Semen quality before and after gonadotoxic treatment

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BACKGROUND: The aim of this study was to analyse the semen quality of patients before and after gonadotoxic therapy. PATIENTS AND METHODS: We evaluated semen quality in 314 patients over a 26 year period. The diagnostic categories were leukaemia (n = 13); lymphoma (n = 128); testicular cancer (n = 102); benign conditions (n = 13); and other malignant neoplasms (n = 58). The degree of azoospermia or oligozoospermia for each disease category was recorded. We then analysed the recovery in semen quality over time for each disease category. RESULTS: The mean patient age was 27.9 years (range 13–65 years). A total of 1115 post-treatment semen samples were analysed from 314 patients. There was a significant reduction in the post-treatment sperm concentration, sperm motility and semen volume compared with pre-treatment levels (P < 0.05) in the entire cohort. However, the sperm movement and motility grade remained unaffected. Patients with testicular carcinoma had the lowest pre-treatment sperm concentrations but also the lowest incidence of azoospermia after cancer treatment. Patients with lymphoma and leukaemia had the highest incidence of post-treatment azoospermia and oligospermia. Patients having the largest reductions in their sperm concentration after treatment required the longest recovery period for spermatogenesis. The diagnostic category was the only significant predictor of post-treatment azoospermia. CONCLUSION: Gonadotoxic treatment results in a significant reduction in sperm quality. The type of cancer or disease, and the pre-treatment sperm concentrations were found to be the most significant factors governing post-treatment semen quality and recovery of spermatogenesis. All categories of patients displayed varying degrees of azoospermia and oligozoospermia, and recovery of gonadal function from these states was not significant. This highlights the importance of ensuring sperm banking before treatment, including for patients with benign conditions. Several factors and associations are discussed further in order to give an insight into the pre- and post-gonadotoxic treatment effects.

Key words: azoospermia/cancer/gonadotoxicity/oligozoospermia/quality

Introduction

Testicular dysfunction is an unwanted and well documented side effect of cancer therapy. Although first reported in 1948, when azoospermia following treatment with nitrogen mustard was described (Spitz, 1948), several reports have since assessed the semen quality in various cancer patients (Whitehead et al., 1982; Viviani et al., 1985; Aubier et al., 1989; Palmieri et al., 1996; Lampe et al., 1997; Tal et al., 2000; Ishikawa et al., 2004).

Improved patient survival rates coupled with advances in reproductive technologies such as IVF and ICSI mean that adult cancer patients can now be offered sperm cryopreservation (Hallak et al., 1999; Bahadur, 2000). However, factors such as the post-treatment semen status, recovery of sperm and safety issues have attracted enormous clinical and medico-legal attention (Byrne et al., 1987; Meistrich, 1993; Robbins et al., 1997; Martin et al., 1999; Chatterjee et al., 2000; Arnon et al., 2001; De Mas et al., 2001; Bahadur et al., 2002; Frias et al., 2003; Codrington et al., 2004; Deane et al., 2004; Seli et al., 2004).

This study assesses the semen quality of patients before and after gonadotoxic treatment. The data were also analysed in order to identify predictors or associations of sperm loss and recovery.

Patients and methods

A retrospective study was conducted of 314 patients who underwent gonadotoxic therapy and were referred for sperm cryopreservation between 1976 and 2002 to the combined Fertility Unit Laboratory at the University College and Middlesex Hospitals. Patients were referred from various clinics and seen within 3 days of their referral. Following counselling and informed consent, semen analyses were
performed, with all samples obtained by masturbation. The semen analyses were carried out in accordance with WHO guidelines (World Health Organization, 1987, 1992) using a Neubauer counting chamber or Makler counting chamber (Menkveld et al., 1980; Imaide et al., 1993; Shiran et al., 1995). Motility grading was 0–4, with 0 as non-progressive and 4 as very good progression. Semen volume measurements were carried out using a sterile graduated syringe. Morphology data were excluded mainly due to changes in the WHO criteria for sperm normality over the study period.

The patient characteristics such as age and disease were recorded. The patients were placed into one of five groups according to their underlying disease: leukaemia, lymphoma, testicular cancer, benign conditions which required chemotherapy (Crohns disease, renal lupus and musculoaponeurotic fibromatosus) and other malignant neoplasms (soft tissue sarcomas, osteosarcoma, prostate and skin cancers).

Two pre-treatment semen samples were collected from each patient. All patient follow-up remained the same in so far as patients chose a convenient time rather than being given a pre-determined fixed appointment. Therefore, results should be viewed in the context of this pragmatic limitation of study design. Each patient was followed-up after undergoing gonadotoxic treatment with an average of three post-treatment visits (range 1–9 post-treatment visits). Mean values of the two pre-treatment semen analyses were compared with the best post-treatment semen analysis. The follow-up period is variable and depended on the rate of recovery of the sperm concentration. Furthermore, for the lymphoma and testicular cancer groups, the first and last post-treatment semen samples were also compared.

Data analysis
Statistical analysis was performed using SPSS® for Windows® (SPSS Inc, Chicago, IL). Data were analysed using Spearman’s rank correlation test, Mann–Whitney U-test, Wilcoxon signed rank test and the Pearson χ² test. A multiple logistic regression analysis was performed using a forward selection model to adjust the existing association between prognostic factors.

Results
The study cohort consisted of 314 patients (leukaemia \( n = 13 \); lymphoma \( n = 128 \); testicular cancer \( n = 102 \); benign conditions \( n = 13 \); other malignant neoplasms \( n = 58 \)). The mean age of the patients was 27.9 years (range 13–65 years). A total of 1115 post-treatment samples were available for analysis over a mean follow-up period of 162 weeks (range 13–789 weeks). The results are summarized in Tables I–IV and in Figure 1a–c.

Combining all the available pre- and post-treatment samples showed that the overall sperm concentration, sperm motility and semen volume were significantly reduced following cytotoxic treatment in the whole cohort (\( n = 314, P < 0.05 \)) (Table Ia). However, the motility grade remained unaffected after gonadotoxic treatment (\( P > 0.05 \)).

Table Ib illustrates the subgroup analyses of semen quality. In the testicular cancer group, only the concentration and volume were significantly reduced. In the leukaemia group, only the concentration was significantly reduced. The lymphoma group revealed a significant reduction in the concentration, motility and motility grade but not the volume, while in the ‘other malignant neoplasm’ group, both the concentration and motility were significantly reduced. In the ‘benign condition’ group, only the sperm concentration showed a significant reduction.

Table II (a–c) illustrates the variation in the concentration, motility grade, motility and semen volume expressed according to the disease category. This shows that the testicular cancer group has the lowest pre-treatment sperm concentration whilst the leukaemia and benign groups have the highest pre-treatment sperm concentrations. The lowest incidence of azoospermia occurred in the testicular cancer and benign groups (Table III). Table IV demonstrates the time taken in order to achieve the best post-treatment sperm concentration for the lymphoma and testicular cancer groups, which represent the groups showing the highest and lowest rates of azoosperma following treatment.

In order to assess the temporal recovery of sperm concentration, Figure 1a–c illustrates that patients who have small changes from their pre-treatment sperm concentrations reached their best post-treatment concentrations at some point within the first 66 months (2000 days, 5.5 years) of their follow-up period. However, those patients who experienced

<table>
<thead>
<tr>
<th>Table Ia. Semen analysis values before and after gonadotoxic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
<tr>
<td>Post-treatment</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
<tr>
<td>( Z )</td>
</tr>
<tr>
<td>( P )</td>
</tr>
</tbody>
</table>

Wilcoxon signed ranks.

<table>
<thead>
<tr>
<th>Table Ib. Semen analysis values before and after treatment for individual diagnostic groups (P-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Leukaemia</td>
</tr>
<tr>
<td>Lymphoma</td>
</tr>
<tr>
<td>Testicular cancer</td>
</tr>
<tr>
<td>Benign condition</td>
</tr>
<tr>
<td>Other malignant neoplasms</td>
</tr>
</tbody>
</table>
a significant deterioration in sperm concentration had to wait longer than 66 months to reach their peak recovery.

Discussion

Although several reports exist in relation to sperm quality before gonadotoxic treatment, very few studies investigate the deterioration in semen quality following such treatment (Aubier et al., 1989; Shafford, 1993; Hieken et al., 1996; Tal et al., 2000; Ishikawa et al., 2004). In our study, the size of the study cohort, duration of the follow-up period and the inclusion of the benign neoplasm group are unique features allowing useful information and inferences to be made for patients undergoing gonadotoxic treatment.

In our analyses, the sperm quality in terms of density, motility and volume was significantly reduced ($P < 0.05$) following gonadotoxic treatment for all groups (Table I). However, only the magnitude of change in concentration appears to be of clinical relevance. This suggests that the damaged or destroyed germinal stem cells may recover from an initial cytotoxic insult and maintain a reasonable sperm quality in terms of motility and motility grading, although sperm concentration remained variable. The lymphoma and leukaemia groups showed the highest incidence of azoospermia following gonadotoxic treatment, at 46 and 59% of their respective groups (Table III). On the other hand, the benign neoplasms and testicular cancer groups showed the lowest incidence of azoospermia at 16 and 12%, respectively, which may be indicative of less severe gonadotoxic treatment regimens in these conditions compared with, for instance, lymphoma.

The testicular cancer group was found to have the lowest sperm concentrations of all the disease categories before treatment (Table II). However, following cytotoxic treatment, the testicular cancer group showed the lowest level of azoospermia (12%), but the highest level of oligozoospermia (38%). The normospermia levels after treatment for the testicular cancer group were also high (50%), second only to the benign group (61%) (Table III). The high level of oligozoospermia in these patients may be due to impaired spermatogenesis pre-treatment, and treatment with less gonadotoxic modalities might have spared them from developing azoospermia. This observation was supported by the findings of recent studies (Bahadur et al., 2002; Gandini et al., 2003). The testicular cancer group had a significant decline in only the concentration and volume. (Table Ib).

Our finding of reduced sperm quality in testicular cancer patients confirms other studies. Patients with testicular cancer have an impaired semen quality at diagnosis which deteriorates further after orchidectomy. Previous studies have shown that 50–70% of patients with testicular cancer after orchidectomy, but before cytotoxic treatments, have impaired spermatogenesis (Hendry et al., 1983; Palmieri et al., 1996). A link between male factor subfertility and testicular cancer is also recognized (Jacobsen et al., 2000). Reduction in sperm concentrations may be linked to disruption of embryonal programming and gonadal development (Skakkebæk

### Table IIa. Semen analysis values in diagnostic subgroups before and after gonadotoxic treatment (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Age (years)</th>
<th>Recovery time (months) between treatment and best post-treatment analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>13</td>
<td>30.4 ± 7.7</td>
<td>51.6 ± 45.2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>128</td>
<td>26.9 ± 6.5</td>
<td>45.06 ± 34.3</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>102</td>
<td>27.8 ± 5.2</td>
<td>31.5 ± 25.6</td>
</tr>
<tr>
<td>Benign condition</td>
<td>13</td>
<td>31.6 ± 8.2</td>
<td>41.3 ± 47.3</td>
</tr>
<tr>
<td>Other malignant neoplasms</td>
<td>58</td>
<td>29.0 ± 10.5</td>
<td>29.3 ± 18.5</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.193</td>
<td>0.007</td>
</tr>
</tbody>
</table>

### Table IIb. Semen analysis values in diagnostic subgroups before gonadotoxic treatment (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment concentration ($10^6$/ml)</th>
<th>Pre-treatment motility grade</th>
<th>Pre-treatment motility (%)</th>
<th>Pre-treatment volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>77.5 ± 62.2</td>
<td>1.79 ± 0.68</td>
<td>46.92 ± 20.87</td>
<td>2.26 ± 1.17</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>63.7 ± 45.7</td>
<td>1.94 ± 0.61</td>
<td>50.12 ± 16.25</td>
<td>2.45 ± 1.13</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>40.6 ± 37.4</td>
<td>1.80 ± 0.60</td>
<td>49.96 ± 15.21</td>
<td>2.80 ± 1.61</td>
</tr>
<tr>
<td>Benign condition</td>
<td>74.4 ± 44.1</td>
<td>1.91 ± 0.51</td>
<td>50.00 ± 18.25</td>
<td>2.19 ± 0.82</td>
</tr>
<tr>
<td>Other malignant neoplasms</td>
<td>49.4 ± 39.4</td>
<td>1.86 ± 0.56</td>
<td>49.57 ± 15.67</td>
<td>2.84 ± 1.53</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.000</td>
<td>0.437</td>
<td>0.980</td>
</tr>
</tbody>
</table>

### Table IIc. Semen analysis values in diagnostic subgroups after gonadotoxic treatment (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Post-treatment concentration ($10^6$/ml)</th>
<th>Post-treatment motility grade</th>
<th>Post-treatment motility (%)</th>
<th>Post-treatment volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>32.2 ± 39.3</td>
<td>2.08 ± 0.49</td>
<td>58.33 ± 14.71</td>
<td>2.92 ± 2.13</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>17.5 ± 31.1</td>
<td>1.79 ± 0.60</td>
<td>43.87 ± 23.30</td>
<td>2.30 ± 1.42</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>32.3 ± 34.5</td>
<td>1.80 ± 0.64</td>
<td>48.45 ± 17.50</td>
<td>2.48 ± 1.58</td>
</tr>
<tr>
<td>Benign condition</td>
<td>41.3 ± 30.7</td>
<td>1.95 ± 0.61</td>
<td>50.00 ± 19.49</td>
<td>1.84 ± 0.77</td>
</tr>
<tr>
<td>Other malignant neoplasms</td>
<td>22.4 ± 35.5</td>
<td>1.92 ± 0.64</td>
<td>42.17 ± 22.70</td>
<td>2.58 ± 1.37</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>0.000</td>
<td>0.580</td>
<td>0.246</td>
</tr>
</tbody>
</table>
et al., 2001), elevated scrotal temperature secondary to neo-
vascularization and HCG production by the neoplastic testi-
cle (Berthelsen and Skakkebaek, 1983). Recovery of sperm
function after treatment has, so far, been unpredictable
(Byrne et al., 1987; Siimes and Rautonen, 1990), While all
patients initially developed azoospermia following bleo-
mycin, cisplatin and etoposide (BEP) chemotherapy, 48% have recovered motile ejaculated sperm after 2 years, and
after 5 years this figure rises to 80% (Lampe et al., 1997). Although the total sperm count may still be reduced, the
potential for fatherhood still exists.

It was reported that impaired pre-treatment semen quality
occurs in lymphoma and leukaemia patients (Botchan et al.,
1997; Hallak et al., 1999). Our previous study (Bahadur et al.,
2002) illustrated that sperm counts before gonadotoxic
treatment were significantly lower in Hodgkin’s and non-
Hodgkin’s lymphoma, and in leukaemia patients than in a
healthy sperm donor population, reflecting the adverse con-
tribution of the disease. This point receives support elsewhere
(Kobayashi et al., 2001; Rueffler et al., 2001). However, nor-
mal semen parameters were also reported in cancer patients
(Rofeim and Gilbert, 2004).

In this study, the leukaemia and lymphoma groups fared
worst following gonadotoxic treatment. There is a body of
information to suggest that the nature of treatment regimens
could explain some effects on the level of gonadotoxicity, and
where sperm prevail, some effect on the sperm function
(Reiter et al., 2002; Chapman et al., 1979; Whitehead et al.,
1982; Anselmo et al., 1990; Longo et al., 1997; Viviani et al.,
1985; Van den berg et al., 2004; Kulkarni et al., 1997; Pryzant
et al., 1993). In our analyses, Table IIa reveals the recovery
time was longest for the leukaemia and lymphoma groups.

Although leukaemia patients had the highest pre-treatment
sperm concentrations (Table II), 46% of patients developed
post-treatment azoospermia (Table III). As there is a high
proportion of viable stem cells which are subjected to gona-
dotoxic agents, this results in a larger overall fall in sperm
concentration. Germ cell dysfunction occurs over a short
interval, as opposed to the situation in testicular cancer. This
may account for the longer recovery time in this group of
patients. It remains difficult to differentiate the contribution
of disease and treatment on semen quality without prospec-
tive randomized controlled studies. Despite its large cohort
size, retrospective analysis of the clinical information has
limited our ability to answer this question completely.

A total of 37% of the cases who had post-treatment azoo-
spermia recovered to a mean sperm concentration of
17.8 × 10^6/ml (0.01–60) during the post-treatment follow-
up period with a mean duration of 48.6 (14–143) months.
Recovery among different diagnostic groups did not differ
significantly (P = 0.25). Information on when sperm recov-
ery first occurs, whilst desirable, would be both unethical and
impractical to obtain as this would involve serial weekly or
monthly semen analyses studies on each patient with azoo-
spermia following a major treatment.

Although future options for patients may become available
with regards to germ cell technology of repopulating the
testes with testicular stem cells, or by generating mature
sperm cells from embryonic stem cells, the safety and ethical
use of these technologies should not be underestimated
(Bahadur, 2004). Cryopreservation of semen prior to gonado-
toxic treatment is the current preferred strategy, but testicular
sperm extraction and ICSI after chemotherapy has also been
employed successfully (Meseguer et al., 2003). Caution

### Table III. Distribution of post-treatment azoo-oligozoospermia in diagnostic subgroups

<table>
<thead>
<tr>
<th>Condition</th>
<th>Azoospermia</th>
<th>Oligozoospermia</th>
<th>Normospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>46%</td>
<td>8%</td>
<td>46%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>59%</td>
<td>14%</td>
<td>27%</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>12%</td>
<td>38%</td>
<td>50%</td>
</tr>
<tr>
<td>Benign condition</td>
<td>16%</td>
<td>23%</td>
<td>61%</td>
</tr>
<tr>
<td>Other malignant</td>
<td>34%</td>
<td>33%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Pearson χ² = 63.19, P = 0.000.

### Table IVa. Recovery period for lymphoma and testicular cancer patients to best post-treatment sperm concentration with different levels of spermatogenetic impairment

<table>
<thead>
<tr>
<th>Post-treatment sperm concentrations (10^6/ml)</th>
<th>Mean duration of recovery period to the best post-treatment sperm concentration (months)</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>49.4 ± 36.4</td>
<td>76</td>
<td>−3.3</td>
</tr>
<tr>
<td>0–20</td>
<td>41 ± 33.9</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>37.5 ± 28.3</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45 ± 34.3</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Testicular cancer</td>
<td>34.7 ± 30.6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>28.9 ± 22.8</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>32.8 ± 26.7</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31.5 ± 25.6</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

### Table IVb. The first and the last semen analyses after treatment in lymphoma and testicular cancer patients

<table>
<thead>
<tr>
<th>Sperm concentration (10^6/ml)</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First post-treatment</td>
<td>7.34</td>
<td>19.89</td>
<td>0</td>
<td>100.00</td>
<td>2.87</td>
<td>0.09</td>
</tr>
<tr>
<td>Last post-treatment</td>
<td>16.14</td>
<td>29.69</td>
<td>0</td>
<td>130.00</td>
<td>2.24</td>
<td>0.14</td>
</tr>
<tr>
<td>Testicular cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First post-treatment</td>
<td>18.33</td>
<td>29.11</td>
<td>0</td>
<td>92.00</td>
<td>2.47</td>
<td>0.01</td>
</tr>
<tr>
<td>Last post-treatment</td>
<td>30.95</td>
<td>36.4</td>
<td>0</td>
<td>100.00</td>
<td>1.67</td>
<td>0.01</td>
</tr>
</tbody>
</table>
needs to be exercised, however, in utilizing sperm obtained after cancer treatment to protect the welfare of the child (Meistrich et al., 1985; Meistrich, 1993; Robbins et al., 1997; Martin et al., 1999; Chatterjee et al., 2000; Arnon et al., 2001; De Mas et al., 2001; Kobayashi et al., 2001; Reiter et al., 2002; Frias et al., 2003; Codrington et al., 2004; Deane et al., 2004; Seli et al., 2004). Even if sperm prevail after treatment, it is prudent practice to avoid use of these in vivo sperm for 1.5–2 years to enable sufficient turnover of cells to expel the mutagenic effect. These estimates may be high, and perhaps 6–12 months grace may be more pragmatic and realistic.

It would be most desirable in our field to have predictive tools and recognizable associations (Fossa et al., 1997; Tal et al., 2000). In our study, we aimed to see if there were any predictors of the semen quality decline and their possible recovery after gonadotoxic treatment. It became clear that the disease category was the single most important predictor of the sperm parameters. This reflects the pathology of the disease and the nature of the gonadotoxic treatment.

As a result of our analyses on our cohort of patients undergoing gonadotoxic treatment, a number of facts and associations emerge, listed below.

(i) There is a positive correlation between the pre-treatment sperm concentration and both post-treatment sperm concentration (Spearman’s $r = 0.181; P = 0.01$) and sperm motility (Spearman’s $r = 0.258; P = 0.000$). However, there is a negative correlation between the pre-treatment semen volume and ‘the interval between toxic therapy and the best post-treatment sperm analyses’ (Spearman’s $r = -0.136, P = 0.017$), such that men with low pre-treatment semen volume tend to require a longer period to achieve the best sperm analyses after their treatment.

(ii) Patients’ age showed a positive correlation with the post-treatment sperm concentrations (Spearman’s $r = 1.24, P = 0.036$) and a negative correlation with the recovery time to achieve the best post-treatment sperm concentration (Spearman’s $r = -0.264, P = 0.000$); hence older patients require a shorter interval to obtain a high post-treatment sperm concentration.

(iii) The time to achieve the best post-treatment sperm concentration showed a positive correlation with the magnitude of change in sperm concentrations following treatment (pre-treatment concentration–post-treatment concentration: Spearman’s $r = 0.140, P < 0.05$). Such a correlation was not observed with the final post-treatment sperm concentrations (Spearman’s $r = -0.067, P = 0.241$), such that men who suffered from large drops in sperm concentrations during their treatment regardless of the final sperm concentration tend to have longer post-treatment recovery periods in their spermatogenesis. This correlation was still valid even after correcting for age ($F = 6.26, P = 0.002$).

We further evaluated the association between the recovery time to the best post-treatment sperm concentration, and the magnitude of change in sperm concentrations. When we subcategorize the cohort into three groups where group A = recovery period up to 33 months, group B = 33–66 months and group C ≥ 66 months, it becomes apparent that

**Figure 1.** (a) Level of decrease in sperm concentrations and recovery period. $R^2 = 0.007; F = 1.35; P > 0.05$. (b) Level of decrease in sperm concentrations and recovery period. $R^2 = 0.007; F = 0.6; P > 0.05$. (c) Level of decrease in sperm concentrations and recovery period. $R^2 = 0.113; F = 5.397; P < 0.05$. 

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the association between the recovery time to the best post-treatment sperm concentrations and the percentage change in sperm concentrations were not significant in groups A and B (Figure 1a–c). In essence, men who have small changes from their pre-treatment sperm concentrations reached their best post-treatment concentrations at some point within the first 66 months of their follow-up period. However, those who experienced significant deterioration in sperm concentration had to wait longer than 66 months to reach their peak recovery ($R^2 = 0.113$, $F = 5.397$, $P = 0.025$). The mean change in sperm concentration was $26 \times 10^6$/ml $(0.01–50)$ in group A, $28 \times 10^6$/ml $(1.5–50)$ in group B and $48 \times 10^6$/ml $(7.7–91)$ in group C.

The negative numbers in Figure 1 indicate an improvement in sperm concentrations after treatment above the pre-treatment levels, reflecting the positive influence of the treatment which eliminates the effect of the underlying disease on spermatogenesis by overpowering its own gonadotoxic potential. Changes in the post-treatment sperm concentrations which revealed significant differences after 66 months of observation stress that there is not only a possible recovery of stem cell spermatogonia, but there is also a non-recoverable effect on the basic reservoir of these cells after gonadotoxic treatment in a subgroup of patients who may be characterized by their underlying disease process and the type of treatment they received.

(iv) A logistic regression analysis to predict the post-treatment azoospermia by using age, diagnostic group and the recovery time revealed that only the diagnostic category is a significant predictor of post-treatment azoospermia. The distribution of patients with oligozoospermia and normospermia also showed significant differences among different diagnostic groups (Table III). Whilst patients with lymphoma had the highest incidence of post-treatment azoospermia, patients with testicular cancer were less likely to be azoospermic after cancer treatment. However, the probability of developing oligospermia in testicular cancer is much higher compared with the other groups. Patients with lymphoma and leukaemia have a higher probability to maintain sperm concentrations $>20 \times 10^6$/ml if they escaped from developing azoospermia. Patients with benign conditions are most likely to be normo-spermic after their gonadotoxic treatment (Table III).

Similarly, patients with different diagnoses also exhibited different time intervals to achieve their best post-treatment sperm parameters. This is a function of the percentage of azoospermia in each group (Table IVa). Patients with lymphoma with the highest level of post-treatment azoospermia have the longest recovery period, in contrast to those with testicular carcinoma who have the lowest percentage of post-treatment azoospermia and shortest recovery period. Although some recovery was evident in sperm concentrations with time, this did not reach statistical significance (Table IVb).

On a broader issue, while our study draws together a number of observations, it also uniquely highlights certain features which may be of clinical use. There were significant differences in semen quality amongst the different disease categories, with the lymphoma and leukaemia group showing the largest change. The benign condition group was least affected, thereby serving as a useful internal control. The testicular cancer group overall remained with the lowest sperm numbers before treatment and appeared reasonably unaffected following treatment, thereby reflecting the testicular pathology of the disease. The question as to how long a patient should wait following treatment before the optimal recovery in sperm quality is achieved remained a challenging question for us to answer, and this is also a frequently asked question during clinical consultations. Apart from those patients who experienced the worst deterioration in sperm concentration and had to wait longer than 66 months to reach their peak recovery, there appears to be no significant benefit in prolonging the post-treatment follow-up with the expectation of significant recovery. This adds a new clinical feature to post-treatment counselling and management of patients.

Approximately 63% of patients may develop irreversible azoospermia. The fact that all categories of patients in our cohort suffered variable levels of azoospermia, together with the unpredictable recovery of azoospermia, reinforces the notion that ejaculated sperm should be banked prior to treatment. The significant decline in overall semen parameters after gonadotoxic treatment should lead clinicians to be more proactive in recommending sperm cryopreservation before treatment. This will help obviate patient distress and medico-legal problems, and above all give each patient a sense of reassurance with the knowledge of heightened safe potential use of sperm not previously subjected to toxic therapy. On a cautionary note, our study does not provide either reassurance or caution in the clinical use of post-treatment sperm, and careful counselling especially on genetic issues must follow.

Conclusion

Patient diagnosis remains a significant factor in predicting post-treatment azoospermia. All patient categories displayed varying levels of azoospermia and oligozoospermia. Given that there was an insignificant recovery in spermatogenesis following treatment, or that azoospermia remained, it is essential to offer sperm banking before any gonadotoxic treatment. This should be allied to dedicated counselling and support to cancer patients.

Several additional observations and associations in relation to semen quality are listed further, thereby giving an insight into pre- and post-gonadotoxic treatment effects and relationships. For example, apart from those patients who experienced the worst deterioration in sperm concentration and had to wait longer than 66 months to reach their peak recovery, there appears to be no significant benefit in prolonging the post-treatment follow-up with the expectations of significant recovery, thereby adding a new clinical feature to post-treatment counselling and management of patients. Patients with lymphoma and leukaemia have a higher probability of maintaining sperm concentrations $>20 \times 10^6$/ml if they escaped from developing azoospermia. Patients with different diagnoses also exhibited different time intervals to achieve their
best post-treatment sperm parameters. The recovery times between treatment and the best post-treatment semen analyses is reflected in the diagnostic category. Finally, the unproven genetic effects of cancer treatment on sperm needs to be kept in perspective.

References


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