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ORIGINAL ARTICLE *Andrology*

Spermatogenesis in Hodgkin's lymphoma patients: a retrospective study of semen quality before and after different chemotherapy regimens

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STUDY QUESTION: Is spermatogenesis impairment caused by Hodgkin's lymphoma (HL) itself or by the various treatments?

SUMMARY ANSWER: HL is not itself the main cause of impaired spermatogenesis, which is instead affected by the treatment; the extent of impairment depends on the type of treatment and the number of cycles.

WHAT IS KNOWN ALREADY: Data in the literature are contradictory, although most studies found poor semen quality in HL patients prior to treatment. The impact of therapy on spermatogenesis depends on the type of treatment, but the time needed to recover testicular function following treatment with chemotherapeutic agents inducing azoospermia is unknown.

STUDY DESIGN, SIZE, DURATION: In a retrospective study, the semen parameters of 519 patients (504 with sperm and 15 who were azoospermic) were investigated.

HL patients were analysed before therapy. A longitudinal study was also conducted of semen quality in 202 patients pre- and post-ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) at T0 (baseline) and 6 (T6), 12 (T12) and 24 (T24) months after the end of treatment, and of 42 patients pre- and post-BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), COPP/ABVD (cyclophosphamide, vincristine, procarbazine, prednisone, doxorubicin, bleomycin, vinblastine and dacarbazine), OPP/ABVD (vincristine, procarbazine, prednisone, doxorubicin, bleomycin, vinblastine and dacarbazine) or MOPP (mechlorethamine, vincristine, procarbazine and prednisone) and inguinal radiotherapy at different observation times (from T0 to 16 years after treatment).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Semen parameters were examined according to World Health Organization 2010 criteria, evaluating sperm concentration, total sperm number, progressive motility and morphology.

MAIN RESULTS AND THE ROLE OF CHANCE: Our data, which pertain to the largest caseload reported to date, indicate that 75% of HL patients are normozoospermic prior to treatment. The results from the HL patients studied pre- and post-therapy demonstrate that spermatogenesis recovery depends on the therapeutic regimen used. After ABVD, there was a statistically significant decrease in sperm concentration and total sperm number at T6 and T12 ($P < 0.001$; $P < 0.01$, respectively). There was a significant drop in progressive motility ($P < 0.001$) and a significant increase in abnormal forms ($P < 0.01$) at T6. The differences in sperm concentration, total sperm number and abnormal forms at T0 and T24 were not statistically significant, indicating that sperm quality had returned to pre-therapy values. The most interesting data in terms of patient management arise from the study of azoospermia induced by other chemotherapeutic agents. A high number of BEACOPP, COPP/ABVD, OPP/ABVD or MOPP cycles (≥ 6) induced a permanent absence of sperm in the seminal fluid, while even following a low number of cycles (< 6), spermatogenesis only recovered after 3–5 years and semen quality was highly impaired.

LIMITATIONS, REASONS FOR CAUTION: The study type (retrospective) and the low caseload and varying time of the follow-up do not permit any firm conclusions to be drawn about the recovery of spermatogenesis after BEACOPP or other combined therapies, or the identification of any risk factors for testicular function in treated patients.

WIDER IMPLICATIONS OF THE FINDINGS: The pretreatment semen parameters of HL patients in this study were better than some results reported in the literature, with a higher percentage of normozoospermic patients. Strengths of this study were the large caseload of HL patients and a high degree of consistency in semen analysis, as all parameters were assessed in the same laboratory. Following the azoospermia induced by different chemotherapeutic protocols, spermatogenesis may take several years to recover. Awareness of this issue will enable oncologists to better inform patients about the possibility of recovering fertility post-treatment and also demonstrates the importance of semen cryobanking before beginning any cancer treatment.

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Key words: Hodgkin lymphoma / semen quality / chemotherapy / ABVD protocol / BEACOPP protocol

Introduction

The treatment of Hodgkin's lymphoma (HL) has advanced considerably in recent decades. The improved survival rates make it ever more important to find treatments that induce fewer and less severe side effects, thus improving quality of life. The ABVD protocol, introduced in the mid-70s (Bonadonna *et al.*, 1975), soon became the standard of care for the treatment of HL. It was preceded by the hybrid protocols OPP/ABVD and COPP/ABVD, now almost completely abandoned. Most recently, the BEACOPP protocol was introduced by the German Hodgkin Study Group (GHSG); it has different active substances and a substantial increase in dose-density compared with ABVD. The BEACOPP protocol was shown to improve the control of neoplasia and increased survival at 10 years by 11% in comparison with the COPP/ABVD protocol. The GHSG therefore recommended the BEACOPP protocol as the new standard of care for suitable patients with advanced HL. However, the BEACOPP protocol is associated with greater toxicity than ABVD or hybrid protocols and can induce acute haematological and non-haematological toxicity, secondary tumours and infertility (Federico *et al.*, 2009; Viviani *et al.*, 2011).

Given their youth, men with HL must be informed about the risk of temporary or permanent sterility, as they may still wish to father children in the future. However, there is conflicting evidence in the literature regarding their potential fertility. Some studies found pretreatment semen quality to be significantly lower than that of healthy subjects, with a decline in fertility of 20–70% (Hallak *et al.*, 2000; Rueffer *et al.*, 2001). Others (Marmor *et al.*, 1986; Padron *et al.*, 1997; Tal *et al.*, 2000; Gandini *et al.*, 2003) found that, on average, HL patients showed quantitatively and qualitatively normal spermatogenesis.

The effect of antineoplastic treatment protocols on the incidence and degree of any testicular dysfunction depends on various factors, including the type of treatment and cumulative dose, etc. Many chemotherapeutic agents can induce depletion of the germ line or halt spermatogonial differentiation. Most chemotherapy drugs are cell cycle-specific and therefore are more cytotoxic for cell systems that proliferate most actively. It is also important to remember that disease progression is not always predictable, and that patients initially treated with less aggressive agents may later undergo more gonadotoxic treatments (Gandini *et al.*, 2006; Trottman *et al.*, 2007).

Given the above, the aim of this study was: (i) to assess the pretreatment quality of spermatogenesis in a large caseload of HL patients and identify any differences in semen parameters in relation to age and clinical stage; (ii) to assess, through a retrospective longitudinal study, the effect of different chemotherapy protocols (ABVD, BEACOPP and other combined chemotherapy protocols) on semen parameters. Another important aim was to analyse any chemotherapy-induced azoospermia, its duration and any recovery of spermatogenesis, even several years after the end of the chemotherapy, to establish if dose or treatment type are risk factors for azoospermia.

Materials and Methods

Patients

The study was approved by the University of Rome 'La Sapienza' institutional review board. Written informed consent was obtained from all participants. A total of 810 HL patients attended our Seminology Laboratory Sperm Bank between 1996 and 2014 for semen cryobanking before beginning treatment. We analysed the pre-therapy semen parameters of 519 of these patients, as they had been entered in a computerized data management system and were thus readily analysable. We also carried out a retrospective longitudinal study of semen quality against therapy type. As not all patients agreed to undergo follow-up post-treatment semen testing for various reasons (forgot, not interested, cured, moved away, too stressful), we assessed the 244 patients who did consent. We divided patients into two groups. Group A consisted of 202 out of 519 patients studied pre- and post-ABVD/Radiotherapy (RT) (according to Canellos *et al.*, 2009; Eich *et al.*, 2010; Engert *et al.*, 2010), at T0 (before beginning cancer treatment) and 6 (T6), 12 (T12) and 24 (T24) months after the end of treatment. Specifically, 202 patients were evaluated at T0, 123 patients at T6, 126 at T12 and 115 at T24. These patients all underwent a baseline semen examination and more than two post-treatment analyses between T6 and T24.

Group B consisted of 42 patients who underwent escalated BEACOPP (doses according to Diehl *et al.*, 1998; Skoetz *et al.*, 2013), COPP/ABVD (doses according to Diehl *et al.*, 1998), OPP/ABVD or MOPP (doses according to Santoro *et al.*, 1987) protocols and inguinal RT.

Semen analysis

Semen samples were collected by masturbation directly into a sterile plastic container after 2–7 days of sexual abstinence and examined by optical microscope according to World Health Organization (WHO) criteria (WHO, 1992, 1999, 2010). The following variables were assessed: sperm concentration ($n \times 10^6$/ml), total sperm number ($n \times 10^6$/ejaculate), progressive motility (%) and morphology (% abnormal forms). In cases of azoospermia (no sperm in the ejaculate), the analysis was performed twice and the diagnosis was made only after having carefully checked the entire post-centrifuge pellet.

Statistical analysis

All quantitative results are expressed as mean, SD and median. The pretreatment semen parameters of the 504 HL patients were categorized by age (13–17, 18–29, 30–39, 40–51 years) and stage (I–II and III–IV). The Kolmogorov–Smirnov test was used to evaluate the normal distribution of

all variables. As some semen parameters were not normally distributed, non-parametric tests (Mann–Whitney, Wilcoxon's signed-rank test) for paired and unpaired data were used to evaluate the differences between two mean values. Multivariate analyses (ANOVA, logistic regressions) were performed for total sperm number, progressive motility, abnormal forms pre-treatment and the independent variables included in the model (age, stage and general symptoms).

Any statistically significant differences in pre- and post-treatment semen parameters were then investigated. Semen parameters were compared at T0/T6, T0/T12 and T0/T24 months. All statistical analyses were performed using GraphPad Prism v.5 (GraphPad Software, Inc., La Jolla, CA, USA). A two-tailed $P < 0.05$ was considered statistically significant.

The effect at T6 of the number of ABVD cycles (2–4 versus 6–8 cycles) on spermatogenesis was also evaluated, calculating the relative efficacy for total sperm number X as $(Xt - X0)/X0$, where $X0$ is the pre-therapy value and Xt is the total sperm number at time T6. Multivariate analyses (ANOVA, logistic regressions) were performed for total sperm number at T6 and the independent variables included in the model (treatment, stage, age and total sperm number at T0).

Results

Semen quality before cancer treatment

The semen parameters of 519 HL patients who cryobanked sperm at the Seminology Laboratory Sperm Bank were evaluated pretreatment. Fifteen patients (2.9%) were azoospermic (mean age 27.1 ± 3.9 years); nine of these had Stage I–II HL and six had Stage III HL. The azoospermic patients were excluded from the statistical analysis, which was therefore performed on 504 patients. Table I shows the mean, SD and median for age and semen parameters for the 504 HL patients before treatment and broken down for the 454 adults and 50 adolescents. These parameters were all normal according to the WHO (2010) values. All patients were divided into two subgroups according to total sperm number, as an index of sperm testicular production, using the 5th percentile (total sperm number $<39 \times 10^6$) as the cut-off (WHO, 2010). A total of 25% of HL patients had impaired spermatogenesis, while 75% had a normal total sperm number.

We also assessed the impact of age and clinical stage on semen quality. The 504 HL patients were divided into four subgroups by age: 13–17 years (50 patients, 9.9%), 18–29 years (284 patients, 56.3%), 30–39 years (142 patients, 28.2%) and 40–51 years (28 patients, 5.6%). The comparative statistical analysis revealed a significantly lower ejaculate volume and lower total sperm number in the 13–17 year age group in comparison with the other groups ($P < 0.05$) (Fig. 1). However, it should be stressed that the mean semen parameters for all groups were normal according to WHO (2010).

Patients were also classified by clinical stage where available (276/504 patients), as shown in Fig. 2. The absence (A) or presence (B) of general symptoms (fever, weight loss, night sweats) was also considered.

Figure 3 shows the pretreatment semen parameters for the 276 HL patients subdivided by stage (early and late). Stage I–II patients were significantly older than Stage III–IV patients (27.5 ± 6.6 versus 26.2 ± 7.4 years, $P < 0.05$), while the semen volume (3.2 ± 1.7 versus 2.6 ± 1.5 ml, $P < 0.01$) and total sperm number (193.8 ± 173.2 versus 146.1 ± 161.3 × 10^6/ejaculate, $P < 0.01$) were significantly lower in the more advanced stages compared with the early stages, respectively. There were no significant differences for the other parameters. Here too, it should be stressed that the mean semen parameters for both groups were normal according to WHO (2010).

We also analysed any differences in semen quality in relation to the absence (A) or presence (B) of general symptoms, finding a significantly lower sperm concentration, total sperm number and progressive motility and a significantly higher percentage of abnormal forms in Stage I–II B versus I–II A ($P < 0.01$) (Fig. 4). In contrast, there were no significant differences between A and B for Stages III–IV, suggesting that systemic symptoms may have a negative effect on semen quality only in the earlier stages. To verify the effect of age, stage and general symptoms on semen quality, we conducted a multivariate analysis, which revealed that the quality of spermatogenesis was affected by general symptoms only (total sperm number $r^2 = 0.038$; $P < 0.001$, progressive motility $r^2 = 0.037$; $P < 0.001$, abnormal forms $r^2 = 0.024$; $P < 0.01$).

Semen quality after cancer treatment

A retrospective longitudinal study was carried out to evaluate the effects of the antineoplastic treatment on semen quality. The patients were divided into two groups (A and B).

Table I Age and pretreatment semen parameters (mean ± SD and median) for all patients with HL and according to age, excluding azoospermic patients.

Patients		Age (years)	Volume (ml)	Sperm concentration ($n \times 10^6$/ml)	Total sperm number ($n \times 10^6$/ejaculate)	Progressive motility (%)	Abnormal forms (%)
All, $n = 504$ (13–51 years)	Mean ± SD	26.7 ± 7.2	2.9 ± 1.6	62.3 ± 59.8	173.4 ± 189.2	38.2 ± 18.0	75.8 ± 12.9
	Median	26.0	2.8	48.0	123.3	45.0	75.0
Adults, $n = 454$ (18–51 years)	Mean ± SD	27.9 ± 6.6	3.1 ± 1.6	62.2 ± 58.7	181.9 ± 195.1	38.0 ± 18.2	75.9 ± 13.0
	Median	28.0	2.8	48.0	135.5	45.0	75.0
Adolescents, $n = 50$ (13–17 years)	Mean ± SD	15.8 ± 1.1	1.8 ± 1.3	63.7 ± 69.9	96.8 ± 94.7	39.6 ± 15.3	74.9 ± 11.9
	Median	16.0	1.3	51.5	72.0	45.0	74.5

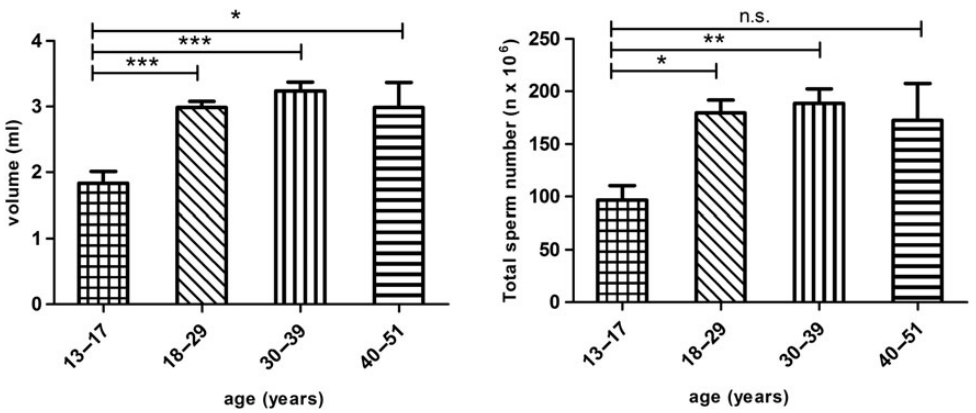

Figure 1 Comparison of mean pretreatment semen parameters of 504 HL patients by age group. Data are presented as mean/SD bars. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s., not significant (Mann–Whitney test).

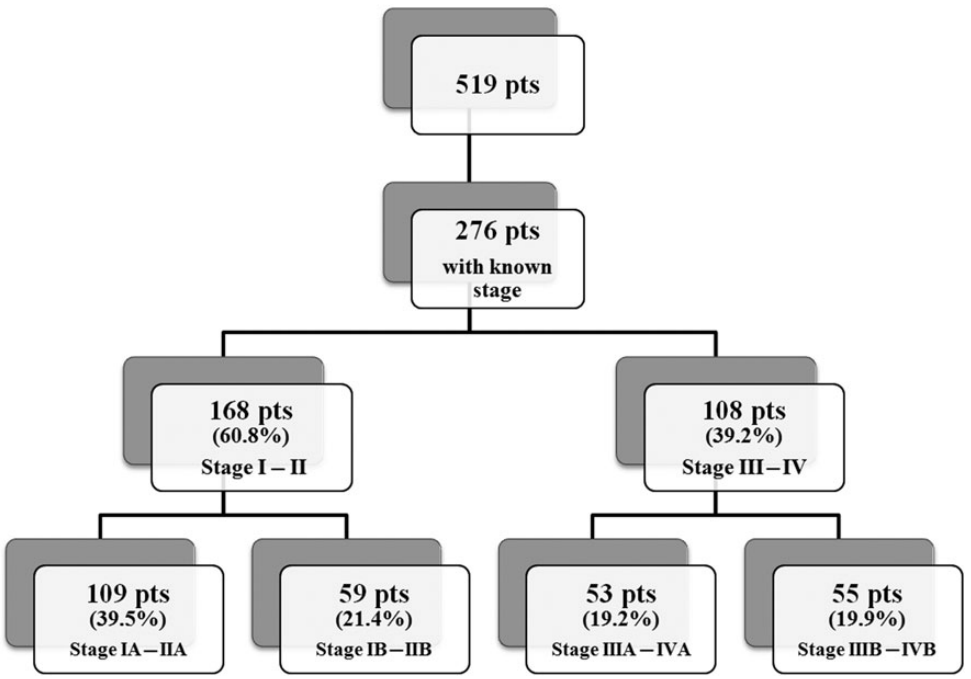

Figure 2 HL patients classified by clinical stage and absence (**A**) or presence (**B**) of symptoms.

Group A

Group A consisted of 202 patients (mean age 27.3 ± 6.8 years) who had undergone standard ABVD cycles of 28 days, with administration on Days 1 and 15. All patients underwent from two to eight ABVD cycles followed by involved field or mantle field radiation, with a total dose of ~30 Gy. None of these patients underwent inguinal RT.

The semen parameters were analysed at T0 (202 patients), T6 (123), T12 (126) and T24 (115 patients). Variations over time in semen parameters after chemotherapy are reported in Fig. 5. There was a statistically significant decrease in sperm concentration and total sperm number at T6 and T12 ($P < 0.001$; $P < 0.01$, respectively). There was a significant drop in progressive motility ($P < 0.001$) and a significant increase in abnormal forms ($P < 0.01$) at T6. No differences in sperm concentration, total sperm number and abnormal forms at T0 and T24 were observed, indicating that sperm quality had returned to pre-therapy values. Further significant improvements in progressive motility were found at T24 ($P < 0.001$). No significant differences in ejaculate volume were found at any follow-up examination. Semen evaluation at T6, T12 and T24 showed that the impact on sperm parameters was most significant 6 months after the end of ABVD therapy. No azoospermic patient

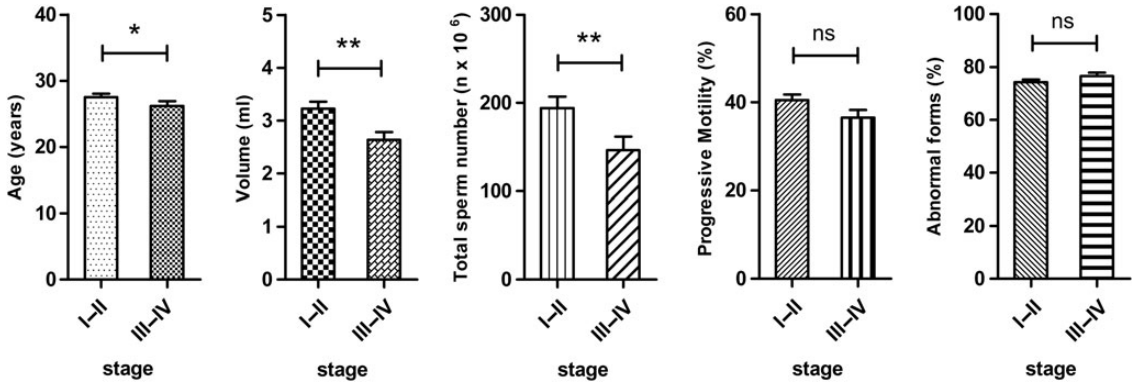

Figure 3 Comparison of mean pretreatment semen parameters of the 276 HL patients (excluding azoospermic patients) by stage (I–II and III–IV). Data are presented as mean/SD bars. *$P < 0.05$, **$P < 0.01$, n.s., not significant (Mann–Whitney test).

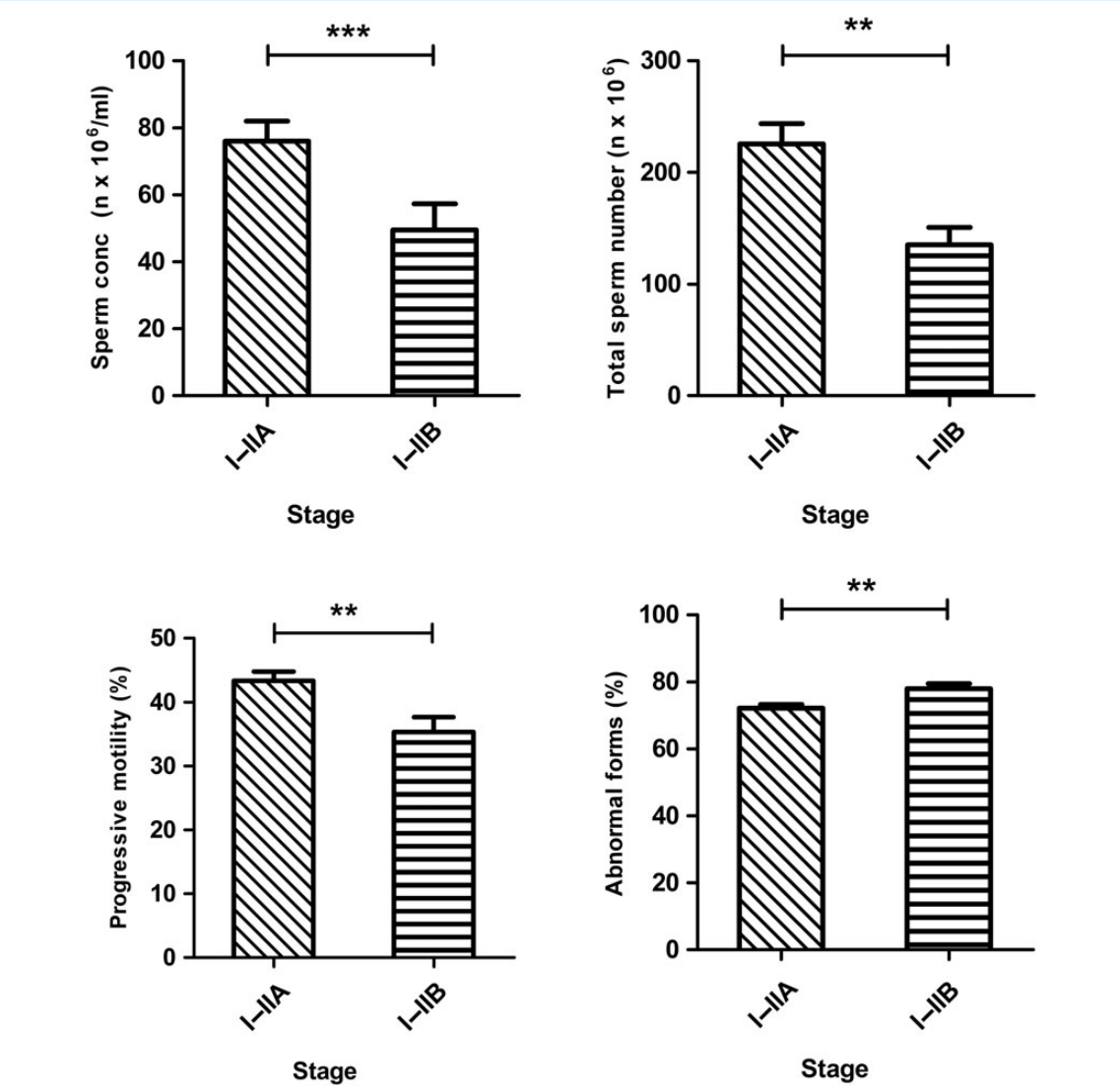

Figure 4 Comparison of mean pretreatment sperm parameters between Stage I–II A and I–II B HL patients. Data are presented as mean/SD bars. **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney test).

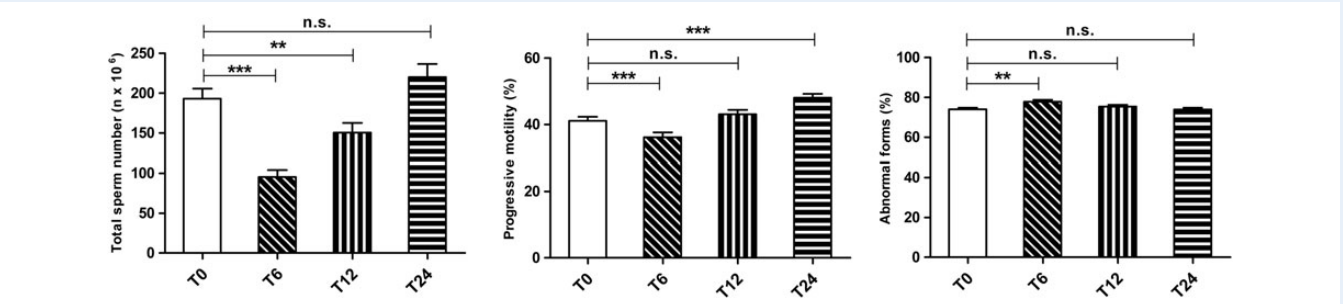

Figure 5 Variation over time in pre- and post-ABVD treatment semen parameters ($n = 202$ patients). Data are presented as mean/SD bars. ABDV, doxorubicin, bleomycin, vinblastine and dacarbazine. $^{**}P < 0.01$, $^{***}P < 0.001$, n.s., not significant (Mann–Whitney test). T0, before beginning cancer treatment; T6, 6 months after the end of treatment; T12, 12 months after the end of treatment; T24, 24 months after the end of treatment.

Table II Decrease in total sperm number (mean ± SD) from T0 to T6 against number of treatment cycles.

Group	Patients (n)	Total sperm number ($n \times 10^6$)		RD	*P*-value
		T0	T6		
L: 2–4 ABVD cycles	51	214.4 ± 212.3	114.2 ± 103.9	−46.7	<0.0001
H: 6–8 ABVD cycles	71	197.6 ± 196.5	78.5 ± 87.1	−60.3	<0.0001
P-value		0.5147	0.0202		

RD, relative difference.
Mann–Whitney test.
L, low cycle group; H, high cycle group.
ABVD, doxorubicin, bleomycin, vinblastine and dacarbazine.
T0, before beginning cancer treatment; T6, 6 months after the end of treatment.

was observed at T6, T12 or T24, except for one patient who was cryptozoospermic at T0 and azoospermic 6 months after six ABVD cycles; however, sperm quality had recovered at T12 (1.0×10^6/ejaculate) and T24 (7.5×10^6/ejaculate).

Total sperm number as an index of testicular sperm production was also considered, using $\geq 39 \times 10^6$/ejaculate as the cut-off (WHO, 2010). At the baseline, 82.2% of patients had a total sperm number $\geq 39 \times 10^6$/ejaculate. By T6, this had dropped to 67.5%, while at T12 and T24, values were similar to the baseline, at 83.3 and 90.4%. Spermatogenesis recovery was also evaluated against the number of ABVD cycles, considering the variation in total sperm number at T6 (lowest value) against T0. A total of 51 patients underwent 2–4 cycles (low cycle group, L) and 71 patients had 6–8 cycles (high cycle group, H). The mean total sperm number at T0 was $190.3 \pm 181.5 \times 10^6$ for Group L and $197.2 \pm 186.0 \times 10^6$ for Group H, dropping significantly at T6 for both groups. The magnitude of the decrease was assessed by calculating the relative difference (RD). Comparison at T6 revealed a greater post-treatment drop in total sperm number for Group H than for Group L (RD 60.3 versus 46.7%, $P < 0.05$) (Table II). These results suggest that the effect on sperm quality is more marked with a greater number of cycles.

Given that the treatment depends on the stage of the disease, we conducted a multivariate analysis of the effect of age, stage and number of cycles. This revealed that spermatogenesis was affected by the number of treatment cycles only ($r^2 = 0.034$; $P < 0.05$).

Group B

Group B consisted of 42 HL patients (mean age 27.0 ± 7.1 years) who underwent other treatments. All patients underwent an annual semen analysis for a varying number of years, beginning at 6 months post-treatment.

BEACOPP group

This consisted of 16 out of 42 patients who underwent the BEACOPP protocol. Of these, three Stage II–III patients (mean age 27.3 ± 7.1 years) recovered spermatogenesis 3 years (1 patient) and 4 years (2 patients) after the end of the treatment. These patients had undergone a low number of cycles [2 cycles (1 patient) and 4 cycles (2 patients)].

The remaining 13 patients, with Stage II–IV (mean age 29.0 ± 7.1 years), who had undergone a greater number of cycles [6 cycles (5 patients) and 8 cycles (8 patients)], were still azoospermic 3 years (6 patients), 5 years (5 patients) and 10 years (2 patients) after the end of therapy.

Combined chemotherapy group

This consisted of 13 out of 42 patients who underwent ABVD/COPP or OPP or MOPP protocols.

Of these, four Stage II–III patients (mean age 17.3 ± 4.9 years) recovered spermatogenesis 3 years (2 patients), 4 years (1 patient) and 5 years (1 patient) after the end of the treatment; these patients had undergone two MOPP cycles (1 patient) or four (1 patient) or five COPP/ABVD

cycles (2 patients). The nine Stage III–IV patients (mean age 24.6 ± 6.2 years) who underwent six OPP/ABVD (5 patients) or six COPP/ABVD cycles (4 patients) were still azoospermic 5 years (1 patient), 8 years (4 patients) and 16 years (4 patients) after the end of therapy.

The recovery of testicular function in patients undergoing BEACOPP, OPP/ABVD or COPP/ABVD protocols was mainly dependent on the number of cycles. The semen quality of those patients who recovered testicular function after both BEACOPP protocol and combined therapy was highly impaired and below the lower limit of WHO reference values (WHO, 2010). The mean semen values and SDs for the seven patients who recovered spermatogenesis were as follows: pre-therapy: total sperm number 117.7 ± 123.5 × 10^6/ejaculate, progressive motility 43.6 ± 10.3%, abnormal forms 74.6 ± 4.1%; post-therapy: total sperm number 0.8 ± 1.2 × 10^6/ejaculate, progressive motility 3.6 ± 2.4%, abnormal forms 97.1 ± 2.7%.

RT group

Thirteen out of 42 (mean age 31.5 ± 8.8 years) patients underwent ABVD (2–6 cycles) followed by inguinal irradiation (30–40 Gy). Most of these patients were azoospermic at 6 months.

Eleven out of 13 Stage I–III patients recovered spermatogenesis at different times, namely 6 months (3 patients), 1 year (2 patients), 2 years (2 patients), 3 years (3 patients) and 5 years (1 patient) after the end of therapy. The semen quality of these patients was highly impaired, with the mean semen values and SDs as follows: pre-therapy: total sperm number 229.6 ± 212.6 × 10^6/ejaculate, progressive motility 43.6 ± 17.2%, abnormal forms 72.9 ± 13.6%; post-therapy: total sperm number 6.3 ± 11.4 × 10^6/ejaculate, progressive motility 9.1 ± 13.0%, abnormal forms 91.6 ± 10.5%.

Two out of 13 Stage II–III patients were still azoospermic 3 years after the end of the therapy.

Discussion

The improvement in treatments for HL has led to a survival rate of above 90%. This improved survival may, depending on the treatment, be accompanied by a variety of medical problems including impaired female and male gametogenesis (Henry-Amar, 1996). The gonadotoxic effect of the treatment is considered a major cause of male infertility, even though some studies have identified spermatogenesis impairment caused by the lymphoma itself.

Semen quality before cancer treatment

The evidence in the literature points to varying degrees of semen quality impairment in HL patients prior to treatment. Some studies (Marmor *et al.*, 1986; Padron *et al.*, 1997; Fitoussi *et al.*, 2000; Tal *et al.*, 2000; Gandini *et al.*, 2003) found normozoospermia in more than 50% of HL patients, while others found a high degree of impaired spermatogenesis (Viviani *et al.*, 1991; Rueffer *et al.*, 2001; Sieniawski *et al.*, 2008; Van der Kaaij *et al.*, 2009) (Table III). There are various other studies that we did not include in this table, because some assess testicular function using only hormone values (van der Kaaij *et al.*, 2007), while others report only mean semen parameters without indicating the percentage of normal sperm production (Bizet *et al.*, 2012).

In any case, it should be stressed that most studies of semen quality in HL patients were carried out on relatively small, disparate caseloads, or with larger cohorts that originated from multicentre studies and for whom semen analyses were carried out by different laboratories that did not always take all semen parameters into consideration. In contrast, this study, which is the largest caseload reported to date, analysed the pretreatment semen parameters of all the recruited HL patients in the same laboratory.

The mean pretreatment semen parameters of the 519 HL patients were normal according to WHO (2010). Only 25% were oligozoospermic, and 2.9% azoospermic. The present study thus found a low level of abnormal spermatogenesis, confirming the results previously reported by the same team (Gandini *et al.*, 2003). There was also a significant reduction in semen volume and total sperm number in the youngest patients (13–17 years); however, this might be because these patients had not yet reached sexual maturity, rather than due to the disease itself (Tinggaard *et al.*, 2012). As noted above, in any case, the mean semen parameters for this group were normal according to WHO (2010).

A comparison of semen parameters in relation to clinical stage (I–II versus III–IV) revealed a significantly lower volume and total sperm number in early versus late stage patients, even though the mean for all

Table III Data from the literature on pretreatment semen quality in patients with HL.

Authors	Year	HL (*n*)	% of patients with normal total sperm number	Azoospermia (%)
Marmor *et al.*	1986	57	66.6	7.0
Viviani *et al.*	1991	92	33.0	/
Padron *et al.*	1997	49	63.0	/
Fitoussi *et al.*	2000	94	53.0	5.0
Tal *et al.*	2000	25	72.7	/
Rueffer *et al.*	2001	158	30.0	8.0
Gandini *et al.*	2003	110	75.5	3.6
Sieniawski *et al.*	2008	202	20.0	11.0
Van der Kaaij *et al.*	2009	474	41.0	3.0
Present study	2015	519	75.0	2.9

semen parameters in both groups was within normal limits. To evaluate the effect of systemic symptoms on semen quality, we compared patients in Group A (no symptoms) and Group B (presence of symptoms), finding a significantly lower sperm concentration, total sperm number and progressive motility and a significant higher percentage of abnormal forms in Group I–II B versus I–II A. For patients with a more advanced stage (III–IV), there were no significant differences between Groups A and B. Systemic symptoms may therefore affect semen quality in earlier stages only, whereas in advanced stages, the diminished semen quality could mainly be due to disease progression rather than to the symptoms. These data confirm, in a much larger caseload, the results obtained in a previous study by the same authors (Gandini *et al.*, 2003), and seem to suggest a higher percentage of normozoospermic patients than found in some other studies.

Semen quality after cancer treatment

Chemotherapy and RT are the main approaches to treating HL and have improved long-term survival. Their various consequences include reports of impaired spermatogenesis. The impact on spermatogenesis depends on the type of treatment. Chemotherapy induces the depletion or arrest of spermatogonial differentiation and mutagenesis in cells at a later developmental stage. In contrast, RT affects the spermatogonia, the most radiosensitive cells due to their intense mitotic activity, as well as spermatids. Spermatids are unprotected due to the loss of their DNA damage repair mechanisms caused by post-meiotic differentiation and chromatin condensation (Gandini *et al.*, 2006; Trottman *et al.*, 2007).

Group A

The recommended regimen for HL is ABVD, an alternative hybrid regimen. Its replacement of MOPP was a therapeutic revolution, increasing survival and reducing toxicity. ABVD is less gonadotoxic, as indicated by one of the first studies by Viviani *et al.* (1985) who reported 97% azoospermia 6 months after the end of MOPP therapy compared with 54% oligozoospermia after ABVD. The results of Viviani *et al.* (1985) have been confirmed by more recent research demonstrating transient azoospermia in a small number of patients treated with ABVD and a subsequent recovery of spermatogenesis 18–24 months after treatment (Sieniawski *et al.*, 2008; Van der Kaaij *et al.*, 2009; Di Bisceglie *et al.*, 2013; Bujan *et al.*, 2014). Comparative studies of the long-term consequences of polychemotherapy revealed that non-alkylating agents show markedly less germinal toxicity than alkylating agents (Schrader *et al.*, 2001), while azoospermia after treatment with ABVD varied from 0 to 4% (Sieniawski *et al.*, 2008). A limitation of all these studies is their small caseload; furthermore, a relatively high proportion (35.8–61%) were lost to follow-up after treatment (Pacey *et al.*, 2012).

Our retrospective longitudinal study comprises a large caseload of 202 HL patients who underwent semen analysis at T0 and 6, 12 and 24 months after the end of ABVD treatment. Azoospermia was not found at any observation time, except in one patient who was cryptozoospermic at T0 and azoospermic 6 months after six ABVD cycles; however, his sperm quality had recovered by T12. In any case, ABVD protocols have the most detrimental effect on spermatogenesis at T6, recovering at T12 and T24. As also reported by Van der Kaaij *et al.* (2009), we found that even in a patient with severely impaired pretreatment spermatogenesis, semen quality can improve after treatment with ABVD. These results also demonstrate that the effect on semen parameters is transient, being most marked at 6 months after the end of chemotherapy and recovering at 2 years. Spermatogenesis recovery is correlated with the time since the end of the therapy; 83.3% of patients treated with ABVD had a normal total sperm number after 12 months and 90.4% after 24 months. Another interesting result was the effect of the number of chemotherapy cycles on testicular sperm production, with a greater number of cycles having a negative impact on total sperm number.

Group B

Various studies have investigated chemotherapy gonadotoxicity in HL patients. Type B spermatogonia, which proliferate actively, are extremely susceptible to cytotoxic agents. However, Type A spermatogonia, which have little mitotic activity, are less affected and could survive polychemotherapy if threshold cumulative cytostatic doses are not surpassed. Patients receiving high doses of alkylating agents are very likely to become azoospermic, although spermatogenesis may recover in the long term (Van der Kaaij *et al.*, 2010). In fact, the effect of chemotherapy on spermatogenesis depends essentially on the combination of agents and the dose. Sieniawski *et al.* (2008) studied 71 HL patients pre- and post-therapy, finding azoospermia in 91% of patients who had received four cycles of COPP/ABVD and in 93% of patients who had undergone eight cycles of BEACOPP. In this study, we found a large number of patients (13/16) with azoospermia up to 10 years after undergoing 6–8 BEACOPP cycles, with spermatogenesis recovery 3–4 years after the end of treatment in just 3 patients who had undergone fewer (2–4) cycles. Testicular function also recovered after 3–5 years in the 13 patients treated with 4–6 OPP/ABVD or COPP/ABVD cycles or 2 MOPP cycles, while 9 out of 13 patients who had undergone 6 COPP/ABVD or OPP/ABVD cycles were still azoospermic after up to 16 years. All these regimens contain cytotoxic agents such as cyclophosphamide and procarbazine. It would appear, therefore, that these agents are mainly responsible for gonadotoxicity, as they are associated with prolonged azoospermia and any recovery of spermatogenesis is slow and, furthermore, semen quality is extremely impaired and below the WHO reference values (WHO, 2010).

A limitation of this study was that the low number of patients in this subgroup ($n = 42$) and varying follow-up times made it difficult to provide conclusive information on the recovery of spermatogenesis after BEACOPP or COPP/ABVD, OPP/ABVD or MOPP protocols or to identify risk factors for testicular function in treated patients. However, it can be postulated that the greater the number of cycles, the more difficult recovery of spermatogenesis.

In conclusion, our data indicate that 75% of HL patients are normozoospermic prior to treatment. Patients with more advanced disease had poorer semen parameters than those in the earlier stages. Comparison of pre- and post-therapy semen parameters demonstrated that spermatogenesis recovery depends on the therapeutic regimen used. ABVD was most detrimental to spermatogenesis at T6, with recovery at T12 and T24; recovery of spermatogenesis thus seems to be a function of time since the end of the therapy. In contrast, a high number of BEACOPP, COPP/ABVD, OPP/ABVD or MOPP cycles led to permanent azoospermia, while even after a low number of cycles spermatogenesis recovered only after 3–5 years and semen quality was highly impaired. Awareness of this issue will enable oncologists to better inform patients about the possibility of recovering fertility post-treatment and also demonstrates the importance of semen cryobanking before beginning any cancer treatment.

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Authors' roles

The trial was conceived and designed by DP and LG. Semen analysis was performed by LG. The article was written by: DP and LG. Data acquisition and statistical analysis was performed by GF, FR and FP. Critical review of the paper was performed by AP, GA, FL and AL.

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Conflict of interest

The authors have no conflicts of interest.

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