Impact of Hodgkin or non-Hodgkin lymphoma and their treatments on sperm aneuploidy: a prospective study by the French CECOS network

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Objective: To assess sperm production and aneuploidy in Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) before and after treatments.
Design: Multicenter, prospective, longitudinal study of lymphoma patients analyzed before treatment and after 3, 6, 12, and 24 months.
Setting: University hospitals.
Patient(s): Forty-five HL and 13 NHL patients were investigated before and after treatment. Treatment regimens were classified in two groups: ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) with or without (±) radiotherapy, and CHOP (doxorubicin, cyclophosphamide, vincristine, prednisone)/MOPP-ABV (mechlorethamine, oncovin, procarbazine, prednisone–doxorubicin, bleomycin, vinblastine). A control group of 29 healthy men was also studied.
Intervention(s): Semen analyses and aneuploidy study by FISH were performed at each time point.
Main Outcome Measure(s): Comparison of mean sperm characteristics and percentage of sperm aneuploidy rates before and after treatment.
Result(s): Before treatment, HL and NHL men had altered semen characteristics and higher sperm aneuploidy rates (median 0.76 [interquartile range 0.56–0.64]) than the control group (0.54 [0.46–0.74]). After treatment, sperm production was significantly lowered 3 and 6 months after ABVD ± radiotherapy or CHOP/MOPP-ABV. After ABVD ± radiotherapy, the aneuploidy rate increased significantly only at 3 months, and values obtained 1 or 2 years later were lower than pretreatment values. In contrast, in the CHOP/MOPP-ABV treatment group, semen characteristics and aneuploidy rate did not return to normal levels until 2 years after treatment.

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Conclusion(s): Lymphoma itself has consequences on sperm aneuploidy frequency before treatment. Moreover, lymphoma treatments have deleterious effects on sperm chromosomes related to treatment type and time since treatment. Patient counseling is essential concerning the transient but significant sperm aneuploidy induced by lymphoma and its treatments. (Fertil Steril® 2017;107: 341–50. ©2016 by American Society for Reproductive Medicine.)
Key Words: Hodgkin, non-Hodgkin lymphoma, sperm aneuploidy, sperm chromosomes, treatment side effects

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Lymphomas are the second most common malignant disease in men of reproductive age after testicular cancer. In 2012, the age-standardized incidence rates for Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) were 2.5 and 12, respectively, per 100,000 men in France, and 1.1 and 6.0 worldwide (1). Depending on the type and stage of lymphoma, conventional treatment usually includes chemotherapy or radiotherapy, associated or not with monoclonal antibodies. Recent advances in antineoplastic treatments allow most patients to achieve remission or cure. Overall survival rates at 5 years are 80% for HL and 55% for NHL. However, treatment often carries side effects such as male gonadal toxicity, and it has a direct impact on posttreatment quality of life.

Spermatogenesis alterations depend on a set of heterogeneous factors, such as the disease itself, the type and dosage of the treatments received, and also individual parameters such as the patient's general condition, history of genital diseases, or previous abnormal sperm quality production. Studies evaluating sperm quality at diagnosis of HL (2–4) showed that 59% to 77% of patients present at least one abnormal sperm parameter before treatment. Similarly, abnormal spermatogenesis is commonly observed at the time of NHL diagnosis (5–7). Several hypotheses have been proposed to explain these pretreatment alterations: disease stage or presence of B-symptoms, in particular fever and night sweats, have been proposed by some authors (2, 4, 8, 9) but not by others (3, 5, 10, 11), as well as elevated erythrocyte sedimentation test values (2, 4). An immune-mediated disorder in HL patients could also explain sperm alterations before treatment (12). Concerning chemotherapy, alkylating agents carry the highest risk of infertility (13, 14). Up to 90% of patients treated with alkylating agents such as mustine, procarbazine, or cyclophosphamide show prolonged azoospermia (15). A study examining the impact of the BEACOPP protocol (doxorubicin, cyclophosphamide, etoposide, bleomycin, vincristine, procarbazine, prednisone), which contains two alkylating agents, found an 89% azoospermia rate after treatment (16). The MOPP (mechlorethamine, oncovin, procarbazine, prednisone) or COPP (cyclophosphamide, vincristine, procarbazine, prednisone)/ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) protocols cause an infertility rate between 60% and 91%, depending on the number of courses received (3, 17). The ABVD protocol, currently the standard reference for HL treatment, is associated with a lower gonadotoxic risk, and spermatogenesis is recovered in 90% of cases (18).

The association of chemotherapy and radiotherapy allows a reduced chemotherapy dose, which decreases treatment toxicity. Conventionally, a total dose of 36–40 Gy is administered fractionally. This dose may be reduced to 20 Gy depending on the cancer stage. Gonads have a high sensitivity to radiotherapy, and although irradiation areas are now precisely defined, the radiation doses received by the gonads are not negligible. Single doses above 400 cGy cause temporary or permanent azoospermia (19). Fractionated irradiation is more harmful than an acute dose (20–22).

In addition to the induction of sperm production alterations by cancer treatment, there are concerns that treatment has possible mutagenic effects on germ cells that consequently could increase the risks of embryo or birth defects, genetic disorders, or cancer among the children of patients treated for cancer.

Before lymphoma treatment, an increase in sperm DNA damage has been reported due to the disease itself (23, 24). After treatment, only a few studies, mostly with small patient series, have reported the effects of treatment on sperm DNA. Sperm from patients who survived their lymphoma present DNA damage after treatment (5, 25). A high DNA fragmentation index was observed until 2 years after the end of treatment and was usually associated with abnormal chromatin compaction (26). Conversely, other studies (27, 28) found no increase of DNA fragmentation index after lymphoma treatment.

Sperm chromosomes have been studied after cancer treatment, first by sperm karyotyping and more recently using FISH. Only a few studies have examined sperm aneuploidy after lymphoma treatment, and most included small patient series. All the studies showed a relationship between antineoplastic therapy and increased rates of sperm aneuploidy (29–36). This effect is described as transient, with a significant increase immediately after the end of treatment and a decline over time. It is noteworthy that discordant results have been published concerning the interval necessary after treatment before a return to pretreatment value, from 100 days to 1 year or more. One study reported an increased rate of aneuploidy 3 to 20 years after HL treated by the very highly gonadotoxic MOPP chemotherapy (29). All chromosomes seem to be affected by this increase, but in different proportions, the sex chromosomes being most affected. It is difficult to compare

results between studies, owing to several factors: the number of lymphoma patients was small, with 12 patients in the largest cohort (36); the studies included different chemotherapy protocols associated or not with radiotherapy. To the best of our knowledge, except for one study (35) that included a small number of patients (n = 11), there has been no longitudinal prospective follow-up of a large series of patients, owing to the difficulty of obtaining repeated samples from the same patient.

Because of the potential risk of severe sperm alterations due to anticancer therapy, patients in France are usually informed about sperm cryopreservation and referred to sperm banking facilities (Centres d'étude et de conservation des œufs et du sperme humains, or CECOS). After treatment, if sperm recovery is sufficient, the use of frozen or fresh sperm is often debated, and particular attention is paid to the quality of the gametes, including sperm aneuploidy.

In this context, the main objective of our prospective, longitudinal study was to determine sperm aneuploidy rates before and after chemotherapy, combined or not with radiotherapy, in the largest prospective and multicenter cohort of lymphoma patients published to date. These data may be of considerable value in improving counseling of patients who wish to become parents after their treatment.

MATERIALS AND METHODS

Patients

This study was part of the collaborative and prospective GAMATOX research project, supported by a French national research grant (Programme Hospitalier de Recherche Clinique PHRC no. 20030222) and conducted in eight CECOS fertility preservation centers (Caen, Clermont-Ferrand, Grenoble, Marseille, Paris Cochin, Paris Tenon, Rouen, and Toulouse). These centers are part of the national network, the Fédération Française des CECOS. The present study enrolled 74 patients who were referred to the CECOS for sperm banking before cancer treatment. As a control group, we recruited 29 healthy men who were candidates for sperm donation or who were referred for sperm preservation before vasectomy. This study was approved by the institutional ethics review board (CCPPRB Toulouse Sud-Ouest II), and all volunteers gave their written informed consent.

All patients and controls provided one semen sample (before treatment), and the patients were also asked to produce four other samples 3 months (T3), 6 months (T6), 12 months (T12), and 24 months (T24) after the end of treatment according to the study design (Supplemental Fig. 1, available online).

Of the 74 patients included, 58 (78%) were followed during the study period. Patients with HL (n = 45) and NHL (n = 13) were treated by the various chemotherapy protocols with or without (±) radiotherapy, depending on diagnosis and the disease stage. The treatments were classified in two groups (Supplemental Table 1): an ABVD ± radiotherapy group (50 patients) and a CHOP (doxorubicin, cyclophosphamide, vincristine, prednisone)/MOPP-ABV group (8 patients).

Semen Analyses

As described previously (5, 37), semen samples were collected in a sterile container after masturbation. Liquefaction was obtained after 30 minutes at 37°C. Semen analysis was performed according to the World Health Organization 1999 guidelines. The eight centers all used similar methodology and participated in external quality control of semen analysis. Because aneuploidy rates are not significantly different in fresh and frozen sperm (38, 39), all samples were frozen so that aneuploidy rates could all be evaluated together at the same time. The remaining semen samples were mixed with a cryoprotectant, frozen in straws, and stored in liquid nitrogen until FISH analysis. All the samples were then included in the certified GERMETHEQUE research biobank (BB-0033-00081) after a standardized procedure of anonymization.

Fluorescence in Situ Hybridization

The FISH procedure was performed as previously described (37). Briefly, for each patient or control, one straw was thawed at 37°C for 10 minutes. Samples were washed twice with phosphate-buffered saline 1× and fixed in a methanol/acetic acid (3:1, vol/vol) solution. Sperm head decondensation was performed in NaOH 1 M solution. Samples were then hybridized with Abbott Vysis centromeric probes (Abbott Laboratories) CEP 18 (18p11.1-q11.1, D18Z1, SpectrumAqua), CEP X (Xp11.1-q11.1, DXZ1, SpectrumGreen), and CEP Y (Yp11.1-q11.1, DYZ3, SpectrumOrange), according to the Vysis protocol in a HYBrite system (Abbott Laboratories). Sperm nuclei were counterstained in a 0.5 μg/mL Hoechst solution for 3 minutes and mounted with antifade.

Aneuploidy Scoring

Sperm chromosome content was blindly scored by three trained technicians in the Grenoble center, following strict reading criteria, and approximately 5,000 cells were counted per sample (37). We previously demonstrated that no difference is observed between standard manual reading and automatic reading (37). Half of the cells were scored on a Metafer Metasystems device as described previously (37), whereas the other half was scored manually on a Nikon Eclipse 80i epifluorescence microscope.

Statistical Analyses

All data were reported on centralized case report forms by web access and were verified by the coordinating center in Toulouse. Data were compared between the control and the lymphoma patient groups using the nonparametric Mann-Whitney test. Sperm aneuploidy data of lymphoma patients were compared before and after treatment by the Wilcoxon signed rank-sum test. Data are presented as median and interquartile range (Q1–Q3) for the tables, and as mean and SD for the figures. Abnormal individual aneuploidy rate was considered significant when it deviated by at least 2× SD from the mean baseline level in controls (40). Statistical analysis was performed using SAS software (9.3, SAS Institute), and $P<.05$ was considered statistically significant.

RESULTS

A total of 1,129,805 spermatozoa were scored by FISH. Each time, a mean of 4,970 (±14) and 4,904 (±457) spermatozoa were scored for controls and lymphoma patients, respectively.

Before Lymphoma Treatment (74 Lymphoma Patients and 29 Controls)

A significant decrease in sperm volume, sperm vitality, and total sperm count per ejaculate was observed in the cancer groups compared with controls (Table 1). The rate of abnormal chromosomal content was significantly higher in the cancer groups, for both types of lymphoma, when compared with controls ($P=.0003$). Hyperhaploid XY spermatozoa were more frequent in the patient group than in controls ($P=.005$ for all lymphomas, $P=.008$ for HL, and $P=.047$ for NHL), as was disomy for chromosome 18 ($P=.005$ for all lymphomas, $P=.10$ for HL, and $P=.025$ for NHL).

After Lymphoma Treatment (58 Patients)

The largest group of patients was treated by ABVD ± radiotherapy (n = 50 before treatment). Patient compliance with the survey was good to excellent (from 56% to 100% according to the different time points and type of treatment).

Table 2 shows that a significant decrease in sperm count and total sperm count was observed in all patient groups 3 months after the end of treatment. The greatest decrease was observed in patients treated by CHOP/MOPP-ABV at this time point. Twelve months after the end of treatment, median sperm counts did not significantly differ from pretreatment values, and they returned to normal values in both groups of patients, although the median total sperm count was fivefold lower than the pretreatment value in the CHOP/MOPP-ABV group. It was noteworthy that in the latter group, median sperm count was 0 at 3 months and near 0 at 6 months. In patients treated by ABVD ± radiotherapy, the total sperm count was significantly higher 24 months after the end of treatment when compared with the pretreatment value.

Before treatment the chromosomal abnormality rate was higher in lymphoma patients than in controls ($P<.001$). Considering all patients independently of treatment regimen, total chromosomal abnormality and disomy rates increased significantly 3 months after the end of treatment ($P=.0004$) (Fig. 1).

In the ABVD ± radiotherapy–treated group of patients, median total chromosomal abnormalities increased 3 months after the end of treatment (0.83 [interquartile range 0.54–1.43] vs. 0.75 [0.56–0.94] before treatment) (Table 3, Supplemental Fig. 2). This increase was mainly due to hyperhaploid 24,XY spermatozoa, whose median increased 1.5-fold. Total chromosomal abnormality and 24,XY sperm rates were significantly lower 12 and 24 months after treatment than before treatment ($P<.001$).

In the CHOP/MOPP-ABV group, some patients were studied 3 and 6 months after treatment (Tables 2 and 3). This chemotherapy protocol resulted in azoospermia or severe oligospermia at these time points. In two patients FISH analysis at 3 months revealed increased median values of disomic and diploid sperm and of total chromosomal abnormalities compared with pretreatment values. Only one patient in this group underwent FISH study 6 months after treatment: sperm aneuploidy was increased, 24,XY, 24X/Y+18, disomic sperm, and total chromosomal abnormalities

TABLE 1

Comparative analysis of sperm characteristics and chromosome content (in %) in the control group and the lymphoma group (HL and NHL) before treatment.

Parameter	Control group (n = 29)		Lymphoma group (n = 74)		HL group (n = 56)		NHL group (n = 18)	
Sperm characteristics								
Volume (mL)	3.50	3.09–5.09	3	2.09–4.09[a]	3.05	2.05–4.55[a]	2.90	2.50–3.70[a]
Sperm count (×10^6/mL)	86.25	19–126	46.39	22–75	45.89	22.50–64.35	48	19–102
Vitality (%)	71	64–82	61	53–73[a]	60	54–72[a]	65	51–80
Motility (%)	45	40–50	40	30–50	40	32–47	35	30–50
Total sperm count (×10^6/ejaculate)	270	157–379	120	60–272[a]	120	59–234[a]	126	76–328
Total motile sperm count (×10^6/ejaculate)	114	56–206	55	19–112[a]	50	19–104[a]	62	35–133
Chromosome content								
Haploid	99.45	99.25–99.53	99.23	99.05–99.43[a]	99.23	99.05–99.41[a]	99.24	99.07–99.47[a]
24,XY	0.26	0.20–0.36	0.36	0.26–0.48[a]	0.36	0.26–0.46[a]	0.36	0.26–0.50[a]
24,YY	0.06	0.02–0.08	0.06	0.04–0.10	0.06	0.06–0.10	0.07	0.04–0.08
24,XX	0.04	0.02–0.08	0.06	0.06–0.08[a]	0.08	0.06–0.08[a]	0.06	0.06–0.08
24,X/Y,+18	0.08	0.06–0.12	0.12	0.08–0.16[a]	0.12	0.08–0.16[a]	0.12	0.10–0.14[a]
Disomy	0.48	0.36–0.61	0.66	0.48–0.80[a]	0.66	0.48–0.80[a]	0.64	0.48–0.80[a]
Diploidy	0.08	0.04–0.12	0.10	0.06–0.14	0.10	0.08–0.14[a]	0.07	0.02–0.12
Total chromosomal abnormalities	0.54	0.46–0.74	0.76	0.56–0.94[a]	0.76	0.58–0.95[a]	0.75	0.52–0.92[a]

Note: Values are expressed as median and interquartile range (Q1-Q3). Haploid = sum of frequencies of 23,X and 23,Y spermatozoa. Disomy = sum of frequencies of 24,XX, 24,YY, 24,XY, and 24,X/Y,+18 spermatozoa. Diploidy = sum of frequencies of 46,XX, 46,YY, and 46,XY diploid spermatozoa. Total chromosomal abnormalities = sum of frequencies of disomic and diploid spermatozoa.

[a] $P<.05$, difference between control group and lymphoma group, HL group and NHL group.

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TABLE 2

Sperm characteristics according to treatment and time points in the 58 lymphoma patients.

Treatment regimen	Before treatment		After treatment: 3 mo		6 mo		12 mo		24 mo	
ABVD ± radiotherapy										
N (%)[a]	50		41 (82)		38 (76)		37 (74)		28 (56)	
Volume (mL)	3.15	2.50–4.59	3.20	2.80–4.40	3.55	2.30–4.80	3.70	2.40–4.90	4.25	3–6.40[b]
Sperm count (10^6/mL)	40.50	22–66	10.80	6.59–20[b]	20.30	4.80–52[b]	40	16–65	42	28.30–75.50
Vitality (%)	60	52–74	64	52–78	66	50–75	66	60–76[b]	70	62–75[b]
Motility (%)	40	30–48	35	30–45	35	30–48	40	30–45	41	35–50[b]
Total sperm count ($\times 10^6$/ejaculate)	130.83	59.78–272.59	36	16.79–89.25[b]	56.86	20.99–146.78[b]	128.95	55–271.82	168.40	106.54–329.79[b]
Total motile sperm count ($\times 10^6$/ejaculate)	53.61	18.34–112.01	15.92	3.99–35.70[b]	17.63	11.34–82.36[b]	51.58	19.73–124.02	97.60	39.24–157.66[b]
CHOP/MOPP-ABV										
N (%)[a]	8		8 (100)		8 (100)		8 (100)		7 (88)	
Volume (mL)	2.85	2.24–3.30	2.60	1.94–3.90	2.09	2.09–4.05	2.50	2.15–3.55	2.59	2.09–3.80
Sperm count (10^6/mL)	83	47.39–114.59	0	0–0.27[b]	0.01	0–7.55[b]	23.10	0.20–61.50	57.59	23–160
Vitality (%)	76	53–80	0	0–40	22	0–61	76	68–82	81	78–85
Motility (%)	48	35–57	0	0–30	16	0–50	50	30–50	55	30–60
Total sperm count ($\times 10^6$/ejaculate)	277.92	115.74–376.15	0	0–0.57[b]	0.04	0–17.42[b]	53.59	0.50–212.23	269.50	48.29–607.99
Total motile sperm count ($\times 10^6$/ejaculate)	115.95	59.49–143.25	0	0–0.34[b]	0.04	0–20.69[b]	26.79	0.07–134.76	107.80	28.97–297.85

Note: Values are expressed as median and interquartile range (Q1–Q3) unless otherwise noted.
[a] N = % of compliance with the survey.
[b] $P < .05$, difference between before-treatment and after-treatment values (3, 6, 12, and 24 months).

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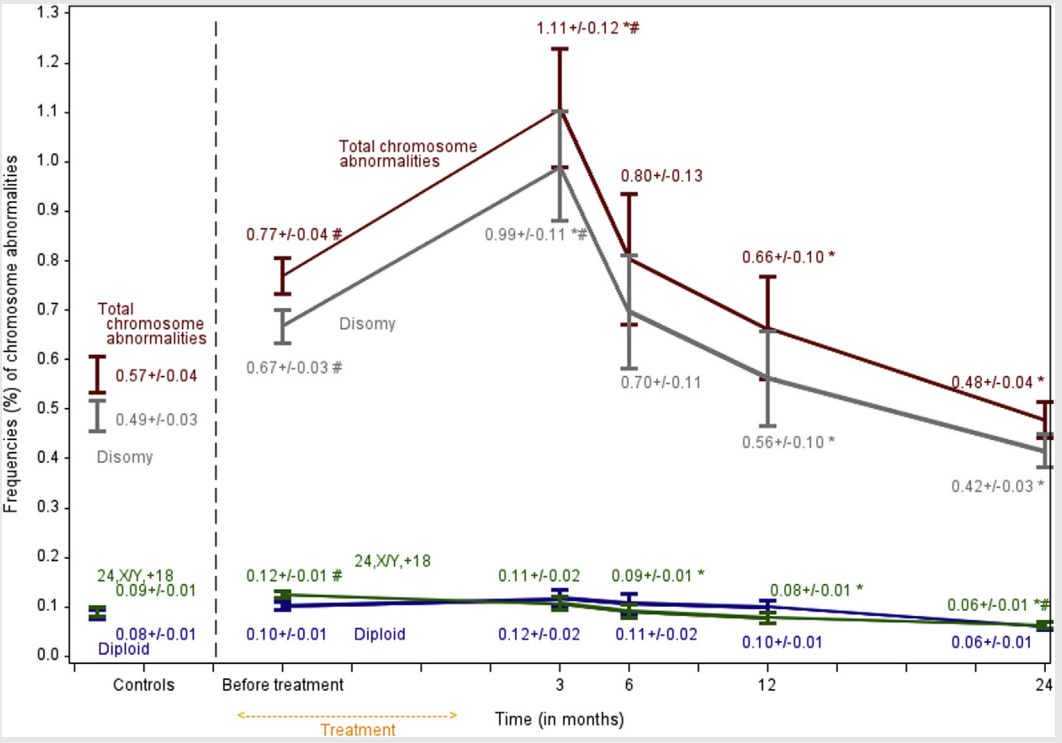
FIGURE 1



Mean percentages of chromosomal abnormalities and SEMs before and during posttreatment follow-up: total chromosomal abnormalities, disomy, 24*X*/*Y*,+18, and diploidy. **P*<.05, pre- and posttreatment difference. #*P*<.05, difference between controls and lymphoma patients. Means and SDs are given at each time point.

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values were the highest found in any patient in our series. At 12 months the median value of 24,XY sperm was significantly higher than the pretreatment median. All aneuploidy parameters returned to pretreatment values at 24 months.

It is important to identify those patients who have a sperm aneuploidy value above the upper limit of the control group (greater than mean + 2× SD of controls [40]). In the ABVD ± radiotherapy–treated group, the number of patients with total chromosomal abnormalities value above normal values increased twofold at 3 months (42%) compared with before treatment (22%). Three times as many patients had abnormal values of 24,XY (47% vs. 14%) or 24,YY (11% vs. 4%) sperm at 3 months. At 6 months, twice as many patients had abnormal values of 24,XY sperm (28%) compared with before treatment (14%).

Although few patients had FISH analysis in the CHOP/MOPP-ABV treated group, all had abnormal disomy and total chromosomal abnormalities rates at 3 and 6 months. The number of patients with abnormal values of 24,XY (50% vs. 25%) and diploid sperm (50% vs. 13%) was increased twofold and threefold, respectively, at 12 months.

DISCUSSION

The present study demonstrates that HL and NHL patients have altered sperm parameters and increased aneuploidy frequency before cancer treatment compared with a control group of healthy men, and that they have abnormal sperm chromosome content from 3 to at least 12 months after the end of treatment. The total sperm abnormality frequency was related to type of treatment and to time since the end of treatment.

To the best of our knowledge, this is the first report based on a standardized, prospective protocol that included the largest lymphoma population in which sperm aneuploidy was followed serially in the same patients before and after treatment (for review of published studies, see Supplemental Table 2). The time points were precisely defined, and sperm was systematically evaluated at all these time points.

Semen parameters followed a similar course before and after cancer treatment, as previously reported in the overall population (5). Before treatment, sperm production (sperm count, total sperm count) and ejaculate volume were lower than in the control group. These sperm alterations before treatment in HL or NHL have been reported by several authors (2–7, 27).

Moreover, we demonstrate that HL and NHL patients had a higher rate of sperm aneuploidy before treatment than healthy controls. Only three studies (30, 33, 35) have reported similar results, but in small series of 2, 5, and 12 patients. The precise mechanisms of sperm chromosome alterations in lymphoma patients before treatment are not known. However, previous studies found increased frequency of sperm aneuploidy in infertile men with sperm

TABLE 3

Sperm chromosome content rates (in %) according to treatment and time point in the 58 lymphoma patients.

Treatment regimen	Before treatment		After treatment: 3 mo		After treatment: 6 mo		After treatment: 12 mo		After treatment: 24 mo	
ABVD ± radiotherapy										
N		50		36[a]		32[a]		34		26
Haploid	99.24	99.05–99.43	99.16	98.56–99.45[b]	99.42	99.11–99.57	99.52	99.35–99.61[b]	99.59	99.45-99.67[b]
24,XY	0.34	0.22–0.46	0.51	0.31–1.02[b]	0.33	0.23–0.56[b]	0.27	0.16–0.40[b]	0.23	0.12–0.32[b]
24,YY	0.08	0.06–0.10	0.04	0.02–0.08	0.02	0.02–0.06[b]	0.04	0.02–0.06[b]	0.04	0.02–0.06[b]
24,XX	0.08	0.06–0.08	0.04	0.03–0.08	0.03	0–0.06[b]	0.04	0.02–0.06[b]	0.04	0.02–0.06[b]
24,X/Y,+18	0.12	0.08–0.16	0.09	0.04–0.13	0.08	0.04–0.10[b]	0.06	0.04–0.08[b]	0.06	0.04–0.08[b]
Disomy	0.65	0.46–0.80	0.70	0.45–1.35	0.52	0.34–0.76	0.40	0.28–0.56	0.32	0.28–0.50[b]
Diploidy	0.10	0.08–0.12	0.08	0.06–0.14[b]	0.08	0.04–0.12	0.10	0.06–0.12[b]	0.06	0.04–0.08[b]
Total chromosomal abnormalities	0.75	0.56–0.94	0.83	0.54–1.43[b]	0.57	0.42–0.88	0.48	0.38–0.64[b]	0.40	0.32–0.54[b]
CHOP/MOPP-ABV										
N		8		2[a]		1[a]		6		6
Haploid	99.24	98.82–99.46	97.80	96.62–98.99	95.36		99.17	98.75–99.19	99.37	99.19–99.49
24,XY	0.36	0.30–0.71	1.41	0.70–2.11	3.16		0.51	0.34–0.92[b]	0.38	0.26–0.62
24,YY	0.06	0.06–0.08	0.11	0–0.22	0.18		0.06	0.04–0.08	0.04	0.04–0.06
24,XX	0.06	0.05–0.09	0.08	0–0.16	0.18		0.05	0.04–0.06	0.04	0.04–0.06
24,X/Y,+18	0.13	0.07–0.17	0.23	0.15–0.32	0.40		0.11	0.08–0.22	0.05	0.04–0.08
Disomy	0.64	0.46–1.09	1.84	0.85–2.82	0.69		0.72	0.64–1.16	0.52	0.40–0.74
Diploidy	0.07	0.05–0.11	0.35	0.15–0.54	3.94		0.13	0.02–0.18	0.08	0.04–0.14
Total chromosomal abnormalities	0.75	0.53–1.17	2.19	1.01–3.37	4.64		0.82	0.81–1.24	0.62	0.50–0.80

Note: Values are expressed as median and interquartile range (Q1–Q3) unless otherwise noted. Haploid = sum of frequencies of 23,X and 23,Y spermatozoa. Disomy = sum of frequencies of 24,XX, 24,YY, 24,XY, and 24,X/Y,+18 spermatozoa. Diploidy = sum of frequencies of 46,XX, 46,YY, and 46,XY diploid spermatozoa. Total chromosomal abnormalities = sum of frequencies of disomic and diploid spermatozoa.

[a] Number of patients fell at 3 and 6 months owing to azoospermia or cryptozoospermia (impossible to perform FISH analysis).

[b] $P < .05$, difference between before treatment and after treatment values (3, 6, 12, and 24 months).

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alterations (41–43). So the hypotheses suggested to explain alteration of semen parameters in lymphoma patients before treatment could also explain increased sperm aneuploidy. It is noteworthy that fever can have a negative impact on sperm chromatin (44, 45), but in our global study (5) we found no link between fever episodes and sperm alterations before treatment in our patients. Further studies are needed to explore the links between cancers and sperm genome alterations before any treatment is given.

Several studies have investigated sperm aneuploidy following treatment of lymphoma. However, these studies included small series of lymphoma patients (from 1 to 12) and used different methods, such as sperm karyotyping (29, 31, 34) or FISH (30–33, 35, 36), whereas one study used single sperm array comparative genomic hybridization (46). Moreover, these were retrospective or case report studies, with the exception of one prospective study (35). The main strength of the present study is that it was prospective, with serial analysis of patients' sperm. It is also the first study in which sperm aneuploidy was examined after treatment with two different regimens.

After ABVD ± radiotherapy and CHOP/MOPP-ABV, sperm aneuploidy frequency was increased 3 months after the end of treatment. The greatest increase of aneuploidy was found after CHOP/MOPP-ABV treatment. For example, XY disomy increased approximately twofold after ABVD ± radiotherapy but threefold after CHOP/MOPP-ABV therapy 3 months after the end of these treatments.

It is noteworthy that 6 and 12 months after ABVD ± radiotherapy, aneuploidy frequency decreased to reach values lower than pretreatment values (i.e., the values found in the control group). This trend was probably due to the eviction of cancer conditions that induced the pretreatment increase of aneuploidy observed in our patients.

Conversely, CHOP/MOPP-ABV treatment increased aneuploidy 6 and 12 months after the end of treatment. For example, the frequency of disomy XY, which was multiplied by 3 at 3 months, was multiplied six- and twofold at 6 and 12 months, respectively, after the end of treatment. Aneuploidy frequency only returned to normal values 24 months after CHOP treatment.

Moreover, after having defined the upper normal values of total chromosomal abnormalities and disomy (i.e., the mean +2 SD of the control group), we showed that nearly 45% and 22% of lymphoma patients had abnormal values at 3 and 6 months, respectively, after the end of treatment. Between 3% and 6% of patients had abnormal values at 24 months.

The ABVD regimen involves a DNA intercalating agent (adriamycin), DNA strand break inductor (bleomycin), spindle disrupter (vinblastine), and an alkylating agent (dacarbazine). The CHOP regimen includes a very strong alkylating agent (cyclophosphamide), an intercalating agent (doxorubicin), and a spindle disrupter (vincristine). Both regimens are suspected of inducing chromosomal abnormalities acting on meiosis or on the postmeiotic phase, particularly in the latter

for structural anomalies, which we did not examine but which have been reported in other studies (29, 34, 46). If such treatment has an effect only during meiosis, one could expect normal results when a spermatogenic cycle was completed. This was not the case with either of the two regimens, suggesting that they have a prolonged action on cells in the meiosis stage or on cells that will reach the meiosis stage (spermatocytes I, differentiated spermatogonia) or on the microenvironment of spermatogenesis (i.e., the germline stem cell niche) (47). Moreover, the more drastic effects of the CHOP/MOPP-ABV regimen, with abnormal sperm aneuploidy frequency persisting at 6 and 12 months, suggest that this regimen has a marked action not only on the cells involved in meiosis at the time of treatment but also a durable effect through spermatogonia defects, impairment of DNA repair capacity, and alteration of the microenvironment of spermatogenesis. The return to normal conditions 2 years after the end of treatment demonstrates that type A spermatogonia were not killed by this chemotherapy regimen.

Although this is as yet the largest study of sperm aneuploidy in lymphoma patients, our study has several limitations. The number of patients in the CHOP/MOPP-ABV group is small, particularly at 3 and 6 months owing to severe impairment of spermatogenesis. The number of ABVD ± radiotherapy patients at these time points was also small, limiting the power to reach statistical significance. This emphasizes the need of further prospective studies with a large number of volunteers. We reported sperm aneuploidy rate using only three specific chromosome probes, and structural chromosomal abnormalities were not investigated. This could underestimate the effects of such treatments on all sperm chromosomes. Some authors have suggested that an interchromosomal effect could exist (30, 48), but there is no consensus on this. Additionally, no other DNA biomarkers were used to detect the long-lasting reproductive effect of lymphoma treatment (49). Sperm chromosomal abnormality thresholds are poorly defined in the literature. We chose as abnormal levels of sperm aneuploidy the values above the mean +2 SD of the control group, as in the study by Neusser et al. (40). The clinical significance of such thresholds has not been demonstrated to date. However, Templado et al. (50) reported an association between a moderate increase in aneuploidy and the risk of fathering aneuploidy offspring or of spontaneous abortion. On the basis of their findings, the moderate increase in aneuploidy observed in our study is probably clinically significant.

Our results may have clinical implications. Routine semen analysis is not sufficient to detect sperm genetic defects after cancer treatment, and it is known that chromosome or sperm DNA lesions do not affect the fertilizing capacity of sperm during natural or artificial procreation (51, 52). Moreover, Frias et al. (30) suggested that the results obtained with four chromosome probes could be extended to the other chromosomes (i.e., an interchromosomal effect). In this case, men who have received lymphoma treatment may have a significant, though transient, risk (for example, at least 1 year for CHOP/MOPP-AVB) of fathering embryos that die prematurely owing to aneuploidy, or, in the worst-case scenario, of having an increased risk of fathering a child with an aneuploidy syndrome. Studies of children of fathers treated for lymphoma have not shown evidence of more frequent abnormalities in offspring. However, it seems there is not sufficient power to detect relative risks of <3–5 (53), and generally the interval between the end of treatment and conception has not been precisely reported, nor were the miscarriage rates.

In the current state of knowledge, couples must be advised to use contraception for 2 years after lymphoma treatment if chemotherapy such as CHOP/MOPP regimens was used. Other published studies also suggest that natural pregnancy should be avoided during a similar period (26, 35, 49, 54). Moreover, because the median aneuploidy rate was increased before and after treatment, FISH analyses could be performed in cryopreserved sperm or sperm probes after treatment, to identify patients at risk (i.e., those who have abnormal values). In the case of abnormal values, reproductive/genetic counseling must be given, and preimplantation genetic screening with IVF or specific ultrasonography monitoring of pregnancy could be proposed in patients who wish to procreate before 1 (ABVD) or 2 years (CHOP/MOPP-ABV) after treatment end or to use cryopreserved sperm with increased aneuploidy.

In conclusion, this prospective study, following our previously published study of spermatogenesis and sperm DNA after lymphoma treatment, demonstrates that lymphoma itself has consequences on sperm aneuploidy frequency before any treatment. In addition, lymphoma treatments have deleterious effects on sperm chromosomes related to type of treatment and to time since treatment. In this context, all patients should be informed of sperm banking before treatment and of the duration of contraception that should be used by the couple after treatment. Moreover, we believe that other prospective studies are necessary using new methods (55, 56) of genome and epigenome investigation in man to evaluate the effects of treatment on sperm quality and the possible risk for progeny (male-mediated developmental toxicology), and also to determine the reproductive safety period after cancer treatment (49).

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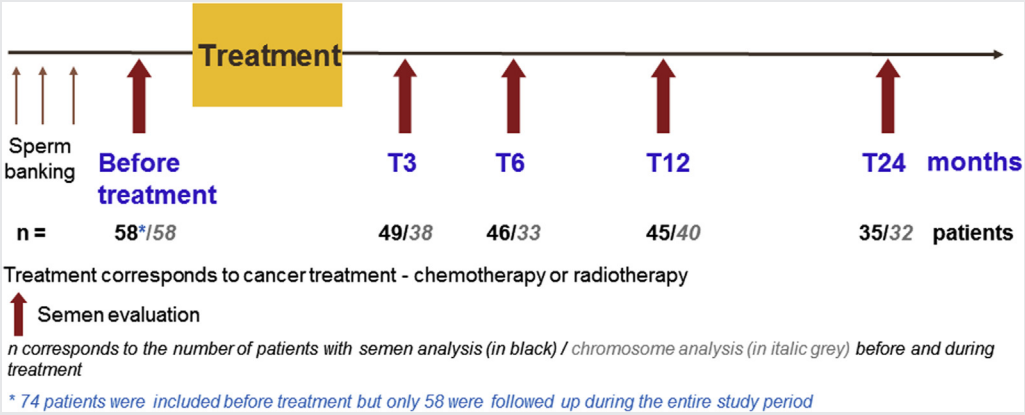
SUPPLEMENTAL FIGURE 1



Design of the prospective study.

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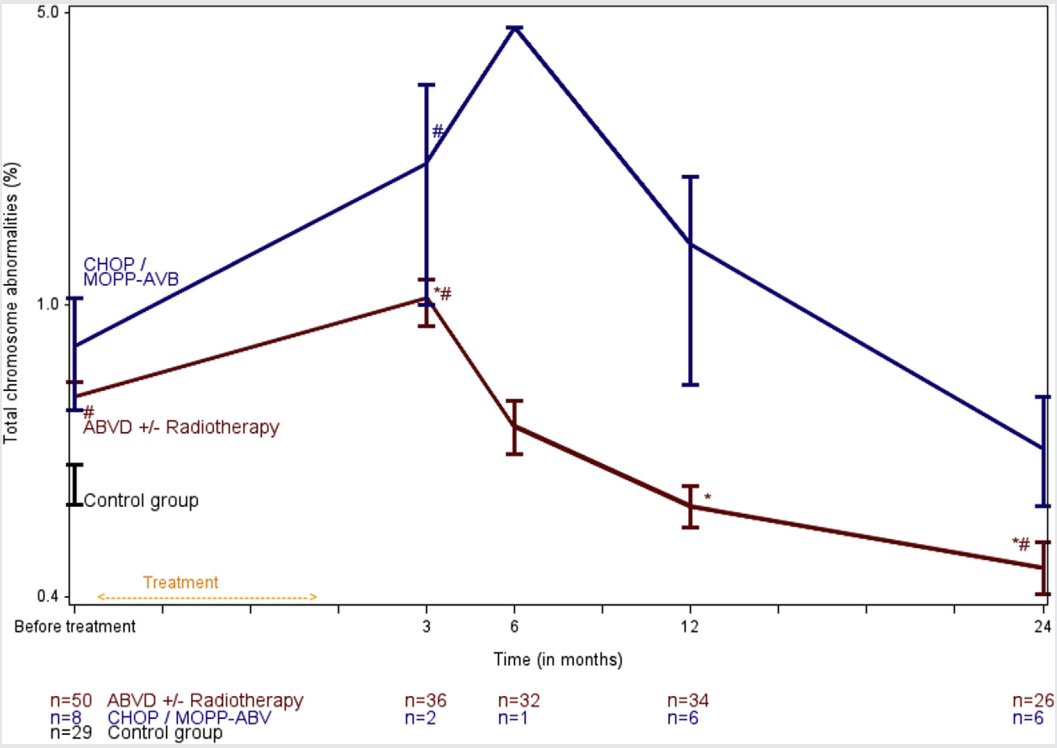
SUPPLEMENTAL FIGURE 2



Mean percentages of total chromosomal abnormalities and SEMs before and during posttreatment follow-up according to type of treatment (ABVD ± radiotherapy, or CHOP/MOPP-ABV) and in the control group. *$P<.05$, pre- and posttreatment difference. #$P<.05$, difference between controls and lymphoma patients.

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SUPPLEMENTAL TABLE 1

Treatment groups according to regimen.

Treatment group	Type of regimen
ABVD ± radiotherapy	ABVD
	R-ACVBP
	ACVBP
	ABVD
	ABVDP
	ABVDP + VABEM
	EBVP
CHOP/MOPP-ABV	CHOP
	R-ACVBP + MTX + VP16 + Cy
	R-CHOP 14
	R-CHOP
	M-CHOP
	BEACOPP + ABV

Note: ABV = doxorubicin, bleomycin, vinblastine; ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; ACVBP = methotrexate, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; BEACOPP = doxorubicin, cyclophosphamide, etoposide, bleomycin, vincristine, procarbazine, prednisone; CHOP = doxorubicin, cyclophosphamide, vincristine, prednisone; CVP = cyclophosphamide, vincristine, prednisone; Cy = cytosine arabinoside; EBVP = epirubicin, bleomycin, vinblastineprednisone; IFM = ifosfamide; M-CHOP = methotrexate + CHOP; MOPP = mechlorethamine, oncovin, procarbazine, and prednisone; MTX = methotrexate; R = rutiximab; VABEM = vindesine, doxorubicin, carmustine, etoposide, methylprednisone; VP16 = etoposide.

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SUPPLEMENTAL TABLE 2

Synthesis of sperm aneuploidy studies of lymphoma patients treated by chemotherapy and/or radiotherapy.

Authors, year	Prospective study	Pathology no. of patients	Methods	Treatment, no. of patients	Results before treatment compared with control group	Time after treatment	Results	Increased aneuploidy rate
Rousseaux et al., 1993	No	HL, 2	Sperm karyotyping	Rx + vinblastine, 2	No data	0 and 38 d	Multiple chromosome rearrangements	Yes
Brandriff et al., 1994	No	HL, 6	Sperm karyotyping	MOPP ± Rx, 6	No data	3 to 20 y	Increase of hyperhaploidy and chromosome structural anomalies	Yes
Martin et al., 1995	No	L, 1	Sperm karyotyping and FISH X Y 1 12	MACOP, 1	No data	3 y	No effect	No
Monteil et al., 1997	No	HL, 1	FISH 1 6 11 X Y	Rx + vinblastine, 1	No data	0 and 38 d	Increased aneuploidy rate	Yes
Robbins et al., 1997	No	HL, 8	FISH 8 X Y	NOVP + Rx, 8	Low increased mean aneuploidy rate in 4 patients before treatment	During and 82 to 999 d after tt	High increased aneuploidy rate with return to baseline values 100 d after treatment	Yes, up to 100 d after treatment
Frias et al., 2003	No	HL, 8 (subset, Robbins 1997 patients)	FISH X Y 18 21	NOVP + Rx, 8	Low increased mean aneuploidy rate in 5 patients before treatment	During and 1 to 2 y after tt	High increased aneuploidy rate during treatment No increase 1 y after treatment	Yes, up to 1 y after treatment
Thomas et al., 2004	No	HL, 10 NHL, 2	FISH X Y 13 18 21	ABVD, 3 EBVP, 2 VIP-ABVD, 1 BEACOPP, 1 MOPP-ABV, 3 VIP-ABVD, 1 COPADAM-CYM, 1 MTX-holoxan-VP16-aracytine, 1 ± Rx	No data	7 mo to 5 y	Increased aneuploidy rate in one patient 7 mo after treatment	Yes, 7 mo after treatment
Tempest et al., 2008	Yes Before and 6, 12, and/or 18–24 mo after tt initiation	HL, 5	FISH X Y 13 21	ABVD, 11	Increased aneuploidy rate before treatment	6 to 24 mo after tt initiation	Increased aneuploidy rate 6 mo after treatment initiation	Yes, 6 mo after tt initiation (4–8 cycles of ABVD)

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SUPPLEMENTAL TABLE 2

Continued.

Authors, year	Prospective study	Pathology no. of patients	Methods	Treatment, no. of patients	Results before treatment compared with control group	Time after treatment	Results	Increased aneuploidy rate
Patassini et al., 2013	No	HL, 3	Single sperm aCGH	ABVD, 3	No data	At end of 3 mo tt	Abnormal molecular sperm karyotyping	Yes, end of tt
Our study	Yes Before and 3, 6, 12, and 24 mo after tt end	HL, 54 NHL, 17	FISH X Y 18	ABVD ± Rx, 50 CHOP/MOPP group, 21	Increased aneuploidy rate before treatment	3 to 24 mo	Increased aneuploidy rate 3–12 mo after end of treatment	Yes 3 mo for ABVD groups 12 mo for other regimens

Note: aCGH = array comparative genomic hybridization; BEACOPP = bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; COPADAMCYM = cyclophosphamide, vincristine, prednisone, adriamycin, cytarabine, methotrexate; MACOP = methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone; NOVP = novantrone, vincristine, vinblastine, prednisone; tt = treatment; VIP = etoposide, ifosfamide, cisplatin.

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