ORIGINAL ARTICLE: FERTILITY PRESERVATION

Impact of lymphoma treatments on spermatogenesis and sperm deoxyribonucleic acid: a multicenter prospective study from the CECOS network

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Objective: To determine consequences of lymphoma treatments on sperm characteristics and sperm DNA, and to evaluate predictors of sperm recovery.
Design: Multicenter prospective longitudinal study of patients analyzed before treatment and after 3, 6, 12, and 24 months.
Setting: University hospitals.
Patient(s): Seventy-five Hodgkin lymphoma and non-Hodgkin lymphoma patients and a control group of 257 fertile men.
Intervention(s): Semen analyses, and sperm DNA and chromatin assessments.
Main Outcome Measure(s): Comparisons of sperm characteristics before and after treatment.
Result(s): Patients already had altered sperm characteristics before lymphoma treatment, with no identified risk factor. Sperm count, total sperm count, motility, and vitality decreased after treatment, with lowest values at 3 and 6 months. Twelve months after treatment, mean sperm count recovered to pretreatment values after doxorubicin, bleomycin, vinblastine, darcarbacine (ABVD) or ABVD + radiotherapy, but not after doxorubicin, cyclophosphamide, vincristine, prednisone (CHOP) or mechlorethamine, oncovin, procarbazine, prednisone (MOPP) chemotherapies. It was noteworthy that 7% of patients remained azoospermic at 24 months. After 24 months, Kaplan-Meier estimates showed that more than 90% of patients will recover normal sperm count after ABVD or ABVD + radiotherapy vs. 61% for CHOP chemotherapies. In multivariate analyses including diagnosis and treatment protocol, only pretreatment total sperm count was related to recovery. Compared with a control group, lymphoma patients had higher sperm chromatin alterations and DNA fragmentation before any treatment. After treatment, DNA fragmentation assessed by TUNEL assay and sperm chromatin structure assay decreased from 3 and 6 months, respectively, while remaining higher than in the control group during follow-up.
Conclusion(s): Lymphoma patients had altered sperm DNA and chromatin before treatment. Lymphoma treatment had damaging effects on spermatogenesis. These data on both the recovery period according to treatment modalities and the pre- and post-treatment chromatin status of sperm are useful tools for counseling patients wishing to conceive. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Lymphoma, Hodgkin, chemotherapy, radiotherapy, spermatogenesis, sperm DNA, sperm chromatin

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Age-standardized incidence rates of Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) are 2.5 and 12.0 per 100,000 in France, 2.3 and 8.8 in Europe, and 2.7 and 14.6 in north America, respectively (1). The treatment of Hodgkin lymphoma and non-Hodgkin lymphoma is based on chemotherapy, radiotherapy, and monoclonal antibodies, according to the specific biological type of the illness and the status of its progression. Moreover, allogenic stem cell transplantation can result in extended survival or relapsed aggressive lymphoma. HL affects mainly young people, and a large proportion of men affected by NHL are also young. Prognosis of these lymphomas has improved very markedly over the past decades, and many young patients wish to procreate. Therefore, semen banking must be recommended before any treatment.

Alterations of sperm characteristics before HL and NHL treatment have been described in several studies (2–8). The causes of such alterations were not clearly identified, but there may be several risk factors for altered spermatogenesis, such as fever (9–11), stress (12, 13), or inflammatory immune-mediated mechanisms (14). In the largest study, which included 474 patients with early-stage HL, sperm characteristics were therefore studied before treatment, and night sweats and fever were found to be related to sperm alterations (7).

Several late adverse effects of chemotherapy or radiotherapy have been described, and sperm banking is recommended before treatment (15). In particular, post-treatment gonadic function could be drastically affected, and the intensity of spermatogenesis alterations is related to the type of drugs used, the cumulative doses of the drug, the radiation doses received by the testis, and the time between the last treatment and sperm analysis (16, 17). The testes are highly affected by alkylating agents, and one of the challenges was to discover drugs that were highly effective in curing the lymphoma but which carried fewer damaging secondary effects on spermatogenesis. For example, after 6 cycles of mechlorethamine, oncovin, procarbazine, prednisone (MOPP) or a comparable regimen containing alkylating agents, 90%–100% of patients experienced prolonged azoospermia, whereas nonalkylating chemotherapy such as doxorubicin, bleomycin, vinblastine, darcarbacine (ABVD) caused only transitory azoospermia (18).

Several studies have examined sperm characteristics after cancer treatment. However, these studies are often limited in value because they include only small series or because they are retrospective. Sperm analysis was carried out at different times before and after chemotherapy/radiotherapy and follow-up designs also differed. Consequently, the dynamics of recovery are not precisely described. To date, only one prospective study, of a small patient series, has been published (19).

Moreover, the consequences of HL and NHL treatment on sperm DNA and chromatin during the recovery periods are a question of paramount importance as damage to the paternal genome may have detrimental effects on progeny. Therefore, post-treatment sperm DNA alterations are still debated (19–21). In this context, our prospective study of 75 lymphoma patients aimed to: (1) evaluate sperm characteristics and DNA damage before and after treatment; (2) evaluate the impact of treatment on spermatogenesis at the various time points during follow-up (3 to 24 months); and (3) identify predictive factors of post-treatment spermatogenesis recovery.

MATERIALS AND METHODS

Patients

This prospective study comprised 75 patients. Patients were assigned to the Centres d'Etudes et de Conservation des Oeufs et du Sperme Humain (CECOS) for sperm banking before cancer treatment. Eight CECOS groups participated: Caen, Clermont-Ferrand, Grenoble, Marseille, Paris Cochin, Paris Tenon, Rouen, and Toulouse. These centers were part of a national network, the Fédération Française des CECOS. Sperm analysis is subject to external quality control in all centers. This study was supported by national research grant PHRC N°20030222 and was approved by the institutional ethics review board. All patients gave their written informed consent. This national program also included a population of patients with testicular germ cell tumors whose results have been previously published (22).

The HL and NHL patients were treated by combination chemotherapy with or without radiotherapy (mainly supradiaphragmatic, mean (±SD) total dose 41.2 ± 15.1 Gy), according to the diagnosis and disease stage. According to treatment protocols, the patients were divided into four groups (Supplemental Table 1): ABVD, ABVD + radiotherapy, CHOP (doxorubicin, cyclophosphamide, vincristine, prednisone), and MOPP groups. Eighteen patients were in the ABVD group, 39 in the ABVD and radiotherapy group, 13 in the CHOP group, and 5 in the MOPP-ABV group.

Usually, two to three semen samples were collected for sperm banking before the initiation of treatment. Patients taking part in the study provided an additional semen sample before treatment (T0). They were asked to repeat semen samples at 3, 6, 12, and 24 months after the end of treatment (Supplemental Fig. 1).

Age, andrological and reproductive histories, tobacco exposure, and febrile episodes were recorded. At each visit, a standard questionnaire was completed about any unusual events since the last visit to the laboratory. Pretreatment sperm characteristics were compared with those of a control group of 257 fertile men. Fifty-one of these fertile men also underwent sperm DNA evaluation (DNA control group).

Semen Analyses

Semen samples were collected by masturbation after a recommended 3–5 days of sexual abstinence. Semen analysis was performed according to WHO guidelines (23) with similar methodology in the 8 laboratories. The characteristics considered were ejaculate volume (ml) and pH, sperm concentration (SC, 10^6/ml), round cell concentration (RC, 10^6/ml), spermatozoa vitality (V, %) and forward motility (M, a+b: %), total sperm count (product of volume by SC = TSC, 10^6/ejaculate), and total motile sperm count (TMSC = TSC × M, 10^6/ejaculate). Duration of sexual abstinence (days) was also recorded. The remaining semen sample was mixed with a cryoprotectant, frozen in straws, and stored in liquid nitrogen until use for the following analyses, performed in the Toulouse CECOS by a single technician.

Sperm Chromatin Structure Assay

Sperm chromatin structure assay (SCSA®) evaluates sperm chromatin integrity. SCSA was performed as described elsewhere (24) and routinely used in our laboratory (10). The extent of DNA denaturation was expressed as the DNA fragmentation index (DFI), which is the ratio of red to total (red plus green) fluorescence intensity. SCSA measured the fraction of highly DNA stainable (HDS) cells, which represent sperm with less condensed/immature chromatin. One aliquot of quality-control sperm was analyzed in pooled samples (results not shown). The analytical coefficient of variation for DFI was <5%, as calculated from values obtained from aliquots of a semen sample.

DNA Fragmentation

Sperm DNA strand breaks were detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL assay), as previously described (10). Sperm DNA fragmentation and propidium iodide (PI) labelling were measured on a Beckman Coulter Epics XL-MCL flow cytometer (Coulter). Each analysis included a minimum of 10,000 stained spermatozoa. The FL1 (green fluorescence from FITC) signals were detected through a 525 ± 25 nm band pass filter and FL2 (red fluorescence from PI) through a 575 ± 25 nm filter. TUNEL analysis consisted of subtracting control (no TdT enzyme) green fluorescence histograms from TdT-positive green fluorescence histograms, yielding the percentage of cells showing DNA strand breaks.

Data Collection and Statistical Analyses

All data were reported on centralized case report forms by web access. Data files were verified by the coordinating center in Toulouse. Data were compared between the control group (fertile men) and the lymphoma groups using the nonparametric Mann-Whitney test. Linear regression was used to adjust data on sexual abstinence and patient age because both these parameters may influence sperm characteristics. Sperm characteristics of HL and NHL patients were compared pre- and post-treatment at 3, 6, 12, and 24 months after start of treatment (T3, T6, T12, and T24) by the Wilcoxon signed rank sum test. Treatment regimens were compared using the Mann-Whitney test.

The Kaplan-Meier method was used to estimate the cumulative rates of successful semen recovery and the 95% confidence interval (CI). Censors were defined as patients who did not succeed in semen recovery, i.e., patients with total sperm count values $<39 \times 10^6$ spermatozoa (25) at their last visit. Statistical differences between groups were calculated by the log-rank test. Univariate and multivariate Cox models were performed to describe factors associated with successful or unsuccessful semen recovery using crude and adjusted hazard ratios (HR) and 95% CIs. Statistical analysis was performed using SAS software (version 9.3, SAS Institute, Inc.), and the significance level was defined as 5%.

RESULTS

Patients

Fifty-seven patients had HL, and 18 had NHL. The mean age was statistically lower in HL (28 ± 6 years old) than in NHL patients (33 ± 7 years old) ($P<.05$). HL patients were mainly treated by ABVD alone (23%) or in combination with radiotherapy (67%). NHL patients were principally treated by CHOP (61%) or ABVD (28%). Treatment by MOPP-ABV represented 7% of HL and NHL patients. The mean number of chemotherapy cycles was nearly 7 for chemotherapy alone and 4.5 for chemotherapy in combination with radiotherapy (ABVD 7.7 ± 3.6, CHOP 7.0 ± 2.2, MOPP-ABV 7.0 ± 1.7, and ABVD + radiotherapy 4.5 ± 1.2 cycles).

Semen Characteristics before Cancer Treatment

Sperm characteristics did not significantly differ according to the frequency of risk factors that could influence sperm characteristics ($P>.05$, results not shown), particularly for history of fever in the last 3 months, which was not found to affect sperm characteristics (Supplemental Table 2). Compared with the control group, HL patients had higher pH and round cell count and lower ejaculate volume, SC, percentage of live sperm and motile sperm, TSC and TMSC ($P<.05$). When comparisons were made after adjustment for abstinence duration and age, parameters known to influence sperm characteristics, increased pH, and decreased percentage of live sperm and motile sperm, and TSC were always noted ($P<.05$). Adjusted for age and abstinence duration and compared with the control group, the HL group had the lowest percentage of live or motile sperm and the lowest TMSC, whereas the NHL group had a decrease only in percentage of motile sperm.

Change in Semen Characteristics after Cancer Treatment

No patient presented azoospermia before treatment, in accordance with the inclusion criteria for the study. A very high percentage of patients became azoospermic after treatment, 26% at T3 and 27% at T6, and although this percentage later decreased, it was still 15% at T12 and 7% at T24. However, no azoospermic patient was noted at T12 with ABVD treatment or at T24 for ABVD or ABVD + radiotherapy treatments. In contrast, at T12, 22% of patients presented azoospermia after CHOP treatment and 75% after MOPP treatment. At T24, the azoospermia rates were 22% and 50% for CHOP and MOPP, respectively.

Compared with pretreatment values, SC and TSC decreased notably 3 months and 6 months after the end of treatment, whatever the type of treatment (Table 1, Fig. 1). The most marked decrease was noted after MOPP-ABV and CHOP therapy, and sperm count was still abnormal 12 months after the end of treatment with these regimens. Moreover, qualitative alterations of sperm, such as percentage of motile sperm, were noted 3 and 6 months after CHOP, and 6, 12, and 24 months after MOPP-ABV treatment. Percentage of live sperm was also altered 3 months after CHOP and 6 months after MOPP-ABV.

In the ABVD group, compared with pretreatment values, the mean ejaculate volume (results not shown, $P<.05$) and the percentage of live and motile sperm were increased 24 months after treatment. In the ABVD + radiotherapy group, TMSC was higher at 24 months than before treatment (Table 1).

TABLE 1

Sperm characteristics at follow-up time points according to treatment.

Charactrtistics	Before treatment	After treatment 3 mo	6 mo	12 mo	24 mo
ABVD, n	18	14	16	14	12
Sperm count, 10^6/mL	45.62 ± 47.08	7.88 ± 7.71[a]	15.56 ± 17.24[a]	28.49 ± 30.32	51.52 ± 52.23
Vitality, %	58.61 ± 13.26	58.09 ± 23.37	54.62 ± 25.45	66.83 ± 23.60	67.33 ± 6.96[a]
Motility, %	30.89 ± 12.64	30.45 ± 16.58	29.00 ± 15.72	35.21 ± 14.29	42.00 ± 13.74[a]
Total sperm count, 10^6/ejaculate	174.41 ± 336.96	33.45 ± 41.48[a]	60.61 ± 103.71[a]	143.91 ± 205.51	242.20 ± 208.85
Total motile sperm count, 10^6/ejaculate	47.48 ± 69.08	15.83 ± 17.11	23.75 ± 33.44	57.37 ± 97.50	104.86 ± 93.50
ABVD + Radiotherapy, n	39	34	28	30	19
Sperm count, 10^6/mL	57.89 ± 47.02	22.07 ± 29.04[a]	33.91 ± 32.33[a]	49.71 ± 52.53	59.08 ± 47.22
Vitality, %	61.10 ± 17.92	62.68 ± 17.68	64.08 ± 21.45[a]	60.48 ± 21.36	68.37 ± 15.13
Motility, %	42.00 ± 13.48	35.63 ± 15.86	37.89 ± 18.14	36.55 ± 18.09	44.58 ± 13.23
Total sperm count, 10^6/ejaculate	217.45 ± 222.05	74.89 ± 130.30[a]	126.25 ± 150.62	184.27 ± 214.18	241.24 ± 220.21
Total motile sperm count, 10^6/ejaculate	88.16 ± 82.12	31.20 ± 50.12[a]	55.78 ± 65.40[a]	82.54 ± 97.38	97.33 ± 74.17[a]
CHOP, n	13	11	13	11	9
Sperm count, 10^6/mL	78.09 ± 74.87	0.59 ± 1.24[a]	9.54 ± 21.73[a]	36.16 ± 78.26[a]	45.78 ± 57.92
Vitality, %	63.33 ± 20.60	19.75 ± 29.67[a]	26.14 ± 34.14	59.00 ± 33.97	62.00 ± 33.34
Motility, %	39.62 ± 15.87	13.75 ± 22.00[a]	18.18 ± 26.39[a]	28.00 ± 27.00	36.43 ± 28.09
Total sperm count, 10^6/ejaculate	215.16 ± 174.23	1.18 ± 2.37[a]	32.09 ± 90.66[a]	126.88 ± 281.71	165.76 ± 222.91
Total motile sperm count, 10^6/ejaculate	83.46 ± 69.00	0.69 ± 1.50[a]	13.79 ± 30.16[a]	75.47 ± 153.30	99.01 ± 109.24
MOPP-ABV, n	5	2	5	4	2
Sperm count, 10^6/mL	45.58 ± 41.24	0.00 ± 0.00	0.02 ± 0.04	7.00 ± 14.00	177.00 ± 250.32
Vitality, %	53.00 ± 9.38	Nm	0.00 ± 0.00	41.00 ± 57.98	86.00 ± -
Motility, %	40.00 ± 9.46	Nm	3.20 ± 7.16	16.67 ± 28.87	30.00 ± 42.43
Total sperm count, 10^6/ejaculate	129.63 ± 122.18	0.00 ± 0.00	0.07 ± 0.16	14.70 ± 29.40	460.20 ± 650.82
Total motile sperm count, 10^6/ejaculate	56.07 ± 60.20	Nm	0.01 ± 0.03	9.80 ± 16.97	276.12 ± 390.49

Note: Values are mean ± standard deviation. Nm = not measurable.
[a] $P<.05$, difference between before treatment and after treatment values (3, 6, 12, and 24 months).

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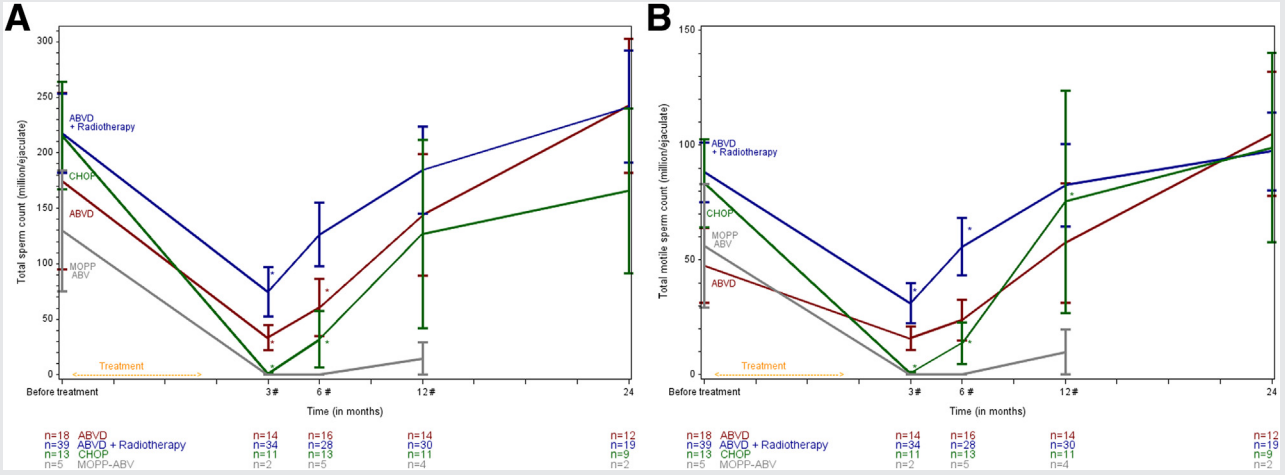
Predictors of Sperm Recovery after Treatment

Stratified according to treatments, Kaplan-Meier estimates showed that at 24 months, cumulative rates of recovery of normal sperm production (TSC $\geq 39 \times 10^6$) reached 92% (95% CI 72%–99%) in patients treated by ABVD + radiotherapy, and 90% (95% CI 76%–98%) in patients treated with ABVD alone, but only 61% (95% CI 35%–88%) in patients treated by CHOP ($P=.017$). A statistically significant

FIGURE 1



(A) Means of total sperm count and standard errors of the mean before and during post-treatment follow-up according to treatment type (ABVD, ABVD + radiotherapy, CHOP, or MOPP-ABV). (B) Means of total motile sperm count and standard errors of the mean before and during post-treatment follow-up according to treatment type (ABVD, ABVD + radiotherapy, CHOP, or MOPP-ABV). *$P<.05$, pre- and post-treatment difference; #$P<.05$, difference between treatments. Means and standard deviations are given at each time point.

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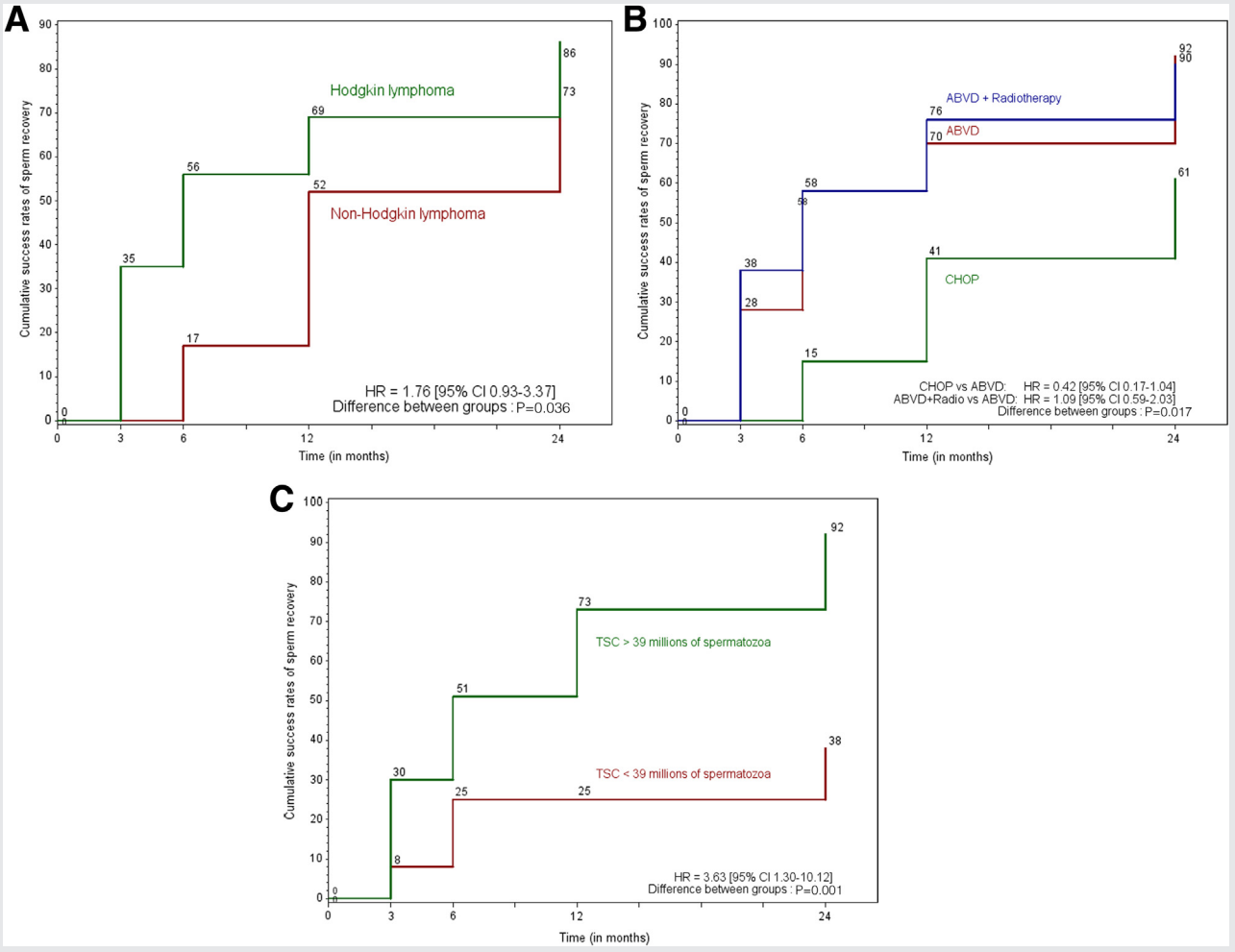
difference in recovery was also observed according to diagnosis: 86% (95% CI 73%–96%) for HL vs. 73% (95% CI 51%–92%; $P=.036$) for NHL. Significant differences in cumulative rates of recovery were also observed between normal and abnormal sperm production before treatment ($P=.001$; Fig. 2). After adjustment for patient age, smoking status, type of diagnosis, and type of treatment in a multivariate Cox model, only TSC before treatment (TSC $<39 \times 10^6$ vs. TSC $\geq 39 \times 10^6$, hazard ratio (HR) = 3.43 [95% CI 1.21–9.65]) remained statistically significant.

DNA Damage

Before treatment and after adjusting for age and abstinence duration, compared with controls, lymphoma patients had significantly higher values of DFI (median = 15.9% [interquartile ranges (IQRs) 9.8%–27.1%] vs. 8.6% [IQRs 6.6%–14.3%], $P<.05$) and of DFI+HDS (22.5% [IQRs 16.4%–34.4%] vs. 14.3% [IQRs 11.3%–20.7%], $P<.05$). TUNEL values before treatment were also higher than those of the control group (12.7% [IQRs 7.1%–15.7%] vs. 7.9% [IQRs 6.6%–13.2%], $P<.05$). According to diagnosis, statistical differences were found between HL and controls for sperm DNA and chromatin values (HL group: DFI 16.3% [IQRs 11.3%–27.6%], DFI+HDS 24.5% [IQRs 18.3%–36.3%], HDS 6.5% [IQRs 5.0%–8.2%], TUNEL 13.9% [IQRs 8.8%–15.8%], $P<.05$), but for chromatin values only (DFI 14.8% [IQRs 7.2%–21.7%], DFI+HDS 21.4% [IQRs 12.5%–28.6%], $P<.05$) between NHL and controls.

To compare results between cancer patients and controls, we defined reference thresholds as the 90th percentiles in controls: DFI 20%, HDS 7.5%, DFI+HDS 26.1%, and TUNEL 16.7%. Before chemotherapy or radiotherapy, DFI and TUNEL

FIGURE 2



Recovery of total sperm count $\geq 39 \times 10^6$/ejaculate after treatment. *P* value indicates the difference between groups using the log-rank test. Hazard ratio (HR) and 95% confidence intervals (CIs) were obtained from a univariate Cox model. The cumulative success rates of semen recovery were estimated by the Kaplan-Meier method, stratified according to: (A) type of lymphoma: 86% (95% CI 73%–96%) for HL and 95% (95% CI 51%–92%) for NHL. (B) Type of treatment: 90% (95% CI 76%–98%) for ABVD; 92% (95% CI 72%–99%) for ABVD + radiotherapy; and 61% (95% CI 35%–88%) for CHOP. (C) Total sperm count before treatment: 38% (95% CI 16%–74%) for TSC $<39 \times 10^6$ and 92% (95% CI 81%–98%) for TSC $\geq 39 \times 10^6$. TSC = Total sperm count.

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values were above normal values in 38% and 19% of patients, respectively.

Compared with pretreatment values, mean values of DFI and DFI+HDS decreased from 6 months to 24 months after treatment, but always remained higher than control group values. At 6 months, 32% of patients had higher than normal DFI values, and 37% of patients had higher than normal DFI+HDS values, whereas at 24 months these percentages decreased to 15% and 32%, respectively. Mean TUNEL values decreased after treatment and were similar to control values from T6 to T24. Table 2 presents results on DNA damage.

DISCUSSION

In this multicenter prospective study, we investigated sperm characteristics and sperm DNA in patients before and after treatment for Hodgkin lymphoma and non-Hodgkin lymphoma, and we studied the predictive factors of sperm recovery. The originality of this study lies in (1) its prospective approach, with serial semen collection before treatment and at 3, 6, 12, and 24 months after the end of treatment; and (2) its inclusion of the largest group of patients in a study with this type of design.

Before treatment, HL and NHL patients had altered sperm characteristics compared with fertile men. Ejaculate volume was reduced, and pH was higher than that in fertile men. Quantitative and qualitative alterations of spermatozoa were observed in these cancer patients. It is noteworthy that sperm DNA alterations were observed before any treatment was started: 38% of patients had abnormal DFI, and 19% had abnormal TUNEL values. Smit et al. (20) found no increase of DFI in 31 HL patients compared with a control group, but an increase in the NHL group (n = 15). Other studies of smaller series than ours reported an increase of sperm DNA alterations before treatment using several tests (21, 26–29); however, this increase was not reported by other authors (30).

Moreover, the spermatozoa alterations, low mean ejaculate volume and high semen pH compared with fertile men could reflect genital tractus modifications due to the disease. It is noteworthy that several authors have reported sperm alterations or high percentages of men with abnormal sperm parameters prior to cancer treatment (2, 3, 5, 8, 21, 31, 32). There could be several explanations for pretreatment sperm alterations: disease stage has been proposed by some authors (5, 33) but not by others (8, 20, 31, 34), as well as elevated erythrocyte sedimentation test values (5) and fever (9). An immune-mediated disorder in HL patients could also explain sperm alterations before treatment (14).

In a recent study of 77 patients who provided semen specimens before HL treatment, poor sperm quality was strongly associated with presence of B-symptoms, in particular fever and night sweats (7). This finding was in accordance with the reported effects of fever episodes on sperm production (10, 11). In contrast, in our study, a history of fever episodes in the months preceding sperm analysis was not associated with altered sperm characteristics. Cancer diagnosis was also a stress factor that could be involved in sperm alterations.

Sperm production values such as SC and TSC were lowest 3 months after the end of treatment, and the intensity of decrease was related to the type of treatment. CHOP and MOPP protocols were the most toxic for sperm production and qualitative alterations, as decreased motility and vitality were observed. Six months after treatment, i.e., after more than 2 spermatogenesis cycles, sperm production was always lower than pretreatment values whatever the treatment modality. For CHOP and MOPP protocols, recovery of mean pretreatment values only occurred 2 years after the end of treatment. CHOP and MOPP contain alkylating agents that are known to be effective in killing spermatogonial stem cells in a dose-dependent manner. However, in most cases, treatment did not kill all stem cells, as evidenced by recovery of spermatogenesis. Treatments without severe alkylating agents probably act on differentiated spermatogonia and/or spermatocytes. In this case, faster sperm recovery would be expected in view of the spermatogenesis cycle (74 days; 35) and epididymal transit duration (mean duration of male genital transit 12 days; 36).

According to Meistrich (37), several hypotheses could explain the long time to recovery of mean sperm pretreatment values: type A spermatogonia do not reinitiate differentiation until their population is regenerated; at the beginning of recovery, if few sperm are produced, these sperm do not survive epididymal transport and do not reach ejaculate. The effects

TABLE 2

DNA fragmentation and abnormal chromatin (%).

		Lymphoma (HL + NHL) group				
			After treatment			
	Control group fertile men	Before treatment	3 mo	6 mo	12 mo	24 mo
N	51	71	38	38	43	34
DFI	8.6 [6.6–14.3]	15.9 [9.8–27.1][a,b]	17.4 [10.2–25.0][a,b]	15.0 [9.6–23.2][a,b,c]	16.7 [8.6–23.4][a,b,c]	14.4 [8.9–24.8][a,b,c]
HDS	5.3 [4.5–6.2]	6.1 [4.9–7.0][a]	6.2 [4.9–8.6][a]	5.4 [4.3–7.5]	5.8 [4.4–6.8]	5.7 [5.0–7.0]
DFI+HDS	14.3 [11.3–20.7]	22.5 [16.4–34.4][a,b]	24.8 [17.1–33.4][a,b]	20.8 [16.3–29.2][a,b,c]	22.2 [14.8–27.9][a,b,c]	21.9 [15.0–31.4][a,b]
TUNEL	7.9 [6.6–13.2]	12.7 [7.1–15.7][a]	7.6 [5.8–12.4][a,c]	9.8 [7.1–13.9][c]	9.3 [6.6–14.7][c]	10.5 [7.3–14.3]

Note: Values are medians [interquartile range: Q1–Q3]. DFI = DNA fragmentation index; HDS = highly DNA stainable cells were assessed by sperm chromatin structure assay; TUNEL = terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling assay.

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

[b] $P<.05$, difference between control group and lymphoma group, adjusted on abstinence duration and age.

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

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of cancer treatment on the micro-environment of spermatogenesis, i.e., the germline stem cell niche (38), could also explain the long-lasting damage. It is noteworthy that in multivariate Cox analysis, the cumulative rate of recovery was related to normal sperm production before treatment: the better the spermatogenesis before treatment, the less the spermatogenesis alterations after. This suggests that altered pretreatment spermatogenesis is more sensitive to therapeutic agents than normal pretreatment spermatogenesis. A total of 7% of our patients remained azoospermic 2 years after treatment (all in the CHOPP and MOPP groups), highlighting the need for sperm banking before treatment.

Very few studies have reported the evolution of sperm DNA damage and sperm quality in lymphoma patients after cancer treatment. Stahl et al. (21) found no increase in DFI after treatment, but semen analysis was done 3 years on average after the end of treatment. Smit et al. (20) found no difference between pretreatment and post-treatment values of DFI measured by SCSA. In one study using the comet assay, an increase of sperm DNA fragmentation was reported up to 24 months after treatment (19). In our study, mean DFI, which was increased before treatment, remained higher compared with the control group, whereas post-treatment values from T6 to T24 were significantly lower than pretreatment values. The percentage of sperm DNA fragmentation, evaluated by the TUNEL test, decreased after treatment to normal-range values. These decreases of mean DFI and TUNEL values after treatment could be explained by an effect of the treatment on the postmeiotic stage of spermatogenesis when male germ cells are DNA repair-deficient, as the risk period for the production of DNA-compromised sperm was in this case shorter than the duration of a cycle of human spermatogenesis (39).

Another hypothesis to explain the decrease of DFI values from 6 to 24 months is that the effects of treatment on the germ cell environment were not persistent, and new germinal cells originated from spermatogonia that were not affected or had DNA repair-competent processes in the pre-meiotic phases of spermatogenesis. Moreover, many cancer-related factors that were present before treatment and that have deleterious effects on spermatogenesis were probably attenuated after treatment. However, it is noteworthy that, compared with the control group, our patients had higher mean DFI values both before treatment and during follow-up. Other studies are needed to explore the causes of chromatin alterations in lymphoma patients.

However, lymphoma treatment could induce genome alterations that were not assessed by the methods used in our study. For example, structural or numerical chromosome sperm damage after lymphoma treatment has been reported by several authors (40–42). Moreover, in recent animal studies, alterations of the sperm proteome after exposure to cyclophosphamide (43), of testis and germ cells gene expression after doxorubicin treatment (44), and of sperm DNA and embryo development after amifostine-doxorubicin treatment (45) were observed.

The question of such effects on humans after cancer treatments is still debated. Studies of children of fathers treated for cancer have not shown evidence of more frequent abnormalities in offspring. However, it seems that these studies do not have sufficient power to detect relative risks of <3–5 (46), and the interval between the end of cancer treatment and conception was not precisely analyzed. Moreover, it seems particularly important to note the paradigm difference ("fundamental mystery"; 47) between the results from animal compared with human studies, underlining the need to conduct further human studies using new technologies.

To date, considering the differences in published results and the drastic effects of cancer treatment on spermatogenesis, counseling is recommended for lymphoma patients, and couples are advised to use contraception for 2 years after lymphoma treatment (19, 42, 48, 49).

Limitations and Bias

Losses to follow-up in the included cohort before treatment could have an impact on our findings. However, statistical differences between time points were calculated in the same population, i.e., on the same number of patients between two time points (before treatments and at T3, 6, 12, or 24). Thus, sperm production recovery after 24 months means a recovery only in patients for whom we had data before treatment and at T24. One limitation of the cytofluorometric methods used (SCSA and TUNEL) was the need for a minimum number of sperm cells (at least 2×10^6) to perform both analyses, thus requiring patients with a relatively compatible recovery of spermatogenesis.

CONCLUSION

Our prospective study demonstrates extremely deleterious effects of lymphoma treatments on spermatogenesis, with recovery of mean sperm values 2 years after the end of treatment. However, it is noteworthy that 7% of patients were azoospermic 2 years after treatment. Treatment type and spermatogenesis status before treatment were linked to recovery. We also found chromatin alteration in lymphoma patients before and after treatment end, although this parameter improved 6 months post-treatment. In this context, all patients must be informed of sperm banking before treatment and of the duration of contraception to be used by the couple after treatment. Consequently, we believe that other prospective studies are necessary using new methods of genome and epigenome exploration in man to evaluate the effects of treatment on spermatozoa quality and possible risk for progeny (male-mediated developmental toxicity).

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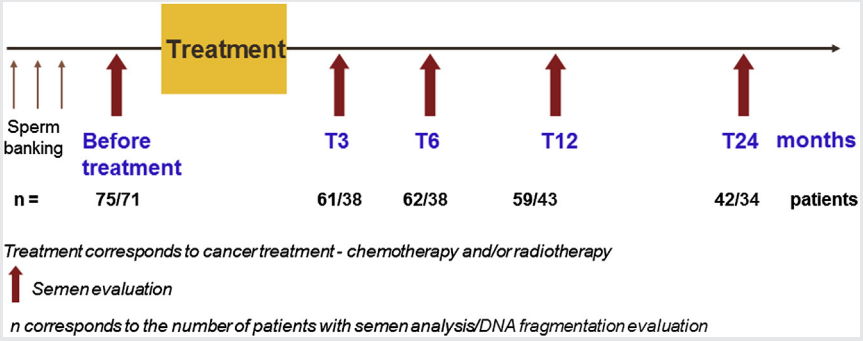
SUPPLEMENTAL FIGURE 1



Design of the prospective study.

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SUPPLEMENTAL TABLE 1

Treatment groups according to different drugs used.

Treatment groups	Type of protocols
ABVD group	ABVD
	R-ACVBP
	ACVBP
ABVD + radiotherapy group	ABVD
	ABVDP
	ABVDP + VABEM
	EBVP
CHOP group	ACVBP
	CHOP
	R-ACVBP + MTX + VP16 + Cy
	R-CVP
	ACVBP R + MTX + IFM + VP16
	R-ACVBP +MTX
	R-CHOP
	R- mini CHVP + interferon
	R-ACVB + MTX+ VP16 + IFM + Cy
	R-CHOP
MOPP-ABV group	BEACOPP + ABV
	BEACOPP

Note: ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; ABV = doxorubicin, bleomycin, vinblastine; ACVB = methotrexate, doxorubicine, cyclophosphamide, vindesin, bleomycin; ACVBP = methotrexate, doxorubicine, cyclophosphamide, vindesin, bleomycin, prednisone; BEACOPP = doxorubicine, cyclophosphamide, etoposide, bleomycine, vincristine, procarbazine, prednisone; CHOP = doxorubicin, cyclophosphamide, vincristine, prednisone; CVP = cyclophosphamide, vincristin, prednisone; Cy = cytosin arabinoside; EBVP = epirubicin, bleomycin, vimblastine, prednisone; IFM = ifosfamide; MTX = methotrexate; R = rutiximab; VABEM = vindesin, doxorubicin, carmustine, etoposide, methylprednisone; VP16 = etoposide.

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SUPPLEMENTAL TABLE 2

Sperm characteristics in the control group and the lymphoma groups.

	Control group fertile men (n = 257)	Lymphoma group (HL + NHL) (n = 75)	Hodgkin lymphoma group (n = 57)	Non-Hodgkin lymphoma group (n = 18)
Volume, mL	3.95 ± 1.90	3.34 ± 1.61[a]	3.41 ± 1.74[a]	3.13 ± 1.09[a]
pH	7.94 ± 0.28	8.05 ± 0.36[a,b]	8.07 ± 0.38[a]	7.99 ±0.28
Sperm count, 10^6/mL	98.67 ± 90.91	57.62 ± 52.57[a]	53.11 ± 46.71[a]	71.90 ± 67.51
Round cells, 10^6/mL	2.10 ± 2.62	2.39 ± 5.09[a]	2.85 ± 3.47[a]	0.98 ± 1.69[a]
Vitality, %	69.87 ± 13.44	67.73 ± 14.78[a,b]	60.05 ± 16.23[b]	61.11 ± 19.04[b]
Motility, %	43.05 ± 13.53	38.78 ± 14.02[a,b]	38.91 ± 14.03[b]	38.38 ± 14.38
Total sperm count, 10^6/ejaculate	361.95 ± 343.25	200.86 ± 240.47[a]	199.31 ± 261.49[a]	205.78 ± 162.58[a]
Total motile sperm count, 10^6/ejaculate	150.49 ± 126.37	75.44 ± 76.41[a,b]	73.80 ± 79.45[a,b]	80.64 ± 67.71[a]

Note: Values are means ± standard deviation.
[a] $P<.05$, difference between control group and lymphoma group, Hodgkin lymphoma group and non-Hodgkin lymphoma group.
[b] $P<.05$, difference between control group and lymphoma group, Hodgkin lymphoma group and non-Hodgkin lymphoma group, adjusted on abstinence duration and age.

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