Testicular cancer and Hodgkin’s disease: evaluation of semen quality

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BACKGROUND: The aim of our study was to establish whether there is a difference in semen quality between patients with testicular cancer (TC) and Hodgkin’s disease (HD). METHODS: We evaluated 342 patients affected by TC (n = 232) or HD (n = 110) who cryobanked sperm before initiating chemo- or radiotherapy. All TC patients were evaluated ~1 month after orchidectomy. RESULTS: A total of 14 patients were azoospermic or cryptozoospermic. In the TC group (n = 222) the mean of the semen parameters was normal according to the World Health Organization (1992). However, dividing the cases into total sperm count >40 x 10⁶/ejaculate and <40 x 10⁶/ejaculate, 35.5% of the patients showed an impaired semen quality. The quality of sperm parameters was higher in seminoma patients than for the other histological groups. A significant difference for all semen variables was observed between patients with serum ßhCG levels classified as pathological (>5 mIU/ml) and those with normal serum ßhCG. Comparison of semen parameters between TC stages I and II showed no significant differences. In the HD group (n = 106), we found that by and large they showed normal spermatogenesis, with only 24.5% having a total sperm count <40 x 10⁶/ejaculate. There was a significant decrease in semen quality in stages III and IV of HD. CONCLUSIONS: Better semen quality was observed in patients with HD than in those with TC. The semen quality observed in our TC and HD groups seems better than previous results reported in the literature.

Key words: Hodgkin’s disease/semen quality/testicular cancer

Introduction

Testicular cancer and Hodgkin’s disease represent two of the most frequent pathologies in young adult males (Meirow and Shenker, 1995). In particular, germ cell tumours derive from the germinal epithelium of seminiferous tubules and have various histological types (seminoma, embryonal carcinoma, choriocarcinoma, teratoma and mixed tumours composed of more than one of the previous histotypes), with different degrees of invasiveness and metastasizing capacity. Hodgkin’s disease is characterized by the presence of large bi-nucleate cells in the lymph node infiltrate. It is classified in four stages of severity: stages I and II indicate the initial stages, while III and IV represent the pathology’s more advanced stages.

The incidence of testicular cancer and Hodgkin’s disease is highest in the age group 20–35 years (Padron et al., 1997). The prognosis of patients with such tumours has improved over the past years following correct staging and utilization of more effective therapies (Laguna et al., 2001). The improvement in prognosis, the young age of the patients and information from the existing literature regarding poor semen quality even before anticancer therapy make semen cryopreservation before treat-ment essential, as such treatment may induce minor or major alterations in spermatogenesis, including possible transitory or irreversible azoospermia (for a review see Bahadur, 2000).

Various authors have reported a decline in semen quality before therapy for both pathologies. In testicular cancer, it has been suggested that this impairment is related to the direct effect of the tumour and the production of ßhCG by some cancer histotypes, and in Hodgkin’s disease to the presence of constitutional symptoms accompanying the disease (fever, weight loss, etc.) (Morrish et al., 1990; Viviani et al., 1991; Fossa et al., 1993; Botchan et al., 1997a,b; Petersen et al., 1998; Tal et al., 2000; Rueffer et al., 2001). It is also necessary to take into account the general stress associated with tumoral illness (Meirow and Schenker, 1995).

The aims of this study were: (i) to examine the quality of semen in patients affected by testicular cancer (after orchidectomy) or Hodgkin’s disease, in both cases before treatment (chemo- or radiotherapy). This was done to establish whether there is a difference between patients affected by cancer pathology involving the sperm production site and those affected by Hodgkin’s disease, which is a systemic pathology;
(ii) for testicular cancer, to evaluate the difference (if any) in semen quality with respect to the histotype and tumour marker (βhCG) production; (iii) for Hodgkin’s disease, to establish if there is a correlation between spermatogenesis and the early (I, II) and late (III, IV) stages and inflammation markers such as erythrocyte sedimentation rate (ESR).

Materials and methods

Patients
The study was approved by the Institutional Review Board of our University Hospital.

We evaluated 342 consecutive patients affected by testicular cancer (TC, n = 232) or Hodgkin’s disease (HD, n = 110) who cryobanked sperm between June 1996 and December 2001 at the Laboratory of Seminology and Immunology of Reproduction of the Department of Medical Pathophysiology, University of Rome ‘La Sapienza’, before initiating chemotherapie or radiotherapy. All testicular cancer patients were evaluated ~1 month after orchidecetomy.

Semen samples were collected by masturbation into sterile plastic jars, after 2–7 days of abstinence. The abstinence period was greater than the above in a few cases due to the urgency in starting therapy (two HD patients and 10 TC patients had an abstinence >7 days). Samples were allowed to liquefy for 60 min at 37°C and were then evaluated according to the World Health Organization (WHO) (World Health Organization, 1992, 1999). Variables taken into consideration were: ejaculate volume (ml), sperm concentration (×10^6/ml), total sperm count (n×10^9), forward motility (%) and morphology (%) of atypical forms. Due to the urgent need for patients to start therapy semen analysis was conducted on only one sample for each patient. All seminal fluid examinations were carried out by the same biologist (L.G.).

Patients signed their informed consent to both cryopreservation and semen quality follow-up. The two groups of patients (TC/HD) were further subdivided by total sperm count, as an index of sperm testicular production, into two subgroups: A: <40×10^6/ejaculate; B: ≥40×10^6/ejaculate. This was on the basis of indications World Health Organization, 1999) to evaluate and compare the quality of spermatogenesis.

TC group patients were classified into clinical stages (I, II, III) and were also subdivided by histological feature: seminoma, embryonal carcinoma, mixed tumours (various associations of seminoma, teratocarcinoma, choriocarcinoma, yolk sac tumour).

During the diagnostic protocol of testicular cancer patients before orchidecetomy, cancer marker (α fetoprotein, βhCG, carcinoembryonic antigen) analysis was conducted. HD group patients were also subdivided by the stage of lymphoma at the time of the diagnosis into stage I–II (early) and stage III–IV (late). In these patients, the ESR had been evaluated during the first examination. These results were later studied for possible correlation with infertility and semen quality (Rueffer et al., 2001).

Statistical analysis
For all variables, means and SD were calculated; for nominal variables, frequencies and % were reported. Comparisons between TC and HD groups were performed by Student’s t-test, or in the absence of normal distribution by Mann–Whitney test. Comparisons among more than two groups were performed by analysis of variance and Bonferroni test.

Results
At the moment of semen analysis, sperm cryopreservation was not possible for 10 patients in the TC group (4.3%) and four patients in the HD group (3.6%). In the TC group, four patients were azoospermic and six were cryptozoospermic (one or two sperm in the pellet), in the HD group three patients were azoospermic and one was cryptozoospermic. These patients were excluded from the statistical analysis, which was performed on 222 TC group and 106 HD group patients only (Figure 1). The mean period of abstinence was 4.1 days for testicular cancer and 4.2 days for Hodgkin’s patients.

Age and semen parameters (volume, sperm concentration, total sperm count, % forward motility and % atypical forms) of the two groups (excluding azoospermic and cryptozoospermic patients) are reported in Table I. TC patients were significantly older than HD patients (P < 0.01). The difference in ejaculate volume between the two groups is not statistically

![Figure 1. Schematic representation of testicular cancer and Hodgkin’s disease patients by histotype and stage of disease.](https://academic.oup.com/humrep/article-abstract/18/4/796/596560/797)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Volume (ml)</th>
<th>Sperm concentration (×10^6/ml)</th>
<th>Total sperm count (×10^9)</th>
<th>Forward motility (%)</th>
<th>Atypical forms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular cancer</td>
<td>222</td>
<td>28.8 ± 5.6</td>
<td>3.4 ± 1.7</td>
<td>30.2 ± 32.3</td>
<td>99.9 ± 108.2</td>
<td>31.1 ± 17.0</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>106</td>
<td>27.3 ± 5.9b</td>
<td>3.4 ± 1.6e</td>
<td>51.5 ± 39.8e</td>
<td>165.9 ± 130.3b</td>
<td>36.9 ± 15.8b</td>
</tr>
</tbody>
</table>

*Not significant. 

bP < 0.01. 

<sup>6</sup>P < 0.001.
significant, but there was a statistically significant reduction in both sperm concentration and total sperm count ($P < 0.001$) and forward motility ($P < 0.01$), and a significant increase in atypical forms ($P < 0.01$) in the TC group compared with the HD group.

The difference between percentages of azoospermic/cryptospermatic patients in the TC (4.3%) and HD (3.6%) groups was not statistically significant. When divided on the basis of the total sperm count, 35.5% (79/222) of TC patients showed a sperm count $<40 \times 10^6$/ejaculate compared with 19.8% (21/106) of HD patients ($P < 0.01$).

Histological data were available for all 222 TC patients. Of these, 118 (53.1%) were affected by seminoma, 50 (22.5%) by embryonal carcinoma and 54 (24.3%) by mixed tumours such as teratoma, embryonal carcinoma, yolk sac tumours, choriocarcinoma in different combinations. Table II reports the age and sperm parameters of testicular cancer patients on the basis of classification by histological type. The mean age of the seminoma group was significantly higher ($P < 0.01$) than that of the other two groups. There was no significant difference in semen volume. The quality of sperm parameters was higher for seminoma patients than for the other groups, but this difference was only statistically significant for sperm concentration and total sperm count (both $P < 0.01$), forward motility ($P < 0.05$) and atypical forms ($P < 0.05$) in seminoma versus embryonal carcinoma.

Testicular cancer stage data were available for 135/222 patients. Ninety-one patients (67.4%) presented with stage I, 37 (27.4%) with stage II and only seven (5.2%) with stage III disease. Comparison of age and semen parameters between stages I and II shows no significant differences. Comparison with stage III was not conducted due to the insufficient number of such patients.

Cancer marker values were available for 177/222 testicular cancer patients. Of these, 34.5% (61/177) showed at least one pathological marker ($\alpha$ fetoprotein, $\beta$hCG, carcinoembryonic antigen). 16.4% of these (10/61) were affected by seminoma, 32.8% (20/61) by embryonal carcinoma and 50.8% (31/61) by mixed tumours (Figure 1). Normal cancer marker values were seen in 66.5% (116/177) of patients: of these, 67.2% (78/116) were affected by seminoma.

Sperm count was $>40 \times 10^6$/ejaculate in 67.2% of patients with normal and 60.6% of patients with pathological marker values. This difference was not statistically significant.

$\beta$hCG assay was performed in 153 patients. Samples with sera $\beta$hCG values $>5$ mIU/ml were considered as positive. Using this criterion, 36 samples with sera levels from 9.2 to 8680 mIU/ml were pathological. A significant difference for all semen variables evaluated was observed between patients with pathological $\beta$hCG values and those with normal values; in particular, total sperm count ($P < 0.05$), sperm motility and atypical forms ($P < 0.01$). However, the concentration of $\beta$hCG detected in the sera was not correlated with any sperm parameter.

Stage evaluation of the 106 HD patients demonstrated that 76 (71.7%) of the patients were in early stages and 30 (28.3%) in late stages. Table III reports their age and semen parameters by stage. Age and volume of the two groups were similar, but there was a significant decrease in sperm concentration and total sperm count ($P < 0.05$) and forward motility ($P < 0.01$) in the later stages.

ESR value was available for 97 HD patients. Seventy of these were in stage I–II and 27 in stage III–IV. Fifty-three patients presented an ESR $>30$ mm after 1 h (the threshold level at which the ESR value was considered pathological). Fifty-three of these were early stage, with five having a total

### Table II. Sperm parameters (mean ± SD) by testicular cancer tumour histotype

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Age (years)</th>
<th>Volume (ml)</th>
<th>Sperm concentration ($\times 10^6$/mil)</th>
<th>Total sperm count ($\times 10^6$)</th>
<th>Forward motility (%)</th>
<th>Atypical forms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminoma (S)</td>
<td>118</td>
<td>30.5 ± 5.2</td>
<td>3.4 ± 1.7</td>
<td>36.5 ± 37.8</td>
<td>122.0 ± 121.4</td>
<td>34.1 ± 16.2</td>
</tr>
<tr>
<td>Embryonal carcinoma (E)</td>
<td>50</td>
<td>26.9 ± 5.8</td>
<td>3.3 ± 1.9</td>
<td>19.2 ± 17.9</td>
<td>65.7 ± 74.5</td>
<td>26.6 ± 16.3</td>
</tr>
<tr>
<td>Mixed tumours (M)</td>
<td>54</td>
<td>26.8 ± 5.3</td>
<td>3.3 ± 1.5</td>
<td>26.7 ± 26.0</td>
<td>83.1 ± 92.9</td>
<td>28.7 ± 18.4</td>
</tr>
<tr>
<td>S versus E</td>
<td>c</td>
<td>a</td>
<td>c</td>
<td>a</td>
<td>b</td>
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</tr>
<tr>
<td>S versus M</td>
<td>c</td>
<td>a</td>
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<td>E versus M</td>
<td>a</td>
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<td>a</td>
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</tr>
</tbody>
</table>

*Not significant.

$^aP < 0.05.$

$^bP < 0.01.$

### Table III. Sperm parameters (mean ± SD) by Hodgkin’s disease stage

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Age (years)</th>
<th>Volume (ml)</th>
<th>Sperm concentration ($\times 10^6$/mil)</th>
<th>Total sperm count ($\times 10^6$)</th>
<th>Forward motility (%)</th>
<th>Atypical forms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I–II</td>
<td>76</td>
<td>27.2 ± 5.9</td>
<td>3.3 ± 1.7</td>
<td>57.4 ± 41.5</td>
<td>182.7 ± 134.8</td>
<td>39.8 ± 14.4</td>
</tr>
<tr>
<td>Stage III–IV</td>
<td>30</td>
<td>27.7 ± 6.1</td>
<td>3.5 ± 1.5</td>
<td>36.7 ± 31.1</td>
<td>123.5 ± 109.0</td>
<td>29.5 ± 16.9</td>
</tr>
</tbody>
</table>

*Not significant.

$^aP < 0.05.$

$^bP < 0.01.$
sperm count <40×10⁶/ejaculate and 28 having ≥40×10⁶/ejaculate. In 20 of the 27 late stage patients, the ESR was pathological; of these, six had total sperm count <40×10⁶/ejaculate and 14 had ≥40×10⁶/ejaculate. It can be seen that of the 53 patients with high ESR, 42 (79.2%) showed a normal sperm count.

Discussion
The prognosis for TC and HD is now greatly improved. In the 1970s, the survival rate was only 10%, while during the 1990s it was 90% for testicular cancer and up to 85–88% for Hodgkin’s patients (Boring, 1994). This impressive increase is due to improvements in both diagnosis and surgical techniques and the pharmaceutical drugs employed (Fossa et al., 1993; Panidis et al., 1999; Dearmley et al., 2001). It is also significant that testicular cancers such as seminoma are particularly susceptible to radiotherapy and various chemotherapy (Schrader et al., 2001; Panidis et al., 1999).

Numerous studies have shown that patients with testicular cancer are affected by oligozoospermia to a greater or lesser extent before starting chemo- or radiotherapy (Hendry et al., 1983; Fritz and Weissbach, 1985; Fossa et al., 1989; Agarwal et al., 1995; Meirow and Schenker 1995; Kliessch et al., 1997; Botchan et al., 1997a; Petersen et al., 1999; Panidis et al., 1999; Fitoussi et al., 2000). It was recently reported by Jacobsen et al. (2000) and de Kretser (2000) in a retrospective study and re-proposed by Skakkebaek et al. (2001) that poor semen quality patients may in fact be at a higher risk of developing testicular cancer. These sperm alterations may be initiated by diverse factors, such as a pre-existing defect in spermatogenesis tied to an anomalous functioning of Sertoli cells in the fetus and newborn baby (Skakkebaek et al., 1998), previous cryptorchidism (Mulder et al., 1984; Giwercman et al., 1989), environmental xenoestrogens (Depue et al., 1983; Sharpe and Skakkebaek, 1993), or substances produced by tumour cells such as βhCG, α fetoprotein (Berthelsen and Skakkebaek, 1983). In particular, data from a testicular biopsies demonstrate a correlation between high βhCG serum levels and spermatogenesis alteration not only in the ipsilateral testis but also in contralateral testicular tissue (Hayashi et al., 2001). In addition, the enormous stress caused by the awareness of having cancer must not be forgotten (Meirow and Schenker, 1995; Panidis et al., 1999). However, contrasting data also exist, such as the study of 178 patients by Lampe et al. (1997) who reported that 53% of the patients were normospermic before chemotherapy.

In our TC patient group, after orchidectomy and before therapy, even after exclusion of the TC patients with azoospermia or cryptozoospermia, the mean of the semen parameters remained in the normal range. Sperm count, forward motility and percentage of atypical forms were in fact normal according to World Health Organisation (1992, 1999) values. However, dividing the cases into total sperm count ≥40×10⁶/ejaculate and <40×10⁶/ejaculate, it is interesting to note that 35.5% of the patients showed an impaired semen quality. Further analysis by stage shows that most patients coming for cryopreservation have early stage cancer. 67.4% of patients presented with stage I, 27.4% with stage II and only 5.2% with stage III, disease. This could in part be responsible for the good mean semen quality observed, although in any case comparison of semen quality in stage I and II patients did not demonstrate a significant difference in sperm parameters. This indicates that there is no deterioration of spermatogenesis as a consequence of disease progression. Analysis by TC histology shows that most of these patients were affected by seminoma and had higher sperm parameter quality than the other TC groups, even though they had a statistically significant higher age. The difference in semen quality is statistically significant only for seminoma versus embryonal carcinoma. Within the TC group, cancer marker βhCG shows a relationship with deteriorated spermatogenesis but not the expected negative correlation between βhCG concentration and semen quality. The role of βhCG in impairing spermatogenesis is not clear. It has been demonstrated that βhCG stimulates aromatization in Leydig cells, although it does not seem to act through a disruption of the hypothalamic–pituitary–gonadal axis (Hayashi et al., 2001).

Various authors have described a more or less severe alteration of semen quality in pre-treatment Hodgkin’s disease patients (Marmor et al., 1986; Viviani et al., 1991; Barr et al., 1993; Padron et al., 1997; Fitoussi et al., 2000; Tal et al., 2000). The percentage of patients affected by oligozoospermia and/or asthenozoospermia and/or teratozoospermia in these studies varies from around 30 to 65%.

Our study included 110 patients with HD. After exclusion of the four patients with azoospermia or cryptozoospermia, we found that on average they showed both quantitatively and qualitatively normal spermatogenesis, with only 19.8% having a total sperm count <40×10⁶/ejaculate. However, seminal characteristics showed decreasing semen quality from early to late stage patients, even though the mean of the seminal parameters in both groups was in WHO’s ‘normal’ range. This may be explained by the fact that these patients, all young, were studied for semen quality very quickly after diagnosis and before the start of chemotherapy.

A recent study demonstrated a correlation between damage to fertility and an ESR elevated as a consequence of inflammation and increase in the negative effect of cytokines on spermatogenesis (Rueffer et al., 2001). The authors suggest the use of ESR as a marker for the severity of HD-related infertility. In contrast, in our patients we found that 72% with an elevated ESR showed normozoospermia, suggesting that this parameter is not a predictive index of semen quality or the potential fertility of HD patients. This difference could be due to the poor correlation between ESR values and HD stage found in our patients.

Our data demonstrate better semen quality in HD than in TC patients, which is confirmed by comparison of groups divided by total sperm count cut off. A higher percentage of TC patients show oligozoospermia, although there was a similar level of azoospermic and cryptozoospermic patients in the HD and TC groups. This lower semen quality could be due to possible local negative effects on spermatogenesis, bearing in mind that TC patients at the time of the study were monorchid and had experienced a very distressing surgical removal of a
neoplastic tests 1 month before analysis, while HD patients, however adversely affected by a systemic pathology, still possessed both testicles and had not been subjected to surgical treatment.

The semen quality observed in our TC and HD patient groups seems better than results reported in current literature. Excluding possible differences in patient group selection and considering the abstinence period as similar to that reported for other groups, this may mean that in the patients with testicular or systemic neoplasia, other factors in addition to the tumoural pathology itself have an effect on spermatogenesis. There is therefore a need to investigate other factors which could act on the pathogenesis of the oligozoospermia in neoplastic patients, such as different influences on spermatogenesis of the same kind of neoplasia, or ethnic and environmental differences in genetic networks controlling spermatogenesis which affect the sensitivity of gonadal function. This last factor has already been suggested to explain differences in the decline in semen quality seen in various countries (Carlsen et al., 1992; Gandini et al., 2000a; Jensen et al., 2002).

Further studies with a larger patient group are necessary to further investigate the effect of neoplastic pathologies on the sperm substructure. Literature evidence already demonstrates that there is a higher percentage of apoptotic DNA fragmentation (Gandini et al., 2000b; Kersemaekers et al., 2002) and a greater frequency of aneuploidy in the sperm of patients with NC (Giwercman et al., 1990; Salanova et al., 1999).

Acknowledgements

The authors wish to thank Marie-Hélène Hayles for assistance in the preparation of the manuscript. This work was supported by a grant of the Italian Ministry of Education University and Research (MIUR-COFIN) and the University of Rome ‘La Sapienza’, Faculty of Medicine.

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