

## FAST-TRACK

# Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients

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**BACKGROUND:** Storing ovarian tissue for fertility preservation in cancer patients carries the risk of the presence of malignant cells that could lead to recurrence of cancer after reimplantation. Methods to exclude presence of cancer cells were used to improve the safety of cryopreservation–reimplantation procedures. **METHODS:** Fifty-eight patients with hematological malignancies were referred for the storage of ovarian tissue for fertility preservation. Investigation included preoperative imaging and histological evaluation of fresh ovarian tissue. After thawing markers to detect minimal residual disease (MRD) were used and compared with patient's disease used as positive control (five patients). **RESULTS:** Preoperative imaging detected disease in the ovaries (two patients). Conventional histology post-tissue harvesting did not disclose malignant cells (56 patients). MRD results post-thawing were negative in Hodgkin's disease (CD30 immunohistochemical staining), in T- and B-cell lymphoma (PCR for T-cell receptor and Ig clones, respectively) and in two chronic myelogenous leukemia patients (RT–PCR for BCR–ABL gene expression). However, highly sensitive real-time RT–PCR was positive in one CML patient and, this alarming result avoided tissue transplantation. **CONCLUSIONS:** Preoperative imaging prevented operations and storage of tissue with cancer. Evaluation of stored ovarian tissue for MRD using sensitive markers is essential to increase safety and to prevent reimplantation of tissue with malignant cells.

**Keywords:** fertility preservation; ovarian tissue; minimal residual disease; cancer; lymphoma; leukemia

### Introduction

Storing ovarian tissue for future grafting is an investigational method of fertility preservation that has been practiced during the last decade (Gosden *et al.*, 1994; Nugent *et al.*, 1997; Oktay *et al.*, 1998) and has recently proved successful in humans. With ovarian cryobanking, abundant primordial follicles containing small, less differentiated oocytes are efficiently stored. In order to restore fertility, the tissue is thawed and surgically grafted to permit follicular growth and oocyte maturation (Oktay *et al.*, 2000; Callejo *et al.*, 2001).

Although considered experimental, hundreds of cancer patients worldwide facing potentially sterilizing chemotherapy have undergone harvesting and cryopreservation of ovarian tissue (Donnez and Bassil, 1998; Meirow *et al.*, 1998; Lee *et al.*, 2006) and transplantation of stored-thawed ovarian

tissue in cured patients has been reported (Oktay and Karlikaya, 2000; Radford *et al.*, 2001; Oktay *et al.*, 2004). With recent reports of pregnancies (Demeestere *et al.*, 2006; Rosendahl *et al.*, 2006) and deliveries (Donnez *et al.*, 2004; Meirow *et al.*, 2005; Demeestere *et al.*, 2007) after transplantation of thawed ovarian tissue, it is anticipated that in the near future an increasing number of cured cancer patients will request reimplantation of stored ovarian tissue (Donnez *et al.*, 2006). However, in cancer patients, there is a concern of the possible presence of malignant cells in the ovarian tissue that could lead to recurrence of the primary disease after reimplantation of the frozen-thawed ovarian tissue.

In order to increase the safety of ovarian tissue cryopreservation–reimplantation procedures, identification of tumor

involvement in the ovaries and detection of small numbers of cancer cells in ovarian tissue is indicated.

In this study, we investigated a cohort of young women suffering from hematological malignancies who were submitted to store ovarian tissue in order to preserve fertility. Clinical and laboratory workup was used to find malignancy in the ovaries and reduce the risk of storage of tissue with cancer. To minimize the risk of transplantation of ovarian tissue contaminated with small number of malignant cells, methods to detect minimal residual disease (MRD) were used.

## Materials and Methods

The study population consisted of 58 young female patients with hematological malignancies who were referred for the storage of ovarian tissue for fertility preservation. Between 1997 and 2007, cancer patients at two tertiary university hospitals had consultations regarding the sterilization risks of imminent chemotherapy. When indicated the various options for preservation of future fertility were discussed including *in vitro* fertilization techniques and ovarian tissue cryopreservation. It was emphasized that in the future, transplantation of stored-thawed ovarian tissue could be performed to restore fertility and the risk of the possible presence of malignant cells in ovarian tissue was discussed. All patients signed an informed consent prior to ovarian tissue collection, and institutional ethical committee approval was available before transplantation of ovarian tissue.

Preoperative workup included imaging of the pelvis (sonography, CT scan and/or CT/PET) to exclude ovarian pathology and possible pelvic metastasis. Ovarian tissue including 1/2 to 2/3 of the cortex was harvested from only one of the ovaries by laparoscopic procedure. A meticulous inspection to look for malignancy in pelvic organs and abdominal cavity was performed.

Ovarian cortical slices of 5 mm × 10 mm with a thickness of 1–2 mm were prepared and cryopreserved as previously described (Newton *et al.*, 1996; Meirow *et al.*, 2007a,b). An additional smaller piece of cortical tissue 3 mm × 3 mm × 1 mm was separately marked and cryopreserved for future investigation of the possible presence of malignant cells using the methods that will be available at the time of tissue thawing and for quality assessment of freezing and storage conditions for each patient prior to transplantation procedure. In all patients, fresh ovarian tissue cortex (2 mm × 2 mm × 1 mm) and the medulla discarded during preparation for cryopreservation were evaluated for the presence of primordial follicles and malignant cells using multiple histological sections selected randomly.

When ovarian tissue was thawed, investigative studies to detect MRD were performed. These included histological evaluation (H&E staining), immunohistochemical staining for anti-CD30 and anti-Ki67 to detect Reed–Sternberg cells in Hodgkin's lymphoma, and searching for molecular markers in B and T cell types non-Hodgkin's lymphoma (NHL) and in chronic myelogenous leukemia (CML). To detect molecular markers in the ovarian cortex PCR, classical RT–PCR and/or quantitative real-time PCR analysis were performed. Patients' bone marrow samples or involved lymph nodes stored prior to treatment were simultaneously evaluated as positive controls (Faderl *et al.*, 1999; Goldberg *et al.*, 2004).

## Results

Mean age of the 58 patients was  $24 \pm 5.7$  years. Preoperative imaging disclosed ovarian and pelvic masses in two patients. A 33-year-old patient diagnosed with high-grade B-cell NHL was referred after suffering a relapse; however, gynecological

**Table I.** Hematology patients storing cryopreserved ovarian tissue after laparoscopy.

	No. of patients	Age	Chemo. before tissue harvesting	BMT after tissue harvesting
Total	56	$24 \pm 5.5$	33	29
Non-Hodgkin's lymphoma	14	$26 \pm 6.4$	9	4
Acute leukemia	5	$26 \pm 3.1$	5	6
MDS	1			
CML	3	$22 \pm 2.5$	3	3
Hodgkin's disease	33 (11)*	$23 \pm 5.6$	16	16

\*Number of high risk patients (11), stage IV (8), disease below diaphragm (6), pelvic lymph nodes (2). Some patients had more than one feature. MDS, the myelodysplastic syndrome; CML, chronic myeloid leukemia; BMT, bone marrow transplantation.

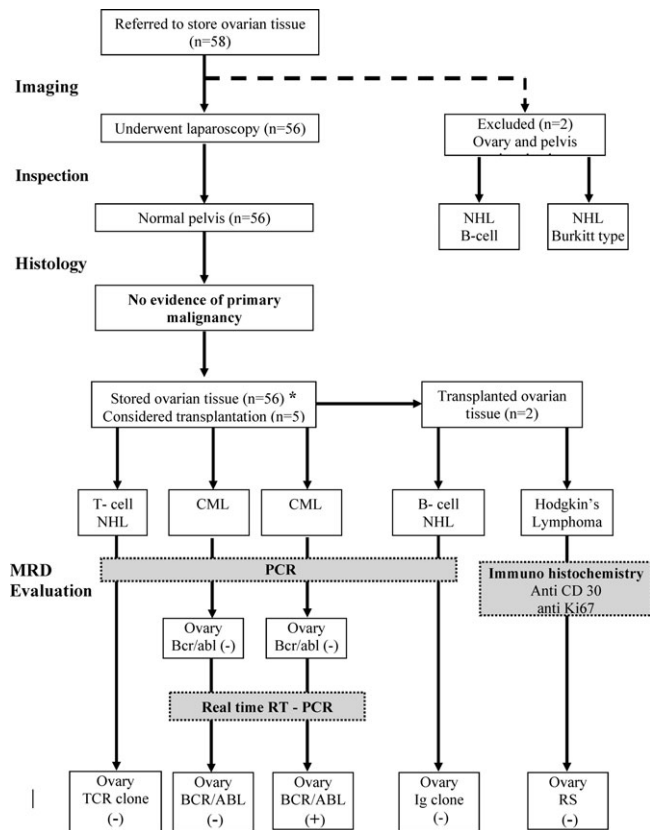
examination and imaging (sonography and PET–CT) identified a large pelvic solid mass involving the ovaries. The second patient was a 14-year-old girl diagnosed with high-grade NHL Burkitt's type, sonography and CT scan showed a large tumor mass in the pelvis and lower abdominal cavity. Ovarian tissue was not collected in either patient.

Imaging of all the other 56 patients who underwent laparoscopy and stored ovarian tissue did not identify disease in the ovary or pelvis and their characteristics are presented in Table I. Direct inspection of the pelvis and abdominal cavity during laparoscopy did not reveal additional organ involvement or tumor masses in any of these patients. Histological sections examined immediately after tissue collection showed that primordial follicles with normal architecture were present in the cortex samples evaluated from all patients including 33 patients who had had previous chemotherapy. The protocol used to increase the safety of storage and transplantation of ovarian tissue in hematological cancer patients is presented (Fig. 1).

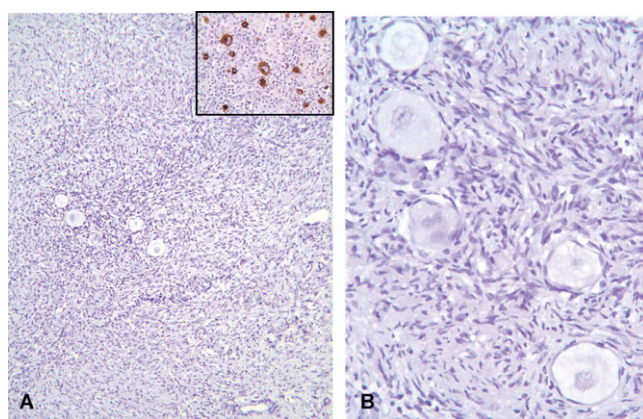
### Hodgkin's lymphoma

Histological sections examined immediately after tissue collection did not identify Reed–Sternberg cells in the ovarian tissue in any Hodgkin's lymphoma patients including 11 women who presented with stage IV disease; disease below the diaphragm or pelvic lymphadenopathy giving these patients a theoretical higher risk of pelvic involvement.

Ovarian tissue from one patient was thawed and tested for MRD. This was a 31-year-old woman suffering from ovarian failure after 6 years of complete remission from Hodgkin's lymphoma. Tissue was harvested prior to high-dose chemotherapy and autologous stem cell transplantation. At the time of ovarian tissue harvesting, the patient had relapsed with disease in the mediastinum with no evidence of disease below the diaphragm. Histology and immunohistochemical staining with anti-CD30 and anti-Ki67 antibodies did not disclose Reed–Sternberg cells in the thawed tissue but staining was positive in patient's mediastinal lymph node removed at the time of diagnosis (Fig. 2a). Primordial follicles with normal architecture were present (Fig. 2b), thus ovarian tissue was thawed and orthotopically transplanted to the ovaries. Two years later, there is no evidence of disease, the



**Figure 1:** The protocol used to detect disease in ovarian tissue stored for fertility preservation in patients with hematological malignancies. Preoperative imaging prevented tissue collection in patients with disease in the ovaries. Ovarian tissue was stored only in patients whose ovaries looked normal at laparoscopy and in whom histology did not disclose malignant cells. Thawed ovarian tissue was evaluated for MRD status in five patients. RS, Reed–Sternberg cells; TCR, T-cell receptor



**Figure 2:** Evaluation of thawed ovarian tissue that was stored for 6 years from a 31 years old patient with Hodgkin's disease. (A) Immunohistochemical staining with CD 30 did not disclose Reed–Sternberg cells in the thawed ovarian tissue (original magnification  $\times 40$ ). Insert—Patient's mediastinal lymph node removed at diagnosis was used as positive control indicating the presence of Reed–Sternberg cells (original magnification  $\times 400$ ). (B) Histology indicating the presence of primordial follicles with normal architecture (original magnification  $\times 400$ )

ovaries are not functioning at this stage and more tissue is available for future transplantation.

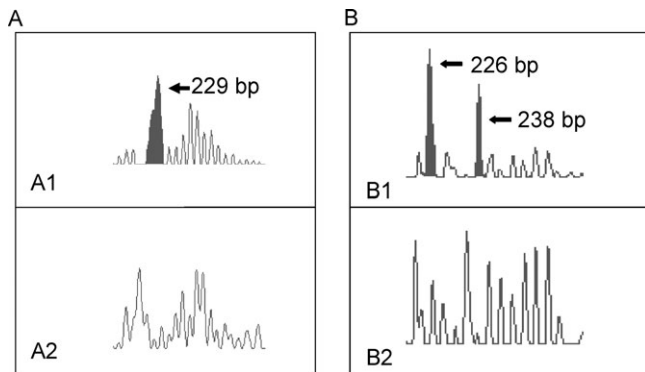
### Non-Hodgkin's lymphoma

In all 16 NHL patients, histological evaluation was negative for lymphoma cells in the ovarian samples examined. NHL is a group of clonal diseases, all the malignant cells in B-cell lymphoma have the same immunoglobulin gene rearrangement. In T-cell lymphoma, the malignant clone carries the same T-cell receptor gene rearrangement. T- and B-cell rearrangement can be detected using PCR techniques, and this method can serve for the detection of minimal residual lymphoma in tissues.

In two patients, we had thawed ovarian tissue that was available for evaluation.

The first patient was a 20-year-old unmarried woman who was diagnosed with NHL stage IV peripheral T-cell. The patient was treated with six cycles of CHOP (cyclophosphamide, adriamycin, vincristine and prednisone). A relapse was detected 3 months after completion of therapy, thus second line combination chemotherapy of MINE/ESHAP (mitoxantrone, ifosfamide, mesna, etoposide, cytarabine, cisplatin and prednisone) was administered followed by high-dose chemotherapy and stem cell transplantation. Prior to high-dose chemotherapy ovarian tissue was harvested, histological evaluation was negative for the presence of malignant cells and the tissue was cryopreserved. In the lymphoma involved lymph nodes and bone marrow, clonal T-cell receptor rearrangement was detected. We have used this rearrangement as a molecular marker that enables the detection of MRD in thawed ovarian tissue. The T-cell receptor PCR was negative, thus increasing the safety of reimplantation (Fig. 3A).

The second patient was a 31-year-old woman who had suffered from primary mediastinal B-cell NHL. At the time of ovarian tissue transplantation, conventional histology did not disclose malignant cells but molecular markers to detect the lymphoma cells were not available. On the basis of clinical judgment, the risk of ovarian involvement was low. More than 3 years post-transplantation, there is no evidence of disease recurrence. The transplant produced a mature oocyte, the patient conceived and delivered a healthy baby (Meirow *et al.*, 2005), and at present, the transplant is inconsistently functioning. Recently, we were able to look for MRD in ovarian tissue for this patient using PCR technique. Paraffin embedded ovarian tissue removed during tissue harvesting was compared with paraffin embedded mediastinal lymph node with NHL cells removed at diagnosis by spectratyping of the repertoire of the rearranged Ig genes using DNA extracted from the tissues. The FR2 primer and a mixture of JH primers were used for the PCR. A clonal pattern was present as two dominant peaks at 226 and 238 bp in the involved lymph node but was absent in the ovarian tissue (Fig. 3B). The results indicated the presence of lymphoma cells in the lymph node but not in the ovarian tissue and were reassuring both for the safety of the previous ovarian tissue transplantation and for the safety of additional future transplantation if indicated.



**Figure 3:** Molecular analysis by PCR for the presence of clonal cell populations carrying specific gene rearrangements using DNA extracted from tissue with evidence of disease and from ovarian tissue. The Y-axes indicate the relative fluorescence intensities and the X-axes the size (bp) of the PCR products.

(A) A 20-year-old patient diagnosed with T-cell NHL that had stored ovarian tissue. Spectratyping of the repertoire of the rearranged TCR $\gamma$  genes using DNA extracted from bone marrow and ovarian tissue. The V $\gamma$ 9 primer and a mixture of J $\gamma$  primers were used for the PCR. (A1) Bone marrow at diagnosis. A monoclonal pattern is present as a single dominant peak at 229 bp. (A2) Thawed paraffin embedded ovarian tissue. A skewed oligoclonal pattern is shown, at 229 bp peak is absent and at 227 bp present but not dominant. The results show that the T-cell receptor rearrangement indicating T-cell lymphoma cells is not identified in the ovarian tissue but is predominant in the bone marrow used as positive control. (B) A 31-year-old patient diagnosed with B-cell NHL that had stored ovarian tissue, underwent successful transplantation of ovarian tissue and recently was evaluated for MRD (see text). Spectratyping of the repertoire of the rearranged Ig genes using DNA extracted from lymph node and ovarian tissue. The FR2 primer and a mixture of JH primers were used for the PCR. (B1) Paraffin embedded mediastinal lymph node removed at diagnosis. A clonal pattern is present as two dominant peaks at 226 and 238 bp. (B2) Paraffin embedded ovarian tissue. A skewed oligoclonal pattern is shown with the 226 and 238 bp peaks absent. The results of the reaction show that the lymph node carries predominantly the clonal Ig gene rearrangement indicating the presence of lymphoma cells which is not present in the ovarian tissue.

### Chronic myelogenous leukemia

Nine patients diagnosed with leukemia had cryopreserved ovarian tissue—three had CML (chronic myeloid leukemia), five AML (acute myelocytic leukemia) and one patient MDS (myelodysplastic syndrome). With conventional histology at the time of tissue collection, leukemic infiltrates, perivascular aggregates or diffuse spreading in the stroma or the cortex were not found in the ovarian tissues of patients with leukemia.

In two patients with CML, ovarian tissue was thawed and evaluated for the presence of malignant cells. CML is characterized by the Philadelphia chromosome which is the result of reciprocal translocation t(9;22) which brings the ABL gene from chromosome 9 together with BCR gene from chromosome 22 creating a fusion gene designated BCR–ABL. The tumor specific breakpoint cluster region–proto-oncogene tyrosine protein kinase ABL1 (BCR–ABL) transcripts can be detected using RT–PCR. We used this method to look for MRD in our CML patients.

A 20-year-old unmarried female was diagnosed with CML. RT–PCR was positive for the BCR–ABL mRNA in the bone marrow. Ovarian tissue was cryopreserved prior to

ablative chemotherapy and allogeneic bone marrow transplantation. Histology did not disclose the presence of hematopoietic tissue in the ovary. RT–PCR performed shortly after tissue collection indicated that there was no evidence of BCR–ABL mRNA product in the ovarian tissue in contrast with the patient's bone marrow that was positive for the translocation (Fig. 4a). After bone marrow transplantation, the patient remained disease free, but suffered from persistent ovarian failure. Five years post-transplantation the patient was married and asked to auto-transplant the stored ovarian tissue in an attempt to restore fertility. The test fragment of ovarian tissue was then thawed and evaluated for the presence of MRD using a more sensitive quantitative real-time RT–PCR that was not available at the time of tissue storage. The results were positive for BCR–ABL transcript indicating the presence of CML cells in the tissue. Re-examination by quantitative real-time RT–PCR of the stored mRNA extracted from ovarian tissue immediately after collection was positive using the more sensitive test (Fig. 4b). At this stage, it was decided not to thaw and transplant the stored ovarian tissue.

The second patient was diagnosed with CML at the age of 23. After blast crisis, she was treated with combination chemotherapy followed by high-dose chemotherapy and bone marrow transplantation. During remission before high-dose chemotherapy ovarian tissue was collected and cryopreserved. Classical RT–PCR was negative for BCR–ABL in ovarian tissue. Recent evaluation of mRNA extracted from patient's ovarian tissue and from the bone marrow by quantitative real-time RT–PCR for BCR–ABL transcript was positive in the bone marrow but negative in the ovary (Fig. 5).

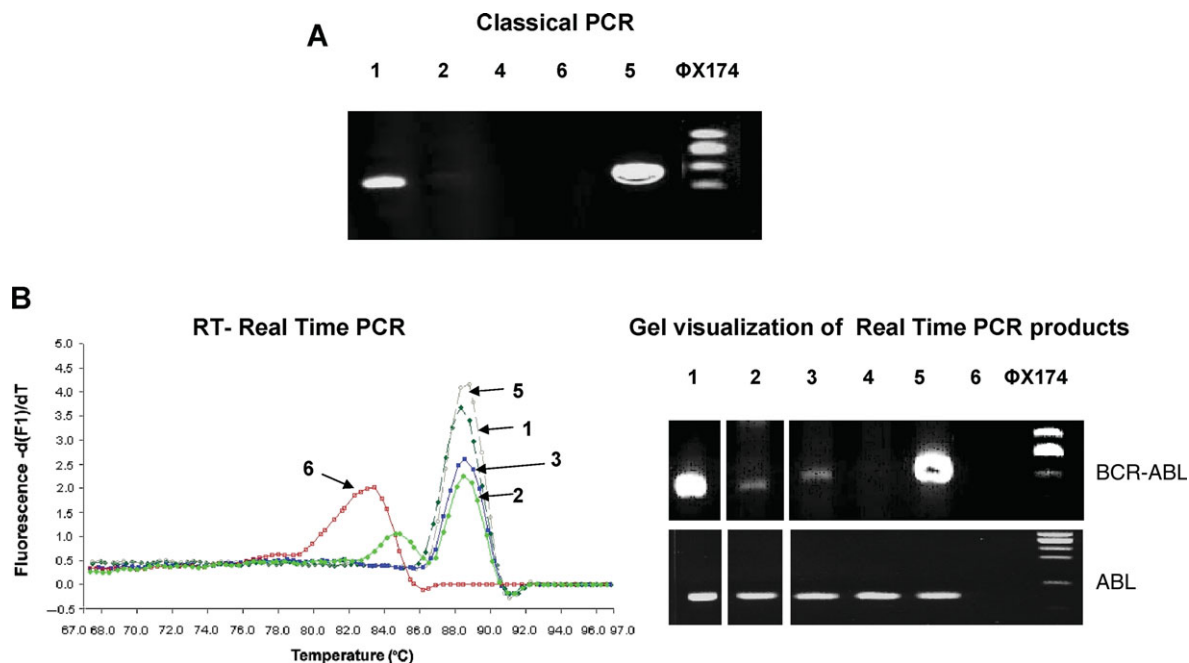
At this stage, none of our AML patients have requested to autotransplant the stored ovarian tissue.

### Discussion

This study of patients with hematological malignancies referred for fertility preservation describes a protocol used to reduce the hazard of harvesting of tissue contaminated by tumor cells and the testing for molecular markers for the detection of MRD in cryopreserved ovarian tissue.

Imaging (sonography, CT scan and/or CT/PET) before ovarian tissue collection revealed macroscopic ovarian pathology related to the primary disease and prevented unnecessary operations. The sensitivity of negative imaging was high as there was no case in which the disease in the ovaries or pelvis was found either during laparoscopy or with histological evaluation at the time of ovarian tissue harvesting. Nevertheless, following the steps of the protocol are important to increase the safety of the procedure.

In all Hodgkin's lymphoma patients including those with stage IV disease, histological evaluation did not find Reed–Sternberg cells in ovarian tissue. Immunohistochemical staining for the detection of Hodgkin's cells in thawed ovarian tissue was also negative and increased the safety of permissible ovarian tissue transplantation. In general, Hodgkin's disease in the ovary is extremely rare (Khan *et al.*, 1986) and probably results from lymphoid tissue that is present at the hilum of the ovary but not in ovarian cortex which is the transplanted



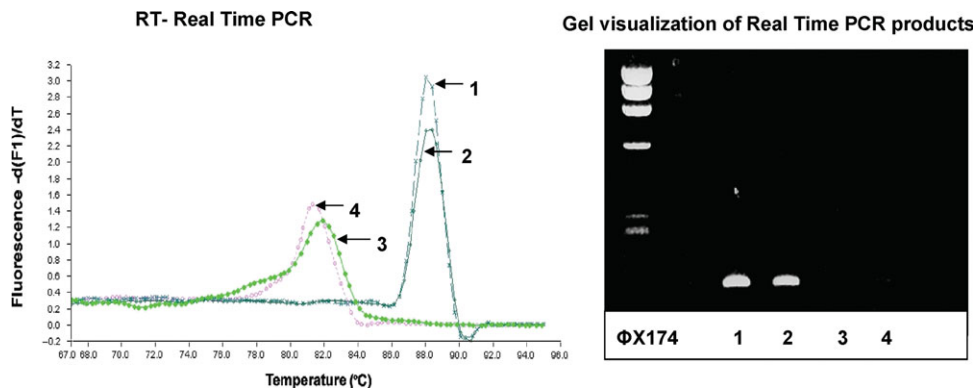
**Figure 4:** RT-PCR for the BCR-ABL mRNA indicating the presence of the Philadelphia chromosome was used as tumor marker and evaluated in a 20-year-old female diagnosed with CML (1999).

Ovarian tissue was cryopreserved prior to ablative chemotherapy and allogeneic bone marrow transplantation. Cryopreserved-thawed ovarian tissue and bone marrow stored prior to chemotherapy were evaluated simultaneously. (A) First classical RT-PCR for BCR-ABL transcript was performed shortly after tissue harvesting, when real-time PCR was not introduced in our laboratory. The results indicate that the bone marrow was involved while there was no evidence of BCR-ABL transcript in the ovarian tissue sent for evaluation. (B) Real-time RT-PCR analysis for the detection of the BCR-ABL transcript performed 5 years post-tissue harvesting when the patient asked to auto-transplant the stored ovarian tissue. Thawed fragment of ovarian tissue was evaluated for presence of MRD using a more sensitive real-time RT-PCR that was not available at the time of tissue storage. The results were positive for BCR-ABL transcript indicating the presence of CML cells in the tissue. Re-examinations by real-time RT-PCR of the mRNA products extracted for the first evaluation of ovarian tissue were now positive. 1, bone marrow; 2, ovarian biopsy mRNA prepared at 1999; 3, ovarian biopsy mRNA prepared at 2004; 4, negative sample; 5, positive control cells with b3a2 translocation; 6, without DNA, no template controls,  $\Phi$ X, HAE III digested DNA size marker

tissue. Previous studies using immunohistochemical staining for Reed-Sternberg cells (Seshadri *et al.*, 2006) and xeno-transplantation into SCID mice (Kim *et al.*, 2001) of cryopreserved-thawed ovarian tissue from patients suffering from Hodgkin's lymphoma did not disclose the presence of Hodgkin's lymphoma in the ovaries. To date, there are no molecular markers for Hodgkin's lymphoma. However, using

the tests described, it appears that there is no increased risk of transferring Hodgkin's cells with ovarian tissue transplantation.

In patients with NHL histological involvement of the ovary is rare, except for those with Burkitt's type, which more commonly involves both ovaries (Osborne and Robboy, 1983). However, in 2 of the 58 patients, the ovaries were macroscopically involved. NHL might be diagnosed during the leukemic phase, and in this



**Figure 5:** Real-time RT-PCR analysis for the detection of the BCR-ABL transcript in a 23-year-old patient who was diagnosed with CML and ovarian tissue was harvested prior to high-dose chemotherapy (1998).

Evaluation (2007) of patient's mRNA extracted from both ovarian tissue and from the bone marrow was positive in the bone marrow but negative in the ovary as were the results of classical PCR analysis performed shortly after tissue collection. 1, positive control; 2, bone marrow mRNA from 1998; 3, ovarian biopsy; 4, no template control;  $\Phi$ X174, DNA size marker

scenario as well as in other leukemias (CML, CLL, ALL, AML), the ovaries might be involved. Leukemic infiltration of the ovaries may be submicroscopic and undetectable.

Specific and sensitive (Gabert *et al.*, 2003) methods for MRD monitoring have been developed over the past years. Currently available methods include PCR amplification of fusion transcripts, rearranged immunoglobulin or T-cell receptor genes and flow cytometric detection of aberrant immunophenotypes. Chromosome aberrations provide tumor-specific PCR targets for MRD detection that are tumor-specific, provide reliable estimates of residual malignant cells and are currently used as markers of molecular response to treatment during clinical remission.

In this study, a few of these methods were tested on thawed ovarian tissue. We studied the T-cell receptor as well as the immunoglobulin gene rearrangement in lymphoid tumors and we studied the BCR–ABL gene which is the hallmark of CML (two patients). In these cases, PCR products were present in the bone marrow but not in ovarian tissue. The sensitivity of tumor markers to identify MRD is high, thus significantly increasing the safety of ovarian tissue transplantation.

Evaluation for MRD in one of the CML patients at different periods using different methods with different sensitivities that were available at the time of evaluation changed the results of the test from negative by classical PCR to positive when quantitative real-time RT–PCR was used. The differences in the test results were due to different method sensitivity and not due to clustering of CML cells in the ovary. The identification of MRD in the bone marrow usually indicates the institution of anti-CML therapy, either by using donor lymphocyte infusion (DLI) or by starting treatment with Imatinib Mesylate (Hughes *et al.*, 2006). However, the clinical significance of MRD demonstration in the ovaries is as yet unknown. It remains questionable whether a positive RT–PCR test should be the threshold for safety of reimplantation of ovarian tissue. In view of our limited knowledge about the clinical significance of this finding, we had uncertainties about this patient who eventually decided not to use the stored ovarian tissue at this stage.

Should molecular studies be performed at the time of tissue harvesting or close to transplantation? As ovarian tissue is stored for many years (for some patients more than 10 years) investigation for MRD prior to transplantation is recommended since new and more sensitive methods are constantly introduced as indicated with the CML patient. In addition, MRD evaluation is expensive, laborious and many of the patients who undergo ovarian tissue storage will not ask to transplant the stored tissue. Thus, for all patients with cancer, a separate vial with samples of ovarian tissue should be stored for final evaluation just prior to ovarian tissue reimplantation (Meirow *et al.*, 2007a,b). Tumor markers do not always exist at the time of transplantation; therefore, in such cases, we should rely on clinical judgment taking into account the type of malignancy, staging and whether remission was induced prior to ovarian tissue collection.

For some malignancies, especially solid tumors, patchy distribution of malignant cells is common and negative evaluation may not rule out the presence of malignant cells in

cryopreserved ovarian tissue. In these cases, other strategies of fertility preservation that do not carry the possible risk of transmitting cancer cells should be considered such as *in vitro* fertilization and embryo (Oktay *et al.*, 2006) or oocyte (Lee *et al.*, 2006) cryopreservation. While preparing ovarian tissue for freezing, attempts should be made to collect immature oocytes from the tissue. These eggs can be matured *in vitro* fertilized and cryopreserved (Revel *et al.*, 2004). If cancer cells are detected in stored ovarian tissue the cryopreserved embryos can be used to restore fertility.

Cryopreservation of testicular tissue or spermatogonia in boys prior to administration of potentially sterilizing cancer treatment is in the early phases of experimentation (Orwig and Schlatt, 2005; Wyns *et al.*, 2007). The same methods and dilemmas presented for ovarian tissue will be applicable for storing testicular tissue if the procedure is to be used in the future.

In summary, the interest in ovarian tissue storage as a real option for preserving fertility in cancer patients has increased. However, genuine concerns regarding the possible recrudescence of the primary disease following reimplantation of stored ovarian tissue with malignant cells exists. To increase the safety of the procedure, methods to detect disease in the ovaries and malignant cells in stored tissue are indicated. The clinical significance of MRD in general and MRD in stored ovarian tissue in particular needs further investigation.

### Author's Role

Dror Meirow—Principal Investigator, treated the patients, conducted the study, wrote the manuscript.

Izhar Hardan MD, Arnon Nagler MD—hematology, treated the patients, evaluated disease spread performed tests to detect MRD and disease status.

Jehoshua Dor, head of Sheba IVF unit, treated the patients, conducted the study. Shai Elizur, Hila Ra'anani evaluated and treated the patients, collected the data.

Eyal Schiff, Head of Obstet Gynecol Sheba—evaluation of results, research meeting whether yes/no transplant the tissue. Participated in writing.

Eduard Fridman MD—pathology evaluated ovarian histology.

Elana Slyusarevsky MsC, Hematology lab—Hadassah medical center, developed the markers and evaluated the tissues for MRD.

Ninette Amariglio PhD, Gideon Rechavi MD, PhD—Sheba cancer research developed the markers for detection and performed the PCR. Evaluated the results.

Dina Ben Yehuda—Principal Investigator, treated the patients, laboratory investigation, participated in writing.

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