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SHORT COMMUNICATION

Ovarian minimal residual disease in chronic myeloid leukaemia

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Abstract The options for fertility preservation include cryopreservation of ovarian tissue. Although transplantation of cryopreserved–thawed ovarian tissue in cancer survivors has resulted in live births, there is evidence of malignancy involvement in ovarian tissue, especially in leukaemia. The objectives of this study were to investigate the involvement of chronic myeloid leukaemia (CML) in ovaries by both pathological/immunohistochemical methods and PCR for the identification of the Philadelphia chromosome (BCR-ABL transcripts). The patient was a survivor of paediatric CML whose ovaries were cryopreserved. The patient became infertile and requested ovarian reimplantation in adulthood. Pathological examinations of ovarian tissue with immunohistochemical stainings, quantitative PCR and two-step nested PCR were applied to identify BCR-ABL transcripts. Despite the lack of positive pathological/immunohistochemical evidence, PCR and two-step nested PCR revealed that the ovary was contaminated by malignant minimal residual CML. Survivors of childhood CML may harbour minimal residual disease in the ovaries. This finding stresses the danger of reseeding cancer by ovarian grafting, especially in patients with leukaemia. If ovarian grafting is considered, reimplantation should be preceded by examination of ovarian samples both pathologically and by molecular techniques. On the basis of molecular findings, ovarian autografting was not recommended in this case report.

KEYWORDS: cancer seeding by ovarian grafting, chronic myeloid leukaemia, ovarian transplantation, pathological/immunohistochemical examination, PCR, Philadelphia chromosome

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Introduction

Chronic myeloid leukaemia (CML) is the most common myeloproliferative disease, although it is relatively rare in paediatric patients (Cotta and Bueso-Ramos, 2007). Originating in abnormal pluripotent bone marrow stem cells, CML usually manifests as a hyperproliferative blood profile and splenomegaly. This includes elevated white blood cells specifically immature cells (blasts) as well as abnormal cells of the myeloid megakaryocyte cell lineages.

Molecular studies have reported a reciprocal chromosomal translocation between chromosomes 9q34 and 22p11 in haematopoietic cells in at least 95% of the patients with CML yielding the Philadelphia chromomsome (Ph; Cotta and Bueso-Ramos, 2007; Ernst and Hochhaus, 2012; Ernst et al., 2011; Kishore and Marin, 2011; Sudoyo and Hardi, 2011). Ph harbours the *Breakpoint Cluster Region* (*BCR*)—*ABL1* (c-*ABL* oncogene) fusion gene which encodes a tyrosine kinase (TK) oncogenic protein. Juxtaposition of the *BCR* domain and the *ABL*'s tyrosine kinase results in deregulated proliferation and apoptosis of haematopoietic progenitors and the clinical and morphologic manifestation of CML (Ernst and Hochhaus, 2012; Kishore and Marin, 2011). *BCR-ABL* transcripts can be rapidly identified by very sensitive techniques such as fluorescence in-situ hybridization or PCR (Ernst et al., 2011; Kishore and Marin, 2011; Saijo, 2012; Sudoyo and Hardi, 2011; Vardiman, 2009), by which the presence of single malignant cells can be detected.

Improvements in anticancer treatment have led to increased survival of patients (Feigin et al., 2008). However, therapy can cause premature ovarian failure in girls and women. The options for fertility preservation are limited, and often the only possibility is cryopreservation of ovarian tissue containing small immature ovarian follicles. So far, autologous transplantation of cryopreserved—thawed ovarian tissue in cancer survivors has resulted in approximately 25 live births (Donnez et al., 2013; Silber, 2012). As far as is known, there is only one report of a leukaemia survivor who underwent ovarian reimplantation (Chung et al., 2013). Indeed, there is increasing although as-yet limited evidence of ovarian involvement in various leukaemic diseases (Chung et al., 2013; Dolmans et al., 2010, 2013; Kyono et al., 2009; Meirow et al., 2008; Rosendahl et al., 2010, 2013).

The aim of this report was to describe a survivor of paediatric CML who requested ovarian grafting for fertility restoration from her frozen—thawed tissue. However, although pathological or immunohistochemical findings were negative, PCR showed evidence of *BCR-ABL1* fusion transcripts in the ovarian tissue. On the basis of these molecular findings, ovarian autografting was not recommended. However, this is a single case, and information from more cases is needed.

Materials and methods

Case description

The study was conducted at a tertiary medical centre. The protocol was approved by the local Human Investigations Committee (reference number 5875, approved 6 July 2010).

The patient was diagnosed with CML by bone marrow biopsy at the age of 12 years (December 1999). Laboratory results included an elevated leukocyte count (486,000/μl) and peripheral blood positive for Ph. She had experienced one menstrual cycle with a single menstrual bleeding 2 months before CML diagnosis. She underwent immediate ovarian cryopreservation for fertility preservation before any form of anticancer therapy was initiated. At surgery, the only finding was an enlarged spleen, but the uterus and ovaries were of normal size and with a few follicles larger than 10 mm. Histological examination of her ovaries did not reveal infiltrations of CML (see Pathological and immunohistochemical evaluation). She was treated with hydroxyurea for 2 months with a decrease of her leukocyte counts (to 5000/μl). In February 2000, she underwent conditioning for bone marrow transplantation with cyclophosphamide (Cytoxan, 120 mg/kg; Bristol-Myers Squibb Co—Mead Johnson and Co, Evansville, IN, USA) and total body irradiation of 1200 centiGrey in six doses with partial lung sparing. She then received bone marrow transplantation from her human leukocyte antigen-identical healthy brother. Post-transplant complications included acute graft versus host (GVH) disease grade II, treated with prednisone at 2 mg/kg/d for six weeks, which eliminated chronic GVH. Her blood count stabilized 14 d after transplantation. Since resolution of acute post-transplantation GVH, she has been well and she continues to have no evidence of *BCR-ABL* transcripts in her peripheral blood. The percentage of Ph-positive cells from 1 year after the transplantation until the time of writing was 3 (false-positive), while PCR in blood remained negative. She continues to be healthy and is checked routinely.

Seven months after the transplantation, she was treated for 7 months with conjugated oestrogens (Premaril 0.625/d; Dexxon, Or Akiva, Israel). Since then she took various forms of oral contraceptives until her wedding at the age of 24. Around that time she stopped the oral contraceptives, became amenorrhoeic, with hot flushes and a single episode of blood spotting. Her hormonal profile included: anti-Müllerian hormone $<$0.4 ng/ml; FSH 103 IU/l and thyroid-stimulating hormone 6.2 μIU/ml. Endometrial thickness measured 2 mm. The dimensions of the right and left ovaries were 27 mm $\times$ 19 mm and 14 mm $\times$ 9 mm, respectively. Upon 17β-oestrogen induction (8 mg/d for nine d), the endometrium thickened to 7 mm.

Histological preparation for light microscopy and immunohistochemistry

The histological preparation method for ovarian tissue has been described in detail previously (Abir et al., 2010). These specimens were examined in the Department of Pathology for leukaemic infiltrates. Tissue samples were immunoassayed using as primary antibodies rabbit polyclonal against human myeloperoxidase (1:3000, A0398; DakoCytomation, Glostrup, Denmark), mouse monoclonal antibodies against glycophorin A (1:400, M0819; DakoCytomation), mouse monoclonal antibodies against CD34 (1:100, Biogenex, Fremont, CA, USA), mouse monoclonal antibodies against CD68 (1: 500, M0876, DakoCytomation), mouse monoclonal antibodies against LCA/CD45 (1:250, M0876; DakoCytomation) and rabbit polyclonal antibodies against human Factor VIII