Cryptozoospermia with normal testicular function after allogeneic stem cell transplantation: A Case Report

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One of the most frequent consequences of allogeneic haemopoietic stem cell transplantation (allo-SCT) in both males and females is gonadal insufficiency. We report the case of a 27-year-old myelodysplastic male who developed azoospermia after allogeneic transplantation of haemopoietic stem cells from his HLA-identical sister. Post-transplant azoospermia was alternated with intermittent severe oligospermia. The patient had a normal endocrine pattern and evidence of mild chronic graft-versus-host disease (cGVHD). Normal intratesticular spermatogenesis was revealed by bilateral fine needle aspiration (FNA) cytology. Inflammation was evident at semen analysis, but no infection was detected by microbiological examination and sperm culture. These findings, together with the reappearance of sperm cells at semen analysis after a low-dose immunosuppressive treatment, suggested the presence of cGVHD of the urogenital tract, causing a reversible obstruction of the spermatic tract and cryptozoospermia. This is the first case report documenting a severe impairment of sperm count because of a reversible obstruction of the seminal tract, likely caused by cGVHD, in a long-term survivor of allo-SCT with normal endocrine pattern. An important practical consequence of this case report is the fact that azoospermia was cured using low-dose immunosuppressive therapy, and this allowed us to avoid expensive stimulatory treatments with gonadotrophins, which remain, however, ineffective if the obstruction of spermatic tracts is not removed. A spontaneous uncomplicated pregnancy occurred in the partner of the patient 3 months after the corticosteroid treatment withdrawal.

Key words: male infertility/testicular function/cryptozoospermia/allogeneic stem cell transplantation/chronic graft-versus-host disease

Introduction

Allogeneic haemopoietic stem cell transplantation (allo-SCT) provides an effective treatment for patients with haematological malignancies, immunological and metabolic disorders. Protocols for the eradication of underlying malignancies and conditioning regimens to transplant include high-dose chemotherapy, irradiation or both. One of the most frequent consequences of myeloablative treatments is gonadotoxicity; indeed, alkylating drugs, which are frequently used in treating protocols and conditioning regimens, have severe gonadotoxic effects (Mertens et al., 1998; Grigg et al., 2000; Brennan and Shalet, 2002). Gonadal damage occurs early after chemotherapy or radiotherapy and may have variable extent and duration (Shalet, 1994; Syrjala et al., 1998).

Germinal epithelium of the testis is more vulnerable to the gonadotoxic effects than the Leydig cells (Shalet, 1994). In fact, decrease of serum testosterone is mostly transient and disappears weeks-to-months after grafting (Littley et al., 1991; Molassiotis et al., 1995); in our experience, testosterone was reduced in about 30% of patients up to 1 year after SCT (Tauchmanovà et al., 2005). On the contrary, germ cell dysfunction induced by allo-SCT appeared to be amenable to recovery only occasionally and never before 24 months from transplantation (Sklar et al., 1984; Jacob et al., 1998; Anserini et al., 2002). In agreement with these data, we observed impaired spermatogenesis in all the 35 patients who underwent seminal fluid analysis 12 months after SCT; among them, 91% were azoospermic (Tauchmanovà et al., 2005). However, an increase in FSH levels, which is an indirect marker of impaired
spermatogenesis, was not observed in about 10% of them. Three plausible hypotheses may be raised to explain the discrepancy between results of seminal fluid analysis (oligozoospermia/azoospermia) and the lack of FSH increase: (i) partial hypothalamic and/or pituitary damage caused by chemotherapy/radiotherapy, leading to impaired gonadotrophin release, (ii) partial or complete arrest of spermatogenesis at the spermatid level and (iii) partial or complete occlusion of the spermatid tract.

Acute or chronic forms of graft-versus-host disease (GVHD) may occur in about half of allo-SCT patients. This condition shares multiple pathogenetic and clinical features with degenerative and autoimmune diseases (Sullivan et al., 1991; Lee et al., 2003). Although the skin, the liver, the eyes and the gastrointestinal system are the sites most frequently involved, virtually any organ or tissue can be targeted by this disorder. Even the genital tract has been identified as a target for chronic GVHD (cGVHD) in women; vulvo-vaginal lesions were described in 4 of 41 women (10%) in a Seattle population (Corson et al., 1982), and in a large recent Italian study on 213 women, a variable degree of gynaecological cGVHD was found in 25% of them (Spinelli et al., 2003). Milder forms consisted in increased propensity to develop vulvo-vaginal inflammation and infections; while more severe forms included vaginal and cervical stenosis, disfigurement of the internal and external genitalia sometimes associated with peritoneal involvement. Conversely, incidence and features of possible urogenital localization of cGVHD in men have not been reported. There are only two studies that observed lower sperm counts in long-term survivors affected by cGVHD when compared with unaffected patients, suggesting a possible influence of this condition on the reproductive status (Grigg et al., 2000; Rovo et al., 2006).

We report the case of a young man with post-transplantation azoospermia, associated with a normal endocrine pattern and a mild cGVHD. Surprisingly, he was found to have normal intra-testicular spermatogenesis by a bilateral fine needle aspiration (FNA) cytology. Clinical, endocrine and cytological features of this patient are discussed and compared with literature data.

Case report

A 27-year-old man underwent peripheral haemopoietic SCT from his HLA-identical sister 3 months after the diagnosis of refractory anaemia with excess of blast-1, according to the World Health Organization (WHO) criteria (Bennett and Komrokji, 2005).

The patient was advised to undergo sperm cryopreservation before starting chemotherapy. At that time, he had a normal sperm count (40 × 10^6/ml) associated with asthenozoospermia [motility classes ‘a’ + ‘b’ = 25% (WHO, 1999)] and reduced percentage of normal sperm morphology (4% evaluated by the Kruger criterion) (Kruger et al., 1986). This finding was in line with a long period (40 days) of sexual abstinence before sampling that was self-referred by the patient.

The transplantation was preceded by a myeloablative conditioning regimen with thiopeta (15 mg/kg) and cyclophosphamide (120 mg/kg); the GVHD prophylaxis was carried out with cyclosporin A (CsA) (1 mg/kg/day by continuous intravenous infusion from day –1 to day +20 and then 4 mg/kg/day orally for 6 months) and short-course methotrexate (MTX) (Lee et al., 2003). The patient received 5 × 10^6/kg of G-CSF-mobilized peripheral blood CD34+ cells and obtained haematopoietic engraftment and full donor chimerism at days +15 and +35, respectively.

Seven months after SCT, the patient experienced a limited cutaneous cGVHD that was successfully controlled by CsA at a daily dose of 2 mg/kg for 6 months. No other post-transplant complication was recorded.

Starting 1 year after transplant, the patient repeated semen analysis several times. This showed a sperm count ranging from azoospermia to severe oligozoospermia, with a reduced volume (0.5–1.5 ml) and maximum sperm count of 0.2 × 10^9/ml. An endocrine evaluation revealed low-normal testosterone levels and normal gonadotrophin, estradiol and prolactin levels. Thyroid and adrenal functions were normal, too (Table I).

During the third post-transplant year, the patient and his partner underwent two attempts of intrauterine insemination (IUI) by using cryopreserved sperm cells and one attempt of ICSI with fresh semen, but pregnancy was not achieved. Fertilization procedures were preceded by hormonal therapy with testosterone enantate 100 mg intramuscularly every 28 days for 8 months, and hMG, one phial intramuscularly twice a week (75 IU of FSH plus 75 IU of LH per phial) for 4 months.

Three years after allo-SCT, the patient consulted our Centre of Infertility. He reported no family history of infertility. He had been affected by unilateral (right) cryptorchidism with spontaneous testicular descent at the age of 7. However, the patient’s pre-transplant fertility is not clear, because he had not attempted to have a child.

The time of evaluation at the Centre, his endocrine parameters (gonadotrophin, testosterone, estradiol and prolactin levels) were normal, in the absence of any hormonal treatment. Both FSH and LH responded well to GnRH stimulation. At physical examination, he had normal secondary sexual features.

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<tr>
<th>Table 1. Endocrine and biochemical evaluation during the post-transplantation follow-up period</th>
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<tr>
<td><strong>Time elapsed after allo-SCT</strong></td>
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<tr>
<td>Testosterone</td>
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<td>FSH</td>
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<td>LH</td>
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<td>Prolactin</td>
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<td>Estradiol</td>
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<td>ESR 1 h</td>
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<td>CRP</td>
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Allo-SCT, allogeneic haemopoietic stem cell transplantation; CRP, C-reactive protein; DHEAS, dehydroepiandrosterone sulphate; ESR, erythrocyte sedimentation rate; TSH, thyroid-stimulating hormone.

All measurements were performed out of any treatment.
(androgenicity), normal penile length and structure and no gynaecomastia. Both testes were retractile at inguinal level; the right testicle had a volume of 7.2 cm$^3$, the left one of 11 cm$^3$; neither epididymis was enlarged. This man reported normal libido and normal erectile function.

Testicular doppler ultrasound examination did not show any morphological or structural abnormality, which excluded varicocele and epididymal enlargement. Semen analysis revealed severe oligospermia ($0.2 \times 10^6$/ml), astenozoospermia (34% motile spermatozoa) and teratozoospermia (normal morphology 9%), according to the Kruger criteria. The presence of leukocytes was evaluated by May–Grünewald and Giemsa staining; sparse granulocytes, lymphocytes and sperm agglutinates indicated inflammation of testicles or spermatic ducts. Infection was excluded by absence of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Chlamydia trachomatis* at a microbiological examination and by absence of growth of any pathogen in sperm culture. Presence of anti-sperm antibodies (ASAs) was excluded by using a commercial test (Sperm Mar Test; Ortho Diagnostic System, Milan, Italy).

**Testicular cytological examination by FNA**

FNA cytology revealed normal spermatogenesis in the left testicle and moderately reduced spermatogenesis in the right one, with a normal bilateral maturation process (Figure 1). This finding suggested obstruction of the seminal ducts.

**Sperm chromosomal analysis by fluorescence in-situ hybridization**

Fluorescence in-situ hybridization (FISH) analysis of sperm chromosomes was performed on 200 consecutive sperm cells for chromosomes X, Y and 18 as previously reported (Foresta et al., 1998) and showed a normal ratio of aneuploid cells (0.69%; normal ≤1.5%) (Figure 2).

At that time, peripheral lymphocyte phenotyping showed a reduced CD4/CD8 ratio that was associated with increased serum interferon-γ (INF-γ), tumour necrosis factor-α (TNF-α) and interleukin (IL)-10 levels (Table II). These findings indicated presence of a persistent, although clinically not evident, cGVHD.

On the basis of these results, we advised the patient not to repeat any hormonal therapy and to start a mild-dose immunosuppressive treatment for 1 month. Semen analysis was repeated at the end of 1-month’s prednisone treatment at a daily dose of 0.5 mg/kg that was administered orally and showed a normal sperm count ($23 \times 10^6$/ml), volume (4 ml) and sperm morphology (23%, according to Kruger criteria), with only mild astenozoospermia [10% of class ‘a’ + 15% of class ‘b’ motile spermatozoa (WHO, 1999)]; lymphocytes or sperm agglutinates did not appear anymore.

A spontaneous uncomplicated pregnancy occurred in the partner of the patient 3 months after prednisone withdrawal.

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**Figure 1.** Testicular fine needle aspiration cytology. Normal spermatogenesis lineage is shown, and presence of all germ cell maturative steps is evident. In this patient, azoospermia with evidence of a large number of intratesticular spermatozoa indicates obstruction of seminal ducts.

**Figure 2.** Evaluation of sperm aneuploidies by FISH analysis. In this patient, the evaluation of 200 consecutive cells revealed a normal percentage of aneuploid spermatozoa (0.69%). Panel a shows normal spermatozoa and panel b shows aneuploid spermatozoa.
Table II. Serum cytokine levels in the patient 3 years after transplantation, compared with the values of healthy donors

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<tr>
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<th>IL-5</th>
<th>IL-4</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>IL-10</th>
<th>CD4/CD8 ratio</th>
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<tr>
<td>Patient’s value</td>
<td>1.8</td>
<td>3.7</td>
<td>2.2</td>
<td>15.3</td>
<td>4.1</td>
<td>8.6</td>
<td>0.56</td>
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<tr>
<td>Controls (n = 20)</td>
<td>2.5 ± 0.6</td>
<td>3.8 ± 0.7</td>
<td>2.5 ± 0.4</td>
<td>6.5 ± 1.2</td>
<td>3.0 ± 0.5</td>
<td>3.4 ± 0.9</td>
<td>1.6 ± 0.7</td>
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IFN-γ, interferon gamma; IL, interleukin; TNF-α, tumour necrosis factor alpha.
Values are expressed as pg/ml with mean ± SEM in the control group. In the patient, measurements were performed out of any treatment.

**Discussion**

During the last decades, efforts have been made to improve lifesaving treatments for haematological disorders, leading to a significant increase in patients’ overall survival. However, patients undergoing allo-SCT are very often young, and the frequent damage to their reproductive function is an important issue in their general health after being cured for the underlying disease. Reproductive axis damage consequent to SCT has been mostly attributed to the effects of two potent gonadotoxic procedures: chemotherapy and radiation therapy. The interpretation and clinical management of post-transplant male reproductive disorders are difficult tasks, because there are no standard diagnostic criteria for gonadal failure, nor parameters of recovery, and longitudinal data regarding the natural history of testicular damage and related symptoms are lacking.

Our patient had undergone a standard myeloablative conditioning regimen with cyclophosphamide, an alkylating agent particularly toxic for gonads. Major gonadotoxicity of alkylating drugs is related to their capacity of damaging both dividing and resting cells, by altering DNA base pairs (Epstein, 1990). Nevertheless, at the evaluation, 3 years had elapsed since the withdrawal of chemotherapy; this time was sufficient enough to explain the presence of intratesticular spermatogenesis and normal chromosome status. The patient was not receiving any immunosuppressive treatment; however, we cannot completely exclude the influence of previous treatment with cyclophosphamide, CsA or MTX in the mild spermatogenic alteration observed in the right testicle. Furthermore, this alteration could also be caused by the previous cryptorchidism at the same site before the age of 7.

It is known that germinal epithelium of the testis is more vulnerable to the effects of chemotherapy than the Leydig cells (Shalet, 1994), independent of age, with a recovery occurring only in some patients (Sklar et al., 1984; Keilholtz et al., 1997). Concerning the Leydig cell function, both normal function (Littley et al., 1991) and persistent subtle dysfunction (Chatterjee et al., 1994; Molassiotis et al., 1995) were described. In our experience, testosterone production was mostly unaffected in long-term survivors from allogeneic SCT who were out of treatments (Tauchmanovà et al., 2002).

Recent evidence suggests that the transplant procedure itself can exert harmful effects on the reproductive function (Grigg et al., 2000; Tauchmanovà et al., 2003). Indeed, allo-SCT represents the only condition in which immunocompetent donor cells are infused into a host. This infusion, which may cause a graft-versus-tumour reaction that is important for disease eradication, is responsible for acute or chronic forms of GVHD occurring in about half of transplanted patients, regardless of their gender (Sullivan et al., 1991; Lee et al., 2003). More data are available on reproductive disorders in women affected by cGVHD; case reports and various series have documented gynaecological manifestation of GVHD in up to 25% of allo-transplanted women (Schubert et al., 1990; Syrjala et al., 1998; Anguenot et al., 2002; Spinelli et al., 2003; Tauchmanovà et al., 2004). GVHD of the reproductive tract may be more easily recognizable in females because of clinical manifestation such as difficult sexual intercourse and haematocolpometra. However, it is probable that a similar percentage of men are affected by urogenital cGVHD, which remains undetected. This condition should be suspected in the presence of azoospermia or severe oligozoospermia, a normal endocrine pattern or a slight increase in circulating FSH levels. After excluding a pituitary deficit of gonadotrophin production by the GnRH stimulation test, cryptozoospermia should be suspected and GVHD origin taken into consideration. Although patients affected by cGVHD in other sites are more likely to be affected, a single urogenital localization of cGVHD cannot be ruled out.

Prevention and treatment of GVHD consist of immunosuppressive drugs such as glucocorticoids, MTX and CsA, at different doses and combinations. All these drugs can negatively affect the reproductive axis. Glucocorticoids at high doses are known to suppress GnRH release, reducing the function of the whole reproductive axis (Nieman and Ilias, 2005). CsA whole blood through levels were shown to be inversely correlated with sperm concentration and motility in men (Handelsman et al., 1982). Moreover, CsA has been shown to cause testicular damage in long-term treatments (Eid et al., 1996; Srinivas et al., 1998). A dose-dependent MTX cytotoxicity on seminiferous tubule cells and spermatogenic maturation were observed in rats (Saxena et al., 2004), whereas in men, MTX administration resulted in severe oligozoospermia that was reversible after the treatment withdrawal (Sussman and Leonard, 1980). Therefore, the observation by Grigg et al. (2000) on a lower sperm count in men affected by cGVHD can be partly attributed to the effects of immunosuppressive treatments on the reproductive axis; indeed, all men evaluated by us during immunosuppressive treatments for GVHD had low testosterone levels (Tauchmanovà et al., 2002). Nevertheless, a possible obstruction of the urogenital tract has not yet been systematically investigated in males.

Because immune system derangement may predispose transplanted patients to infections, microbiological examination and sperm culture are mandatory; in our case, they failed to reveal any infection. Theoretically, a testicular biopsy could confirm the presence of GVHD in seminal ducts, but it was not performed, because it carries a high risk of subsequent testicular fibrosis. Therefore, urogenital cGVHD in our man may only be
suspected by evidence of lymphocytes and sperm agglutinates at semen analysis that were associated with abnormalities at a systemic level such as decreased CD4/CD8 ratio and increased INF-γ, TNF-α and IL-10 levels. Our hypothesis is supported by the finding of negative ASAs; indeed, ASAs were considered in the differential diagnosis for the fertility improvement that was achieved during corticosteroid treatment.

Such an interpretation of the post-transplant reproductive disorder in our patient may lead to the following practical consequence: the spermatic duct obstruction can be reversed by using immunsuppressive treatments at low-to-moderate doses. This treatment was followed by a spontaneous pregnancy in the patient’s partner. Thus, at reproductive counselling, unnecessary and expensive stimulatory treatments with gonadotrophins can probably be avoided in some patients, because they remain ineffective if obstruction is not removed.

To the best of our knowledge, this is the first case report documenting a severe impairment of sperm count because of a reversible obstruction of the seminal tract (cryptozoospermia), probably caused by cGVHD, in a long-term survivor from allo-SCT with normal endocrine pattern.

References


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