agent exposure was estimated using the cyclophosphamide equivalent dose (CED). Odds ratios (ORs) and 95% CIs for oligospermia (sperm concentration >0 and <15 million per mL) and azoospermia were calculated with logistic regression modelling.**

Findings **Azoospermia was noted in 53 (25%) of 214 participants, oligospermia in 59 (28%), and normospermia (sperm concentration ≥15 million per mL) in 102 (48%) participants. 31 (89%) of 35 participants who received CED less than 4000 mg/m² were normospermic. CED was negatively correlated with sperm concentration (correlation coefficient=–0·37, p<0·0001). Mean CED was 10 830 mg/m² (SD 7274) in patients with azoospermia, 8480 mg/m² (4264) in patients with oligospermia, and 6626 mg/m² (3576) in patients with normospermia. In multivariable analysis, CED was significantly associated with an increased risk per 1000 mg/m² CED for azoospermia (OR 1·22, 95% CI 1·11–1·34), and for oligospermia (1·14, 1·04–1·25), but age at diagnosis and age at assessment were not.**

Interpretation **Impaired spermatogenesis was unlikely when the CED was less than 4000 mg/m². Although sperm concentration decreases with increasing CED, there was substantial overlap of CED associated with normospermia, oligospermia, and azoospermia. These data can inform pretreatment patient counselling and use of fertility preservation services.**

Funding **US National Cancer Institute, American Lebanese Syrian Associated Charities.**

Introduction

The treatment of children and adolescents with cancer has become increasingly successful, with about 80% of patients surviving 5 years or more after diagnosis.[1] Irradiation of the testes or treatment with certain classes of chemotherapeutic agents, especially alkylating agents, might impair fertility,[2,3] a risk that increases with cumulative doses of alkylating agents, as estimated by the cyclophosphamide equivalent dose (CED).[4] Published work about the relation between cumulative alkylating agent exposure and semen variables in adult survivors of childhood cancer is scarce, and often confounded by radiation exposure to the testes or hypothalamic–pituitary axis. We undertook the present study to investigate the independent role of alkylating agent exposure to test the hypothesis that increased exposure would be associated with decreased sperm concentration in a cohort of adult male survivors of childhood cancer who were not exposed to radiation therapy for their childhood cancer.

Methods

Study design and participants

Our analysis used data available as of April 30, 2013, for male participants in the St Jude Lifetime Cohort Study (SJLIFE) diagnosed and treated for cancer between 1970 and 2002. The continuing SJLIFE[5,6] study includes patients 0–28 years of age at diagnosis who meet the following criteria: diagnosis of childhood malignancy treated at St Jude Children's Research Hospital; survival for 10 years or more from diagnosis; and a present age 18 years or older. SJLIFE participants undergo risk-based health screening pertinent to the specific treatment received for childhood cancer.[7] Although physical examination included testicular examination, assessment of testicular volume was inconsistently done, and therefore not included in this analysis. Semen analysis was offered to men who had received gonadotoxic treatments (exposure to an alkylating agent, testicular irradiation [any dose], or hypothalamic–pituitary irradiation [≥40 Gy]). We restricted analysis to those

Lancet Oncol 2014; 15: 1215–23

Published Online September 17, 2014 http://dx.doi.org/10.1016/S1470-2045(14)70408-5

See Comment page 1181

See Online for podcast interview with Daniel M Green

Department of Epidemiology and Cancer Control (Prof D M Green MD, K C Shelton MPH, K K Ness PhD, Prof L L Robison PhD, Prof M M Hudson MD), **Department of Oncology** (Prof D M Green, Prof C-H Pui MD, Prof S L Spunt MD, M L Metzger MD, Prof M M Hudson MD), **Department of Biostatistics** (W Liu PhD, Prof D Srivastava PhD), **Department of Pediatric Medicine, Division of Endocrinology** (W Chemaitilly MD), **and Division of Psychology** (J L Klosky PhD), **St Jude Children's Research Hospital, Memphis, TN, USA; Fertility Associates of Memphis, Memphis, TN, USA** (W H Kutteh MD, R W Ke MD); **Department of Obstetrics and Gynecology, Vanderbilt University Medical School, Nashville, TN, USA** (W H Kutteh, R W Ke); **Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA** (Prof C A Sklar MD); **Department of Pediatrics, University of Tennessee Health Sciences Center, Memphis, TN, USA** (Prof S L Spunt, M L Metzger, Prof M M Hudson); **and Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA** (Prof S L Spunt)

Correspondence to: Prof Daniel M Green, Department of Epidemiology and Cancer Control, St Jude Children's Research Hospital, Mail Stop 735, Memphis, TN 38105, USA **daniel.green@stjude.org**

whose exposure to gonadotoxic therapy was only alkylating agent chemotherapy. We excluded from the analyses patients who had undergone vasectomy, received any radiation therapy, or were receiving androgen treatment. Additional details regarding SJLIFE are provided in the appendix. This investigation was approved by the institutional review board in accordance with an assurance filed with and approved by the Department of Health and Human Services. All participants or their guardians gave written informed consent.

See Online for appendix

Procedures

Cumulative doses for 32 specific chemotherapeutic agents (appendix) were abstracted according to a protocol similar to that used in the Childhood Cancer Survivor Study (CCSS).[8] CED was calculated using the following formula: CED (mg/m²)=1·0 (cumulative cyclophosphamide dose [mg/m²]) + 0·244 (cumulative ifosfamide dose [mg/m²]) + 0·857 (cumulative procarbazine dose [mg/m²]) + 14·286 (cumulative chlorambucil dose [mg/m²]) + 15·0 (cumulative carmustine dose [mg/m²]) + 16·0 (cumulative lomustine dose [mg/m²]) + 40 (cumulative melphalan dose [mg/m²]) + 50 (cumulative thiotepa dose [mg/m²]) + 100 cumulative chlormethine dose [mg/m²]) + 8·823 (cumulative busulfan dose [mg/m²]).[4]

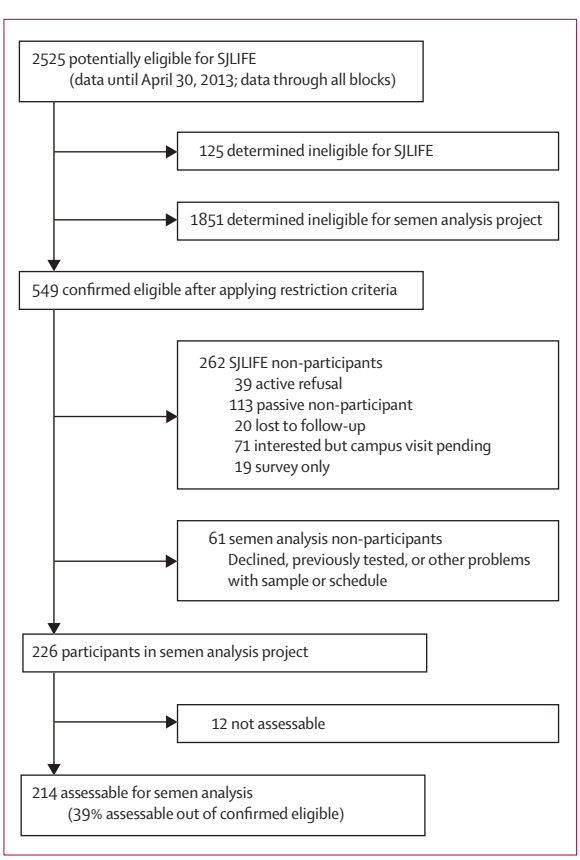

Figure 1: **Study profile**

We did a systematic review of medical records for all participants to ascertain physical and demographic characteristics. Assessment of Tanner stage at diagnosis was not routinely available and therefore not included in the analysis.

Semen samples were collected via masturbation in a private location at the fertility clinic after a planned minimum of 2 days and a maximum of 7 days of sexual abstinence and were processed within 30 min of collection following the 2010 WHO guidelines.[9] Samples were allowed to liquefy and time to liquefaction was recorded. The raw sample was microscopically assessed. The sample was centrifuged and concentrated if no sperm were detected. The concentrated sample was assessed again before being classified as azoospermic. Specimens that contained more than zero and less than 15 million sperm per mL were classified as oligospermic and those with 15 million or more per mL were classified as normospermic. Several additional characteristics, including motility (%),[9] progressive motility (0–4),[10,11] and morphology (≥4% Kruger strict),[9] were assessed in the semen specimens that were not azoospermic. At the time of collection, if there was a history of fever over 38·9°C during the preceding 3 months, any hormonal medication use, or recent genitourinary tract infection or injury, a request for a repeat specimen in 1 month to confirm azoospermia was made.

Statistical analysis

Demographic and treatment characteristics of semen analysis participants, semen analysis non-participants, and SJLIFE non-participants were assessed using descriptive statistics, whereas chemotherapeutic exposures were compared using the Wilcoxon rank-sum test without adjusting for multiple comparisons. Differences across the three sperm concentration groups (azoospermia, oligospermia, and normospermia) with respect to ethnic origin, age at diagnosis, age at semen collection, self-reported health status, and chemotherapy exposures were first assessed using a Kruskal-Wallis test or χ^2 test in a univariate manner. Factors significant at an a of 0·10 (CED and age at diagnosis) were incorporated into the multinomial logistic regression model as continuous variables (age at semen collection was forced into the final model because of the known association between declines in sperm concentration with increasing age). Odds ratios (ORs) and 95% CIs for the final model are reported (appendix). Associations between sperm characteristics (motility, progressive motility, and morphology) with oligospermia and normospermia groups were assessed using Fisher's exact test. Similarly, the association between sperm characteristics and CED (categorised as 0–<4000, 4000–<8000, and ≥8000 mg/m²) were assessed using an exact Pearson χ^2 test,[12] and implemented using the RXC procedure in StatXact. Subgroup analysis in osteosarcoma and neuroblastoma survivors, who also received cisplatin, was done to assess the effect of exposure to this agent on spermatogenesis.

The exact Pearson χ^2 test was used to test the association between treatment with cisplatin (yes or no) and semen category. All other analyses were done using SAS software. p values less than 0·05 were considered significant.

Role of the funding source

The US National Cancer Institute and the American Lebanese Syrian Associated Charities had no role in the study design, collection, analysis or interpretation of the data, the writing of the paper, or the decision to submit the paper for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Of 2400 males eligible for the SJLIFE study as of April 30, 2013, 1851 were excluded from the present analysis because of a history of any radiation treatment, no alkylating agent exposure, vasectomy, or present use of androgens. No patient underwent bilteral orchiectomy. Of the 549 men eligible for the semen analysis project, 226 (41%) participated in a SJLIFE on-campus assessment and agreed to semen analysis. 12 were unable to produce a semen specimen, resulting in 214 assessable participants (figure 1). 27 participants did not strictly follow the WHO guidelines for duration of abstinence (two had 0 days of abstinence, 11 had 1 day, 14 had greater than 7 days; two participants had an unknown number of days abstinent) but were included in the study sample because examination of the sperm concentrations for these patients revealed no consistent pattern related to their non-compliance or unknown status, and multivariable analyses excluding them from the study population provided the same results.

Demographic and treatment characteristics of the semen analysis participants, semen analysis non-participants, and SJLIFE non-participants are shown in tables 1 and 2. SJLIFE non-participants had a higher proportion of non-white than both other groups (p=0·009). Participants who provided a semen sample were younger at cancer diagnosis (p=0·02), less likely to have previously fathered children than the semen analysis non-participants (p=0·0003; appendix), but not different in self-reported present health status (p=0·41), or the cumulative dose of cyclophosphamide received intravenously (p=0·29), orally (p=0·96), or both (p=0·70). Comparison of the semen analysis participants with the SJLIFE non-participants showed that non-participants received significantly less alkylating agent, as estimated by the CED (mean 7221 mg/m² [SD 4545] *vs* 8178 mg/m² [5183]; p=0·0006). None of the survivors included in these analyses was treated with carmustine, lomustine melphalan, or thiotepa, which are included in the CED calculation, or with temozolomide, which is not included in the CED calculation.

Azoospermia was identified in 53 (25%) of 214 participants, oligospermia in 59 (28%), and normospermia in 102 (48%). Mean CED was 10 830 mg/m² (SD 7274) for those with azoospermia, 8480 mg/m² (4264) for those with oligospermia, and 6626 mg/m² (3576) for those with normospermia (figure 2A). Of the 35 patients with a CED of less than 4000 mg/m², 31 (89%) were normospermic (appendix). CED and sperm concentration were negatively correlated (r=–0·37, p<0·0001; figure 2B).

	Study participants		Study non-participants (n=262)
	Semen analysis participants (n=214)	Semen analysis non-participants (n=73)	
Age at diagnosis (years)			
0–4	83 (39%)	13 (18%)	80 (31%)
5–9	44 (21%)	14 (19%)	62 (24%)
10–14	57 (27%)	20 (27%)	65 (25%)
15–19	30 (14%)	26 (36%)	55 (21%)
Ethnic origin			
White	187 (87%)	66 (90%)	206 (79%)
Other	27 (13%)	7 (10%)	56 (21%)
Age at diagnosis (years)			
Median (range)	7·7 (0·01–20·3)	13·3 (0·3–23·6)	8·9 (0·04–28·6)
Mean (SD)	8·0 (5·6)	11·6 (6·2)	9·2 (5·9)
Age at assessment (years)			
Median (range)	29·0 (18·4–56·1)	33·8 (18·9–55·9)	..
Mean (SD)	29·8 (7·3)	34·6 (8·9)	..
Elapsed time from diagnosis to assessment (years)			
Median (range)	21·0 (10·5–41·6)	23·0 (10·4–45·5)	..
Mean (SD)	21·6 (6·7)	23·1 (7·9)	..
Diagnosis			
Acute lymphoblastic leukaemia	70 (33%)	19 (26%)	72 (28%)
Acute myeloid leukaemia	5 (2%)	3 (4%)	9 (3%)
Ewing sarcoma family of tumours	5 (2%)	3 (4%)	5 (2%)
Central nervous system	0 (0%)	2 (3%)	1 (<1%)
Other leukaemias	0 (0%)	0 (0%)	1 (<1%)
Wilms' tumour	0 (0%)	0 (0%)	1 (<1%)
Other malignancy	0 (0%)	1 (1%)	0 (0%)
Hodgkin's lymphoma	2 (1%)	3 (4%)	4 (2%)
Germ-cell tumour	2 (1%)	0 (0%)	5 (2%)
Melanoma	2 (1%)	0 (0%)	3 (1%)
Histiocytosis	2 (1%)	1 (1%)	4 (2%)
Liver malignancies	1 (<1%)	0 (0%)	0 (0%)
Non-Hodgkin lymphoma	53 (25%)	13 (18%)	80 (31%)
Neuroblastoma	26 (12%)	5 (7%)	29 (11%)
Osteosarcoma	32 (15%)	20 (27%)	28 (11%)
Retinoblastoma	7 (3%)	0 (0%)	8 (3%)
Rhabdomyosarcoma	4 (2%)	2 (3%)	4 (2%)
Soft-tissue sarcoma	3 (1%)	1 (1%)	8 (3%)
Present health			
Excellent, very good, good	180 (84%)	57 (80%)*	..
Fair, poor	33 (15%)	14 (20%)*	..

Values are n (%) unless otherwise stated. *Two participants did not complete this section.

Table 1: **Characteristics of eligible participants**

Multinomial logistic regression that included, as continuous variables, age at diagnosis, age at assessment, and CED had ORs for azoospermia of 1·22 (95% CI 1·11–1·34; p<0·0001), and for oligospermia 1·14 (95% CI 1·04–1·25; p=0·006) for each 1000 mg/m² increase in CED compared with those with normospermia. Age at diagnosis and age at assessment were not significant independent predictors of azoospermia or oligospermia (appendix). As a surrogate for pubertal status, we assessed two additional models dichotomising age at diagnosis at either 10 or 12 years; neither was statistically significant (appendix).

Five patients underwent retroperitoneal lymph node dissections. Two were azoospermic, both of whom had testicular yolk sac (endodermal sinus) tumours. Two with retroperitoneal neuroblastomas were oligospermic. One with a paratesticular embryonal rhabdomyosarcoma was normospermic (appendix). Exclusion of these five patients from the logistic regression models did not change the results (appendix).

In patients with neuroblastoma or osteosarcoma who were treated with an alkylating agent, there was no significant difference in the distributions of azoospermia,

	Study participants		Study non-participants (n=262)	Pairwise comparison¶	
	Semen analysis participants (n=214)	Semen analysis non-participants (n=73)		Semen analysis participants *vs* semen analysis non-participants	Semen analysis participants *vs* study non-participants
Cyclophosphamide (intravenous)*	161 (75%)	60 (82%)	215 (82%)	..	..
Median (mg/m²)	7116 (1000–23793)	6871 (1133–25750)	6041 (1000–37685)	..	..
Mean (mg/m²)	7337 (3748)	8032 (4555)	6821 (4008)	0·31	0·29
Cyclophosphamide (oral)*	17 (8%)	0 (0%)	11 (4%)	..	..
Median (mg/m²)	6088 (2100–31894)	..	6645 (4098–11519)	..	..
Mean (mg/m²)	8140 (6753)	..	6977 (2416)	..	0·96
Cyclophosphamide (both oral and intravenous)	17 (8%)	5 (7%)	17 (6%)	..	..
Median (mg/m²)	6961 (4203–14882)	13347 (5231–30968)	7722 (1050–16363)	..	..
Mean (mg/m²)	7898 (3153)	15953 (9573)	8440 (4590)	0·17	0·70
Ifosfamide (intravenous)	26 (12%)	9 (12%)	19 (7%)	..	..
Median (mg/m²)	40000 (14379–72499)	40646 (39750–64859)	39706 (6000–57360)	..	..
Mean (mg/m²)	41532 (15633)	47178 (10620)	32015 (16944)	0·24	0·062
Procarbazine	2 (1%)	3 (4%)	1 (<1%)	..	..
Median (mg/m²)	12469 (4500–20437)	3116 (2363–3405)	3656	..	..
Mean (mg/m²)	12469 (11269)	2961 (538)	..	..	..
Chlormethine	1 (<1%)	2 (3%)	1 (<1%)	..	..
Median (mg/m²)	36	34 (31–36)	38		
Mean (mg/m²)	36	34 (4)	38	..	..
Chlorambucil	0 (0%)	0 (0%)	1 (<1%)	..	..
Median (mg/m²)	..	..	343	..	
Mean (mg/m²)	..	..	343	..	..
Busulfan	3 (1%)	1 (1%)	4 (2%)	..	..
Median (mg/m²)	414 (331–494)	508	558 (369–659)	..	..
Mean (mg/m²)	413 (82)	508	536 (127)	..	..
CED†					
0–<4000 mg/m²	35 (16%)	8 (11%)	46 (19%)	..	..
≥4000–<8000 mg/m²	82 (38%)	26 (37%)	113 (47%)	..	..
≥8000 mg/m²	97 (45%)	37 (52%)	80 (33·5%)	..	..
CED total dose†					
Median (mg/m²)	7400 (1000–41311)	8493 (1133–30968)	6300 (1000–37685)	..	..
Mean (mg/m²)	8178 (5183)	9440 (6333)	7221 (4545)	0·23	< 0·001
Cisplatin only	22 (10%)	11 (15%)	29 (11%)	..	..
Median (mg/m²)	400 (100–580)	400 (300–957)	400 (181–1043)	..	..
Mean (mg/m²)	381 (127)	448 (173)	434 (178)	..	..
Carboplatin only	16 (7%)	4 (5%)	13 (5%)	..	..
Median (mg/m²)	3389 (2456–4677)	3664 (1392–6710)	2770 (1249–5442)	..	..
Mean (mg/m²)	3509 (699)	3858 (2293)	2828 (1170)	..	..

(Table 2 continues on next page)

	Study participants		Study non-participants (n=262)	Pairwise comparison¶	
	Semen analysis participants (n=214)	Semen analysis non-participants (n=73)		Semen analysis participants *vs* semen analysis non-participants	Semen analysis participants *vs* study non-participants
(Continued from previous page)					
Carboplatin and cisplatin	6 (3%)	2 (3%)‡	5 (2%)	..	..
Cisplatin, median (mg/m²)	399 (152–987)	601	600 (403–608)	..	..
Cisplatin, mean (mg/m²)	462 (303)	601	559 (88)	..	..
Carboplatin, median (mg/m²)	2450 (1108–5737)	2073	2029 (632–2100)	..	..
Carboplatin (mg/m²)	2708 (1626)	2073	1778 (641)	..	..
Dacarbazine	3 (1%)	2 (3%)	4 (2%)	..	..
Median (mg/m²)	6250 (2228–6445)	1966 (1674–2259)	2259 (1429–6466)	0·44	0·62
Mean (mg/m²)	4974 (2381)	1966 (414)	3997 (2544)	..	..
Neuroblastoma and osteosarcoma‖					
Cisplatin only	20 (45·5%)**	11 (50·0%)††	22 (50·0%)‡‡	..	..
Median (mg/m²)	400 (100–580)	400 (300–957)	400 (181–1043)	..	..
Mean (mg/m²)	400 (115)	448 (173)	415 (188)	..	..

Data are n of participants (%), median (range) or mean (SD). CED=cyclophosphamide equivalent dose. *22 survivors were missing dose information. †25 survivors were missing CED data: two semen analysis non-participants and 23 study non-participants did not have complete alkylating exposure data so their CED could not be calculated. ‡Cumulative doses for one patient not available. ¶t-test p value. ‖Restricted to participants with neuroblastoma or osteosarcoma (n=112). **44 semen analysis participants were treated with a drug included in the CED with or without DDP. ††22 semen analysis non-participants were treated with a drug included in the CED with or without DDP. ‡‡44 SJLIFE non-participants were treated with a drug included in the CED with or without DDP.

Table 2: **Drug exposure characteristics of participants and non-participants**

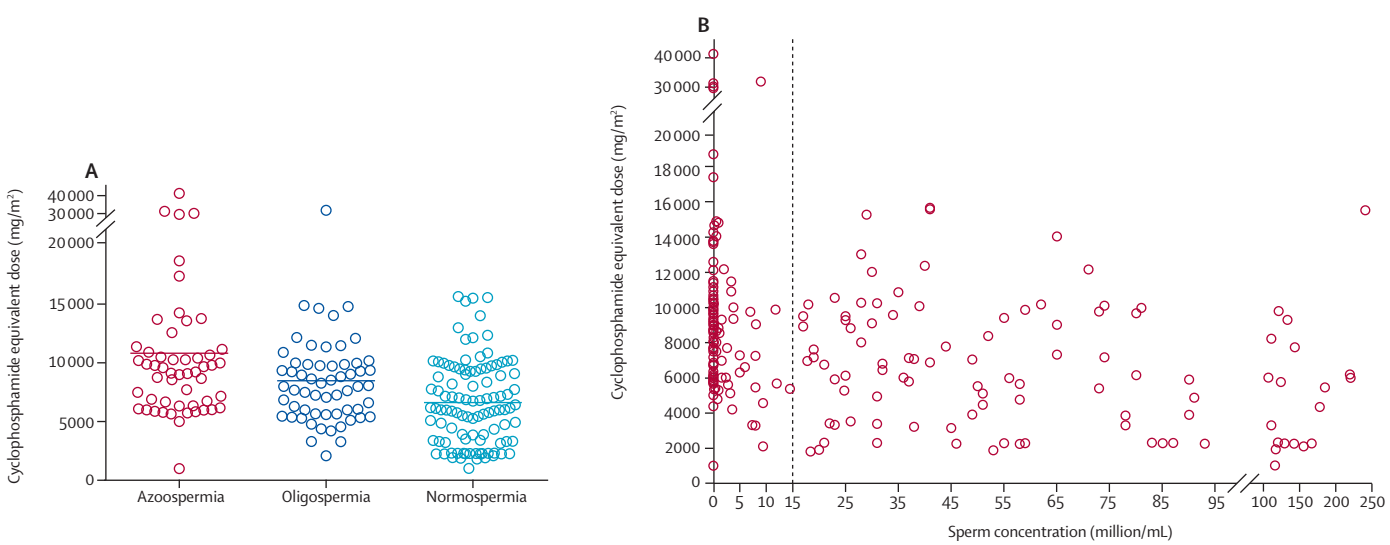

Figure 2: **Relation between cyclophosphamide equivalent dose and semen analysis (A) and sperm concentration (B)**
Means indicated by horizontal lines with each group.

oligospermia, and normospermia between patients who did or did not receive cisplatin (p=0·11 [exact χ^2 test]) (appendix).

Patients with normospermia were more likely to have normal sperm motility and normal sperm morphology than those with oligospermia (table 3). However, there was no evidence that the prevalence of very low or low motility, very low or low progressive motility, or normal morphology was correlated with CED in those with oligospermia or normospermia (table 4).

Discussion

Alkylating agents interfere with spermatogenesis, but there are few data for the effect of host and treatment factors on this risk in survivors of childhood cancer. Using the SJLIFE study, which includes a large cohort of well-characterised, unirradiated male survivors of childhood cancer, we show a correlation between increasing CED and the prevalence of azoospermia (panel). Although impaired spermatogenesis was less likely when the CED was less than 4000 mg/m², we did

	Oligospermia (N=59)†	Normospermia (N=102)	p value*
Motility			
Very low or low (<40%)	24 (42%)	20 (20%)	
Normal (≥40%)	33 (58%)	82 (80%)	0·002
Progressive motility			
Very low or low (≤2·0)	23 (40%)	5 (5%)	
Normal (>2·0)	34 (60%)	97 (95%)	<0·0001
Morphology (% normal)			
Low (0–3%)	14 (36%)	9 (9%)	
Normal (≥4%)	25 (64%)	93 (91%)	<0·0001

Data are n (%). *Exact χ^2 test. †Data are missing for two participants for motility and progressive motility, and for 20 participants for morphology.

Table 3: **Sperm characteristics in oligospermic and normospermic semen analysis participants**

	CED (mg/m²)			p value*
	0–<4000	4000–<8000	>8000	
Oligospermia†				
Motility				
Very low or low (<40%)	1 (33%)	8 (31%)	15 (54%)	
Normal (≥40%)	2 (67%)	18 (70%)	13 (46%)	0·22
Progressive motility				
Very low or low (≤2·0)	1 (33%)	9 (35%)	13 (46%)	
Normal (>2·0)	2 (67%)	17 (65%)	15 (54%)	0·74
Morphology (% normal)				
Low (0–3%)	2 (67%)	7 (37%)	5 (29%)	
Normal (≥4%)	1 (33%)	12 (63%)	12 (71%)	0·61
Normospermia				
Motility (%)				
Very low or low (<40%)	3 (10%)	11 (31%)	6 (17%)	
Normal (≥ 40%)	28 (90%)	25 (70%)	29 (83%)	0·10
Progressive motility				
Very low or low (≤2·0)	0 (0%)	4 (11%)	1 (3%)	
Normal (>2·0)	31 (100%)	32 (89%)	34 (97%)	0·13
Morphology (% normal)				
Low (0–3%)	2 (6%)	4 (11%)	3 (9%)	
Normal (≥4%)	29 (94%)	32 (89%)	32 (91%)	0·9

Values are n (%) unless otherwise stated. *Exact χ^2 test. †Data are missing for two participants for motility and progressive motility, and for 20 participants for morphology.

Table 4: **Distribution of cyclophosphamide equivalent dose (CED) across categorical semen characteristics in oligospermic and normospermic semen analysis participants**

not identify a cumulative dose below which azoospermia did not occur nor one above which azoospermia was uniformly present. Additionally, we report—to the best of our knowledge—the first analysis of sperm motility and morphology in a large number of adult survivors of childhood cancer and record abnormalities of motility and morphology in some normospermic participants.

Previous investigators showed that adult males treated with chemotherapy regimens that included alkylating agents (eg, the MOPP regimen, consisting of chlormethine, vincristine, procarbazine, and prednisone) had a high incidence of azoospermia, with possible recovery in those less intensively treated.[15–18] Few studies report semen analyses in adult survivors of childhood cancer who were treated with alkylating agents, with most including too few exposed patients to assess dose–response relations. Kenney and colleagues[19] observed that spermatogenesis was preserved in survivors of childhood cancer who received 6·0 g/m² or less of cyclophosphamide compared with none of those who received 9·2 g/m² or more. Garolla and colleagues[20] reported a mean sperm concentration of 0·4 million per mL (SD 0·7) in eight patients who received 12·4–18·8 g/m² cyclophosphamide, compared with a mean sperm concentration of 46·8 million per mL (SD 57·2) in 25 patients who had received 21·6–85·0 g/m² of ifosfamide, suggesting that ifosfamide produced less severe damage to spermatogenesis. We did not have a sufficiently large number of patients treated with ifosfamide to assess the relative toxicity of ifosfamide compared with cyclophosphamide to spermatogenesis.

Other studies of adult survivors of childhood cancer, not selected for alkylating agent exposure or diagnosis, reported azoospermia in 23 (17·8%) of 129,[21] 13 (31·0%) of 42,[22] 10 (30·3%) of 33,[23] and nine (42·9%) of 21 participants.[24] In survivors of acute lymphoblastic leukaemia, azoospermia was reported in 17 (36·2%) of 47[25] and five (26·3%) of 19 patients.[26] The only study large enough to support multivariable analyses assessed chemotherapy exposures using predefined sterilising cumulative drug doses rather than examining cumulative drug exposure as a continuous or categorical variable.[21] Data regarding the prevalence of azoospermia in the general population are scarce. Azoospermia was identified in between 1·6% (three of 187) and 2·5% (four of 162) of Danish men 20–35 years of age living with a person of the opposite sex whose partner had no previous pregnancies and neither partner had previous knowledge of fertility,[27] and in 1·9% (10 of 519) of perpetrators of sexual assaults assessed by the Metropolitan Police Forensic Science Laboratory, London.[28]

Treatment with cisplatin did not increase the prevalence of azoospermia in the subgroup treated for neuroblastoma or osteosarcoma, all of whom received an alkylating agent included in the CED calculation. Due to the small number of patients so treated, the statistical power of this analysis is limited. Previous studies of survivors treated with cisplatin provided conflicting results.[29–32] In 129 patients referred to the Centres d'Etudes et de Conservation des Oeufs et du Sperme Humain for sperm banking before testicular germ cell tumour treatment who provided serial semen specimens, spermatogenesis recovered after two or fewer cycles of bleomycin, etoposide, and cisplatin (BEP regimen) at 12 months after the start of treatment, and there was slower, but complete, recovery at 24 months after the start of therapy

in those who received radiation therapy or three or four courses of BEP.[33]

Emerging evidence suggests that genetic variations might be associated with sperm concentrations in the normal population[34,35] and cancer survivors.[36] Genetic polymorphisms could be associated with decreased therapeutic activity (decreased drug activation) or increased toxicity (accelerated drug activation) after treatment with alkylating agents.[37] Other factors that could affect our findings include the use of tobacco, alcohol, or recreational drugs, unreported use of anabolic steroids (participants reporting use of anabolic steroids were excluded from the analysis), obesity, unrecognised or undiagnosed genitourinary abnormalities (eg, varicoceles), and other unknown factors. However, rigorous assessment of the potential effect of some or all of these factors would require a much larger participant population.

No previous study of spermatogenesis after treatment for childhood cancer assessed sperm motility or morphology. In the normal population, both sperm morphology[38–41] and sperm motility[39–42] could be associated with impairment of subsequent male fertility. In the present study, less than half of those with oligospermia had normal morphology. Progressive motility and morphology were at values consistent with impaired fertility in a small percentage of those with normospermia. Thus, in addition to sperm concentration, abnormalities of motility and morphology might be contributing to the decreased fertility seen in adult male survivors of childhood cancer.[3,4]

The strengths of this study include the assessment of semen specimens from a large number of participants treated with alkylating agents who received no radiation therapy, and analysis of all semen specimens in a single, experienced fertility laboratory. Moreover, to control for the effect of other factors that might affect semen parameters, all participants completed a questionnaire that included items regarding genitourinary diseases (eg, epididymitis and urethritis) and fever during the previous 3 months. Additionally, results of a concurrent white blood cell count documented the very low proportion (<4%) with possible infection. Limitations of this study should be considered when interpreting the results. Because of the logistics for the SJLIFE research study, which requires participants to travel long distances to St Jude Children's Research Hospital for an assessment lasting several days (average 3 days), we had to rely on a single semen sample, by contrast with a minimum of two samples recommended when assessing fertility. Moreover, although some participants did not strictly adhere to the WHO recommended period of abstinence before collection of the sample, most reported abstaining within the recommended timeframe and restricting the analysis to this compliant group did not change the results. Our sample size was not sufficiently large to consider statistically the potential effect of factors such as tobacco or recreational drug use, dietary or androgen supplements, or exposure to extremely hot environments (eg, sauna or hot tub), which are known to adversely affect sperm concentration.[41] Our study population represents a highly selected group of long-term survivors diagnosed and treated at our institution, and followed-up over four decades, at a single institution, whose participation was restricted by their previous cancer treatment (ie, alkylating agent exposure, but not radiation), and other factors affecting availability and willingness to provide a semen sample (eg, participant in the SJLIFE cohort, previously known fertility, inability to provide a sample). Specifically, semen analysis participants were younger at cancer diagnosis, less likely to have previously fathered children than the semen analysis non-participants, but not different in the cumulative dose of cyclophosphamide received. The lower rate of previous parenthood in participants could have reflected the higher observed frequency of azoospermia and oligospermia. Overall, care should be taken in generalising our results to the broader population of childhood cancer survivors.

Although exposure data were collected for other drugs used for the treatment of various childhood cancers, we did not assess the effect of antimetabolites (eg, cytosine arabinoside, mercaptopurine, and methotrexate) or tubulin-binding agents (eg, vincristine or vinblastine) on sperm concentration because previous experimental work suggested these agents produce negligible effects on spermatogonial stem cells.[43] Additionally, spermatogenesis

***Panel:* Research in context**

Systematic review

We were familiar with previous work regarding fertility of male childhood cancer survivors compared with their siblings,[3] with reviews done by members of the Male Gonadal Function Working Group of the Children's Oncology Group Guidelines Committee[13] and by the Male Gonadotoxicity Guidelines Group of the International Harmonisation Group.[14] The search terms used in preparation for the Male Gonadal Toxcity Guidelines Group of the International Harmonisation Group are in the appendix.

Interpretation

Our data support earlier work showing that alkylating agents have a negative effect on spermatogenesis. However, by contrast with other studies, our results indicate no protective effect of earlier age at diagnosis against the adverse effect of alkylating agent treatment on spermatogenesis. Our data identify a cumulative exposure below which most patients will experience normal spermatogenesis, although there is substantial overlap above this dose of the outcomes of normospermia, oligospermia, and azoospermia with various exposure levels. Clinicians could use these data for pretreatment counselling and referral of patients for fertility preservation interventions, and for guiding future study design and research regarding the adverse effects of alkylating agent exposure on spermatogenesis.

recovered to normal in most patients treated with combination chemotherapy regimens that did not include an alkylating agent included in the CED.[44–46] Dacarbazine was not included in the formulation of the CED because appropriate data comparing a regimen in which the only substitution was of dacarbazine for another alkylating agent were not available.[4] However, the available data suggest that dacarbazine-containing combination chemotherapy regimens with[44] or without[18] cisplatin have a minimal adverse long-term effect on spermatogenesis. Although the alkylating agent exposures of the entire SJLIFE cohort are diverse,[6] most patients were treated with cyclophosphamide, ifosfamide, or procarbazine, restricting the ability to assess the independent or additive effects of less frequent alkylating agent exposures on spermatogenesis.

The role of semen analysis in the follow-up of adult survivors of childhood cancer is not well defined. Because semen analysis is not always acceptable to young men, investigations of surrogate markers for sperm concentration have been done. Previous data from SJLIFE showed that although sperm concentration is correlated with both follicle-stimulating hormone and inhibin B levels, the specificity and positive predictive value of neither is sufficiently good to support use as a surrogate for sperm concentration.[47] In the SJLIFE analysis, the specificity of the serum level of inhibin B for identifying azoospermic survivors was 45·0% and the positive predictive value was 52·1%, and for follicle-stimulating hormone the specificity was 74·1% and the positive predictive value was 65·1%.[47] Although male survivors of childhood cancer exposed to gonadal toxic therapy are at risk of reduced fertility, it is not recommended that semen analysis be part of routine follow-up care. Rather, it should be used in assessment of fertility for survivors who encounter difficulties in conception and for men who desire information about their potential for paternity.

Our findings will inform communication of treatment risks to parents and male patients before treatment with alkylating agents for childhood or adolescent cancer, facilitate identification of those at greatest risk for fertility impairment who would benefit from pretreatment fertility preservation interventions, and guide the design of future, risk-adapted treatment protocols that include treatment with alkylating agents. Whereas contemporary protocols aim to restrict or eliminate gonadotoxic treatment exposures, alkylating agents are a critical component of therapy for many haematological and solid paediatric malignancies and are likely to remain so, in the immediate future, considering the excellent outcomes achieved with current regimens and the challenges associated with integrating novel, potentially less toxic, agents into first-line therapies.[48,49] Thus, our findings have clinical relevance to the counselling and management of children and adolescents who need alkylating agent chemotherapy to achieve long-term disease-free survival. Additional investigation is needed to address the effect of alkylating agent exposure to define exposure-specific risks related to fertility, the role of genetic factors that modulate the sensitivity of an individual's germinal epithelium to alkylating agents, and interventions to optimise access to and participation in age-appropriate methods of gamete preservation. With continued improvements in reproductive medicine, even men with very low sperm counts might have options to achieve paternity.[13,50]

Contributors

DMG, WHK, CAS, LLR, and MMH designed the study. KCS, WHK, and RWK collected clinical data. WL, DS, KKN, and KCS did statistical analyses. All authors contributed to data interpretation, the writing or revising of the Article, and approved the final version.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank Leontien Kremer, Renee Mulder, and Roderick Skinner, who developed the literature search strategy referenced in this Article. This study was supported in part by National Cancer Institute (grant number CA-21765) and the American Lebanese Syrian Associated Charities.

References

1 Howlader N, Noone AM, Krapcho M, et al. SEER cancer statistics review, 1975–2010, based on November 2012 SEER data submission, posted to the SEER website, April, 2013. http://seer.cancer.gov/csr/1975_2010/ (accessed March, 2014).

2 Robison LL, Hudson MM. Survivors of childhood and adolescent cancer: life-long risks and responsibilities. *Nat Rev Cancer* 2014; **14:** 61–70.

3 Green DM, Kawashima T, Stovall M, et al. Fertility of male survivors of childhood cancer. A report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2010; **28:** 332–39.

4 Green DM, Nolan VG, Goodman PJ, et al. The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure. A report from the Childhood Cancer Survivor Study. *Pediatr Blood Cancer* 2014; **61:** 53–67.

5 Ojha RP, Oancea SC, Ness KK, et al. Assessment of potential bias from non-participation in a dynamic clinical cohort of long-term childhood cancer survivors: results from the St. Jude Lifetime Cohort Study. *Pediatr Blood Cancer* 2013; **60:** 856–64.

6 Hudson MM, Ness KK, Nolan VG, et al. Prospective medical assessment of adults surviving childhood cancer: study design, cohort characteristics, and feasibility of the St. Jude Lifetime Cohort Study. *Pediatr Blood Cancer* 2011; **56:** 825–36.

7 Children's Oncology Group. Long-term follow-up guidelines for survivors of childhood, adolescent, and young adult cancers. 2009. http://www.survivorshipguidelines.org/pdf/LTFUGuidelines.pdf (accessed March, 2014).

8 Robison LL, Mertens AC, Boice JD, et al. Study design and cohort characteristics of the Childhood Cancer Survivor Study: a multi-institutional collaborative project. *Med Pediatr Oncol* 2002; **38:** 229–39.

9 Department of Reproductive Health and Research, WHO. WHO laboratory manual for the examination and processing of human semen, 5th edn, 2010. Geneva: World Health Organization.

10 Comhaire FH, Vermeulen L, Hinting A, Schoonjans F. Accuracy of sperm characteristics in predicting the in vitro fertilizing capacity of semen. *J In Vitro Fert Embryo Transf* 1988; **5:** 326–31.

11 Bollendorf A, Check JH, Lurie D. Evaluation of the effect of the absence of sperm with rapid and linear progressive motility on subsequent pregnancy rates following intrauterine insemination or in vitro fertilization. *J Androl* 1996; **17:** 550–57.

12 Mudholkar GS, Hutson AD. Continuity corrected approximations for and 'exact' inference with Pearson's χ^2. *J Stat Plan Inference* 1997; **59:** 61–78.

13 Kenney LB, Cohen LE, Shnorhavorian M, et al. Male reproductive health after childhood, adolescent, and young adult cancer: a report from the Children's Oncology Group. *J Clin Oncol* 2012; **30:** 3408–16.

14 Kremer LC, Mulder RL, Oeffinger KC, et al. A worldwide collaboration to harmonize guidelines for the long-term follow-up of childhood and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Pediatr Blood Cancer* 2013; **60:** 543–49.

15 Chapman RM, Sutcliffe SB, Malpas JS. Male gonadal dysfunction in Hodgkin's disease. *JAMA* 1981; **245:** 1323–28.

16 Chapman RM, Rees LH, Sutcliffe SB, Edwards CRW, Malpas JS. Cyclical combination chemotherapy and gonadal function. *Lancet* 1979; **1:** 285–89.

17 da Cunha MF, Meistrich ML, Fuller LM, et al. Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy. *J Clin Oncol* 1984; **2:** 571–57.

18 Viviani S, Santoro A, Ragni G, Bonfante V, Bestetti O, Bonadonna G. Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP vs ABVD. *Eur J Cancer Clin Oncol* 1985; **21:** 601–05.

19 Kenney LB, Laufer MR, Grant FD, Grier H, Diller L. High risk of infertility and long term gonadal damage in males treated with high dose cyclophosphamide for sarcoma during childhood. *Cancer* 2001; **91:** 613–21.

20 Garolla A, Pizzato C, Ferlin A, Carli MO, Selice R, Foresta C. Progress in the development of childhood cancer therapy. *Reprod Toxicol* 2006; **22:** 126–32.

21 Romerius P, Stahl O, Moell C, et al. High risk of azoospermia in men treated for childhood cancer. *Int J Androl* 2011; **34:** 69–76.

22 Rendtorff R, Hohmann C, Reinmuth S, et al. Hormone and sperm analyses after chemo- and radiotherapy in childhood and adolescence. *Klin Padiatr* 2010; **222:** 145–49.

23 Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, Wallace WH. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. *Lancet* 2002; **360:** 361–67.

24 van Casteren NJ, van der Linden GH, Hakvoort-Cammel FG, Hahlen K, Dohle GR, van den Heuvel-Eibrink MM. Effect of childhood cancer treatment on fertility markers in adult male long-term survivors. *Pediatr Blood Cancer* 2009; **52:** 108–12.

25 Jahnukainen K, Heikkinen R, Henriksson M, Cooper TG, Puukko-Viertomies LR, Makitie O. Semen quality and fertility in adult long-term survivors of childhood acute lymphoblastic leukemia. *Fertil Steril* 2011; **96:** 837–42.

26 Wallace WH, Shalet SM, Lendon M, Morris-Jones PH. Male fertility in long-term survivors of childhood acute lymphoblastic leukaemia. *Int J Androl* 1991; **14:** 312–19.

27 Jensen TK, Andersson AM, Hjollund NH, et al. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 1997; **82:** 4059–63.

28 Willott GM. Frequency of azoospermia. *Forensic Sci Int* 1982; **20:** 9–10.

29 Longhi A, Macchiagodena M, Vitali G, Bacci G. Fertility in male patients treated with neoadjuvant chemotherapy for osteosarcoma. *J Pediatr Hematol Oncol* 2003; **25:** 292–96.

30 Petersen PM, Hansen SW, Giwercman A, Rorth M, Skakkebaek NE. Dose-dependent impairment of testicular function in patients treated with cisplatin-based chemotherapy for germ cell cancer. *Ann Oncol* 1994; **5:** 355–58.

31 Brydoy M, Fossa SD, Klepp O, et al. Sperm counts and endocrinological markers of spermatogenesis in long-term survivors of testicular cancer. *Br J Cancer* 2012; **107:** 1833–39.

32 Reinmuth S, Hohmann C, Rendtorff R, et al. Impact of chemotherapy and radiotherapy in childhood on fertility in adulthood: the FeCt-survey of childhood cancer survivors in Germany. *J Cancer Res Clin Oncol* 2013; **139:** 2071–78.

33 Bujan L, Walschaerts M, Moinard N, et al. Impact of chemotherapy and radiotherapy for testicular germ cell tumors on spermatogenesis and sperm DNA: a multicenter prospective study from the CECOS network. *Fertil Steril* 2013; **100:** 673–80.

34 Aston KI, Krausz C, Laface I, Ruiz-Castane E, Carrell DT. Evaluation of 172 candidate polymorphisms for association with oligozoospermia or azoospermia in a large cohort of men of European descent. *Hum Reprod* 2010; **25:** 1383–97.

35 Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. *J Androl* 2009; **30:** 711–25.

36 Romerius P, Giwercman A, Moell C, et al. Estrogen receptor alpha single nucleotide polymorphism modifies the risk of azoospermia in childhood cancer survivors. *Pharmacogenet Genomics* 2011; **21:** 263–69.

37 Pinto N, Ludeman SM, Dolan ME. Pharmacogenetic studies related to cyclophosphamide-based therapy. *Pharmacogenomics* 2009; **10:** 1897–903.

38 Eggert-Kruse W, Schwarz H, Rohr G, Demirakca T, Tilgen W, Runnebaum B. Sperm morphology assessment using strict criteria and male fertility under in-vivo conditions of conception. *Hum Reprod* 1996; **11:** 139–46.

39 van der Merwe FH, Kruger TF, Oehninger SC, Lombard CJ. The use of semen parameters to identify the subfertile male in the general population. *Gynecol Obstet Invest* 2005; **59:** 86–91.

40 Zinaman MJ, Brown CC, Selevan SG, Clegg ED. Semen quality and human fertility: a prospective study with healthy couples. *J Androl* 2000; **21:** 145–53.

41 Jouannet P, Ducot B, Feneux D, Spira A. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. *Int J Androl* 1988; **11:** 379–94.

42 Larsen L, Scheike T, Jensen TK, et al. Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First Pregnancy Planner Study Team. *Hum Reprod* 2000; **15:** 1562–67.

43 Meistrich ML, Finch M, da Cunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res* 1982; **42:** 122–31.

44 Meistrich ML, Chawla SP, Da Cunha MF, et al. Recovery of sperm production after chemotherapy for osteosarcoma. *Cancer* 1989; **63:** 2115–23.

45 Meistrich ML, Wilson G, Mathur K, et al. Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease. *J Clin Oncol* 1997; **15:** 3488–95.

46 Hill M, Milan S, Cunningham D, et al. Evaluation of the efficacy of the VEEP regimen in adult Hodgkin's disease with assessment of gonadal and cardiac toxicity. *J Clin Oncol* 1995; **13:** 387–95.

47 Green DM, Zhu L, Zhang N, et al. Lack of specificity of plasma concentrations of inhibin B and follicle-stimulating hormone for identification of azoospermic survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. *J Clin Oncol* 2013; **31:** 1324–28.

48 Green DM, Kun LE, Matthay KK, et al. Relevance of historical therapeutic approaches to the contemporary treatment of pediatric solid tumors. *Pediatr Blood Cancer* 2013; **60:** 1083–94.

49 Hudson MM, Neglia JP, Woods WG, et al. Lessons from the past: opportunities to improve childhood cancer survivor care through outcomes investigations of historical therapeutic approaches for pediatric hematological malignancies. *Pediatr Blood Cancer* 2012; **58:** 334–43.

50 Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol* 2006; **24:** 2917–31.