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A review: the essentials of informed consent for cancer patients before autologous transplantation of ovarian tissue

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Abstract: Purpose: To highlight the imperative of informed consent in the fertility preservation of cancer patients before ovarian tissue autotransplantation. Methods: Three papers of tumor recurrence after autotransplantation of frozen-thawed ovarian tissue were compared with the main papers before tumor recurrence was reported in the cancer patients. Results: Histology was performed before autotransplantation in cases 1 and 3, but not in case 2. Histology alone is insufficient for the detection of minimal residual disease (MRD). Furthermore, transplanted ovarian tissue is different from that examined for MRD detection, and how much of the resected ovarian tissue was examined for MRD detection is unclear. The possibility that grafted tissue caused tumor recurrence cannot be ruled out in any of the three cases, because autotransplantation in cancer patients, at present, uses ovarian tissue different from that examined for MRD. Conclusions: We recommend informed consent for cancer patients, because: 1) the transplanted ovarian tissue is different from the ovarian tissue examined for MRD detection; 2) the amount of resected ovarian tissue analyzed for MRD is very small; and 3) MRD detection methods vary. In conclusion, freezing and storage of ovaries should be encouraged, transplantation must be performed carefully, and informed consent is essential.

Key words: Minimal residual disease, Transplantation, Fertility preservation, Cancer, Recurrence

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Introduction

At the World Congress on Fertility Preservation (2009) [1], we asserted that it is important to "actively preserve fertility, but autotransplant cautiously," based on an analysis of autopsy findings of 5,571 females under the age of 40 in Japan [2].

Recently, more than 60 babies have been born after transplantation of frozen-thawed ovarian tissue (FTOT) [3]. However, Minimal Residual Disease (MRD) is a significant problem in FTOT for cancer patients. The International Society of Fertility Preservation (ISFP) Practice Committee has recommended FTOT for patients with lymphoma, leukemia, and breast cancer, for fertility preservation (FP) [4]. Rosendahl *et al.* (2013) [5] reported MRD in 7% (31/422) of cases of ovarian tissue cryopreservation; 2/58 in imaging, 1/367 in histology, 1/220 in immunohistochemistry, 21/43 in PCR, and 5/101 in human-animal (human ovarian tissue transplanted into immuno-deficient mice experiments). But in 33 clinical cases of FTOT, no recurrence occurred until June 2012.

PCR results were positive in 20/33 of leukemia cases and 1/8 of Ewing sarcoma cases. PCR is a very sensitive assay, especially in case of leukemia; however, patients shown to be positive by PCR were all negative histologically. Dr. CY Andersen and Dr. DA Gook suggested the possibility of recurrence from reimplanted ovarian tissue in a personal communication at the end of April 2015.

We reviewed three recent papers about tumor recurrence after transplantation of FTOT and compared them with six previous papers on successful births after transplantation of FTOT in cancer patients (Table 1).

Table 1 Summary of three cases with recurrence and primary articles of birth following autotransplantation in patients with malignancy of frozen-thawed ovarian tissue

Author/year[a]	Disease age[b] (years)	MRD[c] exam.	Excision method[d] Cryo. volume[e]	MRD exam. volume[f] Autotransplanted volume[g]	Explanation: MRD exam. and Graft[h]	Recurrence[i]
Stern, C.J., *et al.* (2013, 2014) (Case 1)	Granulosa cell tumor 25	Histology	One entire ovary removed 15 slices	No[j] 60+30 slices 1×2×4 mm	No	cannot be excluded with 100% certainty
Ernst, E.H., *et al.* (2013) (Case 2)	Breast cancer 36	No exam.	One entire ovary removed 29 pieces	0 (Zero) 12 pieces	No	most unlikely to have been related to the transplantation
Andersen, C.Y., *et al.* (2014) (Case 3)	Ewing sarcoma 9	Histology	One entire ovary removed Ten pieces	No (80 examined after death) 20	No	cannot be excluded with 100% certainty
Donnez, J. (2004)	Hodgkin 25	Histology	Five biopsies from one ovary 12–15×5 mm 70 cubes from 4 biopsies	No One strip of 12×4 mm and 35 cubes of 2×2 mm and remaining 32 cubes 60/40	No	No
Meirow, D. (2005)	Non-Hodgkin 28	Histology	Partial resection Three strips and small fragments	No 100	No	No
Demeestere, I. (2007)	Hodgkin 24	Histology Three pieces for MRD and follicular density	One entire ovary removed 40 pieces	Three fragments/ ? + four pieces + two fragments	No	No
Andersen, C.Y. (2008)	Hodgkin 25 Ewing Sarcoma 27	Histology One small biopsy histology	One entire ovary removed 5×5×1–2 mm	Yes One small biopsy 50/50 50	No	No
Sanches-Serano, M. (2010)	Breast cancer 36	No	One ovarian cortex extraction No	No	No	No
Dittrich, R. (2012)	Hodgkin 25	Histology; Only follicular density was written	Partial resection (2/3) from Both ovaries	No Six fragments (1–2 mm)	No	No

a) Year published; b) Age (years) at cryopreservation; c) Method of detection of Minimal Residual Disease (MRD); d) Methods of removal of ovarian tissue; e) Cryopreservation volume; f) Proportion of tissue used for detection of MRD (%); g) Proportion of tissue autotransplanted (%); h) Explanations of different tissues for MRD detection and graft ; i) Possibility of reimplantation of malignant cells after graft; j) No=not reported.

Methods

We compared three papers of tumor recurrence after autotransplantation of frozen-thawed ovarian with the six papers published before tumor recurrence was reported in the cancer patients.

Results

Case 1: Granulosa tumor

A delivery of twins following heterotopic grafting of FTOT was reported [6, 7]. One entire ovary was removed and histology was normal before cryopreservation and before FTOT.

The laparoscopic grafting procedure (a total of 90 slices) was performed twice.

There was macroscopic evidence of a tumor at the site of Cesarean section, but there was no evidence of a tumor in the graft sites. Consequently, the possibility that tumor recurrence resulted from the grafted tissue cannot be excluded with 100% certainty.

Case 2: Breast cancer

The legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue was performed due to cancer recurrence [8]. The patient's left ovary was excised by laparoscopy, and 29 pieces of ovarian tissue were cryopreserved. No histological assessment of the tissue was performed prior to cryopreservation, nor was it transplanted to immunodeficient mice. Twelve pieces of frozen-thawed ovarian tissue were transplanted. Six months after transplantation, the patient conceived naturally. However, during the first trimester of pregnancy the patient was diagnosed with a new left-side breast cancer in the same quadrant as the breast cancer of 8 years earlier. The recurrence was most unlikely to have been related to the transplantation.

Case 3: Ewing sarcoma [9]

A patient with Ewing sarcoma died at 18 years of age due to relapse 6 years after FTOT. She had had 10 pieces of one of her ovaries frozen at 9 years of age. Histological examination confirmed normal conditions. Two of ten pieces of ovarian cortex were grafted at 13 years of age. The eight pieces of ovarian tissue were thawed and examined for the EWS/FL1 translocation after death. These data indicate that the ovarian tissue did not contain malignant cells. However, since the two originally transplanted ovarian tissue pieces of cortex were not analysed for the EWS/FL1 translocation before transplantation of FTOT, the possibility cannot be excluded with 100% certainty.

Other cases: Hodgkin disease, non-Hodgkin lymphoma, Ewing sarcoma and breast cancer [10–15]

Dr. CY Andersen [13] reported their clear policy for ovarian tissue cryopreservation in detail. All patients had one entire ovary removed laparoscopically. The cortex was isolated, cut into fragments of 5 × 5×1 mm^3 and cryopreserved by the slow freezing method. One small biopsy was taken for histological evaluation. The numbers of fragments cryopreserved from the six patients were 34, 29, 20, 20, 13 and 19.

Histology was examined performed on samples from 9/10 patients and reported in 8/9 papers. The amount of resected ovarian tissue analyzed for MRD is unclear. The explanation that the transplanted ovarian tissue was different from the ovarian tissue examined for MRD detection was not reported at all. The only MRD detection method used was histology.

Discussion

Histology was performed before autotransplantation in cases 1 and 3, but not in case 2.

Rosendahl *et al.* (2013) [5] reported MRD in 7% (31/422) of cases of ovarian tissue cryopreservation, and the MRD detection rate was very low (1/367) in histology. Histology alone is insufficient for diagnosis of MRD. Fundamentally, the transplanted ovarian tissue is different from the ovarian tissue examined for detection of MRD. So the possibility that tumor recurrence resulted from the grafted tissue cannot be excluded with 100% certainty. In the near future, it may be possible to autotransplant ovarian tissue with only normal cells after killing cancer cells by Boron Neutron Capture Therapy (BNCT) [16]. It may also be possible to obtain metaphase II oocytes following *in vitro* maturation of germinal vesicle oocytes from resected fresh ovarian tissue or following *in vitro* culture of primordial follicles from frozen-thawed ovarian tissue in the near future. At present, the amount of the resected ovarian tissue examined for the detection of MRD is probably less than 5%. We need to determine what kind of examinations should be performed for the detection of MRD, and how much they cost. We must also consider the balance of effects and costs. In conclusion, it is extreamly important that informed consent be obtained from cancer patients, because: 1) the transplanted ovarian tissue is different from the ovarian tissue examined for MRD detection; 2) the amount of resected ovarian tissue analyzed for MRD is very small; and 3) MRD detection methods vary. Freezing and storage of ovarian tissue should be encouraged, transplantation must be performed carefully, and informed consent is essential.

We hope for the development of MRD detection methods and much more useful data for patients and medical staff.

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