No Long-Term Increase in Sperm Aneuploidy Rates after Anticancer Therapy: Sperm Fluorescence *In situ* Hybridization Analysis in 26 Patients Treated for Testicular Cancer or Lymphoma

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ABSTRACT

Purpose: **Lymphomas and testicular cancers are the most frequent malignancies among young men. With recent improvement of survival rates, for many patients, the question is raised of the consequences of the anticancer treatments on their fertility and more specifically of a potential genetic risk for the offspring. This article presents the study of sperm aneuploidy rates in the largest population of cancer-treated patients studied thus far.**

Experimental Design: **In the present study, 38 patients were initially included 7 months to 5 years after a cancer treatment by chemotherapy and/or radiotherapy for testicular cancer ($n = 19$) or lymphoma ($n = 19$). Twelve of them were azoospermic. Sperm aneuploidy rates of chromosomes X, Y, 13, 18, and 21 were analyzed by multicolor fluorescent *in situ* hybridization in the 26 other patients.**

Results: **In most cases, the disomy/diploidy rates after cancer therapy did not significantly differ from those observed in the group of control healthy donors. Only five patients (one lymphoma and four testicular cancer) showed significant but still moderate increases in disomic and/or diploid sperm. For the lymphoma patient, the short post-therapeutic delay after the treatment could explain the elevated aneuploidy rates, whereas no risk factor in the clinical, biological, or therapeutic records could be identified in any of the four testicular cancer patients with elevated sperm aneuploidy rates.**

Conclusions: **These data suggest an absence of long-term effect of anticancer therapy on sperm aneuploidy rates, and therefore, no long-term increased risk of aneuploidy for the offspring obtained either spontaneously or after assisted reproductive techniques.**

INTRODUCTION

Testicular cancer and lymphomas are the most frequent malignancies among adolescents and young men (1, 2). Important improvements have recently been achieved in the management of these diseases. After radical orchidectomy, the adjuvant treatment for testicular cancer differs according to the tumor's histology (3–6). Approximately 95% of malignant tumors arising in the testis are germ-cell tumors and are classified as seminomatous or nonseminomatous (7, 8). Patients with low-stage seminoma are usually treated with radiation of the retroperitoneal and ipsilateral pelvic lymph nodes (4, 7, 9), whereas patients with nonseminomatous tumors or advanced seminomas receive a cisplatin-based chemotherapy, with standard protocols, including cisplatin, etoposide, and bleomycin (3, 6, 10). For Hodgkin's disease and non-Hodgkin's lymphomas, the treatment is usually based on chemotherapy with or without radiotherapy (11–18). These new treatment protocols have greatly increased the patients' survival rates, which now exceed 90% for testicular cancers (7, 19) and for most cases of Hodgkin's disease (20, 21). Most of these treatments have many side effects, including gonadal toxicity. Indeed, chemotherapy or radiotherapy has been shown to inhibit spermatogenesis (22–24), and although spermatogenesis is often recovered after a few years, there is no real predictive factor of posttreatment fertility (25, 26). Another concern is the fact that due to their biological mechanism of action, anticancer treatments could also induce germ-cells genetic damage. Hence, sperm chromosomal abnormalities in these patients may increase the risk of fetal death and congenital abnormalities in their offspring. It has been quite well documented that during the 3 months after cancer treatments by chemotherapy and/or radiotherapy, the rates of DNA breaks and abnormal meiotic segregations are increased (27–31), leading to increased structural and numerical chromosomal abnormalities in spermatozoa. However, potential long-term effects of these treatments on the sperm aneuploidy rates have not been well established yet. Numerous reports have been published evaluating the health of cancer patients' offspring (32–37), and no increase of the risk of congenital malformations has been observed. However, these studies involve

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Table 1 Clinical and biological data of the 12 patients showing post therapeutic azoospermia

Patients	Disease	Sperm count	Teratospermia	Chemotherapy	No. of courses	Radiotherapy	Dose (Gys)	Time between treatment and sperm analysis
A1	NHL	63 million/mL	75%	COP-COPADAM-CYM		Retroperitoneal and splenic	30	10 mo
A2	NHL	170 million/mL	57%	CEOP	2			20 mo
				CEOPVP	2			
				TI	1			
A3	NHL	230 million/mL	56%	COPADAM-CYM		Above and under diaphragm	9	2 y
A4	NHL	88 million/mL	31%	MOPP-ABV	6	Above diaphragm	40	2 y
A5	HD	22 million/mL	50%	ABVD	4			3 y
				TI	1			
A6	HD	54 million/mL	61%	MOPP-ABV	4	Above and under diaphragm	36	5 y
A7	HD	4 million/mL	NE	ABVPP	8			5.5 y
A8	S	32 million/mL	70%			Under diaphragm	26	11 mo
A9	NS	45 million/mL	60%	BEP	1			20 mo
				EP	3			
				TI	1			
A10	NS	0.3 million/mL	NE	EP	6			3 y
A11	S	2 million/mL	71%	BEP	3	Above and under diaphragm	30	4 y
A12	NS	22 million/mL	49%	BEP	3			4 y

Abbreviations: NHL, non-Hodgkin's lymphoma; COP, cyclophosphamide, vincristine, prednisone; COPADAM-CYM, cyclophosphamide, vincristine, prednisone, Adriamycin, cytarabine, high dose of methotrexate; CEOP(VP), cyclophosphamide, epirubicin, vincristine, prednisone, (etoposide, cysplatin); MOPP, mustine, vincristine, procarbazine, prednisone; HD, Hodgkin's disease; ABVPP, Adriamycin, bleomycin, vindesine, procarbazine, prednisone; TI, treatment intensification; NE, nonevaluated; S, seminoma; NS, nonseminomatous tumor; (B)EP, bleomycin, etoposide, cisplatin.

small size and heterogeneous populations and are therefore unable to detect any increased relative risks $<$ 3-fold.

The reported studies of sperm chromosome analysis by fluorescence *in situ* hybridization (FISH) or sperm karyotyping in cancer-treated patients, a few months or years after chemotherapy or radiotherapy, show contradictory results. Indeed, elevated (38–40) or normal (28, 31, 41–44) rates of chromosomal abnormalities have been found. Furthermore, these reports concern only very small populations of patients (6 at most), often with different diseases and treatments. Therefore, other studies are necessary with standardized groups of patients to determine the middle-term consequences of cancer treatments on sperm chromosomes and evaluate a potential risk of chromosomal abnormality for the offspring.

In the present study, 38 patients were included 7 months to 5 years after a cancer treatment by chemotherapy and/or radiotherapy for testicular cancer ($n = 19$) or lymphoma ($n = 19$). Sperm parameters analysis showed that 12 of them were azoospermic. Sperm chromosome analysis was performed by FISH in the other 26 men treated for testicular cancer ($n = 14$) or lymphoma ($n = 12$). The segregation of chromosomes 13, 21, 18, X, and Y was analyzed, respectively, by two- and three-color FISH. These chromosomes are indeed those involved in most aneuploidies observed at birth. The sperm aneuploidy rates of the patients were compared with those of 12 healthy donors.

MATERIALS AND METHODS

Cancer Patients and Samples. The design of our study was approved by the Comité Consultatif de Protection des Personnes en Recherche Biomedicale. Patients $>$ 18 years old were recruited who had had sperm cryopreservation before treatment for testicular cancer or lymphoma in the Centre d'étude et de Conservation des Oeufs et du Sperme humain of Grenoble University Hospital between January 1995 and December 2000. Of 133 patients who had been informed about the research protocol, 69 gave a response. Thirty-one patients were not included because (*a*) they had not received any chemotherapy or radiotherapy (10 patients), (*b*) they had recently died accidentally (2 patients), (*c*) collecting sperm was impossible because of posttreatment ejaculation (3 patients), (*d*) they were being treated for cancer reoccurrence (1 patient), and (*e*) they refused to participate in the study (15 patients). Thirty-eight patients (19 treated for testicular cancer and 19 treated for lymphoma) were finally included in the study and gave a sperm sample. The clinical and biological data of the patients are presented in Table 1 (for the 12 azoospermic patients) and Table 2 (for the 26 other patients: 12 with lymphoma and 14 with testicular cancer). Semen specimens from 12 healthy fertile donors aged between 25 and 45 years with a normal karyotype and normal sperm parameters were used as controls. They were found among Centre d'étude et de Conservation des Oeufs et du Sperme humain sperm donors, which means they had at least one healthy child each and had signed a written agreement for their sperm to be used for research, after it had been fully exploited for sperm donation. Semen samples were collected at the laboratory and processed after liquefaction (30 minutes at 37°C). The volume, number of sperm cells per mL, motility, and morphology were analyzed according to the WHO recommendations. Each sperm was cryopreserved in liquid nitrogen according to the standardized procedures of sperm banking.

Preparation of Sperm Nuclei. After thawing for 10 minutes at 37°C, the spermatozoa were washed three times in

Table 2 Main clinical and therapeutic data in the nonazoospermic patients

Patients	Age at diagnosis (y)	Disease	Chemotherapy	No. of courses	Radiotherapy	Dose (Grays)	Time between treatment and FISH analysis
Lymphoma							
L1*	27	HD	ABVD	3	Retroperitoneal and splenic	30	7 mo
					Above diaphragm	40	
L2	29	HD	EBVP	6	Above diaphragm	40	11 mo
L3	34	HD	EBVP	6	Above diaphragm	20	12 mo
L4	19	HD	ABVD	3	Retroperitoneal and splenic	30	13 mo
					Above diaphragm	40	
L5	31	HD	ABVD	3	Retroperitoneal and splenic	30	16 mo
					Above diaphragm	40	
L6	25	HD	BEACOPP	4	Above diaphragm	30	16 mo
L7	35	HD	VIP-ABVD	6	Above diaphragm	40	21 mo
L8	40	HD	MOPP-ABV-Hybrid	6	Above diaphragm	40	2 y
L9	18	NHL (Burkitt type)	COPADAM-CYM	3 2	Above diaphragm	40	2 y
L10	37	NHL (diffuse large B-cell)	ACVBP MTX-HOLOXAN-VP16-ARACYTINE	4			4 y
L11	38	HD	MOPP-ABV-Hybrid	6	Above diaphragm	40	5 y
L12	36	HD	MOPP-ABV-Hybrid	4	Above diaphragm	40	5 y
Testicular cancer							
T1	31	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	19 mo
T2	27	NS	BEP	4			20 mo
T3*	24	NS	BEP	4			21 mo
T4*	35	S	EP	4			23 mo
T5	26	S			Retroperitoneal and ipsilateral pelvic lymph nodes	35	2 y
T6*	38	S	EP	4	Retroperitoneal and ipsilateral pelvic lymph nodes	30	2 y
T7	42	NS	BEP EP	3 1			2 y
T8	41	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	2 y
T9	40	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	4 y
T10*	39	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	4 y
T11	46	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	4 y
T12	31	NS	BEP	4			4 y
T13	40	S			Retroperitoneal and ipsilateral pelvic lymph nodes	25	5 y
T14	35	S			Retroperitoneal and ipsilateral pelvic lymph nodes	30	5 y

* Patients with increased aneuploidy rate(s).

Abbreviations: HD, Hodgkin's disease; ABVD, adriomycin, bleomycin, vinblastine, dacarbozine; EBVP, epirubicine, bleomycin, vinblastine, prednisone; BEACOPP, bleomycin, etoposide, Adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone; VIP, etoposide, ifosfamide, cisplatin, MOPP, mustine, vincristine, procarbazine, prednisone; NHL, non-Hodgkin's lymphoma; COPADAM, cyclophosphamide, vincristine, prednisone, Adriamycin, CYM cytarabine, high dose of methotrexate; ACVBP, Adriamycin, cyclophosphamide, vindesine, bleomycin, prednisone; MTX, methotrexate; S, seminoma; NS, nonseminomatous tumor; (B)EP, bleomycin, etoposide, cisplatin.

PBS (pH 7) and fixed during 30 minutes in a methanol-acetic acid (3:1) solution at 4°C. After centrifugation, the sperm cells were finally resuspended in a volume of 200 μL of the same fixative and deposited onto clean glass slides. The sperm nuclei were then decondensed by dipping the slides into a NaOH 1 N solution, for 1 to 5 minutes. The slides were then washed twice in 2× SCC and dehydrated in ethanol (70, 90, and 100%) before being processed for FISH.

FISH Experiments. Three-color FISH experiments were performed using α-satellite fluorescent centromeric probes specific for chromosomes X (CEP X Spectrum Orange; Vysis, Downers Grove, IL), Y (CEP Y Spectrum Green; Vysis), and 18 (CEP 18 Spectrum Aqua; Vysis). For two-color FISH experiments, locus-specific probes for chromosomes 13 (LSI 13 Spectrum Green; Vysis) and 21 (LSI 21 Spectrum Orange; Vysis) were used. FISH was performed according to the manufacturer's recommendations (Vysis). Briefly, after the hybridization solution was applied to the slides, they were placed in a dark chamber for 5 minutes at 75°C for denaturation, then incubated for 12 hours at 37°C. The slides were then washed in 0.4× SSC/0.3% NP40 during 2 minutes at 73°C and in 2× SSC/0.1% NP40 for 1 minute at room temperature. The sperm nuclei were finally counterstained with 200 ng/mL DAPI in Vectashield (Vector, Burlingame, CA).

Scoring of Sperm Nuclei and Analysis. FISH preparations were observed under a fluorescent microscope (Zeiss Axiophot, Heidelberg, Germany) equipped with filters for FITC, Texas Red, 4′-6 diamidino-2′-phenylindole dihydrochloride, and aqua and spectrum orange (Vysis). For scoring, the following criteria were used: (*a*) only sperm nuclei with a well-defined boundary were taken into account; and (*b*) two signals were scored when they were of approximately equal size and separated from each other by a distance larger than the diameter of one signal. A minimum of 5000 sperm nuclei per set of chromosome probes were examined if possible. The scoring was performed blinded by two individuals (each counting approximately half of the nuclei on each slide).

The disomy and diploidy rates observed in each patient were compared with the corresponding confidence intervals of the rates calculated from the data found in the control population (mean +/− 1.96 SD). Groups of patients were additionally compared using Mann-Whitney's test or exact Fisher's test. When testing the differences by subgroups (unilateral testing), it must be highlighted that type II error was always <20% up to six patients in each group.

RESULTS

Sperm Parameters. Thirty-eight patients treated for lymphoma ($n = 19$) or testicular cancer ($n = 19$) 7 months to 5 years earlier were included in the study for sperm parameters and sperm cytogenetic analysis. Twelve (32%) were azoospermic, including 7 lymphoma and 5 testis cancer patients (Table 1). Interestingly, this outcome did not seem to correlate with specific parameters such as sperm counts before treatment, types of cancer, therapeutic protocols, or posttherapeutic delays. Among the others, 19 of 26 had oligozoospermia (sperm count, $<20 \times 10^6$/mL) and/or teratospermia (abnormal sperm form, >75%) after treatment (Table 3).

Sperm Cytogenetic Analysis. Sperm cytogenetic analysis was performed on the 26 nonazoospermic patients. The individual results for the frequencies of disomy X, Y, XY, 13, 18, and 21 and diploidy for each patient are shown in Tables 4 and 5. Between 1500 and 5000 spermatozoa were scored for each patient and in each control men in both X, Y, 18, or 13 21 FISH experiments. The patients disomy/diploidy rates were individually compared with the 95% confidence interval of each of the corresponding disomy/diploidy rates observed in the control healthy donors population. The patient values in bold exceed this 95% confidence interval.

Among the lymphoma patients (Table 4), only one patient (L1), had elevated sperm rates for disomy XY (1.45 *versus* 1.01), disomy 13 (0.79 *versus* 0.63), and diploidy (1.38 *versus* 1.14).

Among the 14 testicular cancer patients, 4 (T3, T4, T6, and T10) presented at least one value higher than that of the 95% confidence interval of the controls (Table 5). Spermatozoa of patient T10 showed elevated aneuploidy rates for X and Y disomies (24XX rate of 0.75 *versus* 0.29 and 24YY rate of 0.96 *versus* 0.49). The disomy 18 rate was also moderately elevated (0.48 *versus* 0.44) in this patient, whereas the diploidy rate showed an abnormal value (2.08 *versus* 1.14). Patient T6 showed slightly abnormal values for X and 21 disomy rates (0.36 *versus* 0.29 and 0.61 *versus* 0.56, respectively), and patient T3 only showed an abnormal diploidy rate (1.63 *versus* 1.14). Sperm analysis by FISH for patient T4 only revealed an elevated XY disomy rate (1.21 *versus* 1.01).

Several groups of patients were constituted according to the type of cancer (lymphoma or testis cancer), staging of cancer (I or T_1 *versus* III or T_3 of international classifications, respectively, for lymphomas or testicular cancer), treatment including chemotherapy *versus* no chemotherapy, treatment including radiotherapy *versus* no radiotherapy, treatment with underdiaphragmatic radiotherapy *versus* other radiotherapy, posttherapeutic delay (<1 year *versus* >1 year; <2 years *versus* >2 years), sperm counts (< *versus* >5 million spermatozoa/mL), teratozoospermia (> *versus* <80% abnormal spermatozoa), age of patients (>35 *versus* <35 years), tobacco, and toxic exposure. No significant increases in disomy or in diploidy rates were found when comparing the data observed between these different groups and with those observed in the control group (Mann-Whitney's test, $P > 0.05$). When the same parameters defining the above groups were compared between the patients with elevated aneuploidy rates and those with normal rates, no significant parameter was found different in these two groups (exact Fisher's test, $P > 0,05$).

Discussion and Overview of the Current Literature. Chemotherapy and radiotherapy are known to be responsible for gonadal damage in men. With recent chemotherapy protocols (without alkylating agents) and radiotherapy improvements, the impairment of spermatogenesis is transient in most cases (45, 46). In some patients, spermatogenesis recovery is sufficient enough for them to father children. Several studies have shown reassuring results concerning the health of these children (32–37). However, when spermatogenesis is only partially recovered, it is possible to help these patients to conceive by using assisted reproductive technologies. The question remains open as to the possibility that the spermatozoa produced in this

Table 3 Semen analysis before and after treatment

	Before treatment			After treatment		
Patients	Sperm count ($\times 10^6$/mL)	Sperm motility (%)	Teratospermia (%)	Sperm count ($\times 10^6$/mL)	Sperm motility (%)	Teratospermia
Lymphoma						
*L1**	*140*	60	65	*56*	60	71
L2	*54*	70	74	***10***	50	78
L3	22	**49**	**87**	26	60	77
L4	**15**	50	**87**	**10**	70	**84**
L5	*73*	50	68	***15***	50	73
L6	*110*	70	63	*28*	80	69
L7	*88*	60	**87**	*49*	**35**	68
L8	*133*	**40**	40	***0.6***	50	**95**
L9	*154*	60	23	*28*	50	**93**
L10	*72*	70	47	***12.3***	80	71
L11	*80*	60	12	***8.5***	**40**	**85**
L12	*60*	**30**	39	80	50	59
Testicular cancer						
T1	**1.5**	**5**	**98**	20	**40**	**92**
T2	*32*	60	66	21	**40**	71
*T3**	**15**	80	58	***2***	60	95
T4*	**9.5**	60	76	**8.5**	50	79
T5	*165*	50	61	***12***	50	60
*T6**	*35*	70	50	***5***	60	69
T7	*32*	60	49	***14***	**30**	56
T8	**10**	70	53	20	70	**90**
T9	*29*	**30**	52	***5.6***	**25**	**89**
T10*	**12**	50	74	32	50	**92**
T11	**14**	80	47	25	60	63
T12	*86*	70	70	***15***	60	72
T13	35	60	48	35	70	60
T14	36	50	66	25	**30**	**91**

NOTE. Patients who have decreased their sperm count after the treatment are mentioned in italic.
The abnormal sperm parameters are written in bold.
* Indicates the patients with increased aneuploidy rates.

context of posttherapeutic spermatogenesis impairment might have increased chromosomal abnormalities, which could be transmitted to the offspring.

Twenty-six patients were analyzed for sperm aneuploidy rates. The main observation is that most of the disomy/diploidy rates after cancer therapy did not significantly differ from those observed in the group of control healthy donors. Only five patients showed significant but still moderate increases in disomic and/or diploid sperm.

It is also noteworthy that none of our data suggest a correlation between elevated sperm aneuploidy rates and abnormal sperm parameters. Indeed, all lymphoma patients who showed oligozoospermia and/or teratospermia had no increase of aneuploidy rates. Among testicular cancer patients who had abnormal sperm aneuploidy rates, patients T3, T4, and T6 presented oligospermia with or without teratospermia, but patient T10 had normal sperm parameters. This observation is in contrast with many studies reporting an increased risk of sperm chromosomal abnormalities in infertile men with constitutive oligospermia or/and teratospermia (47–50). However, although the largest of its kind, this study still involves small numbers of patients, and definite conclusions could not be drawn concerning the relationship between sperm aneuploidy rates and sperm parameters in this population.

Within the group of lymphoma patients, only one patient (L1) showed increased sperm aneuploidy rates, including disomy XY, disomy 13, and diploidy. This patient had been treated for Hodgkin's disease 7 months before by three courses of Adriamycin-bleomycin-vinblastine-imidazole carboxamide (ABVD) therapy followed by lomboaortic irradiation (total radiation dose: 30 Gy over the course of 15 days). Patients L4 and L5, who had received similar treatments, respectively, 13 and 16 months before, showed no significant increases in their sperm aneuploidy rates. These data suggest that the short delay between the end of the treatment and sperm cytogenetic analysis could partly explain the increased aneuploidy rates in patient L1. Moreover, this 7 months delay was the shortest of all 38 patients in the study. Table 6 shows published studies about sperm chromosomal aneuploidy after treatment for lymphoma, classified according to the posttherapeutic delay. When time between treatment and analysis is <3 months, all authors agree to find a significant increase in the incidence of numerical and structural sperm chromosome aberrations (27, 28, 31). When the posttherapeutic delay exceeds 3 months, most studies do not show any increase in sperm aneuploidies (28, 31, 42, 51). Frias *et al.* (31) find ~18% of sperm carrying a numerical abnormality 35 to 50 days after Novantrone, Vincristine, Vinblastine, and Prednisone treatment for three lymphoma patients, but these elevated abnormality rates do not persist 1 to 2 years after treatment. In another study (28), sperm defects that were observed 35 to 50 days after the end of Novantrone, Vincristine, Vinblastine, and Prednisone treatment also declined to pretreatment levels within ~3 months (three patients studied). Brandriff *et al.* (38) found a

Table 4 FISH analysis for patients treated for lymphoma

Patients	No. of sperm nuclei scored	Disomies 13, 13, 21 (%)	Disomies 13, 21, 21 (%)	No. of sperm nuclei scored	Disomies X, X, 18 (%)	Disomies Y, Y, 18 (%)	Disomies X, Y, 18 (%)	Disomies X or Y, 18, 18 (%)	Diploidy (%)
L1*	2,673	**0.79**	0.22	4,075	0.17	0.37	**1.45**	0.25	**1.38**
L2	3,126	0.29	0.22	5,304	0.02	0.08	0.38	0.02	0.21
L3	4,717	0.28	0.25	5,094	0.04	0.10	0.16	0.06	0.43
L4	5,049	0.46	0.14	4,463	0.07	0.18	0.45	0.02	0.33
L5	5,005	0.36	0.20	4,316	0.19	0.19	0.30	0.07	0.39
L6	3,533	0.40	0.40	5,489	0.15	0.20	0.38	0.09	0.37
L7	3,831	0.16	0.55	5,064	0.12	0.49	0.22	0.08	0.12
L8	1,569	0.38	0.25	4,246	0.16	0.00	0.40	0.00	0.34
L9	4,023	0.40	0.32	4,096	0.12	0.07	0.51	0.17	0.36
L10	1,537	0.52	0.33	5,333	0.17	0.17	0.15	0.15	0.31
L11	3,834	0.31	0.13	5,150	0.06	0.14	0.43	0.02	0.36
L12	3,688	0.24	0.24	3,342	0.09	0.05	0.96	0.09	0.61
Controls (12 men)	Mean + 1.96SD	0.63	0.56	Mean + 1.96SD	0.29	0.49	1.01	0.44	1.14
Mean	38,749 sperm nuclei scored	0.40	0.30	59,962 sperm nuclei scored	0.16	0.18	0.45	0.15	0.54

NOTE. Patients with decreased sperm counts after the treatment are mentioned in italic. Abnormal aneuploidy rates are written in bold.
* Indicates patients with abnormal aneuploidy rates.

significant increase of sperm aneuploidy rates in 6 patients 3 to 20 years after nitrogen mustard-vincristine-procarbazine-prednisone therapy for Hodgkin's disease. However, nitrogen mustard-vincristine-procarbazine-prednisone treatment contains two alkylating agents, which are known to have a drastic effect on the genetic content of germ cells (52). Therefore, although the follow-up of the patients was not part of the present study, another sperm chromosome analysis would be interesting to perform in patient L1, 1 or 2 years after his therapy.

ABVD is currently designated as the standard chemotherapy for early and intermediate stages of Hodgkin's disease (12, 18). The effect of this treatment on sperm aneuploidy rates had not been published before. In the past, the most widely used treatment for Hodgkin diseases was nitrogen mustard-vincristine-procarbazine-prednisone, which contained two alkylating agents associated with radiotherapy. This combination is nowadays abandoned after demonstration that the nonalkylating-agent-containing protocol ABVD plus radiotherapy was superior to nitrogen mustard-vincristine-procarbazine-prednisone plus radiotherapy, with significantly fewer posttherapeutic azoospermia and lower carcinogenesis risks (26, 53–55). In the present study, 7 patients (L1, L4, L5, L7, L8, L11, and L12)

Table 5 FISH analysis for patients treated for testicular cancer

Patients	No. of sperm nuclei scored	Disomies 13, 13, 21 (%)	Disomies 13, 21, 21 (%)	No. of sperm nuclei scored	Disomies X, X, 18 (%)	Disomies Y, Y, 18 (%)	Disomies X, Y, 18 (%)	Disomies X or Y, 18, 18 (%)	Diploidy (%)
T1	4,824	0.19	0.29	7,413	0.15	0.28	0.53	0.03	0.38
T2	3,361	0.15	0.24	5,010	0.08	0.34	0.20	0.06	0.26
T3*	2,990	**0.70**	0.43	2,273	0.04	0.00	1.01	0.18	**1.63**
T4*	4,049	0.49	0.17	4,638	0.09	0.32	**1.21**	0.09	0.65
T5	3,119	0.26	0.29	2,768	0.07	0.14	0.18	0.11	0.11
T6*	1,627	0.55	**0.61**	1,685	**0.36**	0.06	0.77	0.00	0.12
T7	3,666	0.19	0.22	4,196	0.02	0.05	0.41	0.00	0.07
T8	2,461	0.16	0.08	4,060	0.07	0.00	0.15	0.12	0.05
T9	4,660	0.26	0.36	4,539	0.07	0.31	0.95	0.09	1.06
T10*	3,242	0.31	0.37	5,200	**0.75**	**0.96**	0.77	**0.48**	**2.08**
T11	3,620	0.52	0.25	4,671	0.04	0.21	0.36	0.11	0.73
T12	3,558	0.22	0.22	5,430	0.07	0.07	0.26	0.07	0.04
T13	3,848	0.26	0.23	5,639	0.04	0.05	0.11	0.02	0.02
T14	4,857	0.43	0.37	3,974	0.15	0.23	0.93	0.10	0.38
Controls (12 men)	Mean + 1.96SD	0.63	0.56	Mean + 1.96SD	0.29	0.49	1.01	0.44	1.14
Mean	38,749 sperm nuclei scored	0.40	0.30	59,962 sperm nuclei scored	0.16	0.18	0.45	0.15	0.54

NOTE. Patients with decreased sperm counts after the treatment are mentioned in italic. Abnormal aneuploidy rates are written in bold.
* Indicates patients with abnormal aneuploidy rates.

Table 6 Chromosome abnormalities after treatment for lymphoma: results of the published studies

Reference no.	Disease	No. of patients	Treatment	Type of analysis	Chromosomes studied by FISH	No. of sperm nuclei scored per patient	Increased chromosome abnormalities	Time between treatment and analysis
Rousseaux *et al.* (27)	HD	2	RT + vinblastine	Caryotype		# 40	Yes	1 day
Frias *et al.* (31)	HD	4	NOVP +/− RT	FISH	X,Y,18,21	10,000	Yes	1–2 mo
Robbins *et al.* (28)	HD	4	NOVP	FISH	8,X,Y	10,000	Yes	1–2 mo
Robbins *et al.* (28)	HD	3	NOVP	FISH	8, X, Y	10,000	No	3 mo to 3 y
Monteil *et al.* (51)	HD	1	RT + vinblastine	FISH	1,6,11,X,Y		No	1 y
Frias *et al.* (31)	HD	3	NOVP	FISH	X,Y,18,21	10,000	No	1–2 y
Martin *et al.* (42)	NHL	1	MACOP-B	Caryotype		193	No	3 y
				FISH	1, 12	10,000	No	
					1, X, Y	10,000	No	
Present study	HD (10) NHL (2)	12	CT +/− RT	FISH	X, Y, 13, 18, 21	Mean 4,660/ patient	Yes (1/12) No (11/12)	7 mo to 5 y
Brandriff *et al.* (38)	HD	6	MOPP +/− RT	Caryotype		571 for 6 patients	Yes	3–20 y

Abbreviations: HD, Hodgkin's disease; RT, radiotherapy; NOVP, novantrone, vincristine, vinblastine, prednisone; NHL, non-Hodgkin's lymphoma; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone; CT, chemotherapy (several protocols—please see text); MOPP, mustine, vincristine, procarbazine, prednisone.

were treated by ABVD associated with radiotherapy or other chemotherapy protocols. Interestingly, in all patients but L1 (with short posttherapeutic delay), the frequency of sperm chromosomal abnormalities was not increased compared with that of control donors.

Patient L9 was treated for Burkitt's lymphoma and patient L10 for diffuse large B-cell lymphoma, which are both non-Hodgkin's aggressive B-cell lymphomas. Chemotherapy for non-Hodgkin's lymphoma usually involves several drugs and frequently combines cyclophosphamide, doxorubicin, vincristine, and prednisone (13, 56). The two patients received these types of drugs combined with others and presented sperm aneuploidy rates similar to the controls. One other study (showed in Table 6), using sperm karyotyping and FISH techniques, demonstrated no increased frequency of numerical chromosomal abnormalities in one patient treated for non-Hodgkin's lymphoma by methotrexate, doxorubicin, cyclophosphamide, vincristine, and prednisone 3 years prior (42). Taken together, our study and the data presented in 1995 by Martin *et al.* (57) give information about the chromosomal effects of non-Hodgkin's lymphoma treatment for only three patients, despite non-Hodgkin's lymphoma being the fifth most frequent cancer. There are two explanations for this. First, this disease is rare in people <30 years old, and its frequency increases with age. Second, young men affected by aggressive non-Hodgkin's lymphoma are most commonly treated by high doses of chemotherapy containing alkylating agents such as cyclophosfamide, which can be responsible for definitive azoospermia. It is noteworthy that the other four non-Hodgkin's lymphoma patients included in the present study were azoospermic (Table 1). However, our results seem to be reassuring for the few non-Hodgkin's lymphoma patients whose spermatogenesis is preserved after chemotherapy.

In the testicular cancer group of patients, 10 of 14 showed no increase in their sperm aneuploidy rates compared with the control population. The other four showed one (T4 and T6) or

Table 7 Chromosome abnormalities after treatment for testicular cancer: results of the published studies

Authors	Histology	No. of patients	Treatment	Type of analysis	Chromosomes studied by FISH	No. of sperm nuclei scored	Increased chromosome abnormalities	Time between treatment and analysis
De Mas *et al.* (40)	Nonspecified	5	BEP	FISH	18/X/Y	10,000/patient	Yes	6–17 mo
					7/16	10,000/patient		
Jenderny *et al.* (41)	S	1	BVP	Caryotype		63	No	9 mo
Martin *et al.* (39)	NS	1	BEP	FISH	1/12	10,000	Yes	1 y
Genesca *et al.* (58)	NS	2	BEP or BVP	Caryotype		100–118/patient	Yes for structural abnormalities but not for numerical abnormalities	2–5 y
This study	S (10) NS (4)	14	RT (8) (B)EP (5) EP + RT (1)	FISH	X, Y, 13, 18, 21	Mean 3563/patient	No (10) Yes (4)	19 mo to 5 y
Martin *et al.* (43)	NS	4	BEP	FISH	1/12	10,000/patient	No	2–13 y
					1/X/Y	10,000/patient		
Martin *et al.* (44)				Caryotype		46–187/patient (mean: 137/patient)	No	

Abbreviations: BEP, bleomycin, etoposide, cisplatin; S, seminoma; NS, nonseminomatous tumor; BVP, bleomycin, vinblastine, cisplatin; RT, radiotherapy.

more (T3 and T10) increased aneuploidy rates. For these patients, neither the posttherapeutic delays nor the type of cancer or treatment could be correlated with these increased rates. Table 7 presents a synthesis of five published studies on sperm aneuploidies after bleomycin or bleomycin vinblastine cisplatin chemotherapy for testicular cancer. In an early study, the cytogenetic analysis of ~100 sperm chromosome metaphases in two patients 2 and 5 years after treatment (58) showed a significant increase of sperm chromosome structural aberrations but no increase in numerical abnormalities. In two studies, the authors found abnormal sperm aneuploidy rates in six patients 6 to 17 months after treatment by bleomycin (39, 40). In two other studies, the authors found no increased sperm aneuploidy rates in one patient 9 months after a bleomycin vinblastine cisplatin treatment (41) and in four patients 2 to 13 years after bleomycin treatment (43, 44). These results seem contradictory, but it is difficult to conclude with only 13 testis cancer patients studied thus far with different clinical backgrounds and posttherapeutic delays.

For the testicular cancer patients, no direct link could be found between the parameters of the treatment and the abnormal sperm aneuploidy rates. It is possible that the elevated aneuploidy rates observed in some patients could have preceded the treatment. However, none of these four patients had lifestyle or toxic risk factors, which could be associated with increased chromosomal abnormalities in sperm nuclei (59–61), such as cigarette smoking or professional toxic exposure. Considering a possible effect of the cancer itself on sperm aneuploidy rates, it has been published that spermatozoa from untreated testicular cancer patients do not show an increased chromosomal instability (39, 43, 62).

Our results suggest that chemotherapy and radiotherapy used for testicular cancer or lymphoma might not induce long-term aneuploidies in sperm. However, although a population of 26 patients is analyzed here, which is more than any published studies thus far, the number of patients for each disease still remains small. In addition, exposure to anticancer therapy may also induce other types of genetic defects in sperm such as structural chromosomal abnormalities or DNA damages (63), which were not explored here and would need to be investigated with appropriate methods. Taken together, our data combined with those of previous studies suggest that the risk of aneuploidy in the offspring of patients treated for testis cancer or lymphoma is not much higher than in the general population. However a 2-year posttherapeutic delay before conceiving should be highly recommended in these situations.

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