

In male Hodgkin lymphoma patients, impaired fertility may be improved by non-gonadotoxic therapy

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Received 22 May 2021; accepted for publication 28 July 2021

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Summary

The outcome of patients with Hodgkin lymphoma (HL) has improved significantly in recent years, and now attention is increasingly being focused on the well-being of these young patients. This study aimed to analyse the influence of HL and its treatment on the spermatogenic status of 46 male HL patients with available spermograms, treated between 2008 and 2016. Analysing prognostic factors at diagnosis, we found that the number of spermatozoa was reduced in stage III–IV; motility and vitality were reduced in stage III–IV and in the presence of B symptoms; and abnormal forms were increased in patients with elevated erythrocyte sedimentation rate (ESR) and low albumin. Furthermore, we found that haematopoietic stem cell transplantation (HSCT) was associated with a severe impairment of fertility in terms of sperm motility. In HL-treated patients who did not undergo HSCT we found a statistically significantly improved fertility in terms of motility. In this study, we found that HSCT induced infertility in the majority of male patients with HL, but that first-line treatment could improve the impaired fertility status caused by disease. Further studies are needed in larger case series to investigate risk factors for impaired fertility at HL diagnosis and after treatment.

Keywords: Hodgkin lymphoma, semen quality, chemotherapy, adriamycin, bleomycin, vinblastin and dacarbazine regimen, autologous stem cell transplantation.

Introduction

Most males diagnosed with Hodgkin lymphoma (HL) are of reproductive age, and as the average paternal age in many industrialized countries rises, so the proportion of men diagnosed with HL before having children will increase.¹

Furthermore, reproductive function is impaired in a considerable number of patients at the time of diagnosis, compromising both the possibility of spontaneous fertilization and the chance for sperm cryopreservation before treatment.

Improvements in survival and the availability of less gonadotoxic treatments have led to an increasing number of survivors of HL who wish to have children.^{2,3} Previous studies found a reduced fertility in patients with HL prior to therapy, indicating that HL-associated factors contribute to infertility. Other authors, instead, found that, on average, HL patients showed normal spermatogenesis, both quantitatively and qualitatively.^{4–7}

In general, gonadal damage is attributed to toxic effects on the germinal epithelium caused by chemotherapy

containing alkylating agents, especially cyclophosphamide and procarbazine. The effect of antineoplastic treatment protocols on the incidence and degree of any testicular dysfunction depends on various factors, including the type of treatment, 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 to those cell systems that proliferate most actively.

Given the clinical importance of this issue, and the current lack of empirical evidence, we investigated men with HL treated with modern regimens in order to evaluate a possible impairment of fertility. Fertility status was correlated to patients' clinical and biological features at the time of diagnosis.

Patients and methods

Between 2008 and 2016, 100 cases of HL were diagnosed at the University Hospital of Bari (Italy). All patients had

signed informed consent to undergo treatment, after the risks of chemotherapy and the various treatment options had been explained to them. All patients presented for counselling on fertility preservation methods before receiving cytotoxic chemotherapy; 46 requested semen cryopreservation (Table I), while 54 refused cryopreservation (25 were already fathers and they did not want further children, 29 refused on the grounds that they wanted to start chemotherapy treatment as soon as possible).

We analysed pre-therapy semen parameters in these patients; not all patients agreed to undergo follow-up post-treatment semen testing for various reasons (forgot, not interested, cured, moved away, too stressful), so we finally assessed 24 patients (Table II).

All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable. The Independent Ethical Committee (IEC) of 'Policlinico' Hospital of Bari approved this study.

All patients were clinically staged according to the Ann Arbor system, including medical history, complete physical examination, blood counts, biochemical profile, chest X-ray films, total body positron emission tomography/computed tomography (PET/CT), chest, abdomen and pelvis CT, and unilateral bone marrow biopsy.

Clinical characteristics are listed in Table I. Median age at diagnosis was 25 years (range 15–37), 18 patients (39%) presented advanced-stage disease (III–IV), 15 (33%) bulky disease, 16 (35%) presented B symptoms, 9 (20%) extranodal disease.

First-line chemotherapy was adriamycin, bleomycin, vinblastin and dacarbazine (ABVD) in all patients. The median number of chemotherapy lines was one (1–5); 33 (72%) patients had undergone prior supradiaphragmatic radiotherapy but none pelvic radiotherapy.

The ifosfamide, gemcitabine and vinorelbine (IGEV) regimen was used as salvage chemotherapy; 12 patients (26%) underwent autologous haematopoietic stem cell

transplantation (HSCT). Carmustine, etoposide, cytarabine and melphalan (BEAM) were used as high-dose chemotherapy. Three patients (6%) underwent allogeneic HSCT.

Semen analysis

After a sexual abstinence of at least three days, semen samples after masturbation were collected. An average total of 10 straws per patient was cryopreserved before the treatments (range 2–17). All assays were evaluated for sperm volume, count, forward motility and morphological spermatozoa criteria, according to WHO guidelines (fifth edition, 2010).

Disordered sperm quality conditions included oligozoospermia (O, sperm concentration $<15 \times 10^6/\text{ml}$), asthenozoospermia (A, sperm with $<40\%$ total motility) and theratozoospermia (T, sperm with $<4\%$ normal morphology). These parameters define single damage or disorder of one or more of these variables, severe damage like an OAT-syndrome (disorder of all three variables), or azoospermia, with no spermatozoa in the ejaculate.

Statistical analysis

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 differences between mean values. Analyses were performed pre and post treatment for total sperm number, progressive motility, abnormal forms and clinical and biological parameters.

Any statistically significant differences found between pre- and post-treatment semen parameters were then investigated.

Results

Patients characteristics

During the study period, 46 newly diagnosed cases of HL were treated with the ABVD regimen. Clinical characteristics are shown in Table I.

At the end of first treatment, 42 patients (91%) were in complete remission (CR), and four patients (9%) in partial remission (PR).

After a median follow-up of 104 months (range 53–168 months), eight had relapsed; 12 patients (26%) underwent autologous HSCT. Three patients (6%) underwent allogeneic HSCT. At last follow-up, 45 patients (98%) were alive and in CR, one (2%) had died of a second malignancy (diffuse large B cell non-Hodgkin lymphoma) that occurred three years after HSCT.

Pre-treatment semen analysis

The median sperm concentration was $22 \times 10^6/\text{ml}$ (0–125), the median percentage of normal-motility sperms was 40%

Table I. Patients' characteristics.

	First line alone	Stem cell transplantation
Total 46	34 (74%)	12 (26%)
Median age (range)	26 (15–37)	25 (16–32)
Stage III–IV	11 (32%)	7 (58%)
B symptoms	12 (35%)	4 (33%)
Bulky disease	10 (29%)	5 (41%)
Extranodal disease	5 (15%)	4 (33%)
Elevated erythrocyte sedimentation rate (ESR)	25 (73%)	9 (75%)
WBC $>12 \times 10^3/\mu\text{l}$	7 (21%)	1 (8%)
Albumin $<4 \text{ g/dl}$	11 (32%)	5 (42%)
Lymphocytes $<600/\mu\text{l}$	4 (12%)	2 (17%)

WBC, white blood cell count.

Table II. Characteristics of the 24 patients with semen availability before and after treatment.

ID	Age	Stage	B symptoms	Treatment	Follow-up (months)	Dyspermia before treatment	Dyspermia after treatment	Birth of a child
1	20	2	No	First line only	146	O	O, A	No
2	27	2	No	First line only	55			No
3	16	2	Yes	First line only	84			No
4	28	3	Yes	HSCT	87		AZO	No
5	30	2	No	First line only	56	T	O, A	No
6	22	2	No	First line only	66	O		No
7	28	3	Yes	First line only	69	OAT	A	No
8	21	2	Yes	First line only	85			No
9	26	3	Yes	HSCT	111	OAT		Yes
10	23	2	No	HSCT	71	OAT	AZO	No
11	29	4	No	First line only	65	O, A	OAT	No
12	19	4	No	HSCT	123	A	OAT	No
13	24	2	No	First line only	109	A		No
14	16	4	No	HSCT	171	O	A	No
15	26	3	No	First line only	91			No
16	36	2	No	First line only	80	A	O, A	Yes
17	26	2	No	First line only	67		AZO	No
18	27	4	Yes	First line only	107	A		No
19	23	2	No	First line only	94	O, A		No
20	17	4	Yes	First line only	158	O, A		No
21	37	2	No	First line only	103	O, A	A	No
22	27	3	Yes	First line only	62	O, A		No
23	37	3	Yes	First line only	87			No
24	19	4	Yes	First line only	87			No

A, asthenozoospermia; AZO, azoospermia; HSCT, haematopoietic stem cell transplantation; O, oligozoospermia; T, theratozoospermia.

(0–75), and the median percentage of normal-morphology sperms was 15% (0–48). Severely damaged sperm was observed in eight patients (17%). Of these, two were azoospermic and six had an OAT-syndrome; seven patients (15%) were found to have combined damage, seven patients (15%) presenting OA but none AT. Single damage was found in 18 patients (39%), while 13 (28%) patients showed a normal sperm analysis at the time of diagnosis.

Analysing prognostic factors, we found that the number of spermatozoa at diagnosis was reduced in disease stage III–IV ($19 \times 10^6/\text{ml}$ vs $49 \times 10^6/\text{ml}$; $P = 0.001$); motility and vitality were reduced in stage III–IV (motility: 29% vs 43%, $P = 0.015$; vitality: 49% vs 71%, $P = 0.011$; Fig 1) and in the presence of B symptoms (motility: 30% vs 44%, $P = 0.016$; vitality: 53% vs 69%, $P = 0.05$; Fig 1); abnormal forms were increased in patients with elevated ESR (84% vs 75%, $P = 0.017$; Fig 2) and albumin <4 g/dl (87% vs 75%, $P = 0.002$; Fig 2).

Post-treatment semen analysis

Of the 24 patients analysed after treatment, 12 patients were analysed once, 10 twice and two patients underwent multiple analyses. The median time of sperm analysis after the end of treatment was 54 months (range 26–137 months).

The median sperm concentration was $27 \times 10^6/\text{ml}$ (0–210), the median percentage of normal-motility sperms was 45% (0–85), and the median percentage of normal-morphology sperms was 8% (0–28). Severe damage was observed in five (21%) of the patients. Of these, three were azoospermic and two had an OAT-syndrome; three patients (12.5%) showed combined damage, and three (12%) OA. At the post-treatment analysis, single damage was found in three patients (12.5%), while 13 (54%) showed a normal sperm analysis. In the group of patients who underwent HSCT, four had oligospermia (three of them azoospermia), two OAT and one single damage (A). In the group of patients not undergoing HSCT one had azoospermia, three OA, two A, and 12 a normal sperm analysis.

Pre- and post-treatment semen analysis

Sperm collected at diagnosis was compared with the first check performed 24 months after the end of treatment. We observed that in eight patients (33%) the number of spermatozoa dropped (of these, 50% underwent HSCT), while in 16 the spermatozoa increased (of these, 87% did not undergo HSCT).

The number of normal forms was reduced in 20 patients; nine had normal motility (of these 56% underwent HSCT) and 15 normal vitality (of these 33% underwent HSCT).

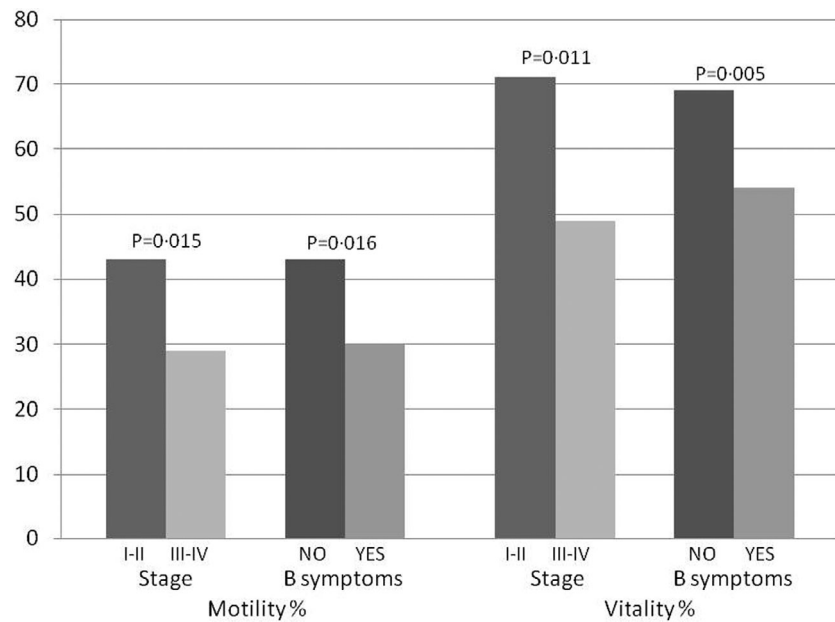


Fig 1. Median percentage of motility and vitality of semen analysis and correlation with clinical stage and B symptoms.

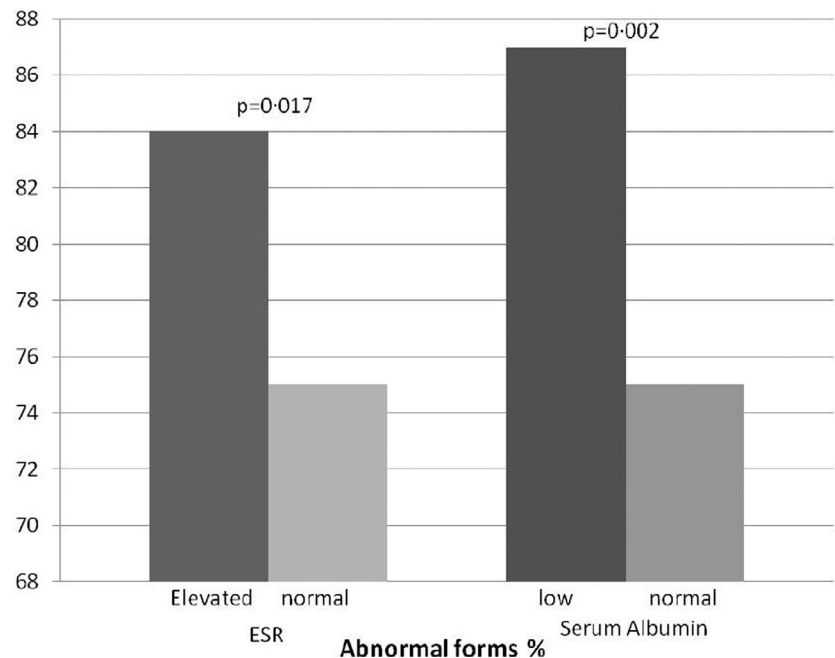


Fig 2. Median percentage of abnormal forms in semen analysis and correlation with erythrocyte sedimentation rate (ESR) and serum albumin levels.

Furthermore, we found that HSCT was associated with severe fertility impairment in terms of sperm motility (74% at diagnosis vs 22% after HSCT; $P = 0.025$). In patients who did not undergo HSCT, fertility was statistically significantly better in terms of number of spermatozoa ($39 \times 10^6/\text{ml}$ vs $53 \times 10^6/\text{ml}$; $P = 0.001$) and motility (35% at diagnosis vs 50% after treatment; $P = 0.009$).

To date, no patient has procreated using the sperm bank [two refractory patients who underwent HSCT and are being treated with checkpoint inhibitor (CPI) are currently trying], while two patients (one who underwent an autologous transplant and one treated with a single-line therapy) have

become fathers of a healthy child, without the need for assisted fertilization, at 110 and 79 months, respectively, from the last chemotherapy (Table II).

Discussion

For advanced Hodgkin lymphoma, first-line chemotherapy, alone or in combination with radiotherapy, leads to five-year progression-free survival (PFS) rates of 70–85%, regardless of the chemotherapy regimen applied.¹

Standard of care for relapsed or refractory HL is second-line chemotherapy followed by autologous HSCT, which can

induce long-term remission in approximately 40–50% of patients.^{8–10}

However, these improved treatments for HL can, depending on the treatment, be accompanied by a variety of medical problems, including reduced female and male gametogenesis.^{3–7} The gonadotoxic effect of treatment is considered a major cause of male infertility, although some studies have found that the impaired spermatogenesis was caused by the lymphoma itself.

In our analysis the median sperm concentration in all patients was $22 \times 10^6/\text{ml}$ (range 0– $125 \times 10^6/\text{ml}$), the median percentage of normal-motility sperm was 40% (range 0–75%) and the median percentage of normal-morphology spermatozoa was 15% (range 0–48%). Compared to healthy controls, we found a significant decrease in fertility parameters. In a previous study, the mean sperm concentration in 1 351 healthy men was twice that in our series ($98.8 \times 10^6/\text{ml}$), the mean percentage of normal-motility sperm was 66% and the mean percentage of normal-morphology sperm was 61%; in another study of 238 normal men the mean sperm concentration was $133.9 \times 10^6/\text{ml}$.^{11,12}

Chapman *et al.* found that 16 of 37 (43%) patients with HL had adequate sperm counts before starting treatment. Trial biopsies were taken from nine patients. Eight of these biopsies showed pathological changes ranging up to sclerosis of the germinal epithelium.¹³

In addition to HL, reduced fertility rates at diagnosis have also been identified in patients with non-Hodgkin lymphoma, testicular cancer and other malignancies.^{14,15}

Systemic symptoms, particularly fever, have been considered to be associated with higher rates of infertility.¹⁶

Patients with more advanced disease had poorer semen parameters than those in earlier stages.

In our study we found that the number of spermatozoa at diagnosis was reduced in stage III–IV; motility and vitality were reduced in stage III–IV and in the presence of B symptoms; abnormal forms were increased in patients with elevated ESR and albumin <4 g/dl. Clinical and biological parameters may be associated with the influence on fertility of several cytokines, as reported by many authors in recent publications.^{17–19}

Thus, since HL is a biologically active disease, several cytokines (IL-1, IL-6, TNF- α and soluble interleukin receptor sR IL-2 and sR IL-6) have sparked research interest as a possible mechanism contributing to infertility.

If HL itself induces the observed changes, successful HL therapy can lead to the recovery of fertility, but only if the chemotherapy regimen does not contain toxic gonadal drugs.^{4–7}

This is important because patients with HL are primarily younger, and family planning may not have been completed at disease onset. Chemotherapy and radiation therapy (RT) are the main approaches to HL treatment and have improved long-term survival but among the various consequences there have been reports of impaired spermatogenesis. The impact

on spermatogenesis depends on the type of treatment. Only with ABVD alone was a high recovery rate observed after treatment. Chemotherapy induces depletion or arrested differentiation and mutagenesis of spermatogonia in cells at a later stage of development. Conversely, RT affects spermatogonia, which are the most radiosensitive cells due to their intense mitotic activity, as well as spermatids. Spermatids are unprotected due to loss of their DNA damage repair mechanisms caused by postmeiotic differentiation and chromatin condensation.²⁰ However, regarding DNA sperm damage, it was demonstrated that DNA alterations were present also before any treatment. Bujan *et al.* reported that the DNA fragmentation index was 8.6% in the control group of fertile men, 15.9% in lymphoma patients before treatment and 17.4% at three months after treatment.²¹ Instead, another study did not find differences between the HL group and the control group in terms of the DNA fragmentation index.²²

The recovery of spermatogenesis is related to the time lapse since the end of therapy; 83.3% of ABVD-treated patients had a normal total sperm count after 12 months and 90.4% after 24 months. Another interesting finding was the effect of the number of chemotherapy cycles on testicular sperm production, showing that a higher number of cycles had a negative impact on the total sperm count. In this study we found that HSCT was associated with severe fertility impairment in terms of sperm motility. Furthermore, in patients who did not undergo HSCT we found a statistically significantly better fertility in terms of motility.

In our study, only two HL survivors had children after therapy and none of them used cryopreserved semen. For this reason, it should be taken into account that most of them already had children at the time of the HL diagnosis. Nevertheless, it is extremely important to cryopreserve sperm before any treatment, given that sperm cannot later be cryopreserved until at least 6 months after the last chemotherapy and that a second-line therapy with HSCT could be needed.

In conclusion, our data indicate that in a large number of HL patients at diagnosis, the sperm count was reduced, there was a low percentage of normal motility and numerous abnormal forms were present. Unfavourable clinical and biological parameters present at diagnosis were associated with worse spermatogenesis. Comparison of semen parameters before and after therapy showed that the recovery of spermatogenesis depends on the therapeutic regimen applied. Further investigation is needed to clarify the mechanisms underlying the HL high initial infertility rate and to identify potent new chemotherapy regimens that can preserve gonadal function.

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