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Follow-up of sperm concentration and motility in patients with lymphoma

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Lymphomas are a group of diseases, prevalent at reproductive age. Fertility is notoriously reduced among lymphoma patients. This study evaluates pre- and post-treatment semen concentration and motility, and factors associated with semen quality deterioration. We followed-up 33 patients with non-Hodgkin's lymphoma or with Hodgkin's disease during the years 1987–1997 who were referred for semen cryopreservation. Pretreatment semen analysis, and hormonal profile were recorded at diagnosis and at least 1 year after completion of the treatment, and compared. Medical records for disease type, disease stage and treatment protocols were related to long-term sperm outcome. Hormonal concentrations were not predictive of post-treatment sperm concentration. In patients with localized disease, initial sperm concentration and motility tended to be preserved, compared with patients with widespread disease ($P = 0.016$). In Hodgkin's disease patients, treatment with the adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) protocol was superior to the mechloretamine, vincristine, procarbazine and prednisone with ABV protocol regarding germinal toxicity ($P = 0.0008$). The post-treatment sperm outcome was better in patients treated with local irradiation than in those who did not undergo irradiation ($P = 0.0027$). No predictive tools for post-treatment fertility were found and, therefore, every patient with a lymphoma should have his semen cryopreserved at diagnosis.

Key words: fertility/Hodgkin's disease/lymphoma/Non-Hodgkin's lymphoma/semen

Introduction

Lymphomas are defined as a group of malignant diseases characterized by infiltration of lymph nodes with malignant cells that destroy their architecture. Hodgkin's disease (HD) is identified histologically by large, binucleated Reed–Sternberg cells in lymph node biopsies. Non-Hodgkin's lymphomas (NHL) are a large group of diseases, defined as any lymphoma that is not HD. HD and NHL are biologically and clinically distinct, the former being the most common type of lymphoma during the reproductive period of life, with a peak incidence at 15–35 years of age. The incidence of HD is increasing, especially among patients aged 20–44 years (Chen *et al.*, 1997). NHL is one of the 10 most common malignant diseases. The annual incidence of NHL is increasing (Wingo *et al.*, 1998), especially among young males (aged <55 years). The detrimental effect of HD, as well as its treatment, on male fertility are well known: fertility is reduced in up to 70% of patients with HD (Viviani *et al.*, 1991), but the cause thereof is not completely understood. Fever, general state of a patient suffering from a malignant disease and psychological factors were all suggested as possible causes. Infertility is also possibly immune-mediated (Barr *et al.*, 1993). Chemotherapy and irradiation further contribute to infertility. Testicular damage may be irreversible, but even if reversed, repair may take as long as 20 years (Marmor and Duyck, 1995). New treatment regimens have increased patient survival substantially, so that more patients at the reproductive age are living and are confronted with infertility.

Semen cryopreservation is an element of management of lymphoma patients. This is due to the growing awareness of physicians and patients themselves regarding the potentially devastating effect of the disease and its treatment on spermatogenesis and future fertility. Patients who had a lymphoma were referred to our institute for semen cryopreservation before commencing treatment. Previously, we studied the effect of lymphomas on spermatogenesis and assessed pretreatment sperm quality (Botchan *et al.*, 1997). The present study was conducted to determine the factors, either the disease characteristics themselves, or different treatment protocols, directly related to semen concentration and motility deterioration. In this study we assessed pre- and post-treatment sperm quality, reflected by sperm motility and concentration. Patients were categorized by their disease (HD or NHL), disease stage and treatment. Staging included distinction between patients with and without systemic symptoms: fever, weight loss and night sweats ('B symptoms'). We related these factors to the change in sperm concentration and final total motile count (TMC). Knowledge of the modifiable factors associated with poor outcome assists in the choice of more appropriate treatment protocols, and minimizes the risk of infertility among lymphoma patients.

Materials and methods

The study population comprised 33 patients, derived from 112 consecutive lymphoma patients referred to our centre for semen cryopreservation prior to initiation of treatment between the years

1987 and 1997. Follow-up, at least 1 year after completion of treatment, was carried out on these 33 patients. The remaining patients were not re-evaluated: 12 patients died as a result of the disease, 34 were lost to follow-up, 31 refused re-evaluation, and for the remaining two patients, medical data concerning exact diagnosis and treatment were missing. Informed consent was obtained from all the patients in our study group. The mean follow-up period was 36 months (range: 12–93 months). We tried to find a relationship between follow-up period and sperm quality as reflected by sperm concentration. Blood samples for testosterone and FSH concentrations were taken before commencing treatment and at the end of the follow-up period. Semen samples (range: 1–20, mean: 5.4 samples per patient) were collected for analysis and cryopreservation was performed immediately after the diagnosis of the lymphoma was established. Multiple samples were collected from each patient due to the desire to cryopreserve more than one sample per patient. One sperm sample was collected for analysis at the end of the follow-up period. The samples were collected after 72 h of sexual abstinence, with the exception of three patients who required urgent treatment. We reviewed the medical records and obtained the age, exact diagnosis (HD or NHL), stage of the disease at diagnosis according to the Ann Arbor Staging System (Ultmann and Moran, 1973), evidence of B symptoms and treatment protocols.

Our study group of 33 patients consisted mainly of young men aged 16–42 years (mean 28.5 years). Among these, eight were diagnosed as having NHL and 25 as having HD. Staging was recorded for 24 of our 33 patients: 14 patients had localized disease at diagnosis (stage I or II), and 10 had widespread disease (stages III or IV). Treatment comprised irradiation ($n = 3$), chemotherapy ($n = 16$) or both ($n = 13$). One patient refused treatment and therefore received neither chemotherapy nor irradiation. The most common chemotherapy protocols among HD patients were adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) and mechloretamine, vincristine, procarbazine and prednisone with ABV (MOPP-ABV). Among the 29 patients treated with chemotherapy, 10 received ABVD, nine received MOPP-ABV and 10 were treated with other protocols.

Patients were classified into three categories according to sperm concentration, before and after treatment: severe oligozoospermia ($<5\times10^6$/ml), mild oligozoospermia ($5–20\times10^6$/ml) and normal sperm concentration ($>20\times10^6$/ml). Azoospermia was defined as complete absence of spermatozoa in the ejaculate. Improvement in sperm concentration was defined as moving from one category of sperm concentration to a higher category, while deterioration was classified as the reverse. No change was defined as remaining in the same category.

The change in sperm concentration was compared between HD and NHL patients, between different stages of disease and between different treatment protocols. In addition, patients were classified into two groups according to TMC post-treatment: group A (TMC = 0) and group B (TMC > 0). Total motile count was compared according to the above classification criteria. The statistical analysis was repeated, considering only the first pretreatment semen sample for each patient, and comparing the results with the analysis, taking into consideration an average of concentration and motility of all the pretreatment samples taken for a given patient.

Statistical analysis

Forward stepwise logistic regression, with sperm concentration as the dependent variable, was used to evaluate the importance of the different variables measured. Statistical analysis was performed by the Statistical Package for Social Science/Personal Computers (SPSS/PC+) version 8.0. The χ^2 test was used to evaluate the effect of stage, chemotherapy treatment protocol and irradiation on change in sperm concentration and on the existence of motile spermatozoa. One-way analysis of variance (ANOVA) was used to evaluate the effect of hormonal profile on change in sperm concentration and on the existence of motile spermatozoa; $P < 0.05$ was considered to be statistically significant.

Table I. Patient distribution according to sperm concentration

	Severe oligozoospermia $<5\times10^6$/ml	Mild oligozoospermia $5–20\times10^6$/ml	Normal $>20\times10^6$/ml
Pretreatment (%)	2 (6.1)	7 (21.2)	24 (72.7)
Post-treatment (%)	17 (51.5)	1 (3.0)	15 (45.5)

Table II. Patient distribution according to changes in sperm concentration after treatment as a function of diagnosis, disease stage, treatment protocol and irradiation. Values in parentheses are percentages

	Total	Improvement	No change	Deterioration	*P*
Diagnosis					
HD	25	2 (8)	12 (48)	11 (44)	NS
NHL	8	1 (12)	3 (38)	4 (50)	
Disease stage at diagnosis					
Stages I, II	14	1 (7)	10 (72)	3 (21)	0.016
Stages III, IV	10	0 (0)	2 (20)	8 (80)	
Chemotherapy protocol					
ABVD	10	1 (10)	9 (90)	0 (0)	0.0008
MOPP-ABV	9	0 (0)	1 (11)	8 (89)	
Irradiation treatment					
No irradiation	17	0 (0)	4 (24)	13 (76)	0.0027
Irradiation	16	3 (19)	10 (62)	3 (19)	

HD = Hodgkin's disease; NHL = Non-Hodgkin's lymphoma; ABVD = adriamycin, bleomycin, vinblastine and dacarbazine; MOPP-ABV = mechloretamine, vincristine, procarbazine and prednisone; NS = not significant.

Results

The mean time to re-evaluation was 36 months (range: 12–93 months). No statistically significant relationship was found between the time to re-evaluation and sperm concentration, when the follow-up period was >12 months. Therefore, we did not subdivide the patients into subgroups by length of follow-up period.

Patient distribution by sperm concentration before and after treatment is shown in Table I. Since we dealt with patients referred for sperm cryopreservation, none of those who entered the study was azoospermic before treatment; however, at the time of follow-up 11 patients (33.3%) had become azoospermic after treatment. Serum testosterone and FSH concentrations prior to treatment were not predictive of the change in sperm concentration ($P > 0.05$). There was no statistically significant difference between HD and NHL patients regarding improvement or deterioration in sperm concentration (Table II). Most of the patients with localized disease (stages I or II) preserved their initial sperm concentration, while deterioration in sperm concentration was detected in patients with widespread disease (stages III or IV) ($P = 0.016$), as shown in Table II. No

Table III. Pre- and post-treatment hormonal concentrations, and comparison between patients with and without motile spermatozoa

	Motile spermatozoa = 0	Motile spermatozoa >0
Pre-treatment		
FSH (IU/ml)	4.70	4.86
Testosterone (ng/ml)	5.45	5.95
Post-treatment		
FSH (IU/ml)	16.35	11.00
Testosterone (ng/ml)	4.98	3.99

There were no significant differences between the patients with and without motile spermatozoa.

statistically significant difference was found in the change in sperm concentration when comparing patients with and without B symptoms. Two of the most common treatment protocols among our HD patients were ABVD and MOPP-ABV. The detrimental effect for sperm concentration was less for patients treated with ABVD ($P = 0.0008$, Table II). Stepwise regression analysis revealed that poorer post-treatment sperm concentrations in advanced-stage disease were mainly attributed to the chemotherapy regimen used, followed by the disease stage itself. Patients with localized disease were treated with local irradiation. A better outcome was found in patients who were treated with irradiation rather than those who were not treated ($P = 0.0027$; Table II). Forward regression analysis was used to demonstrate that the better outcome reflected the effect of stage, rather than the effect of irradiation.

Post-treatment TMC was calculated for each of our patients. We sought factors that were related to deterioration of sperm quality, reflected by complete absence of motile spermatozoa after treatment. FSH and testosterone concentrations before and after treatment were not predictive for complete absence of motile spermatozoa ($P > 0.05$, Table III). Furthermore, differences in the existence of motile spermatozoa between HD and NHL patients were not statistically significant (Table IV). Widespread disease (stage III or IV), chemotherapy with MOPP-ABV protocol and no irradiation treatment were all related to TMC = 0 post-treatment (Table IV). There was no significant relationship between existence of B symptoms and TMC = 0 after treatment. Similar results were found either using the first pretreatment semen sample or the average of all pretreatment samples collected per patient (data not shown), leading to the same conclusions.

Discussion

The detrimental effect of lymphomas and their treatment is well-established in the medical literature. Up to 30% of lymphoma patients have pretreatment semen anomalies, which is reflected by a sperm count of $<20\times10^6$/ml and reduced sperm motility (Marmor *et al.*, 1986). In another study, oligozoospermia, asthenozoospermia and/or teratozoospermia were found in 67% of HD patients (Viviani *et al.*, 1991). These anomalies were attributed to emotional stress, elevated body temperature and immunological influences (Barr *et al.*, 1993). A comprehensive study, encompassing 12 years' experience of semen cryopreservation for cancer patients, revealed reduced pretreatment sperm motility (pretreatment motile sperm count was 7.6×10^6/ml and motility was 33%), among HD patients (Padron *et al.*, 1997). In the present study, we also demonstrated reduced pretreatment sperm concentrations: values $<20\times10^6$/ml were found in nine (27.3%) patients, values $<5\times10^6$/ml, defined as severe oligozoospermia, were found in two (6.1%) of the patients. Sperm count after treatment revealed further damage to spermatogenesis so that 51.5% of the patients had severe oligozoospermia. This could not be predicted by the pretreatment hormonal profile, within the limitation of this study population size.

Table IV. Patient distribution according to the existence of motile spermatozoa as a function of diagnosis, disease stage, treatment protocol and irradiation. Values in parentheses are percentages

	Total	Motile spermatozoa = 0	Motile spermatozoa >0	*P*
Diagnosis				
HD	25	10 (40)	15 (60)	NS
NHL	8	3 (37)	5 (63)	
Disease stage at diagnosis				
Stages I, II	14	3 (21)	11 (79)	0.017
Stages III, IV	10	7 (70)	3 (30)	
Chemotherapy protocol				
ABVD	10	0 (0)	10 (100)	0.001
MOPP-ABV	9	7 (78)	2 (22)	
Irradiation treatment				
No irradiation	17	11 (65)	6 (35)	0.007
Irradiation	16	3 (19)	13 (81)	

HD = Hodgkin's disease; NHL = Non-Hodgkin's lymphoma; ABVD = adriamycin, bleomycin, vinblastine and dacarbazine; MOPP-ABV = mechloretamine, vincristine, procarbazine and prednisone; NS = not significant.

The question of infertility and its management should be addressed in HD, as well as in NHL patients. The current study found that patients with localized disease (stages I or II) were more likely to have their sperm concentration preserved (in up to 71% of the patients), than those with widespread disease (stages III or IV), in whom deterioration of sperm concentration was almost the rule (up to 80% of patients). Absence of motile spermatozoa, 1 year or more after completion of treatment, was more prevalent among patients with advanced-stage disease (21 versus 70% in stages I or II, versus stages III or IV respectively). These differences may be attributable to more aggressive disease or its treatment. The effect of the disease and its treatment in these patients should be studied further. Systemic manifestations of lymphomas include fever, weight loss and night sweats ('B symptoms'). We found no statistically significant differences in sperm quality reflected by sperm concentration and motility, in patients with and without B symptoms.

Treatment protocols are determined by the oncologist, unlike predetermined factors such as disease type (HD or NHL), stage, or hormonal state. The first priority in choosing the treatment regimen should be its effectiveness and the possibility to achieve a cure. When treatment protocols are equally effective, the impact upon spermatogenesis should be taken

into consideration. Chemotherapy protocols have been studied extensively. The effects of MOPP and ABVD for HD patients are similar. In up to 97% of cases, treatment with MOPP has been shown to result in azoospermia, while ABVD-induced azoospermia occurred in only 54% of patients (Viviani *et al.*, 1985). In a review of the literature, MOPP was found to cause azoospermia in 300 of 373 HD patients (Marmor and Duyck, 1995). This high testicular toxicity of the MOPP protocol was attributed to alkylating agents (mechloretamine and procarbazine). Recovery of spermatogenesis following MOPP chemotherapy may take up to 10 years (Marmor and Duyck, 1995). We found no statistically significant relationship between follow-up period and sperm concentration, when the follow-up period was >12 months. In our study, sperm concentration deteriorated among most patients treated with MOPP (88.9%), while in the majority of the patients treated with ABVD (90%) there was no change in sperm concentration. Up to 78% of our patients who were treated with MOPP had no motile spermatozoa at the end of the follow-up period, while among those who received ABVD motile spermatozoa could be found in all cases. These results should be considered when choosing the treatment protocol for HD patients.

Of our patients, ~50% were treated with local irradiation; none received local irradiation directed to the testes. Inclusion of pelvic irradiation in the treatment regimen for NHL patients was found to be an independent determinant of reduced spermatogenesis recovery (Pryzant *et al.*, 1993). Recovery of spermatogenesis after irradiation varies widely with irradiation apparatus used, the irradiated field and the total delivered dose. Among our patients, those who received local irradiation had a better outcome. They demonstrated a tendency for pretreatment sperm quality to be preserved and only a few had immotile spermatozoa following treatment. These findings are consistent with the fact that none of the patients received local irradiation directed to the testes. Irradiation was used mainly to treat patients with early-stage disease and the better outcome among these patients probably reflects the effect of stage, rather than irradiation.

Similar results and conclusions were obtained when considering only the first pretreatment sample per patient, compared to an average of concentration and motility of all the pretreatment samples collected.

In conclusion, lymphoma patients may face infertility caused by the disease itself, or by its treatment. In this study we demonstrated that in patients with widespread disease the outcome was less favourable than that of patients with localized disease. The treatment further affected spermatogenesis, and protocols differed in their toxicity. In this respect, superiority of the ABVD protocol over the MOPP-ABV protocol was well observed. Nevertheless, since no predictive criteria exist as yet for sperm quality deterioration in individual patients, as reflected by sperm concentration and motility, cryopreservation of semen is recommended for every lymphoma patient once the diagnosis is established, regardless of disease stage or given treatment regimen. Published data (Naysmith *et al.*, 1998) support no minimal criteria for semen cryopreservation as long as spermatozoa are present in the ejaculate. With semen cryopreservation, the need for advanced assisted reproductive techniques, e.g. intracytoplasmic sperm injection (ICSI), can be markedly reduced. The need for donor insemination can also be reduced, and a very reasonable chance of conception can be offered to all cancer patients.

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