Comment

Impaired Spermatogenesis in Patients Treated for Hodgkin’s Lymphoma

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Recent advances in the diagnosis and treatment of malignant diseases has brought into focus certain quality of life issues, such as the problem of infertility [1]. The impact of these problems is magnified in malignant diseases that predominantly affect patients in the reproductive age group, of which Hodgkin's lymphoma is one of the most common in young men [2]. Currently, the disease has a 5-year survival rate of 75–90% [3].

Hodgkin's lymphoma can seriously affect the male’s fertility potential, and these deleterious effects may be due to the disease itself and/or its treatment (Figure 1). Other concerns, such as the transmission of genetically compromised material to the offspring, and the subsequent increase in congenital anomalies have become a reality due to the recent expansion in assisted reproductive techniques (ARTs) for the treatment of infertility in patients with malignant conditions.

Impact of Hodgkin's Lymphoma on Testicular Function
The gonadotoxic effects of drugs and irradiation used for the treatment of Hodgkin's disease were previously considered to be the only cause of infertility due to such circumstances. However, several studies have documented that a decline in fertility can occur in 20–70% of patients with Hodgkin's disease prior to the initiation of treatment [4,5]. Although Hodgkin’s disease does not involve local tumors or metastases in the testicles themselves, structural abnormalities in the testicular parenchyma have been reported, e.g. tubular hyalinization [6]. Relatively higher impairment of spermatogenesis was noted in patients with Hodgkin's lymphoma compared with healthy donors and men with non-Hodgkin's lymphoma [7].

The decrease in fertility potential may be attributed to an immunological imbalance that leads to alterations in lymphocytic cell populations and cytokine profiles (e.g. interleukins and tumor necrosis factor) and, in turn, impaired testicular function [8,9]. In support of the immunological imbalance theory, sperm agglutinins were found in 31% of patients with Hodgkin's disease [10]. Another potential cause of infertility with Hodgkin's lymphoma is the deterioration in the patient's general condition, which may indirectly affect the reproductive capacity. Weight loss due to increased catabolism and malnutrition is one the most frequent manifestations that may negatively impact fertility. Hypothalamic dysfunction and subsequent hormonal imbalance in the form of elevations in stress hormones and prolactin may be another contributing factor [11,12].

Several attempts have been made to correlate the impairment in spermatogenesis with the systemic manifestations of the disease. Fever, elevated erythrocyte sedimentation rates, low hemoglobin levels, and advanced Hodgkin's disease stage have all been reported as prognostic indicators for decline in fertility [5,8,13]; however, these relationships are not yet established as other studies failed to detect any correlation.
between sperm abnormalities and disease stage or symptomatic symptoms [4,13].

**Gonadal Toxicity**

**Following Treatment**

Chemotherapy, radiotherapy, and bone marrow transplantation are the main modalities for the treatment of Hodgkin’s lymphoma [14]. The impact of cytotoxic agents on male fertility is more frequently encountered as the survival rates increase. Traditionally, Sertoli cells form a barrier between the blood and the testicular germ cells in order to protect the testicles from noxious agents. Nonetheless, many chemotherapeutic drugs can severely interrupt the integrity of this barrier and enter the testes via the blood vessel plexus in the interstitial region [15].

Patient age and pubertal status greatly affect the extent of damage exerted by cancer therapy on testicular architecture. Initially, it was thought that the testicles of pre- and peripubertal males were less vulnerable to the toxic effects induced by treatment; however, it is now evident that the damage to testicular structure following chemo/radiotherapy is comparable between these patients and adults [16]. Differentiating germ cells are more vulnerable to drug-induced cytotoxicity and subsequent necrosis, while Leydig and Sertoli cells display functional rather than structural anomalies. The effect of chemotherapy on germ cells takes two forms: depletion/arrest of spermatogonial differentiation and mutagenesis in late-stage cells, which may transmit DNA mutations to the next generation [17].

Somatic cells are more resistant to chemotherapy and radiation-induced damage than are germ cells. Doses as low as 0.1–1.2 Gray (Gy) can have detectable effects on spermatogenesis in adult men, with doses of >4 Gy causing more permanent effects [18].
In contrast, Leydig cell dysfunction is not observed until doses of 20 Gy are administered to prepubertal boys and up to 30 Gy in sexually mature males [19]. Therefore, testosterone production is relatively preserved below these doses, and many patients present with normal secondary sexual characteristics despite severe impairment of spermatogenesis.

Germ cell depletion initiates a series of events that disturbs the hypothalamic–pituitary–gonadal axis; the depletion reduces the testicular size, causing a reduction in blood flow, and as a direct consequence, a reduction in the amount of testosterone distributed into the circulation [20]. This decrease in testosterone results in a significant increase in luteinizing hormone, the primary stimulator of testosterone synthesis by Leydig cells, in order to maintain a constant serum testosterone level.

**Strategies to Minimize Gonadotoxicity**

The negative effects of therapy for Hodgkin’s lymphoma on fertility differ according to the treatment regimen used. For patients in the reproductive age group, it is prudent to select agents that have minimum toxicity as well as maximum therapeutic effects. Sperm production has been reported to recover rapidly within 3–4 months following NOVP (mitoxantrone, vincristine, vinblastine, and prednisone) therapy, despite its marked effect on spermatogenesis. The effect of radiation therapy on sperm counts following NOVP is equal to the effects of radiation alone [21]. This rapid recovery is expected since NOVP chemotherapy damages spermatogenic germ cells rather than inhibiting stem cells [22]. In this context, NOVP may be preferred over MOPP (mustine, vincristine, procarbazine, and prednisone) for the treatment of Hodgkin’s lymphoma. ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) is also preferred to MOPP since it has less gonadal toxicity [23].

In general, MOPP therapy has severe detrimental effects on spermatogenesis and should be avoided. MOPP has been reported to permanently impair spermatogenesis in long-term survivors of pediatric Hodgkin’s disease, and can lead to constant azoospermia for at least 14 months after completion of treatment in adults [24,25]. Similarly, ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisolone) used in the treatment of Hodgkin’s lymphoma can lead to irreversible damage to the germinal epithelium. Persistently high levels of follicle-stimulating hormone were detected in patients who received ChlVPP up to 17 years after the completion of therapy [26].

Protection of the gonads during irradiation is one of the most important measures for minimizing toxicity during therapy. The gonads must be excluded from the field of radiation or shielded from the direct radiation beam. Although gonadal shields can reduce the amount of radiation by 2–5-fold, some radiation may still get through. The inverted Y field used for Hodgkin’s disease typically results in 2–3 Gy reaching the gonads [27].

Preventive medical treatment with gonadal steroids, gonadotrophin-releasing hormone analogues, and anti-androgens may be used to enhance the recovery of spermatogenesis following cytotoxic treatment, as documented in an animal model [28]. These testosterone suppressors may work by enhancing the potential of the somatic cells in the testes to support the recovery of spermatogenesis [3]. However, the recovery of stem spermatogonia cells after prolonged periods of iatrogenic azoospermia remains subject to controversy; only one of eight clinical trials reported that hormonal treatment given before and during cytotoxic therapy was able to protect spermatogenesis [29].

**Fertility Following Hodgkin’s Lymphoma**

The reproductive capacity of individuals undergoing malignancy treatment can be conserved by cryopreserving the gametes and using ARTs when pregnancy is desired [30]. Cryopreserved spermatozoa from cancer patients results in complete pregnancies in 18% of cycles [31]. Cancer patients who have recovered sperm production following cytotoxic therapy are still capable of having...
children, even with below normal sperm counts [24].

The spontaneous recovery of spermatogenesis following gonadotoxic treatment will depend on the ability of spermatogonia to resume their mitotic activity [32]. Men should not be considered sterile despite prolonged azoospermia after undergoing chemotherapy. Spermatozoa could still be recovered from those men who remain azoospermic long after chemotherapy by testicular sperm extraction (TESE), which, when combined with intracytoplasmic sperm injection (ICSI), may result in pregnancy [33].

Risk of Genetic Mutations
Patients with Hodgkin’s diseases have a significantly higher prevalence of sperm DNA damage than healthy men [34]. Chemo/radiotherapies can potentially produce single-gene mutations and chromosomal translocations in spermatogonia [35]. Although sperm DNA damage can be assessed using a variety of techniques [36], none can definitively determine whether mutations will be passed onto any offspring. Mutations that occur early in stem spermatogonia will produce mutation-carrying sperm for the lifetime of the male, whereas those occurring in the later stages of spermatogenesis will only lead to mutation-carrying sperm for a few months. Since meiotic and post-meiotic germ cells are more susceptible to mutations than are stem spermatogonia, the mutational risks are highest when a pregnancy occurs within one spermatogenic cycle after the male is exposed to the damaging agent [17].

The high prevalence of sperm DNA damage and mutations indicates that children born from a pregnancy occurring either before or after treatment may be at risk of increased congenital anomalies. The frequency of sperm aneuploidies have been reported to persist for >18 months after chemotherapy [37], which indicates that patients should be advised to use contraception for 18 months after the completion of therapy. Nevertheless, the rate of congenital anomalies in children born to fathers with cancer was comparable with the general population [38]. Similarly, these rates were comparable in children born using cryopreserved spermatozoa from patients with Hodgkin’s disease [10]. It is also reassuring that, in most instances, no major congenital abnormalities were seen in the offspring of males who received radiation therapy [39].

Options for Fertility Preservation
There are a number of ways in which fertility can be preserved, and these are summarized in Table 1. Currently, semen cryopreservation represents a feasible option for fertility preservation in patients who are to be subjected to cytotoxic therapy [40]. Cryopreservation of sperm after the start of therapy could damage the chromosomal structure, causing de novo mutations; therefore, it is crucial to cryopreserve sperm before the initiation of therapy [41]. Current consensus is that three semen samples should be cryopreserved with at least 2 days of abstinence. Additional samples are usually favored, since they increase the number of banked sperm [42]. Unfortunately, the pressing need to begin therapy may not always allow for this traditional approach. In these circumstances, sperm cryopreservation during treatment may be considered until azoospermia occurs [43]. However, the risk of damage to the sperm genetic material should be seriously considered for fear of transmission to the offspring.

Lower quality spermatozoa has been reported in pre-freeze and post-thaw samples in men with Hodgkin’s lymphoma [44,45]; however, recent advances in the field of ARTs have enabled the retrieval and storage of sperm from all cancer groups [46]. Sperm retrieval may even be attempted in azoospermic patients. TESE, in this case termed “Onco-tese” may be successfully performed, and the retrieved sperm may be cryopreserved for future use [47]. The effect of cryodamage on spermatozoa from patients with cancer is similar to that of normal donors [48,49], and so the percentage decline in semen quality from pre-freeze to post-thaw is comparable in cancer patients.

Sperm cryopreservation is considered to be a feasible option for fertility preservation,
even in cases where pregnancy is sought after prolonged durations. Thawed sperm combined with ARTs resulted in live births following long-term semen banking prior to chemotherapy and radiotherapy for Hodgkin’s disease [50]. However, it has been reported that only a small percentage of patients (<10%) who bank their spermatozoa before chemotherapy or radiotherapy return for assisted reproduction [51–53]. The main reasons for this low utilization rate are recovery or waiting for possible resumption of spermatogenesis, short period from original illness, anxiety regarding potential risks for the children, and uncertainty about their long-term health [48].

Testicular tissue harvesting is another possible alternative for fertility preservation, and is a technique that may be of particular importance in prepubertal patients in whom no ejaculated spermatozoa can be cryopreserved. Although prepubertal testes lack mature spermatozoa, they do contain the diploid stem germ cells from which haploid spermatozoa can be derived. Testicular tissue can be harvested from a biopsy and stored either as a tissue section or as isolated germ cells prior to cancer therapy. Following cure and on entering adulthood, this tissue can be thawed and re-implanted into the patient’s own testes to restore natural fertility, a procedure known as germ cell transplantation. Alternatively, the stored stem cells can be matured in vitro until they are able to achieve fertilization via ICSI [54]. Although these two measures have been the subject of intensive research in the last decade, further refinements in the protocols used may still be needed before they can be used routinely in clinical practice [55].

In general, the preservation of fertility using germ cell transplantation raises some concerns. There is a potential risk that the harvested germ cells would contain malignant cells that may reintroduce the disease. Also, the proportion of spermatogonia is low in testicular tissue suspension – estimated at 1/5000 – which may not be enough to reintiate future spermatogenesis [56,57].

In patients who suffer from severe gonadotoxicity that affects the ability of Sertoli cells to support spermatogenesis, in vitro germ cell maturation may be considered. It has been reported that mouse spermatogonia can survive up to 4 months in culture and retain their ability to commence spermatogenesis following transplantation [58]. However, the lack of well standardized methods for in vitro maturation of diploid stem cells into haploid spermatozoa currently limits this option.

Finally, it is important to note that any option for fertility preservation following cancer treatment must be considered in the patient’s best interests. Any procedure must have a sound evidence base, as well as moral provenance, before it can be considered [59]. It is also prudent to consider the fate of the cryopreserved cells in the event of divorce or the patient’s death. It is still a controversial issue whether the cells should be destroyed or if the parents should be allowed to decide their future [60].

Conclusion

Patients with Hodgkin’s lymphoma may suffer from impairment of spermatogenesis, both directly due to the disease itself and indirectly as a result of cytotoxic treatment. The degree and duration of impairment depends mainly on the nature of the
treatment. A variety of measures can be used to minimize the deleterious effects of cytoxic treatment on spermatogenesis. Several methods are currently available for fertility preservation in patients with Hodgkin’s lymphoma. Among them, sperm cryopreservation (particularly before the initiation of therapy) is the most advised method. ARTs have increased the chances of fertility restoration following treatments for malignancy. All men in the reproductive age group diagnosed with Hodgkin’s lymphoma should be counseled regarding the options for preservation of their fertility.

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